ACHEMS - 1993

ABSTRACTS

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of the
Association for Chemoreception Sciences

ABSTRACTS

This book contains abstracts of the volunteer papers and posters of ACHEMS 1993. 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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1

Differential Effects of Topical Anesthesia on Taste Qualities

F. CATALANOTTO, P. HOVLIARAS*, Y. LECADRE (New Jersey Dental School) AND L. BARTOSHUK (Yale University)

We investigated effects of topical anesthesia on taste. Following a 1 minute rinse with 5 cc's of 1% dyclonine, 21 subjects were given a random order sequence of taste stimuli applied to the tongue tip with a cotton swab. Subjects provided suprathreshold scaling, magnitude estimates of the intensity of solutions of NaCl(.10M, .32M); sucrose(S)(.10M, .32M), citric acid (CA)(.0032M, .010M); and quinine HCl(Q)(.001M, .00032M). Subjects were tested with 2 or 5 minute intervals between sets of stimuli on 2 separate days. A "0" intensity estimate was interpreted as onset of anesthesia; all subjects gave "0" responses to all taste stimuli immediately after the anesthetic rinse. Any response other than "0" was interpreted as recovery from anesthesia; recovery started 2 minutes after anesthesia rinse. ANOVA showed that time courses of recovery from anesthesia were different for the 4 stimuli ($F(10\text{-}3)=23.783$; $p<.00001$). Follow-up Newman-Keuls tests ($p<.05$) revealed that recovery for Q was slower than other three stimuli and recoveries for NaCl and CA were slower than S. Results suggest that some taste qualities are differentially affected by topical anesthetics. This model might provide an excellent method to probe quality coding of taste.

Supported by the Foundation of UMDNJ and the NJDS Alumni Association

2

Differential Loss of Sensitivity to Bitter Compounds in Aging.

YOSHIKO YOKOMUKAI[1,2], BEVERLY J. COWART[1] & GARY K. BEAUCHAMP[1] ([1]Monell Chemical Senses Center; [2]Kirin Brewery Co., Ltd.).

We previously demonstrated individual differences in sensitivity to the bitterness of quinine sulfate (QS) and urea (U) among young adults (AChemS, 1991). In a sample of 52 young subjects, 36% judged selected suprathreshold concentrations of these compounds (see below) to be equally bitter, 33% found QS to be more bitter than U and 31% found U more bitter than QS. These differences were highly reliable and related to threshold sensitivity to QS. In the present study, 50 elderly adults (>65 years) also used a 13-point category scale to rate the perceived bitterness of intermixed series of U (0.06-0.30 M) and QS (0.002-0.015 mM). In contrast to young subjects, only 2% of the elderly found QS to be more bitter than U; 48% found U to be more bitter than Q, and 48% found the two compounds to be equally bitter. Direct comparisons of the ratings assigned by the young and elderly indicate there was no substantial difference between the groups in their judgments of the bitterness of U, whereas the elderly found higher concentrations of QS to be significantly less bitter than did the young. Parallel results were observed in measures of absolute sensitivity to U and QS. Specifically, U detection thresholds were comparable in young and elderly subjects, but the elderly had significantly higher thresholds for QS than did the young. Age-related loss of sensitivity to the bitterness of QS, at both threshold and suprathreshold levels, has been reported previously (e.g., Cowart, Ann. NY Acad. Sci. 561: 39-55, 1989). However, the present data indicate that this loss does not extend to all bitter compounds, and that sensitivity to U may be relatively stable across the adult life span. The basis for this phenomenon is not known, but it may reflect differential effects of aging on different bitter transduction sequences.

Supported by NIH DC-00882 and Kirin Brewery Co.

3

Influence of Stimulus Area, Subject Age and Tongue Region on a Nonparametric Signal Detection Measure of Taste Sensitivity to NaCl.

TOSHI MATSUDA & RICHARD L. DOTY (Smell and Taste Center & Dept. of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA)

Twelve young (20 to 29 years) and 12 elderly (70 to 79 years) subjects (half of each sex) were tested for their sensitivity to three concentrations of NaCl (0.01M, 0.1M & 1.0M) on two localized regions of the tongue using a signal detection paradigm and a microprocessor-controlled gustometer. Stimulus duration was 2 seconds and the tongue areas stimulated were 12.5 mm^2, 25 mm^2, and 50 mm^2. The tastants were presented via a glass stimulation device held to the tongue by a mild suction surround, and were focused at two loci: the right tongue tip and a region 3.0 cm posterior to the tongue tip 1.5 cm to the right of the midline. Overall, the young subjects evidenced greater sensitivity to all three concentrations of the NaCl at both loci, as determined by the R-index, a nonparametric signal detection measure of sensitivity. The young, but not the elderly, were more sensitive to stimulation presented to the tip of the tongue than to that presented to the more posterior region. In the young, but not the elderly, detection performance improved as a function of the size of the tongue area stimulated, although this relationship was observed only at the posterior site. No sex differences in sensitivity at either site were found. These data indicate that (i) older persons have a significant decrement in the ability to taste NaCl solutions presented to localized regions of the tongue and (ii) functional relations between the size of the tongue region stimulated and taste sensitivity to NaCl depend upon both the subject's age and the region of the tongue stimulated.

Supported by NIDCD Grant PO 00161.

4

Documenting Taste Deficits Resulting from Rinsing with Chlorhexidine Gluconate in Humans.

MARION E. FRANK, MARY ANNE DELLA-FERA, JILL HELMS and APRIL E. MOTT (University of Connecticut Health Center, Farmington, CT)

Chlorhexidine gluconate is used to control bacteria in the oral cavity. Daily rinses with a chlorhexidine solution result in transient losses in tasting NaCl (Lang et al., 1988). Validity of the whole-mouth taste test (Bartoshuk, 1989) used by the Connecticut Chemosensory Clinical Research Center (CCCRC) and the potential chlorhexidine has for treating salty dysgeusias were addressed by studying the chlorhexidine effect. The taste test was given to 16 healthy subjects, who rinsed with 0.12% chlorhexidine for three minutes twice a day. Each subject was tested three times: the week preceding mouth-rinsing, after four days of rinsing, and 3-6 days after termination of rinsing. Chlorhexidine rinses reduced the perceptual intensity of NaCl and quinine.HCl, not sucrose or citric acid. For example, the mean magnitude estimate (ME) for 0.1M NaCl was 24 before and 6.5 during the rinse period ($p<.001$, t test); the mean ME for 0.1mM quinine.HCl was 22 before and 10 during the rinse period ($p<.01$, t test). In 75% of the cases, experimental MEs for NaCl and quinine.HCl fell below the 15th percentile for normal subjects; only 10% of MEs for citric acid and sucrose were that low. No effects on taste perception were detected 3-6 days after the chlorhexidine rinse was terminated. Besides showing quality-specific alterations in taste intensity, the subjects did not recognize sucrose as sweet, citric acid as sour, or NaCl as salty as frequently while rinsing with chlorhexidine than pre- or post-rinse (chi-square tests, $p<0.05$). We conclude that the CCCRC whole-mouth taste test is sensitive to taste deficits and that chlorhexidine predominantly alters salty taste perceptions but other taste qualities are not completely spared.

5

Development of Olfactory Evoked Potentials for Functional Assessment in the Elderly. CLAIRE MURPHY (San Diego State University and UCSD Medical Center, San Diego, CA), STEVEN NORDIN (UCSD Medical Center and San Diego State University), RENE de WIJK (John B. Pierce Laboratory and San Diego State University), WILLIAM S. CAIN (John B. Pierce Laboratory), and JOHN POLICH (The Scripps Research Institute, La Jolla, CA)

Subject bias, experimenter effects, criterion shifts, and memory problems in older people may limit the interpretation of some psychophysical tasks for functional assessment of olfaction in the elderly clinic patient. The olfactory evoked potential (OEP) shows considerable promise as an objective, non-invasive measure of sensory function that can be applied in clinical settings. Methods similar to those of Kobal were employed to record OEPs in young and elderly subjects. Stimuli were presented olfactometrically in a stream of air heated to 36.5 °C and humidified to 80% RH. Stimulus rise time did not exceed 20 msec. EEG activity was recorded from the Fz, Cz, and Pz electrode positions of the international 10/20 system, amplified, and filtered using a commercial recording system. Electro-ocular activity also was monitored. Using Kobal's technique of velopharyngeal closure, breathing was restricted to the mouth during trials, keeping nasal flow rate constant. Stimuli were applied randomly during breathing. Subjects produced occasional magnitude estimates to monitor odor strength. Latency from stimulus onset as well as base-to-peak and peak-to-peak amplitudes of N1 and P2 of the OEP were assessed. Older subjects displayed longer latencies of N1 and P2 than younger subjects. In addition, consonant with findings of Hummel et al (ECRO, 1992), OEP amplitude varied with concentration of the stimulus and with age of the subject: older subjects demonstrated lower amplitudes of N1, P2, and N1/P2. These data are encouraging for the use of the OEP in studies of aging and as a clinical tool.

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6

Age-Related Changes of Chemosensory Event-Related Potentials after Trigeminal and Olfactory Stimulation

T. HUMMEL, S. BARZ, and G. KOBAL (Dept. of Pharmacology, Univ. of Erlangen-Nürnberg, Erlangen, FRG)

The aim of this study was to investigate whether it is possible to trace age-related changes in the perception of olfactory and trigeminal chemical stimuli by means of chemosensory event-related potentials (CSERP). 3 groups of healthy volunteers participated in the experiments (age 15-34 [group A], 35-54 [B], and 55-74 yrs [C]). Each group was comprised of 8 female and 8 male subjects. Stimulants were chosen to either stimulate fibers of the trigeminal (CO_2) or the olfactory nerve (vanillin, H_2S). Within one session each of the 3 stimuli was applied 16 times to the left nostril (200 ms, interval ~ 40 s). In additional 3 short sessions, olfactory (session 1: standard H_2S, target vanillin; session 2: standard vanillin, target H_2S) and acoustical stimuli (standard 1.5 kHz, target 2 kHz) were presented in an odd-ball-paradigm. The probability of the target stimuli was p=0.16. For olfactory stimuli the interstimulus interval was 6 s, for acoustical stimuli it was 2 s. Moreover, the subjects' ability to discriminate (8 pairs of odorants) and to detect odorants (pyridine, phenyl ethylalcohol) was tested within a triple-forced choice paradigm. Odor identification was investigated by means of 8 odors. CSERP amplitudes in response to CO_2 decreased in an age-related manner, whereas responses to olfactory stimuli sharply decreased for group C. The decrease of P3 amplitudes to olfactory stimuli was also most pronounced for group C. This finding was accompanied by a similar change of the subjects' ability to discriminate odors. In contrast, P3 amplitudes to acoustical stimuli linearly decreased in an age-related manner. Measurements of both, odor identification and thresholds revealed that there was nearly no difference between groups A and B, whereas performance declined for group C. The results establish that the age-related perception of olfactory stimuli changes in a different manner when compared to the perception of trigeminal stimuli. This may be due to differences between the two systems regarding receptor and transduction mechanisms.

7

Assessment of Individual Olfactory Sensitivity in Aging: Role of Threshold Variability.

JOSEPH C. STEVENS (John B. Pierce Laboratory)
AASHISH D. DADARWALA (John B. Pierce Laboratory)

Individual thresholds of elderly persons average one to two orders of magnitude higher than those of young adults, but with overlap of about three orders of magnitude. The present study shows that such overlap results mainly from brief threshold tests that exaggerate individual differences. A better assessment of individual threshold (for 1-butanol) was achieved by averaging 2AFC detection thresholds from two to eight brief tests spread over four or more days. The spread of each group's thresholds (12 young and 12 elderly subjects) narrowed strikingly with increased number of tests averaged up to four; further tests accomplished no more. Based on only one test, thresholds of young and elderly overlapped in the usual way; but based on the averages of four or more tests thresholds overlapped negligibly. Weakened smell sensitivity thus does characterize the individual as well as the average older person.

Supported by NIA Grant AG04287.

8

Odor Detection and Recognition Memory in Alzheimer's Disease.

STEVEN NORDIN (UCSD Medical Center and San Diego State University), CLAIRE MURPHY (San Diego State University and UCSD Medical Center), RANI NIJJAR and CARLO QUINONEZ (San Diego State University)

Absolute odor detectability for n-butyl alcohol, and short-term odor recognition memory for various common household odorants was studied in 65 patients diagnosed with probable Alzheimer's disease (AD) and 82 aged-matched healthy elderly controls. As task comparison modalities, taste thresholds for sucrose were determined as well as visual recognition memory performance for various faces of presidents and vice presidents and for engineering symbols. The detection thresholds were obtained by a two-alternative, forced-choice, ascending procedure. For the recognition tasks, ten stimuli for each modality were initially inspected by the subjects, followed by five old and five new stimuli to be evaluated as either old or new. The results demonstrated significantly elevated odor, but not taste, detection thresholds for the AD patients as compared to the controls. For the AD patients, the odor thresholds correlated significantly with degree of dementia (DRS score), and, interestingly, average annual increase in threshold correlated significantly with average annual progression of dementia (decrease in DRS score). In addition, the AD patients showed a significantly poorer odor recognition ability (proportion correct responses) than the controls, but did so also for the faces and symbols. Due to the normal taste thresholds in AD, the elevated odor thresholds suggest a sensory olfactory decline in AD rather than a difficulty in performing the detection task. One the other hand, the poor odor recognition memory in AD may predominantly be referred to a general memory decline rather than an olfactory decline in specific, since a poor recognition memory was also found for faces and symbols. It was also found that the correlation between degree of dementia (DRS score) and odor recognition memory tends to increase with severity of the disease. The findings imply that the assessment of absolute odor detection sensitivity may be a more valuable tool than odor recognition memory in the diagnosis of Alzheimer's disease, but that odor recognition memory may prove useful in charting its course.

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9

Pre- and Post-Operative Studies of Olfactory Function in Patients with Anterior Temporal Lobectomy. STEVEN E. WEST, RICHARD L. DOTY, MICHAEL J. O'CONNOR, and MICHAEL R. SPERLING (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA, and Departments of Neurosurgery and Neurology, Graduate Hospital, Philadelphia, PA USA)

Temporal lobectomy has been associated with various olfactory deficits. However, to date, no study has evaluated the same temporal lobectomy patients in a pre-/post-operative design. In this study, we administered the following olfactory tests to each side of the nose of patients with intractable epilepsy one day before and four to six days after anterior temporal lobectomy: (i) the University of Pennsylvania Smell Identification Test; (ii) a single staircase forced-choice odor detection test using the odorant phenyl ethyl alcohol; (iii) a 16-item odor discrimination test; and (iv) a 12-item odor memory test incorporating 10-, 30- and 60-sec delay intervals. Half of the these right-handed subjects had received left temporal lobectomies, wheras half had received right temporal lobectomies. Control subjects were given the same battery of tests at equivalent time periods. The primary finding of this study is that the major portion of the olfactory dysfunction attributed by others to temporal lobectomy is present prior to the operative procedure and that lobectomy, per se, accounts only for a small proportion of such dysfunction. Additional findings related to (i) the side of the operation (i.e., left vs. right), (ii) the side of hemispheric dominance (language dominant vs. language non-dominant), and (iii) the type of olfactory test will be discussed.

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10

Chemosensory Event-Related Potentials in Temporal Lobe Epilepsy and First Recordings of Olfactory Event-Related Magnetic Fields

G. KOBAL, T. HUMMEL, B. KETTENMANN (Dept. of Pharmacology and Toxicology, University of Erlangen) E. PAULI, P. SCHÜLER, , H. STEFAN (Dept. of Neurology, University of Erlangen, FRG)

The aim of the study was to investigate chemosensory functions in patients with temporal lobe epilepsy in order to find out, whether both olfactory and trigeminal stimuli applied either ipsilaterally or contralaterally to the focus are processed differently. Twenty-two patients were investigated (12 patients with a focus located in the left, 10 patients with a focus located in the right temporal lobe). Investigation of the trigeminal system was performed by the use of carbon dioxide. The olfactory system was tested using vanillin and hydrogen sulphide. Chemosensory functions were assessed by means of chemosensory event-related potentials (CSERP). In both groups of patients prolonged CSERP latencies were found after stimulation of the left nostril with carbon dioxide when compared to stimulation of the right nostril. In contrast, for olfactory stimuli a different pattern emerged. Latencies after left-sided olfactory stimulation were prolonged in patients with left-sided epileptical foci. Similarly, when the right nostril had been stimulated in patients with a right-sided focus CSERP latencies were prolonged. Thus, it may be assumed that the neocortical processing of olfactory, but not trigeminally mediated information is affected by functional lesions of the temporal lobe. After olfactory stimulation in patients with a right-sided focus the distribution of amplitudes was different from normal. Moreover, analyses revealed non-overlapping 95% confidence intervals for latency N1 when vanillin was applied to the right nostril. These results indicate that the right temporal lobe possibly plays a different role in the processing of olfactory information. Additionally first recordings of event-related magnetic fields and source localization techniques will be demonstrated combining simultaneous recordings of electric and magnetic responses with MRI.

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11

PET Scan Representation of Central Olfactory Processing in Phantosmia

DONALD LEOPOLD, M.D. (Johns Hopkins Medical Institutions)
GARY E. MEYERROSE, M.D., ZSOLT SZABO, M.D. and SAMUEL SOSTRE, M.D. (Johns Hopkins Medical Institutions)

A 21 year old Caucasian female who suffered from right sided nasal phantosmia was evaluated with positron emission tomography (PET) using (F-18)2-fluoro-2-deoxy-D-glucose (FDG). The patient complained of an almost daily "burning rubber" odor which disrupted her normal activities and had increased in frequency since its onset four years previously. Baseline head and neck evaluation was unremarkable except for a right sided septal deformity. Gustatory functional testing was normal while olfactory functional testing indicated severe hyposmia on the left and mild hyposmia on the right. Evaluations with computerized tomography, magnetic resonance imaging, and electroencephalography failed to provide an explanation for the olfactory dysfunction. Prior to surgery a PET scan was performed on the patient while she was experiencing the phantosmia. Regions of interest on the PET scan were analyzed employing both visual inspection and quantitative measurement of activity. Activity in the various regions of interest was expressed as a ratio between activity in the region of interest and activity in the cerebellum. The PET scan showed slightly increased activity involving the entire frontal region of the brain, the anterior portion of the left temporal lobe contralateral to the affected side of the nose, and a focal area of increased activity within the left temporal lobe. The patient's olfactory hallucinations resolved following excision of all the olfactory epithelium from the right nasal cavity, including transection of the fila olfactoria. Ten months after surgery a second PET scan was performed while the patient again attempted to induce an episode of phantosmia with forced nasal breathing. The postoperative scan demonstrated a 10-20% decrease in the relative activity of the left temporal, left frontal, and left insula areas of the brain and a 5-20% increase in activity in the right temporal area of the brain when compared to the preoperative PET scan. The focus of increased activity seen in the left temporal lobe on the preoperative PET scan demonstrated a 16% decrease in activity on the postoperative PET scan. The activity in the cerebellar regions of the brain demonstrated less than a 3% difference between the postoperative and preoperative PET scans. Although it is not possible to draw conclusions from the results of these two PET scans performed on a single patient, the findings suggest either primary or secondary CNS involvement in patients with phantosmia. Whether the metabolic patterns seen on the PET scans in this single patient represent true findings can only be clarified through further study.

12

An Overview of Electrogustometry, A Simple Way to Test Taste. MARION E. FRANK, (University of Connecticut Health Center, Farmington, CT)

Taste, a chemical sense, can be readily activated by weak electric current applied to regions of the oral epithelium that have taste buds. Neurophysiological studies in animals indicate that the chorda tympani, a taste nerve, but not the lingual nerve, a general sensory nerve innervating the same regions of the tongue, is activated by direct anodal currents ranging from 1uA to 100uA. These currents specifically stimulate the taste system. Activation of taste nerves by anodal current occurs via taste-bud receptor cells that are sensitive to ionic stimuli. In humans, anodal currents elicit sour-metallic sensations, for which thresholds and magnitude estimates can be mapped to identify areas of compromised taste function. The strengths of electrogustometry, a clinical tool for nearly 40 years, include precision in localization and duration of stimuli, specificity for the taste system, and portability. Electric stimuli are more convenient than chemical stimuli and superior to chemical stimuli for studies of taste-evoked electroencephalographic potentials. However, electrogustometry cannot readily assess whole-mouth taste function, nor can it identify taste deficits that occur in specific submodalities of taste. It is unsuitable for evaluation of the nonionic sweet and bitter tastes. For electrogustometry to compete with "chemogustometry" in general clinical taste evaluations, electrogustometers must be developed that deliver defined current pulses through stably and reproducibly positioned electrodes that are easily sterilized. With multipolar electrodes, the separate taste-bud fields could be simultaneously tested.

13

Clinical Use of The Electrogustometry
- Its Strengths and Limitations -

H. TOMITA (Department of Otorhinolaryngology, Nihon University, School of Medicine / Tokyo)

Electrogustometer of decibel scale (Rion TR-05 type), a method developed by the author, is widely used in Japan.

The strengths of this testing method are that, provided that a few requirements are met, anybody can operate it with ease and that it takes only a short time for measurement with high reliability of the measured values. Whereas the threshold of electric taste varies by individuals, the bilateral difference invariably remains within 4dB: a value of 6dB or more can be regarded as pathological. It is therefore possible to diagnose a slight taste disorder of which the patient is not aware. Seven types of taste disorders can be distinguished by measuring the threshold of the electric taste in six sites in the mouth (i.e., the symmetrical sites of the front half of the tongue: chorda tympani nerve, the rear half of the tongue: glossopharyngeal nerve, and the soft palate: the greater petrosal nerve). Furthermore, it is possible to estimate not only each taste nerve disorder but also whether it is a disorder of the central nerves system or that of the taste bud level. These features make the method useful for determining the lesion location of acute peripheral facial palsy and for establishing prognosis of the paralysis. In the case of a new patient with abnormal taste, it is possible to forecast the effects of zinc treatment by using both the electrogustometry and qualitative and quantitative clinical gustometry using filter-paper discs.

In case of a patient with diabetic neuropathy, the threshold of electric taste deteriorated in one-third of all cases even in cases where the awareness of abnormal taste was absent. In cases of decreased or totally lost knee jerk, the frequency of the abnormal taste increased significantly.

Limitations of the Electrogustometry: The taste quality of electric taste is a single taste (metallic taste or sour taste in many cases) for that particular individual. The method is therefore useless for diagnosing dissociated taste disorders such as that for sweetness only. Another disadvantage is that the recovery of the taste disorder does not always go in parallel with the process of electric taste and, consequently, the method is inferior in this regard compared with the filter paper disc method.

14

Advantages and Limitations of Electrogustometry for Clinical Assessment of Taste Function. CLAIRE MURPHY (San Diego State University and UCSD Medical Center, San Diego, CA)

Patients with complaints about taste often present a challenge to the Otorhinolaryngologist in terms of both diagnosis and treatment. Few present with taste loss; many report dysgeusia. Of patients seen in chemosenory clinics, patients with dysgeusia are among the most difficult to treat successfully. A better understanding of the nature of the problem in these patients is necessary before clinical success will be achieved. This presentation will explore the advantages and limitations of electrogustometry for assessment of taste loss and suggest avenues for further study. For example, electrogustometry may be especially useful in localization of taste loss since the discreet nature of the stimulus allows for precise control. One known limitation of the electrogustometry method is the inability of the electrical stimulus to differentially stimulate all taste qualities. Because it appears that electrogustometry is not an equally effective stimulus for all taste modalities, the limitations of this type of testing in clinical assessment need to be clearly understood. It is not known whether electrical stimulation will mask or enhance the persistent taste of a dysgeusic. The usefulness of electrogustometry in this domain is largely unexplored. A better understanding of the mechanism of stimulation may facilitate placing the electrogustometer in the proper place in the clinical battery for testing taste. Clinical testing of the sense of taste has usually meant presentation of a series of concentrations of more than one taste quality with water blanks. Preparation and storage of appropriate batteries of chemical solutions make psychophysical testing with solutions appear daunting for most physicians in private practice. A rapid, portable, easily administered, clinical test for assessing taste function is sorely needed. The potential for electrogustometry to satisfy this need will be discussed.

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15

Electrogustometry and Taste Bud Prevalence I. MILLER, JR., (Dept. of Neurobiol. and Anat., Bowman Gray Sch. Med of Wake Forest University., W-S, NC 27103)

Electrogustometry should assess the integrity and the sensitivity of the peripheral taste system in order to be a useful diagnostic method. It should provide evidence about the presence and functional status of taste buds and sensory nerves provided that pathways in the central nervous system are intact. Our recent studies are intended to assess relationships between numbers of taste buds on the human tongue and taste perception. It is assumed that normal taste perception requires the presence of functional taste buds, and that chemical stimuli induce responses by contact with the taste pores. Taste buds on the human tongue are located exclusively on specialized papillae, but the number, locations and appearance of papillae vary. We estimate that there is an avg total of about 320 fungiform papillae/ tongue with an average of about 3.5 taste pores/ papilla for an estimated avg. total of 1120 fungiform taste buds. But, we find a wide variation among healthy subjects from an est. total of a few hundred to several thousand fungiform taste buds. On the tongue tip, taste pore densities may range from <10 - >500 tb/cm^2. We estimate an average density of about 30 fungiform papillae/cm^2 on the tongue tip with a range from 20 to more than 60 pap/cm^2. Fungiform papillae average about .75 mm in diameter. Studies suggest that taste perception is more acute for individuals with more abundant taste buds. Stimulation of the tongue with electrical current applied to different sized areas has yielded steeper slopes on the tongue tip than on other regions. We hypothesize that taste perception elicited by electrical stimulation on the anterior part of the tongue will be related to the number of stimulated taste buds in healthy subjects. Some diseases may have normal numbers of taste pores but diminished sensitivity.

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16

Detection and Choice of Males in the Female Lobster, *Homarus americanus*. PAUL BUSHMANN and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

Mature Homarus americanus females enter shelters of mature males to molt and mate. In this study females of various molt stage and males were tested in a Y-shaped flume. The two upstream arms of the flume each contained an artificial male shelter; seawater flowed at 1 cm/sec by and through the shelters downstream to the female start position. Two equal sized donor males were housed together separately and allowed to establish dominance. A trial consisted of placing the dominant and subordinate donor males into a shelter (DS), placing only the dominant (D) or subordinate (S) male into a shelter while the other remained empty, or two empty shelters (B). Females or males were placed in the start position and allowed to move about the flume for 15 minutes, while position in the flume and behavior near each male shelter were recorded on video. Females and males made initial shelter choices from the start position, and attempted to enter some shelters. Males showed no significant differences between shelters under any trial condition. Female initial choice did not differ between shelters in B trials, which acted as a control, nor did they discriminate between shelters in DS trials. Females did make initial choices of dominant and subordinate males under the D and S trial conditions, respectively. In the D and S trials females entered inhabited shelters more than uninhabited shelters; this difference was significant for D trials only. No difference between shelter entering was seen for females in the DS trials. Molt stage of tested animals did not appear to play a role in choice or entering behavior. These data indicate that females are capable of detecting males from a distance, will move upstream to approach these males, and can determine the dominance status of a male when close. Additional data suggest that female initial choice may be correlated with male body size, and that the presence of male urine may be important for finding and entering male shelters.

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17

Whole-Cell Recording from Local Interneurons in the Olfactory Pathway of the Spiny Lobster. C.E. DIEBEL and B.W. ACHE. (Whitney Laboratory, and Depts. Zoology & Neuroscience, Univ. Florida, St. Augustine, FL 32086).

Whole-cell recording from an isolated, perfused lobster brain preparation with biocytin-filled electrodes revealed two types of local interneurons in somata cluster #9 (Sandeman et al., Biol. Bull., 183: 304, 1992) that innervate the columnar glomeruli of the olfactory neuropil (ON). One type branched exclusively in the ON. These cells entered the core of the ON and branched extensively in the basal region of many glomeruli. A few of the branches extended into the cap region of the innervated glomeruli. A second type also innervated multiple ON glomeruli, but many fewer and branching was confined to the basal region. The dendritic arbors of the latter type of cells extended into the central zone of the neighboring accessory neuropil (AcN). Both types of interneurons responded to electrical stimulation of the olfactory nerve with a depolarization that subserved a burst of spikes and EPSPs. Depolarization persisted for up to 10 sec post-stimulation. The latency of the first type (28 ± 10 msec) contrasted with the significantly longer latency (124 ± 12 msec) of the second type. Subthreshold electrical stimulation evoked graded EPSPs in cells of both types with no evidence of an after-hyperpolarization, producing a pattern of response in these cells not unlike that of anaxonal olfactory interneurons in vertebrates and insects. The arborization pattern of the cells is consistent with our earlier proposition (Schmidt and Ache, Abst., 10th ECRO Congress, 1992) that the cap and base are distinct regions of synpatic processing within the columnar glomeruli. The physiological similarity of these local olfactory interneurons with their counterparts in at least two other types of animals begins to suggest the existence of a fundamental strategy for processing olfactory information at the first synaptic level.

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18

Modulation of Chemosensory Behavior in the Spiny Lobster: the Influence of Nutritional State. PETER C. DANIEL (Hofstra University), CHARLES DERBY, AND CAROLE ALLEN (Georgia State University)

The Florida spiny lobster has proved to be a valuable model for studying the mechanisms of processing of odorant information. Considerable progress has been made in understanding anatomical organization, mechanisms underlying activation/inactivation of chemosensory cells, and the processing of complex odorants by populations of neurons. How the lobster responds behaviorally to chemosensory input likely depends on a number of internal variables including molt stage, reproductive state and nutritional state. We are interested in elucidating the mechanisms by which internal state can modulate the influence of chemical cues on behavior. We report here preliminary findings showing that fasting state can modify some behaviors associated with the detection and location of food odorants. Six spiny lobsters were tested with artificial seawater and with artificial crab mixture at three concentrations (0.0005, 0.005, 0.5 mM) after one, four and eight days of fasting. Two behavioral variables were measured: antennular flick rate and number of antennular wipes. As expected, antennular flick rate was dose-dependent with an increase in flick rate even at the lowest concentration of crab mixture tested. In contrast, a significant increase in the number of antennular wipes occurred only at the highest crab mixture concentration. Flick rate significantly increased after eight days of fasting but not after four days relative to the one day control. There was no observable change in antennular wipe response with fasting. These results suggest that the threshold for detection of odorants (antennular flicks) may decrease with fasting while a behavior associated with the removal of odorants from the olfactory surfaces (antennular wipes) is unaffected. In future studies, we will search for hormones that may induce changes in behavior similar to those observed with fasting. Then we can investigate how internal signals can influence neural pathways involved in the detection and processing of external chemical cues.

Supported by NSF.

19

Whole-cell Recording from Multi-glomerular Projection Neurons in the Olfactory Lobe of the Spiny Lobster. M. WACHOWIAK and B.W. ACHE (Whitney Laboratory and Dept. Neuroscience, Univ. Florida, St. Augustine, FL, 32086).

Whole-cell, current clamp recordings from projection neurons in the olfactory lobe of the spiny lobster were obtained by patching the cell somata in an isolated, perfused brain preparation. The somata of the projection neurons are confined to single, accessible cell cluster (#10, Sandeman et al., Biol. Bull., 183:304, 1992). Rapid, complete fills of neurons could be obtained by including biocytin in the patch pipette. All projection neurons innervated multiple glomeruli in the olfactory lobe, although the number of glomeruli innervated and the extent of branching within the glomerulus varied. Most (75%) cells sparsely innervated each of many OL glomeruli, while the remainder (25%) sparsely innervated many fewer OL glomeruli and densely innervated several other OL glomeruli. In response to maximal electrical stimulation of the olfactory nerve, both types of projection neurons typically responded with a rapid, brief (50 ms) depolarization underlying a burst of spikes and EPSPs, followed by a slow, 1-2 sec duration after-hyperpolarization. Subthreshold electrical stimulation hyperpolarized the cell with no evidence of EPSPs. With a nose/brain preparation, odors elicited graded responses from the cells at moderate concentrations, but could suppress output at higher concentrations. The cells had different, but overlapping response spectra. The response of the projection neurons in the lobster OL is similar to that of projection neurons in the olfactory CNS of insects and vertebrates, where the output of projection neurons is thought to reflect shaping of excitatory receptor input through inhibitory connections among glomeruli. The two patterns of glomerular innervation in the lobster OL suggest that weighing input from a few, selected glomeruli relative to that averaged across many glomeruli may be fundamental to this process.

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20

Mechanisms of Olfactory Mixture Interactions: Whole Cell Patch Clamp Studies of Olfactory Receptor Neurons of the Spiny Lobster. TED SIMON and CHARLES DERBY (Department of Biology, Georgia State University)

Whole cell patch clamping was used to investigate mechanisms of mixture interactions in olfactory receptor neurons (ORNs) of the spiny lobster *Panulirus argus*. We used a set of odorants previously used to identify mixture interactions based on spike trains from axons of ORNs and on behavior. These odorants were artificial oyster mixture (AOM), adenosine 5'-monophosphate (5'AMP), betaine (Bet), L-cysteine (Cys), L-glutamate (Glu), taurine (Tau), ammonium chloride, DL-succinate, and binary mixtures of these compounds. Forty odorant-sensitive ORNs were studied. These ORNs had a variety of response specificities. These stimuli more frequently elicited inward than outward currents. 5'AMP, Glu, Tau and Bet evoked the largest and most numerous outward currents; CYS most commonly evoked outward currents. Na^+ or Na^+/K^+ were the charge-carrying ions for Glu-, Tau-, and Bet-evoked responses; Ca^{2+} was the charge-carrying ion for one AOM-excited ORN. We observed both rapid and delayed odor responses. Responses rarely desensitized during the 5 sec of chemostimulation. In 5 ORNs, mixture suppression was observed between two excitatory compounds, apparently due to an electrotonic shunting mechanism: in 4 ORNs excited by both Glu and 5'AMP and in 1 ORN excited by both Tau and Glu, binary mixtures of the 2 excitants produced inward currents smaller than those evoked by either component. We hypothesize that this occurs because the conductances for the 2 components of a mixture shunt each other, establishing a local circuit in the dendrite which diminishes or eliminates the current at the soma. One case of mixture suppression apparently occurred due to inhibition of binding between 2 components of a binary mixture: Tau had no effect by itself but addition of Tau to Bet suppressed the inward current generated by Bet. These 2 mechanisms of mixture interactions, together with a previously defined mechanism of mixture suppression -- activation of opposing ionic conductance by the components of a mixture (Michel et al. 1991) -- allow shaping of responses to odorant mixtures.

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21

Ionic Selectivity and Ligand Specificity of IP3-gated Channels Mediating Excitatory Transduction in Lobster Olfactory Receptor Neurons. FADOOL, D.A. and B.W. ACHE (Whitney Laboratory and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086).

Previously, we reported that inositol 1,4,5-trisphosphate (IP_3) directly gates a 74 pS and a 30 pS channel in the plasma membrane of cultured lobster olfactory receptor neurons, and suggested that one or both mediate excitatory transduction (Fadool and Ache, *Neuron* 9: 907, 1992). To further implicate the IP_3-gated channels in excitatory transduction, we determined their ion selectivity and ligand specificity. Both the larger conductance (n=2) and the smaller conductance (n=5) channels were modulated by Ca^{2+}; the Pr_{open} increased 2 fold between [Ca^{2+}] 10 μM and 3 mM, with a corresponding 50% decrease in conductance that was further reduced at holding potentials of +30 mV and above. Over time, both the larger (n=2) and the smaller (n=3) conductance channels could enter a long open mode ($Pr_{open} \geq 0.91$) that was followed by a closed mode that flickered to the open state ($Pr_{open} \leq 0.15$) and then returned to the most observed state ($Pr_{open} = 0.27$) for as long as 30 min. The smaller conductance channel conducted $Ba^{2+} > Ca^{2+} \geq Sr^{2+} > Na^{+}$ and reversed independently of E_{Na+}, E_{K+}, and E_{Cl-}. The larger conductance channel reversed independently of E_{Ca2+}, but not E_{Na+}. The smaller conductance channel was blocked by TTX (n=2 of 4) and CoCd (n=4 of 4), whereas the larger conductance channel was not blocked by TTX (n=4 of 4) but was blocked by CoCd (n=3 of 3). Correspondingly, odor-evoked inward macroscopic currents were reduced to 15.92 ± 7.51 (n=10) percent of control by 5 mM CoCd; they were either not reduced (n=3) or reduced to 50.07 ± 4.07 (n=6) or 11.04 ± 7.8 (n=2) percent of control by 10 μM TTX; and they were reduced to 86.52 ± 9 (n=5) percent of control by 10 mM TEA. Neither micromolar inositol (n=7), cyclic IP (n=7), IP_2 (n=5), nor IP_6 (n=6) elicited channel activity when applied to the inside face of cell-free patches. Inositol 1,3,4,5-tetraphosphate (IP_4) activated channels in 19 of 34 (56%) cell-free patches. This channel inactivated within seconds, and had a larger conductance (191.97 ± 13.2 pS), a more dense distribution (0.56 to 10.08 μm^{-2}), and a shorter mean open time (5.03 ± 1.02 msec) than did either of the channels activated by IP_3. When an equal mixture of micromolar IP_3 + IP_4 was applied to the inside of the patch, the Pr_{open} decreased from 0.32 ± 0.07 (IP_4 alone, n=6) and 0.27 ± 0.05 (IP_3 alone, n=5) to 0.04 ± 0.01 (IP_3 + IP_4, n=8) and complex subconductance states were frequently observed. The data suggest that IP_3 acts selectively on a primarily calcium permeant and a primarily sodium permeant channel, which would be consistent with the proposition the either or both channels are the effectors for excitatory transduction. IP_4 appears to gate a distinct type of channel, whose role in transduction, if any, remains to be established.

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22

Sequence of a Partial cDNA Encoding a Putative Cyclic Nucleotide - Gated Ion Channel from Lobster Olfactory Organ. S.D. MUNGER[1,3], R.M. GREENBERG[1], H.G. TRAPIDO-ROSENTHAL[1] AND B.W. ACHE[1,2,3] ([1]Whitney Lab and Depts. of [2]Zoology and [3]Neuroscience, Univ. of Florida, St. Augustine, FL).

Previously, we reported that cAMP is an olfactory second messenger in lobster olfactory receptor neurons (ORNs) (Michel and Ache, *J. Neurosci.*, 12:3979, 1992). Preliminary evidence suggests that cAMP directly activates its effector channel (Michel, et al., *Chem. Senses* 17:669, 1992). In contrast to the situation in vertebrate ORNs, however, cAMP appears to gate a novel, K^+ selective ion channel in the lobster that suppresses rather than excites the cell (Michel, et al., J. Neurophysiol., 65:446, 1991). To further characterize this channel as well as to provide a probe to study the known heterogeneous expression of the inhibitory transduction pathway in lobster ORNs, we have cloned a partial cDNA from lobster olfactory organ that is homologous to vertebrate cyclic nucleotide gated channels. To isolate this fragment, we designed degenerate oligonucleotide primers to the highly conserved cyclic nucleotide binding region of known cyclic nucleotide-gated channels and used them to screen genomic DNA from the lobster olfactory organ (the lateral antennule) by the polymerase chain reaction (PCR). A PCR product of predicted size (122 bp) was obtained which exhibited 50-65% amino acid identity to the published sequences of other cyclic nucleotide-gated channels. Exact primers were then designed to the known genomic sequence and used to screen a cDNA library constructed from lobster olfactory organ mRNA. A 78 bp cDNA that exactly matched to the corresponding portion of the genomic clone was amplified. We are currently using a variety of strategies to obtain the complete sequence of this product.

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23

Biochemical Analysis of Binding Characteristics of A Putative Glutamate Receptor in the Olfactory Organ of the Spiny Lobster MICHELE F. BURGESS, CHARLES D. DERBY, KIRBY OLSON (Dept. of Biology, Georgia State University)

Excitatory amino acid systems occur in the vertebrate central nervous system as well as the invertebrate neuromuscular junction, major ganglionic pathways, and chemosensory systems. The excitatory amino acid L-glutamate is of special interest due to its involvement in vertebrate synaptic plasticity as well as human degenerative mental disorders There are two general classes of glutamate receptors - ionotropic and metabotropic - and several identified subclasses. In previous behavioral and electrophysiological studies, the olfactory organ of the spiny lobster has been shown to respond to L-glutamate. In our preliminary experiments using dendritic membrane of the aesthetasc (olfactory) sensilla, the binding characteristics of the putative glutamate receptor were studied utilizing an established radioligand binding assay. An association curve demonstrated that the putative receptor is rapidly saturable and a dissociation curve demonstrated an equally rapid and completely reversible dissociation, which are characteristics of an odorant-binding receptor site. A single site analysis of the Rosenthal transformation of a saturation experiment yields a K_D value of 7.6 μM and a B_{max} value of 23.0 fmol/μg protein. This affinity and receptor density resemble that of previously studied taurine receptors and AMP receptors. Further studies will include self-inhibition as well as inhibition involving other known odorant molecules in order to understand the role of this receptor in detecting mixtures. In addition, similarities between this glutamate receptor and ionotropic and metabotropic excitatory amino acid receptors in other systems will be examined using analogs of glutamate.

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24

Binding Behavior of Mixtures of Odorant Molecules to the Taurine Olfactory Receptor Sites of the Spiny Lobster. KIRBY OLSON and CHARLES DERBY (Dept. of Biology, Georgia State University)

This work centers on the role of odorant binding behavior at olfactory receptor sites in the ability of lobsters to detect and discriminate odor mixtures. First the behavior of unlabeled taurine competing for labeled taurine sites was characterized. This inhibitory behavior showed a significantly better fit to a two site model: 81% of the sites are high affinity (IC_{50} = 7 μM) and 19% are low affinity sites (IC_{50} = 0.007 μM). The inhibition of binding of 1 μM tritiated taurine by six compounds (betaine, ammonia, glutamate, succinate, cysteine, and 5'adenosine monophosphate) known to stimulate olfactory neurons was also evaluated. Only betaine produced complete, competitive inhibition of taurine binding; this is consistent with electrophysiology results showing that betaine excites most taurine-best cells, though to a much lesser extent than taurine itself does. Low affinity sites showed greater affinity for these inhibitors than for taurine, indicating that these sites may represent receptor sites for the other compounds with some affinity for taurine. The maximum inhibition of binding by the highest concentration (10^{-3} M) of any other compound was 40%. A number of binary mixtures of the six inhibitors were also tested; inhibition never exceeded 40% unless the mixture contained betaine. Inhibition in excess of that accounted for by the low affinity sites may represent noncompetitive inhibition of taurine binding by the other compounds. Mixtures which contained betaine completely inhibited labeled taurine binding though only at higher concentrations than betaine alone would. Actual inhibition at all concentrations of mixtures of odorants fell substantially short of predicted values generated by a single site receptor model, including mixtures with betaine. Our results demonstrate that mixture suppression can occur in binding events at olfactory receptors.

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Biochemistry of olfactory uptake systems: taurine enhances the uptake of glycine. HENRY G. TRAPIDO-ROSENTHAL, LISA R. GENTILCORE, RICHARD A. GLEESON, and WILLIAM E.S. CARR. (Whitney Laboratory, University of Florida, St. Augustine, Florida).

The amino acids taurine and glycine are odorants that activate specific chemosensory cells in the olfactory sensilla of the spiny lobster, Panulirus argus. These amino acids are also present in the receptor lymph that bathes the dendrites of the chemosensory cells, where they are maintained at concentrations of about 10 nM for taurine, and up to 500 nM for glycine. Previous work has shown that a background concentration of an odorant amino acid directly affects the sensitivity of receptor cells. In this work, we show that background concentrations of one amino acid (taurine) has a significant effect on a perireceptor event, the uptake system for another amino acid (glycine). When present at a concentration of 10 nM, taurine approximately doubles the amount of radiolabeled glycine taken up by the lobster olfactory organ. Kinetic studies demonstrate that taurine exerts its effect by increasing the rate of uptake, rather than by altering the affinity of the glycine transporter for its substrate. The ability of background concentrations of amino acids in the receptor environment to affect both perireceptor and receptor events, emphasizes the importance of understanding the composition of the chemical milieu in which chemoreception takes place.

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Cloning, sequencing, and activity of a cytochrome P450 from the olfactory organ of the spiny lobster. HENRY G. TRAPIDO-ROSENTHAL, SEAN M. BOYLE, STEVEN D. MUNGER, JAMES C. NETHERTON, MARGARET O. JAMES, 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, as well as the processes of a number of auxiliary cells. Our prior biochemical studies demonstrated that sensilla have an enzymatic activity that is capable of metabolizing compounds that are substrates for the cytochrome P450 class of monooxygenase enzymes. In the work described here, we show that the lobster olfactory organ contains receptor cells that respond to cytochrome P450 substrates such as quinine when those substances are present in seawater. A complementary DNA (cDNA) library was constructed in the bacteriophage vector λgt11, from messenger RNA (mRNA) isolated from the lobster olfactory organ. The polymerase chain reaction (PCR) was used to screen this library for cDNA sequences encoding odorant-metabolizing enzymes. We show here that the olfactory organ cDNA library contains sequences identical to those from the lobster hepatopancreas that code for a cytochrome P450. The deduced amino acid sequence suggests that the encoded enzyme is a member of the CYP2B family of phenobarbital-induced cytochromes P450.

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Effects of Stimulus Concentration on Frequency Coding in Lobster Chemoreceptor Cells.
GEORGE GOMEZ, RAINER VOIGT and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543).

Chemical stimuli in the environment are distributed in a turbulent fashion that results in a chaotic series of patches of varying concentrations; the intensity and encounter frequency of these patches decrease with increasing distance from the source. A patch of odor moving over a stationary point is measured as a pulse of odor occurring in time. If chemoreceptors mediate distance orientation then they might be designed to encode pulsatile information occurring at varying stimulus concentrations in an odor plume. It has been shown that chemoreceptors on the lateral antennule of *Homarus americanus* can accurately encode pulses of Hydroxyproline (Hyp) up to 2 Hz (Gomez et al. 1992. Chem. Senses 17:631-632). To determine how stimulus intensity affects these frequency filter properties, lateral antennules of intermolt lobsters were excised and situated in an olfactometer. Aesthetasc sensilla bearing Hyp-sensitive receptors were localized using a concentric pipette pressure injection system. The In Vivo Electrochemistry Computer System (IVEC-5) measured tracer molecule (dopamine) concentrations mixed in to the stimulus solution to verify each stimulus presentation; errant pulse series were repeated to eliminate possible effects of stimulus variability (for details see Gomez et al. 1992. Biol. Bull. 183(2):353-354). Receptor cells were stimulated with ten 100 ms pulses of 10^{-5}, 10^{-4} and 10^{-3} M Hyp at rates of 0.5, 1 and 2 Hz. Responses were recorded extracellularly. Individual receptor cells have different frequency following capabilities. At concentrations close to cell threshold (10^{-5} M) following capabilities (i.e., synchronization coefficient, Goldberg and Brown 1969) for higher frequencies were generally poor. Higher stimulus concentrations improved the frequency following. Response magnitude (number of spikes) and cumulative adaptation increased while first spike latency decreased with increasing stimulus concentration.

Functional Architecture of Three Glomerular Neuropiles in the Olfactory System of the Crayfish. DE FOREST MELLON, JR. (University of Virginia)

I have compared the anatomical organization of three different regions of glomerular neuropile in the crayfish olfactory system: the olfactory lobe, the accessory lobe, and the hemi-ellipsoid body. Each neuropile has a basic structure that can be generalized as consisting of an input surface, a plexiform layer, and an output surface. In all three neuropiles the respective input surfaces each consists of a peripheral reticulum of afferent fibers that forms a cortex surrounding the neuropile. The plexiform layers exhibit differing degrees of complexity. That of the hemi-ellipsoid body is simple, consisting of a thin shell beneath the fibrous cortex that conforms to the shape of the regional structure. This layer contains spherical microglomeruli, loci of synaptic contact between the rosette terminals of input fibers and fine dendritic branches of the output neurons. The output neurons branch extensively to contact all of the microglomeruli, and their axons exit the hemi-ellipsoid body at a confined point as a compact stalk. The plexiform layer of the accessory lobe is structurally more complex, in that the afferent fibers form bifurcate, extensively-branched and cylindrically shaped dendritic trees that are arranged radially beneath the cortex of afferent fibers. Output fibers branch extensively among the cylindrical glomeruli and leave the accessory lobe in concert as a compact fascicle. In the olfactory lobe the plexiform layer is relatively thick and probably contains several functionally distinct zones. The afferent fibers penetrate each columnar glomerulus at its broad cap region and branch modestly within the column . Afferent-to-interneuron synapses are most prevalent in the cap zone, whereas in the central and apical zones of the column, higher-order synapses are more numerous. In the olfactory lobe as in the other two neuropiles, output neurons leave the neuropile as a single compact bundle of fibers. Each of the three neuropiles can be considered as a distorted flat sheet, in which evolutionary expansion of the input surface has forced its overgrowth and envelopment of a compact, fasciculated pedestal of output fibers.

Structure of the Olfactory Sensilla of the Blue Crab, Callinectes sapidus. RICHARD A. GLEESON[1], LORRAINE M. MCDOWELL[2], HENRY C. ALDRICH[2]. ([1]The Whitney Lab. and [2]Dept. Microbiol. and Cell Sci., Univ. of Fla.)

The olfactory sensilla (aesthetascs) of the blue crab are arranged in a dense tuft located on the outer flagellum of the antennule. Each aesthetasc contains the dendritic processes of many chemosensory neurons. These processes are separated from the external seawater environment by a thin cuticle that is permeable to odor molecules. Because the euryhaline blue crab is found living in salinities ranging from seawater to freshwater, these dendrites can be exposed to variable osmotic/ionic conditions. In this study, transmission electron microscopy was used to provide a detailed description of the internal organization of the aesthetascs in order to: (1) compare and contrast the morphological features with those present in the aesthetascs of marine and freshwater crustaceans; and (2) identify structural changes that may occur following acclimation to various salinities. This work is part of an overall project designed to elucidate the structural and functional mechanisms important in maintaining chemosensory function under varying osmotic/ionic conditions.

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A Comparison of Information Currents in Decapod Crustaceans

THOMAS BREITHAUPT, GEORGE GOMEZ, BARBARA HERR, TASLEEM KACHRA, JAMES DIPALMA and JELLE ATEMA
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Chemical stimuli in the aquatic environment are dispersed primarily by bulk transport; diffusion processes are effective only over short distances (at most 1 mm, Atema J., 1987 in *Comparative Physiology: Life in Water and on Land.* Dejours L. *et al.*, eds.). Thus any nearby water movement has a strong influence on the chemosensory input by directing chemical stimuli towards or away from the receptor. Decapod crustaceans are capable of producing powerful water currents by beating specialized appendages. These currents occur close to the antennules, the major chemoreceptors. Currents produced by American lobsters (*Homarus americanus*), hermit crabs (*Pagurus pollicarus*), and green crabs (*Carcinus maenas*) were studied using video analysis of appendage motion and flow visualization (single particle tracking) in a static water tank. Two major currents were produced by the animals: gill currents (produced by scaphognatite beating) and exopodite currents (beating of the exopodites of the maxillipeds). While scaphognathite beating in all animals produced a rather stable anteriorly projecting current, exopodite movements could redirect this current in lobsters (Atema J., 1985, Soc.Exp.Biol.Symp. 39: 387-423) and could produce an independent variable current in green crabs and hermit crabs. Stimulation of the animals with food odors from different sides was followed by increased exopodite activity in all species tested. With the aid of the exopodites all animals could fan a current that drew water from the odor source towards their antennules. Obviously the animals are capable of manipulating their environment in order to get chemical information from desired locations. Details on 3-D spatial distribution of the currents, their active modulation and the interaction of the currents with the chemosensory sampling organs will be discussed.

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IMP And Glutamate As Stimuli In *Paramecium* Chemoreception

WANQING YANG and JUDITH VAN HOUTEN (University of Vermont, Burlington, VT 05405)

Paramecium is repelled by inosine monophosphate, as measured by T-maze assays. Two purine nucleotides (AMP and GMP) interfere with *Paramecium's* behavioral response to IMP, while CMP and cAMP do not, indicating that the IMP-induced response is purine nucleotide-specific. More surprisingly, glutamate, an amino acid neurotransmitter and a key substance in umami taste, inhibits *Paramecium's* behavioral response to IMP, but IMP does not inhibit the response to glutamate. Synergism of glutamate and 5'-ribonucleotides was found in hamster chorda typani (Yamamoto et al, Brain Res. 451: 147-162, 1988). In *Paramecium*, synergism of glutamate and 5'-AMP is exhibited in behavioral T-maze assays. ^{3}H-Glutamate binding to whole cells saturates by 60 minutes and the Kd is ~103 μM. The 5'-ribonucleotides IMP, AMP and GMP all partially displace glutamate binding, but CMP and cAMP do not interfere with glutamate binding at all. These results are consistent with the T-maze assays. Motion analysis of *Paramecium* swimming behavior shows that IMP reduces *Paramecium* swimming speed and increases the frequency of turning. This behavior is typical of repellents which depolarize the cells. Intracellular cAMP RIA measurements show that IMP decreases the cAMP level by 50% and glutamate increases it about three fold, compared to controls.

This work was supported by NIH and the VCC.

Contributions of Synaptic Integration to the Spike Patterns Evoked by Odor Blends in Central Olfactory Neurons of Insects.

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JOHN G. HILDEBRAND (ARL Div. of Neurobiol., U. Arizona, Tucson 85721)

The precise execution of odor-guided behaviors in many animals depends on the ability of olfactory circuits to extract temporal information from the odor plume. In insects, the best-studied example of this discriminative task comes from studies on the perception of sex pheromones; blends of chemicals that together comprise a species-specific message that attracts males to females. One of our models, the large sphinx moth *Manduca sexta*, relies primarily on 2 odorants to serve as its mating signal. This simple binary mixture has been used to characterize the response properties of different types of neurons found in the insect's primary olfactory center, the antennal lobe (AL). A subset of AL projection (output) neurons responds in a unique fashion with a triphasic (-/+/-) response to the binary pheromone blend. By stimulating the antenna with only one or the other component, we can "dissect apart" the different phases of this complex postsynaptic response. One pheromone component mediates an excitatory postsynaptic potential (EPSP) associated with spike activity, and the other mediates an inhibitory postsynaptic potential (IPSP) associated with spike suppression. Most importantly, when the two components are combined in the species-specific ratio, the neuron can encode each stimulus pulse with a discrete burst of spikes, thus synchronizing the projection neuron's activity to the antennal input. If the ratio of the 2 components is altered significantly, however, this synchronization is lost. There is a strong correlation between the IPSP amplitude and the ability of a neuron to encode the duration of a pheromone-blend stimulus. Neurons that receive little or no inhibitory input cannot accurately encode changes in stimulus duration. In those neurons exhibiting a strong (5-10 mV) IPSP, however, there is a linear correlation between stimulus duration and the duration of the evoked spike train with stimulus pulses from 50 to 500 msec. Furthermore, these neurons can encode a completely random sequence of stimulus pulses of different durations with considerable accuracy. The varied integrative functions of different pheromone blend-processing neurons are reflected further in their dendritic morphologies. In male *Manduca*, information about each of the 2 pheromone components is processed in a different glomerular region. Neurons that can track stimuli of varying duration and frequency have extensive arborizations in both regions. Neurons that cannot track these stimuli show few arborizations in the region that receives information about the component that mediates the IPSP. Behavioral evidence shows that insects in flight must be able to detect temporal fluctuations in an odor plume in order to locate the odor source. The integration of excitatory and inhibitory input in some projection neurons therefore permits them to monitor with considerable accuracy the rapid and periodic temporal changes in the odor stimulus.

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33

A Neural Networks Model of an Insect's Food Choice System

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Feeding decisions made by the insect brain depend largely on input from the chemosensory system. A computational model is being developed that simulates feeding decisions by a caterpillar choosing among acceptable and unacceptable plants. Inputs to the model are electrophysiological recordings from gustatory sensory neurons of the caterpillar. The objectives are to determine which components of the sensory data are important in the decision process, and to elucidate the rules by which these decisions are made. The three-stage model employs neural network algorithms for (1) acquiring, processing and classifying action potentials; (2) integrating data from multiple sensory organs; and (3) determining the likelihood of feeding. This third "decision module" is "trained" to associate chemosensory data with behavioral responses to the same stimulus. When trained with responses to a variety of acceptable and unacceptable plants, the model can be challenged with sensory responses to a novel stimulus and asked to predict the feeding decision. These simulated decisions can then be compared with experimentally derived behavioral data to verify the accuracy of the model. Analysis of the structure (e.g., the "synaptic weights") of a successfully trained network provides information about which activities and interactions of the chemosensory neurons are important to the decision-making process. The model is being tested using the tobacco hornworm as the experimental insect; the sensory responses are from the eight chemoreceptive neurons in the two styloconic sensilla, the most important gustatory organs involved in food selection. Behavioral data are from food choice experiments using sensory-impaired animals in which styloconica are the only functional chemosensory organs, thus ensuring that the data which are inputs to the model represent the only chemosensory information used in the observed feeding decisions.

34

A Model of Dendritic Signal Transmission in Insect Taste Hairs

KAI HANSEN (Zoological Institute , University of Regensburg, Germany).

The basic physiology of taste hairs is less well understood than their specificity pattern and their biological meaning, presumably as only extracellular recording techniques are available. As a consequence of TEM studies here a model of the receptor cell is developed. An important part of it is the dendritic outer segment (DOS) as signal transferring link between the transduction zone at the tip and the spike generator at the soma. It shows an extraordinary length of up to .4 mm combined with a sub-micron diameter. The model implies the following interpretations: 1. Assuming the normal cytoplasmic resistance (100 $\Omega.cm^2$) for the longitudinal resistance of the DOS 3 GΩ are calculated. As a result only receptor currents below 20 pA can flow through the DOS with a tolerable voltage drop. Similar low current values for high stimulus concentrations can be deduced from extracellular receptor potentials. In a rough estimation such a current of 20 pA corresponds to about 20 simultaneously open tranduction channels of 10 pS. 2. The DOS membran surface is more than two decades larger than that of the receptive area. To avoid attenuation of the receptor current and of the resulting depolarization of the soma membrane the DOS membrane resistance has to be extremely high ($> 40k\Omega.cm^2$). 3. At the onset of the stimulation the membrane conductance changes fast at the DOS tip leading to a reloading of the membrane capacities; consequently the receptor current shows transiently higher values than predicted from the longitudinal resistance of the DOS. This transient has a parallel in the first phasic portion of the spike train and might be one factor responsible for the adaptation phenomenon.

35

Behavioral and Chemosensory Responses of Adult *Diabrotica* Beetles to GABA$_A$ Receptor Agonists and Antagonists

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A number of compounds isolated from host and non-host plants have proven to be strong feeding inhibitors for adult *Diabrotica virgifera virgifera* (western corn rootworm). Many of these compounds are known to be effective on vertebrate GABA receptor. In contrast, agonists of this membrane protein have been shown to be feeding stimulants. This feeding stimulation or inhibition by selected agonists and antagonists of GABA receptor suggests there may be much similarity between the central and peripheral GABA receptors in gustatory cells. We have identified a group of chemosensilla located on the maxillary galea of adult *Diabrotica* beetles with taste cells that are responsive to the feeding inhibitors bicuculine, hydrastine, strychnine, and picrotoxinin and also to the feeding stimulants GABA and glycine. Cucurbitacin B, a very potent feeding stimulant from an alternative host plant (squash) evokes a strong chemosensory response and has a proposed binding site on GABA receptor. For all compounds dose responses of the sensory cells overlap with the effective behavioral ranges. These results raise the possibility that there are peripheral chemosensory receptors with unique affinities and specificities for plant neurotoxicants that parallel closely those same features for central nervous system GABA receptors. Such parallel pharmacological specificity at peripheral and central sites could hold important implications for the evolution of insect-plant interactions.

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36

Isolation of *Drosophila* Olfactory Genes Using Enhancer Trap Technology.

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Using enhancer trap technology as a means to isolate new olfactory genes, we have screened 6,500 *Drosophila* lines, each containing a unique transposable element enhancer-detector insertion. If the insertion site for this element is near an olfactory gene, the tissue specific enhancer for that gene will drive reporter gene expression in the olfactory system. A total of 12 enhancer trap lines, which show reporter gene expression in both larval and adult olfactory organs but relatively little staining elsewhere, have been identified. The reporter gene expression pattern of these lines suggests that most of these lines identify genes which are expressed in subsets of the adult antenna. Some lines show reporter gene expression patterns similar to the distribution of different classes of olfactory sensilla. One line shows a sexual dimorphism and one line has an expression pattern which becomes increasingly restricted with adult age. Also, some lines show reporter gene expression in other chemosensory organs as well. We have isolated genomic DNA flanking the enhancer-detector from these lines and are in the process of identifying the corresponding transcripts. In addition we have created deletions of flanking genomic DNA of these lines to determine whether any of these lines identifies an olfactory function specific to a subset of odorants.

37

Molecular Genetics of Olfaction: Towards cloning *pentagon*, a *Drosophila* gene required for response to a specific odorant.

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Behavioral and molecular genetic approaches are being used 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.

A number of ethyl methane sulfonate-induced *Drosophila* mutants were isolated using a T maze behavioral paradigm. One of these mutants, *pentagon (ptg)*, exhibits an odorant-specific defect – it is defective to benzaldehyde, but normal to several other odorants, using two different behavioral assays. Electrophysiologic studies confirm this specificity and suggest that the defect may be manifested in the antenna. The specificity of the defect suggests that *ptg* defines a molecule required in the reception, transduction or processing of a specific subset of chemical information in the olfactory system.

Genetic analysis reveals that *ptg* maps between 8A1-2 and 8A4-5 on the X chromosome. Three *ptg* alleles have been isolated as a result of a hybrid dysgenic cross, which results in the mobilization of P transposable elements. A P-element has been directly visualized in the correct chromosomal position in one of these, *ptg-P-3E*, by *in situ* hybridization to polytene chromosomes. Libraries were generated from all three mutants and screened with a P-element probe in an attempt to isolate genomic DNA flanking the sites of insertion. Four classes of fragment homologous to the probe were identified, and these were used as probes for *in situ* hybridization to wild type chromosomes containing no P-elements. DNA from one of these classes hybridized to the 8A region, suggesting that a portion of the *ptg* locus is contained within this fragment. We are currently using this fragment to clone the corresponding wild-type sequences. The cloning and characterization of the *ptg* locus should provide information about this gene's role in olfaction, and also molecular markers permitting a developmental examination of the olfactory system.

38

Fructose and Glucose Non-tasters in the Hawaiian, *Drosophila adiastola*. JASON E. POSKANZER and LINDA M. KENNEDY (Department of Biology and Neuroscience program, Clark University, Worcester, MA 01610).

Study of taste receptor variants in fruitflies could elucidate transduction mechanisms for sweet taste. Variants showing altered behavioral and receptor cell responses to trehalose, glucose and pyranose sugars are known in the small fruitfly, *Drosophila melanogaster* [Tanimura (1991), Tanimura, et al. (1982), Tanimura, et al. (1988), Rodrigues and Siddiqi (1981), Bhavsar, et al. (1983)], but no fructose or furanose variants have been reported. In the Hawaiian, *D. adiastola*, behavioral and neurophysiological data indicate separate receptor cell mechanisms for fructose and glucose [Her and Kennedy (1991), Poskanzer, et al. (1992)]. Our behavioral tests were adapted from Her and Kennedy (1991). After food deprivation (water ad lib) for 48 h, groups of 43-55 flies (separated by sex) were allowed to drink water and then given a choice between differently colored solutions (glucose or fructose) at concentrations such that the preference index = 0.6 [Her and Kennedy (1991)]. After 4.5 h feeding, flies were placed into individual vials containing colorless sucrose, and allowed to feed for two posttest days. Flies were separated according to color(s) excreted on the sides of the vials: only red, only blue, and both red and blue. Those showing only one color were retested with reversed colors. Thus, any with only red in the first test and only blue in the second were designated "fructose non-tasters", and vice versa for "glucose non-tasters". Both types occurred, at frequencies of 6.1% for fructose non-tasters and 4.0% for glucose non-tasters. There were no significant differences in frequencies according to sex (X^2 test, $p > 0.05$), suggesting that the variance is not sex-linked. Currently, we are breeding a fructose non-taster line and characterizing the defects genetically to determine if they are autosomal or sex-linked in nature. We are also using neurophysiological recordings of receptor cell responses to sugars to characterize these defects.

39

Adaptation-promoting Effect of IP3, Ca2+, and Phorbol Ester on the Sugar Taste Receptor Cell of the Blowfly, Phormia regina

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The fly has a receptor cell highly specialized for the taste of sugars. We introduced IP3, Ca2+, or a phorbol ester (DPBA), into the cell and investigated their effects on the response to sucrose. The sugar receptor cell generates impulses during constant stimulation with sucrose, but the impulse frequency gradually declines as the cell adapts to the stimulus. Thus, this gradual reduction of the impulse frequency is a direct manifestation of adaptation of the cell. These reagents accelerated the gradual reduction of the impulse frequency, although the initial impulse frequency was little affected. In contrast to these reagents, EGTA retarded the gradual reduction of the impulse frequency. Moreover, when IP3 and DPBA were applied together, the gradual reduction of the impulse frequency was more accelerated than when either IP3 or DPBA was applied. When IP3 and EGTA were applied together, however, the accelerating effect of IP3 tended to be canceled.

40

Molecular Mechanisms Underlying Olfactory Specifity in the Moth Antenna.

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The antenna of the moth *Manduca sexta* is an olfactory epithelium organized in a clear mosaic. This epithelium includes 2 major classes of olfactory sensilla: pheromone specific and general-odorant sensitive. Each sensillum further includes 2 types of cell: sensory neuron and support cell; at the onset of development some 100,000 mother cells give rise to discrete clusters of sister neurons and support cells. Previous studies have identified protein molecular markers which are expressed in specific cellular patterns among these various cell types. Three classes of odorant binding protein (OBPs) are differentially expressed in support cells of the respective sensilla classes suggesting that they are markers of sensilla and neuron class specificity. Certain odorant degrading enzymes (ODEs) are expressed in support cells in either pheromone specific alone or in both pheromone specific and general-odorant sensilla. An abundant membrane protein RP11, a putative receptor protein, is expressed in olfactory neurons. We are using this system to identify mechanisms regulating developmental pathways, establishing temporal and spatial patterns of expression in the olfactory epithelium. Studies will be presented which further characterize the moth olfactory proteins with respect to their function and distribution.

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41

Olfactory Reception and Inactivation of Insect- and Host Plant Volatiles by the Beet Armyworm Moth, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae).

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Olfactory reception and inactivation of insect and host plant volatiles were investigated in the beet armyworm moth, *Spodoptera exigua*, using electroantennograms (EAGs) enhanced by novel computer techniques, and single sensillum recordings. Parameters of the EAGs investigated included *rise* of the negative phase, and *decay* following stimulation, both at several time intervals, and a novel parameter called *level*. Level was considered to be a measure of the number of molecules arriving at the receptor sites minus the number of molecules removed. Male moths were more responsive to plant odors than females based on normalized peaks. While none of the five components of female volatile emissions were detected by female moths, receptors in male moths responded to the four behaviorally-active components. Green leaf volatiles (GLVs), benzaldehyde, linalool, and myrcene, elicited the largest normalized peaks in both sexes. Values for rise for pheromone components in males were significantly greater than for GLVs. Decay values were the same for plant odors in both sexes. Decay values for insect-produced compounds were greater than those for plant odors thus indicating specificity of deactivation processes. Differential level values were found for: pheromone components (low); GLVs, benzaldehyde, pentanol, and myrcene (intermediate); and linalool and heptanol (high). Recordings from the trichoid sensilla of males revealed two receptor neurons, one which was reliably stimulated by *Z*-9, *E*-12-tetradecenyl acetate, while the other neuron responded to *Z*-9-tetradecen-1-ol. These neurons also responded with increasing spike frequencies to a GLV and a monoterpene. These results may help to explain not only the larger EAGs recorded from male antennae, and synergism of the female pheromone by GLVs, but also may indicate a role for plant volatiles in the cessation of pheromone-mediated flight.

42

Effect of differing proportions of two 14-carbon acetates on discrimination and upwind flight by the cabbage looper moth, Trichoplusia ni (Hübner).

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The male cabbage looper has three concentration-tuned specialist receptor neurons on the antenna that detect (Z)-7-dodecenyl acetate, (Z)-7-tetradecenyl acetate, and (Z)-7-tetradecenyl acetate at levels that are normally emitted by females. Conventional thought maintains that a mixture of these three compounds and three additional female-emitted compounds is more effective in eliciting sexual behaviors than any single component or other mixture. It would seem that such differences would be discriminated using paired stimulus sources. Thus, the male cabbage looper should be able to discriminate a difference between two stimulus sources, one disseminating a mixture of four 12-carbon acetates and two 14-carbon acetates and one disseminating the same four 12-carbon acetates, but differing proportions of the two 14-carbon acetates. If the differences between such mixtures are discriminable, the concept of the importance of the total mixture could be attributable to discrimination. However, if the male cannot discriminate differences in such mixtures, both the importance of complete mixtures in eliciting sexual behaviors and/or the relatedness of discrimination to perception of single stimuli must be further investigated.

43

The Effects of Developmental Conditions on Peripheral Receptor Neuron Response in The Cabbage Looper Moth, *Trichoplusia ni*.

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The cabbage looper moth is an important agricultural pest of many crusiferous plant crops. As is characteristic of many Lepidoptera, several aspects of reproduction, including long range orientation to mates, are mediated by pheromones. The female cabbage looper produces and releases a blend of pheromone compounds, including the major component, Z-7, dodecen-1-ol acetate (Z-7,12:AC). The male moth possesses a morphologically distinct set of antennal sensilla, which contain receptor neurons sensitive to Z-7,12:Ac. In the southern part of the United States, this insect is multivoltine and consequently successive generations are exposed to different environmental conditions during their development. Since environmental conditions are know to influence pheromone communication, we tested the effect of different temperature and light regimes during development on the response characteristics of the peripheral receptor system in adults. Eggs from a single clutch were divided and raised on the same artificial diet under different temperatures and photoperiods; (Cold reared) at 64.4°F, 12:12 L:D or (Warm reared) at 80°F at 14:10 L:D. Over 90% of the animals emerged as adults under both conditions, however warm reared insects completed their development in 4 weeks as compared to 9 weeks for cold reared moths. Pupal weights of the two groups were similar; 197±12 mg (Mean±SEM) for cold reared and 204±6 mg for warm reared animals. Following emergence, we recorded responses from pheromone sensitive receptor neurons to stimulation with graded amounts of Z-7,12:AC. The response functions of receptor neurons from the warm and cold reared insects were similar in both their threshold of sensitivity and overall dose response relationships to Z-7,12:AC. However, the bulk frequency measures recorded here do not provide any information about the temporal pattern of action potential discharge which may play an important role in information coding. Further analyses are under way to compare these temporal patterns of responses in the two sets of insects. In addition, responses to some of the minor pheromone components will also be examined.

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44

Olfactory Learning In the Honey Bee, Apis mellifera: Perception of Components After Conditioning to Mixtures

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The olfactory systems of both vertebrates and invertebrates are designed to detect potentially thousands of odors that vary in terms of chemical composition and temporal patterning of the signal. Proboscis extension conditioning of the honey bee, during which a 5 sec pulse of odorant is paired with brief a sucrose feeding, can be used to explore how this processing takes place when bees are conditioned to mixtures of two odorants. When tested with the components after conditioning to some mixtures, responses are equally strong to each of the components. In contrast, binary mixtures containing an aliphatic aldehyde and alcohol reliably show asymmetric overshadowing of the latter; that is, subjects respond to hexanal more frequently than they do to 1-hexanol. One explanation for this effect is *generalization decrement*. If the compound is perceived at least in part as a unique stimulus, then stronger responding to one component may be due to a greater perceptual similarity of that component to the compound. To test for such mixture-unique perceptual properties, honey bees were trained to two binary mixtures presented over 12 trials in a pseudorandom sequence. Extinction tests were performed in a pseudorandom sequence with the training mixtures and all four possible novel combinations. If mixture-unique perceptual effects occur, then responding to the novel mixtures should be lower than that to the training mixtures. This trend was never observed in four repetitions using different combinations of odorants. These results suggest that processing of each odorant in a mixture takes place at least in large part independently; for some binary mixtures, there is no need to invoke strong mixture-unique perceptual effects. Such effects may become stronger for mixtures of larger numbers of odorants. Other models (e.g., blocking) may be needed to provide a more complete explanation of olfactory processing.

HPLC Analysis of Squid Ink Reveals Olfactory Stimuli, L-Dopa and Dopamine.

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Squid ink is an alarm substance that both confuses predators and alerts conspecifics to the presence of danger. Although the ejection of ink is a powerful visual stimulus, studies also indicate a chemical component to the signal. Squid ink is mainly composed of inert melanin pigments, however, the nonpigmented portion of the ink contains the enzymes and precursors of melanin synthesis. Our previous behavioral studies showed that squid olfactory organs detect L-dopa[1], a key chemical in melanogenesis. Squid olfactory neurons also respond to dopamine, a biogenic amine not previously described in squid ink. We performed HPLC on ink taken from the ink sacs of adult *Loligo opalescens*. Oxidation of the fresh ink was minimized by including 10mM ascorbic acid and gassing all solutions with nitrogen. The ink was conjugated with ortho-phthaldialdehyde, injected into the HPLC, and amine containing compounds were detected fluorometrically. Standard curves constructed for L-dopa and dopamine allowed quantitation from individual ink sacs. We found that L-dopa was present in undiluted ink at a mean concentration of 1.42 $\pm$ .95 mM (s.d., n=9) and was significantly greater than the mean dopamine concentration of 0.23 $\pm$ 0.15 mM (s.d., n=8). These values are close to those at which both compounds are effective in behavioral[1] and electrophysiological[2] experiments.

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Na^+-Restricted Rats Lack Functional Na^+ Channels in Taste Cell Apical Membranes: Proof by Membrane Voltage Perturbation.

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Rats raised on a low sodium diet during pre- and postnatal development give subnormal chorda tympani (CT) responses to NaCl, but normal responses to other taste stimuli. Evidence, based on the established pharmacology of amiloride, suggests that Na^+-restricted rats do not develop the normal density of apical membrane Na^+ channels in taste cells. However, the validity of this has not been established directly using methods independent of pharmacological agents. We have recorded the CT responses of Na^+-restricted and control rats to NaCl under lingual epithelial current clamp, and positive and negative voltage clamp. The CT responses of Na^+-restricted rats were virtually insensitive to perturbations in apical membrane voltage. In contrast control rat CT responses to voltage perturbations changed in a manner consistent with the presence of taste cell apical membrane Na^+ ion conductance pathways. In the case of controls, CT responses to a given NaCl concentration recorded at -50 mV and +50 mV were respectively significantly enhanced and suppressed relative to current clamp. The voltage sensitivity function defined as R(C,-50) - R(C,+50), where R is CT response, and C is NaCl concentration permits a quantitative estimate of the channel density deficit for Na^+-restricted rats. The maximum voltage sensitivity for control rats was 3.4 in CT response units, but only 0.3 for Na^+-restricted rats. Thus voltage perturbation shows that in the case of Na^+-restricted rats less than 10% of the normal taste cell apical membrane Na^+ conduction pathways are in a functional state.

Vasopressin modulates amiloride-sensitive Na and proton conductances in hamster fungiform taste cells.

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The peptide hormone arginine^8-vasopressin (Arg^8-VP) and the mineralocorticoid aldosterone (ALDO) have been shown to increase the density of amiloride-sensitive (AS) Na channels in transporting epithelia. Because the AS-Na channel has been implicated in both salt and acid transduction in hamster (Gilbertson et al. J. Gen. Physiol. *100*, 803-824, 1992), we have investigated the effects of Arg^8-VP and ALDO on NaCl and acid taste. We have used the perforated patch technique to monitor AS-Na currents (I_{Na}) and proton currents (I_{H+}) in isolated hamster fungiform taste cells. Following a 10-20 min treatment with Arg^8-VP (10 mU/ml), I_{Na} and I_{H+} were each enhanced several fold. The enhanced I_{Na} and I_{H+} following Arg^8-VP treatment were completely blocked by amiloride (30 μM). A membrane-permeant form of cAMP (8-bromo-cAMP; 0.25 mM) mimicked the effects of Arg^8-VP, causing a 2-to-3 fold increase in I_{Na} and I_{H+}. On the other hand, ALDO had no effects on taste cells when applied for as long as 1.5 hr. Following ALDO treatment, I_{Na} and I_{H+} were unchanged. Longer applications of ALDO were not tested. These results are consistent with the reported effects of these hormones in epithelia. Arg^8-VP (via cAMP) leads to incorporation into the plasma membrane of preformed AS-Na channels from submembrane vesicles, a process requiring 10-30 minutes. ALDO, on the other hand, requires 3-24 hr to act since it is believed to modify characteristics of existing apical channels through a process requiring *de novo* protein synthesis. The modulation of AS-Na channel density by Arg^8-VP suggests that the initial events in gustation may be under hormonal control. Under certain conditions (hypovolemia, salt deprivation), this hormone is released into the bloodstream. We speculate that Arg^8-VP may reach the taste cells via the lingual artery, cause an increased density of AS-Na channels, and, in turn, enhance the perception of salty and acid stimuli.

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Biochemical and Physiological Evidence for Dual Transduction Pathways in Lobster Olfactory Receptor Neurons.

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Previously, we reported that both cAMP and IP_3 are putative second messengers in lobster olfactory receptor neurons (ORNs) and proposed that cAMP and IP_3 mediate distinct transduction pathways in the same cell (Michel and Ache, *J. Neurosci.*, 12:3979, 1992; Fadool and Ache, *Neuron*, 9:907, 1992). Evidence implicating IP_3 was obtained from cultured somata of lobster ORNs; IP_3 has yet to be localized to the outer dendrites of lobster ORNs. We now report that odors rapidly and transiently elevate levels of cAMP and IP_3 in outer dendritic membranes *in vitro*. Consistent with their physiological effects, the odors proline and taurine (100 micromolar) elevated both cAMP and IP_3. Proline, however, preferentially elevated cAMP while taurine preferentially elevated IP_3. In other experiments, 100 micromolar cAMP and 0.2 micromolar IP_3 applied to the inner face of cell free patches pulled from vesiculated outer dendrites of lobster ORNs activated at least two different types of channels that could be discriminated by their relative permeability to K^+. Most patches contained ligand activated channels, and at least several patches yielded channels of both types. The biochemical and physiological evidence are consistent with the proposition that cAMP and IP_3 are olfactory second messengers in lobster ORNs. The presence of cAMP and IP_3 activated channels in the same membrane is direct evidence that cAMP and IP_3 mediated transduction pathways can occur in the same cell.

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Co-Existence of Cation and Anion Components in the Odorant-Induced Current of Vertebrate Olfactory Receptor Neurons

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Odorant-induced currents were recorded in isolated olfactory receptor neurons under the whole-cell voltage clamp mode, and their ionic dependence was investigated. In addition to the well-known cationic conductance, it was found that odorant-induced currents contained Cl^- component. The Cl^- component appeared only when the bathing solution contained Ca^{2+}. Since the odorant-activated cationic conductance has been shown to be permeable to Ca^{2+} and since the anionic current trailed the cationic current, we concluded that the anionic inward current was activated by an influx of Ca^{2+} through the odorant-activated cationic channel. This idea is further supported by the finding of Ca^{2+}-activated Cl channel in the isolated ciliary preparation (Kleene & Gesteland, 1991). Using SITS as a selective blocker, we have estimated that Cl^- carries roughly half of the inward current at -50 mV. Perforated-patch experiments have also confirmed that the Cl^- equilibrium potential is more positive than resting membrane potential, suggesting that the activation of Cl^- channel contributes to the depolarization. We speculate that the co-existence of cation and anion components in the olfactory transduction current may serve to compensate against changes in mucosal ionic concentration.

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Origin of the Chloride Component of Olfactory Receptor Current.

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Recent evidence in amphibians suggests that much of the receptor current during an odorant response may be carried by chloride ions. It is known that the olfactory cilia have a substantial calcium-activated chloride conductance. The following model explains how the known odorant transduction pathway can give rise to a secondary chloride current. Initially, odorants cause activation of the ciliary cAMP-gated channels. This allows cations, including calcium, to flow into the cilium. When sufficient calcium accumulates inside the cilium, it activates the calcium-activated chloride conductance. Evidence for this model was obtained in single cilia excised from frog olfactory receptor neurons. In the presence of cytoplasmic cAMP, voltage jumps from 0 mV to negative potentials resulted in a biphasic inward current. Initially, the cilium passed a steady current due primarily to the influx of cations through the cAMP-gated channels. Subsequently, current increased to a second plateau. The additional inward current in the second phase was due to a chloride efflux through the calcium-activated chloride conductance. This chloride phase was eliminated when cytoplasmic chloride was replaced by methanesulfonate, or on addition of the chloride-channel blockers niflumic acid or 3',5'-dichlorodiphenylamine-2-carboxylate. These blockers had no effect on the cAMP-activated conductance. Elimination of extracellular calcium by intrapipette perfusion also abolished the second phase of the current. In the presence of a cytoplasmic calcium buffer (2 mM BAPTA), the second phase often took 2-3 sec to appear. When calcium buffering was reduced tenfold, the onset of the second phase was much earlier. With 100 μM cytoplasmic cAMP present, the amount of secondary chloride current corresponded to an intraciliary calcium concentration of >10 μM, which saturates the channels. Current through the chloride conductance was greater than that through the cAMP-activated cationic conductance by about threefold.

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Rapid Application and Removal of Second Messengers Reveals Integrative Properties of Olfactory Signal Transduction.

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Signal transduction in vertebrate olfactory receptor neurons involves the G-protein dependent activation of a second messenger cascade that leads to the production of the cyclic nucleotide cAMP and a subsequent direct gating of a non-specific cation channel (cN channel) by cAMP and possibly also by cGMP. We previously described single-channel properties of the cN channel under steady state properties of agonist application. In addition, regulation of adaptation and blockage of this channel by divalent cations has been investigated.
A long standing problem in olfactory physiology is the difference in time course between the odor-induced second messenger accumulation, which is very fast and transient (peak within 50 ms) and the much slower electrical response of the cell. We therefore initiated a series of experiments in which rapid pulses (rise time < 1 ms) of second messengers were applied to inside-out patches containing either the native salamander cN channel at low density or a cloned rat cN channel at high density. Agonists were applied using a piezo-switch device so that the effect of agonist concentration and pulse length on both rate of rise and rate of decay could be tested.
Surprisingly, these experiments showed that both the onset and the offset kinetics of the olfactory cN channel were rather slow. The activation kinetics of the channel seemed to be especially rate-limited by the binding reaction of the second messenger even at a concentration as high as 1 mM cGMP. Several tests were undertaken to assure that the kinetic behavior was due to channel gating and not to restricted diffusion in the patch. Therefore, the traditional idea that the cN channel behaves like a static sensor of second messenger concentration does not seem to hold true in olfactory cells. Instead, it is the slow intrinsic time course of channel gating that determines the time course of the electrical response, giving the ion channel the role of an integrator for the rapid second messenger pulses.

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Isolation of Human Olfactory Neurons and First Electrophysiological Charcterization

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Relative little is known about the mechanism of olfactory transduction at the molecular or cellular level in man. Biopsy material from the epithelium of the middle turbinates of patients, who suffered from polyposis was obtained during surgery. Additional biopsy material from the upper part of the first turbinate was examined to investigate the distribution of olfactory neurons in the periphery of the nose. Immediately after biopsy the tissue was suspended in a special culture medium. The tissue was cut into small pieces and digested with trypsin, which resulted in the release of single cells. Olfactory neurons could be easily recognized by their charcteristic morphology: the presence of cilia protruding from the olfactory knob, the bipolar form and mostly the presence of a piece of axon. We developed a culture medium which allows transport and storage of the isolated cells. Recordings from excised inside out patches could be made up to seven hours after isolation of these olfactory neurons. Responses from whole cell patches after application of mixtures of perfumes could be recorded. Using inside out patches we started to investigate the effects of cAMP and cGMP and other purine nucleotides in the millimolar range.

Sweet Taste Transduction in the Hamster: The Role of Cyclic Nucleotides and Cations. THOMAS A. CUMMINGS & SUE C. KINNAMON (Colorado State University and the Rocky Mountain Taste and Smell Center).

In mammals sweet stimuli are thought to be transduced by two different receptor-mediated mechanisms. In one model, cAMP has been implicated as a second messenger (Striem et al., Biochem. J. 260:121-126, 1989), yet its precise role remains unclear. In the alternative model, sweeteners are thought to activate a ligand-gated cation channel (DeSimone et al., Science 214:1039-1041, 1981; Mierson et al., J. Gen. Physiol. 92:87-11, 1988) permitting an influx of Na^+ to depolarize the membrane. In this study we have used *in situ* recording of hamster taste buds to delineate further the role of cyclic nucleotides and cations in sweet taste transduction. Three control solutions were used: A 30 mM N-methyl-D-glucamine ($NMDG^+$) control devoid of permeant cations, an artificial hamster saliva (in mM; 6.6 Na^+, 43 K^+, 1.5 Ca^{++}, and 1.3 Mg^{++}), and a 100 mM KCl solution. The following stimuli (in mM) were dissolved in these controls and perfused through a recording pipette placed over fungiform taste buds: Sucrose (200), a high-potency artificial sweetener NC-00274-01 (0.1), 8 cpt-cAMP (2), and dibutyryl-cGMP (2). One in four taste buds responded to both artificial and natural sweeteners with rapidly adapting trains of action currents. All sweet-responsive taste buds responded to cAMP. Of 8 sweet-responsive taste buds tested, 7 also responded to cGMP. No sweet-unresponsive buds responded to cyclic nucleotides. When sweeteners or cyclic nucleotides were applied with cation-containing controls there was no enhancement of the response compared to those in the $NMDG^+$ controls. These data are consistent with the hypothesis that cAMP and/or cGMP act as second messengers in the transduction of both natural and artificial sweeteners, and suggest that ligand-gated cation channels are not involved. Studies are underway to determine the precise role these nucleotides play in the transduction process.

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Non sugar sweeteners activate cellular formation of Inositol triphosphate (IP_3) in circumvallate (CV) taste buds sheets of the rat.

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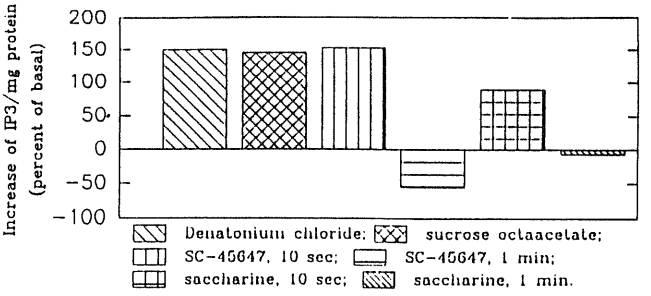
Studies with lingual taste membranes and with intact CV taste bud sheets have suggested that cAMP is involved with sweet taste transduction induced by sugars. SC-45647 is a potent synthetic guanidine sweetener for humans and rats. Unlike sucrose, SC-45647 and sodium saccharine did not stimulate intracellular formation of cAMP in CV taste bud sheets of rats. To investigate a possible role of IP_3 in the transduction of sweet taste induced by these sweeteners, intact epithelial and CV tissue sheets were isolated. Four halves (one from each rat) of each tissue were pooled and then incubated (35°C) with a taste stimulus. Incubation of the corresponding non stimulated pool indicated the basal (control) level. Positive controls were bitter taste stimuli such as denatonium chloride and sucrose octaacetate, which activate the phosphoinositide pathway in the rat. Stimulation for 1 minute by 1μM denatonium chloride or 1mM sucrose octaacetate resulted in 150 and 145% increase of the IP_3 level, respectively. Stimulation for 10 seconds with 1μM SC-45647 and 20 mM saccharine significantly increased the formation of the signal molecule IP_3 in the CV taste bud sheets 152 and 89%, respectively. Stimulation during 1 minute with SC-45647 resulted in a significant decline in the content of IP_3 compared to the basal level, while saccharine induced IP_3 levels were not statistically different from the basal. IP_3 levels in non sensory epithelial tissue were not affected by these sweeteners. The decline in IP_3 levels that occurred after 1 minute is probably due to accelerated degradation. The present results suggest that more than one second messenger may be involved.



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We thank the NutraSweet Co., Dr. C. Nofre and Dr. J-M. Tinti for providing samples of SC-45647.

Taste Cell Expression of G-proteins

SUSAN K. McLAUGHLIN, ALAIN ROBICHON, NANCY SPICKOFSKY AND ROBERT F. MARGOLSKEE

Our laboratory is studying the molecular and cellular biology of mammalian taste transduction. Our approach has been to clone taste cell homologues of known signal transduction proteins. Toward this end we prepared a cDNA library from rat circumvallate and foliate taste papillae. We initially focused our efforts on cloning guanine nucleotide binding regulatory proteins (G proteins) since these proteins mediate signal transduction in olfactory, visual and taste systems. The polymerase chain reaction (PCR) was used to amplify and clone taste cell G protein α subunit cDNAs. Sequence analysis followed by RNase protection and *in situ* hybridization demonstrated elevated expression of α_{i-3} and α_{14} in taste tissue vs. non-taste tissue. We are examining other G protein α clones for elevated expression in taste tissue.

Role of Beta-adrenergic Receptor Kinase, Beta-arrestin , and Cyclic GMP in Olfactory Desensitization.

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TED M. DAWSON (Johns Hopkins U. School of Medicine)
ROBERT E. BAKIN (Johns Hopkins U. School of Medicine)
HELEN H. CHO (Johns Hopkins U. School of Medicine)

Beta-adrenergic receptor kinase (B-ARK) and beta-arrestin (B-ARR) function in agonist-activated or homologous desensitization of G-protein-coupled receptors. We have localized the isoforms B-ARK-2 and B-ARR-2 as highly enriched in the dendritic knobs and cilia of the olfactory receptor neurons (ORN's), where the initial events of signal transduction occur. Preincubation of isolated rat olfactory cilia with neutralizing antibodies raised against B-ARK-2 and B-ARR-2 fusion proteins increases the odorant-induced elevation of cAMP and attenuates desensitization. Using rat B-ARK-2 and B-ARR-2 clones, probes have been designed to identifiy olfactory-specific isoforms.

Using primary cultures of ORN's, odorants are shown to cause a slow (10 sec) and sustained elevation in cGMP . This rise is dose-dependent on odorant concentration, while the percentage increase varies for each odorant. Manipulation of cGMP levels with inhibitors of guanylyl cyclase or cGMP phosphodiesterase enhances or diminishes the odorant-induced cAMP response, respectively, suggesting cGMP may function in desensitization.

57

Stimulus-Response Relations of Individual Olfactory Receptor Neurons Determined With Controlled Odor Stimuli.
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Vertebrate olfactory receptor neurons respond to a wide range of odor molecules with an inward cationic current that can be measured with the whole cell patch clamp. Although the broad outlines of the transduction cascade are now understood, an important quantitative measure, the relationship between the stimulus intensity and the cellular response, is missing. This is largely due to the difficulty of delivering a controlled stimulus to olfactory receptors. We have utilized a "concentration clamp" technique in which cells are immersed in known concentrations of odor solutions for carefully controlled durations while the odor elicited current is measured. Applying mixtures of odors and then individual components of the mixtures over a wide concentration range we found cells that responded to one or more odors with different affinities. More than half the cells tested responded to more than one of the three odors presented suggesting that most cells have generally broad odor specificities. The dose-response curves over a large number of cells indicated generally low affinities and relatively narrow dynamic operating ranges. We also found that over a 1.2 second time period cells integrate stimulus information and both stimulus duration and concentration are critical to determining the magnitude of the odor response. Taken together these features enable the olfactory system to detect a large number of ligands with adequate sensitivity.

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58

Standing Calcium Gradients in olfactory receptor neurons: patch clamp and calcium imaging analysis
F.W. LISCHKA AND D. SCHILD (Univ. Göttingen/FRG)

We have used a digital imaging technique in conjunction with the patch clamp technique to investigate the intracellular calcium concentration and its spatial distribution in isolated olfactory receptor neurons of Xenopus laevis using the Ca^{2+} indicator dyes fura-2 and fura-2/AM. The cells were held under voltage clamp whole-cell conditions throughout the experiments. Resting calcium concentrations in cells loaded with fura-2/AM were between 10 and 200 nM and depended only weakly upon the extracellular calcium concentration. In cells which were loaded with the penta-potassium salt of fura-2 through the patch pipet, calcium concentrations were in the same range if ATP was added to the pipette solution. Otherwise Ca^{2+} reached concentrations of about 500 nM. Most of the observed cells showed a standing gradient of calcium, the calcium concentrations in the distal dendritic end of the cell being higher than in the soma. In some cells, the gradient was markedly reduced or abolished by adding either Amiloride or Ruthenium Red to the bath solution. In a few cells, neither drug had any effect upon the gradient. It is suggested that the inhomogenous spatial distribution of intracellular calcium in olfactory cells of Xenpus laevis is brought about by an influx of calcium ions through at least two different calcium permeable conductances in the peripheral compartments of the cells. In 18 cells we observed that low seal resistances can be a conspicuous source of Ca^{2+} in patch clamped olfactory neurons. The resulting, highly increased Ca^{2+} concentrations were correlated with a destruction of the neurons' normal morphological shape.

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59

Intracellular-messenger-dependent calcium channels open before Ca^{2+}-dependent cation channels in cultured insect olfactory receptor neurons.
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After 2-3 weeks in culture, pupal olfactory receptor neurons (ORNs) from Manduca sexta males respond to their species-specific sex pheromones with opening of cation channels (Stengl et al., J. Neurosci. 1992). Relatively long delays suggested an indirect opening of the pheromone-dependent cation channels via intracellular messengers. Apparently directly Ca^{2+}-dependent cation channels as well as protein kinase C-dependent cation channels occur in cultured ORNs with properties similar to the pheromone-dependent cation channels (Stengl, J. Exp. Biol. 1992 submitted). Since no intracellular Ca^{2+} stores are apparent in the outer dendrite where the transduction cascade is thought to start, it was examined, whether Ca^{2+} influx from outside through intracellular-messenger-dependent channels might initiate cation currents. Because transient rises of inositol trisphosphate (IP_3) precede pheromone-dependent receptor potentials (Breer et al., Nature 1990) it was tested whether IP_3-dependent Ca^{2+} channels occur in cultured ORNs.

Here I report that IP_3-dependent Ca^{2+} currents occur in cultured ORNs. These currents always initiate Ca^{2+}-dependent cation currents. Furthermore it is shown that after pheromone application transient inward currents, which share properties with the IP_3-dependent Ca^{2+} currents, occur before pheromone-dependent cation currents.

This work was accomplished in the laboratories of Drs. J.G. Hildebrand and R.B. Levine (University of Arizona, Tucson, USA) and was supported by DFG grant STE 531 and in part by NIH grant AI-23253 to J.G. Hildebrand.

60

Intensity and Hedonics of Gustatory and Olfactory Stimuli In Taste-Smell Mixtures.
LORI A. WHITTEN & H.P WEINGARTEN (McMaster University).

We examined whether the hedonics of gustatory and olfactory stimuli could be influenced by one another. In Experiment 1, undergraduates rated taste intensity and hedonics of sucrose-strawberry mixtures (0.0, 0.1, 0.2, 0.3, & 0.4 M with 0.0 or 5.0 ml/L strawberry). Odor enhanced taste intensity, but not hedonics. In Experiment 2, undergraduates rated odor intensity and hedonics of sucrose-strawberry mixtures (0.0, 0.1, 0.5, 2.5, & 5.0 ml/L with 0.0 or 0.4 M sucrose). Taste enhanced both intensity and hedonics of strawberry odour. All these effects were larger when taste and odor were both presented in the mouth compared to presentation of taste in the mouth and odor in the nose using a Two-Module Delivery System. These data indicate that taste and smell may interact to influence hedonic judgements and that the hedonics of smell are more labile than those of taste.

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61

Taste-Smell Interactions With Multiple Sweeteners.
N. J. VAN DER KLAAUW and R. A. FRANK (University of Cincinnati)

Previous research in our laboratory has demonstrated that taste-odorant interactions are tastant and odorant dependent. Continuing investigation revealed that odor-induced changes in sweetness judgments were dramatically influenced by instructions. Several odorants enhanced sweetness when sweetness alone was judged, while sweetness was suppressed for the same stimuli when total intensity ratings were broken down into ratings for the sweetness, saltiness, sourness, bitterness and fruitiness of each solution. Similar interaction results were observed using different target tastes, and heterogeneous taste mixtures.

The odor-induced enhancement of sweetness of sucrose may be due to the conceptual overlap between the sweetness of sucrose and the fruitiness of the odorants. If subjects are asked to judge sweetness only, these "fuzzy" concepts are combined, causing enhancement. When subjects are given an opportunity to make separate assessments of sweetness and fruitiness, this enhancement disappears. If indeed the cognitive confusion of sweetness and fruitiness causes the enhancement effect, similar results should be observed when other sweeteners are used. The present experiment investigated mixtures of fructose, aspartame and saccharine with strawberry and wintergreen odor (the latter is rated as sweet, but not as fruity). Preliminary results show that strawberry odor indeed enhanced the sweetness of all the sweeteners when sweetness alone was judged, whereas it suppressed the sweetness when the same subjects made multiple ratings. As expected, wintergreen odor produced no enhancement of sweetness.

62

Oral Sensitivities and Saliva Flow/Composition
CHRISTINE E. ZOUMAS (The Pennsylvania State University)
JEAN-XAVIER GUINARD (The Pennsylvania State University)

Studies of oral sensitivities have been limited mostly to threshold measurements and scaling of suprathreshold levels of taste stimuli, and the role of saliva has seldom been addressed. The objectives of this study were (1) to examine the temporal perception of both taste and texture attributes in young adults using time-intensity (TI) measurements; (2) to simultaneously collect saliva to study inter-individual differences in saliva flow and composition; and (3) to examine the relationship(s) between saliva flow/composition and oral sensitivities. A computerized system was designed for simultaneous TI and saliva flow data collection. Twenty subjects between 18 and 30 years of age participated in the study. Seven sensory attributes were rated in duplicate for three stimulus concentrations as follows: sweetness of lemonade with 0.39, 0.56 and 0.79 g/L aspartame; umami flavor of chicken broth with 1, 5.5 and 10 g/L monosodium glutamate; astringency of white wine with 0, 350 and 700 mg/L added tannic acid; bitterness of beer with 0, 15 and 30 mg/L added isohumulones; viscosity of sweet water with methyl cellulose at 25, 400 and 1500 centipoise; adhesiveness of peanut butter with three levels of peanut oil; and cohesiveness of mass of crackers with three moisture levels. Saliva was analyzed for Na^+, K^+, Ca^{++}, Mg^{++}, total protein, glucose and ∂-amylase activity. Significant inter-individual differences were found for saliva flow and composition. Stimuli with a low pH and stimuli requiring mastication before swallowing produced a significantly higher salivary flow than the other stimuli. In turn, the main determinant of variations in saliva composition was salivary flow. The relationships between TI and saliva parameters are examined using correlation coefficients, multiple regression and principal component analysis.

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63

PROP Supertasters and the Perception of Ethyl Alcohol. L.M. BARTOSHUK, E. CONNER, D. GRUBIN, T. KARRER, K. KOCHENBACH, M. PALCSO, D. SNOW, (Yale University School of Medicine), M. PELCHAT (Monell Chemical Senses Center), S. DANOWSKI (San Diego State University).

Threshold and suprathreshold criteria can be used to classify taste responses to PROP (6-n-propylthiouracil). The suprathreshold criteria used were based on ratios of PROP intensities to NaCl intensities using the following concentrations: .001 P/.32 N and .0032 P/1.0 N. The groups were as follows. Nontasters: average ratios ≤ .4, thresholds ≥ .0002; medium tasters: average ratios between .4 and 1.2; thresholds ≤ .0001; supertasters: average ratios ≥ 1.2 and thresholds ≤ .0001. When 10% ethyl alcohol was applied to the tip of the tongue, it tasted more bitter and felt more irritating to supertasters than to nontasters. When a series of ethyl alcohol concentrations (10%-50%) were tested with the whole mouth (sip and spit method), supertasters and medium tasters perceived more bitterness than nontasters did for all concentrations. Supertasters and medium tasters perceived more irritation than nontasters for 30-50% alcohol. The concentrations used in these studies are encountered in alcoholic beverages (e.g., 100 proof vodka is about 50% ethyl alcohol). There has long been evidence linking PROP nontasting and alcoholism but the significance of this was unclear since exposure of taste receptors to alcohol might have produced damage that made subjects appear to be nontasters. However, recent work (Pelchat and Danowski, 1992) showing that nonalcoholic children of alcoholics show a higher proportion of nontasters than nonalcoholic children of nonalcoholics suggests a genuine nontaster-alcoholism link. We suggest the possibility that medium tasters and supertasters might be protected from alcoholism because ethyl alcohol is less palatable to them than it is to nontasters.

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64

Relationships among Papillae, Taste Pores, and 6-n-Propylthiouracil (PROP) Suprathreshold Taste Sensitivity. F.E. REEDY, Jr.[1], L.M. BARTOSHUK[2], I.J. MILLER, Jr.[1], V.B. DUFFY[2], K. YANAGISAWA[2] (Bowman Gray School of Medicine, Winston-Salem, NC[1]; Yale University School of Medicine, New Haven, CT.[2])

We hypothesize that suprathreshold taste responsiveness to PROP (6-*n*-propylthiouracil) may be related to anatomical variations among taste receptor organs in humans. Eighteen subjects (Ss) (8 males, 10 females) aged 11-59 years, participated in suprathreshold scaling of PROP and sodium chloride (NaCl) taste intensities and in anatomical studies of their tongues. Taste pores (tp) and fungiform papillae (pap) were quantified with videomicroscopy. Ss with higher PROP responsiveness also had higher pap densities (Spearman rho (r)=0.64, p<0.01), tp densities (r=0.79, p<0.01), % pap with ≥ 8 tps (r=0.76, p<0.01), and a higher number of tp/pap (r=0.69, P<0.01). Ss with higher PROP responsiveness also had fewer pap lacking tps (r=0.62, p<0.01). Pap of individuals with higher PROP responsiveness were smaller in surface area (r=-0.54, p<0.05). Ss were classified into nontasters (NT), medium tasters (MT) and supertasters (ST) using thresholds and the average of two ratios of PROP/NaCl: 0.001P/0.32N and 0.0032P/1.0N. NTs had thresholds ≥0.0002 and ave ratios ≤ 0.4. MTs had thresholds ≤ 0.0001 and ave ratios between 0.4 and 1.2. STs had thresholds ≤0.0001 and ave ratios ≥1.2. For NT, MT and ST pap/cm^2 were 61, 78, and 106; tp/cm^2 were 96, 184, 425; and ave pap areas were .67, .47, .38 mm^2, resp. Ring-like structures were present around 16, 61, and 78%, resp. of the pap. Papillae with ≥ 8 tps were found in 0, 3.4, and 17% of the NTs, MTs, and STs, resp. These results tend to support the hypothesis that variations in responsiveness to PROP are related to anatomical differences among fungiform papillae and to the relative number of fungiform taste buds that subjects have.

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Statistical Analysis of Taste Clinic Outpatients Examined Between Years 1976-1990

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Our "Taste Clinic" established especially for patients with taste disorders, has examined a total of 2,801 patients between the frame of 1976-1990. A statistical analysis of all outpatients examined during this 15 year period was conducted and it's results are reported herein. Our study found the sex ratio of patients to be male 2: female 3, with the former totaling 1,081 patients and the latter 1,720. An examination of the age composition exhibited two peaks, the first being in the 50's and the second being in the 70's. An increase in the ratio of older patients from the 1980's was also observed. The time from illness to actual examination was investigated in 3 five- year time frames and it was determined in all time frames that 3 months was the peak length of time for patients to take before coming in forexamination 19% of the total appear to visit our clinic within 3 months; by 6 months, the number rose to 50%. Early diagnosis and treatment of taste disorders are vital for effective treatment; accordingly, these figures seem to sadly suggest the lack of public understanding in Japan regarding taste disorders. Taste disorders were classified using the filter paper disc method and accordingly, 5.2% of all examined cases were found to be severe, 22.7% of medium severity, 46.8%, mild, and 7.7%, healthy. No difference was seen between respective years on this matter.

Correlation and Multifactorial Analysis of the Threshold of the Electrogustometry

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(Department of Otolaryngology, Nihon University, School of Medicine, Tokyo JAPAN)

Electrogustometry was carried out on 106 non-smoking healthy subjects under 30 years of age. The taste threshold was determined in the chorda tympani nerve, the greater petrosal nerve and the glossopharyngeal nerve areas, respectively. Between both sides the threshold was significantly correlated in all nerve area ($r>0.625$). Correlations between the three nerve areas were also significant. At the same time, threshold was analysed into principal components. The whole component was explained in 75% by the first and second principal components. For the sake of comparison, we also conducted whole mouth gustatory test on 18 of the 106 subjects. Correlation between the principal component from the threshold of electrogustometry and those of the whole mouth test was not significant.

Assessing the Value of Electrogustometry as a Clinical Tool.

CHRISTOPHER T. SAMPSON, MARION E. FRANK, APRIL E. MOTT (University of Connecticut Health Center, Farmington, CT)

In order to assess the appropriateness of the Rion TR-06 Electrogustometer for use at the Connecticut Chemosensory Clinical Research Center (CCCRC), anterior tongue thresholds were obtained on 6 male and 6 female, age-matched subjects between the ages of 25 and 52 (mean = 37.54). Subjects were assigned to two groups based on the side of the tongue to be tested first. Anodal current was applied to the anterior edge of the tongue, approximately 1.5cm to either side of the median furrow. Each subject was initially presented with a forced-choice between a -6dB (4.0ua) signal and a blank stimulus, and asked to choose the stronger of the pair. Assignment of the blank stimulus to the first or the second of the stimuli within a pair was randomized. The signal strength was incremented by 2dB with each incorrect choice, until the stimulus at a particular intensity was chosen over the blank five times consecutively. Subjects' responses were compared with respect to sex and side tested first (t test), and also with respect to age (Pearson r). No correlation between threshold response and age was found. Neither was there observed a difference attributable to sex. Left anterior thresholds were found to be lower in those subjects tested on the right side first than in those tested on the left side first, possibly suggesting the presence of a learning effect. Finally, it was found that the thresholds were grouped near the lowest end of the instrument's scale (combined mean=-1.00dB, SD=6.02), possibly an indication that the range of the instrument -- 4.0ua (-6dB) to 400ua (34dB) -- does not adequately extend to include the weaker signals.

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Taste Performance of the Anterior Human Tongue Varies With Fungiform Taste Bud Density

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Humans may demonstrate individual diversities of taste sensitivity on the anterior tongue due, in part, to variations in fungiform taste bud density. The purpose of this study was to determine the relationship of discriminative taste performance for citric acid with a known number of taste buds. A two-alternative forced choice, modified staircase procedure was used to derive detection thresholds for citric acid in 84 subjects. A visual analog scale was used to measure judgment for 5 concentrations of citric acid. Citric acid solutions were delivered to an isolated, spatially matched low rate (10cc/min) flow chamber on the anterior surface of the tongue. The taste buds bathed by stimuli within the chamber were distinguished by methylene blue stain and recorded by videomicroscopy. The sip and spit method was used to contrast the spatially-matched condition with whole mouth stimulation. The results demonstrated: (i) an inverse relationship between taste bud density and detection threshold; (ii) a minimum number of taste buds are reqired to scale suprathreshold concentrations; (iii) y-intercept approaches zero intensity as the number of fungiform papillae decreases; (iv) spatially matched threshold was elevated 2-3 log units above whole mouth threshold; and (v) averaged spatially-matched visual analog scaling functions were decreased by the same proportion across the dynamic range of concentrations compared to whole mouth stimulation.

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69

Gustatory and Appetitive effects of Cannabinoids. RICHARD MATTES (Monell Center), LESLIE SHAW and KARL ENGELMAN (Hospital of the University of Pennsylvania).

Anecdotal evidence suggests cannabinoids may stimulate appetite and enhance chemosensory function. The present study evaluated these properties in an age and gender stratified sample of 57 healthy adults in a double-blind, placebo-controlled trial. Subjects reported to the hospital at 8:00 AM, stimulated saliva was collected, physiological measures (e.g., heart rate, blood pressure) were determined, a battery of sensory tests (magnitude estimation and hedonic ratings of 5 concentrations of NaCl in tomato juice, sucrose in fruit-flavored beverage, citric acid in lemonade, urea in tonic water, optimization of these beverages) was completed and health and dietary information collected. A standard breakfast was then served and placebo or active medication (15mg for males, 10mg for females) provided. Measurements were repeated 2, 4 and 6 hours later. A snack tray of preweighed foods was available after 10:00AM and lunch was self-selected. Total daily intake was monitored. The procedure was repeated about 3 weeks later with the other form of treatment. Subjects evidencing drug absorption on the active treatment day had significantly higher energy intake, desired level of intake, heart rate, diastolic blood pressure and reported thirst. Significant consistent changes were not noted for intensity or hedonic responses or salivary flow rate. These data confirm the appetite stimulating property of cannabinoids and suggest this action is not related to enhanced sensory function. Studies are underway to determine whether route of drug delivery (e.g., smoking, sublingual, rectal suppository) may alter these findings.

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70

Investigation of the relationship over time between oral glucose concentration and perceived sweetness intensity.
B. GUGGENBUHL and A.C. NOBLE (University of California, Davis, CA 95616)

To examine factors influencing temporal sweetness perception, twenty trained subjects (S) continuously rated sweetness of aqueous glucose solutions of 20, 80 and 140 g/L by time-intensity methodology (TI) from ingestion, through expectoration at 7 sec until extinction of the sensation. While the Ss rated sweetness, expectorated saliva was collected at 7 sec and at five subsequent 30 sec intervals. Salivary flow was collected unilaterally from the parotid gland to assign S to salivary flow groups. Glucose concentration in the expectorated saliva samples, analyzed using an automated immobilized enzyme assay, was highly correlated with that of the glucose stimuli. When Ss were grouped on the basis of accumulated saliva flow, the concentration of glucose in the expectorated saliva was highest for low-flow S. Within the low-flow S,although there was considerabile variablity, the slopes of the sweetness intensity decay curve were correlated with those of the glucose saliva concentration. Comparison of the decay responses of high and low flow S, however, failed to show a systematic relationship between perceived duration of sweetness and salivary glucose concentration, despite previous efforts to train the S to standardize their time-intensity responses. These data will be discussed in detail to show the relationship over time between perceived sweetness and oral glucose concentration.

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71

Multivariate Analysis of the Time-Intensity Profiles of Sweet and/or Bitter Stimuli
DOREEN Y. HONG (The Pennsylvania State University)
JEAN-XAVIER GUINARD (The Pennsylvania State University)

The time-intensity (TI) methodology is being used extensively to investigate the sensory properties of taste stimuli. The purpose of this study was to evaluate the usefulness of various TI parameters in the investigation of taste chemoreception. The TI profiles of 23 sweet and/or bitter stimuli were compared across 25 subjects at a concentration equi-intense to that of a 200 mM NaCl solution. Subjects rated the overall taste intensity of the solutions in triplicate using a computerized system for TI measurements. Various methods of TI curve normalization and averaging were compared. The following parameters were extracted from TI curves: (1) maximum intensity, (2) total duration, (3) time to maximum intensity, (4) persistence (time for the intensity to drop to half its maximum value), plateau intensity (5) and duration (6), (7) area under the curve, and maximum rates of onset (8) and extinction (9). The data for each TI parameter was analyzed by analysis of variance (AOV), principal component (PCA) and cluster (CA) analyses. Significant differences were found for all TI parameters across the stimuli ($p<0.001$). Replications were not a significant source of variation except for total duration and persistence ($p<0.05$). In the PCAs and CAs of intensity-related parameters, stimuli were clustered based on their taste quality (sweet vs. bitter) thereby suggesting the existence of two distinct receptor mechanisms for sweet and bitter tastes. Total duration was found to be unidimensional and independent of suprathreshold quality.

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72

Sweetness Intensity Ratings of Binary Mixtures of Various Sweeteners. SUSAN S. SCHIFFMAN[1], ELIZABETH SATTELY-MILLER[1], BREVICK G. GRAHAM[1], SUZANNE D. PECORE[2], BARBARA J. BOOTH[2], and MICHAEL L. LOSEE[2] ([1]Duke University and [2]The NutraSweet Co.)

The purpose of this study was to determine the intensity of mixtures of sweeteners varying in chemical structure. A trained panel tasted fourteen sweeteners: 3 sugars (fructose, glucose, sucrose), 2 polyhydric alcohols (mannitol, sorbitol), 2 terpenoid glycosides (rebaudioside-A, stevioside), 2 dipeptide derivatives (alitame, aspartame), 1 sulfamate (sodium cyclamate), 1 protein (thaumatin), 2 n-sulfanylamides (acesulfame-K, sodium saccharin), and 1 dihydrochalcone (neohesperidin dihydrochalcone). These fourteen sweeteners were tested at three concentrations that were isointense with 3%, 5%, and 7% sucrose. The intensity of mixtures of each of these sweeteners mixed with an identical intensity of alitame, aspartame, sodium saccharin, sorbitol, stevioside, and sucrose was rated by the panelists. Both synergism and suppression were found depending on the chemical identity of the two compounds in the mixture. The greatest synergism was observed when two artificial sweeteners were mixed together. The least synergism was observed at the highest concentration (isointense to 7% sucrose). When a sweetener of one type of chemical structure was added to another sweetener of the same structural type, sweetness intensity ratings were suppressed.

The Effect of Tannic Acid on Sweetness Intensity Ratings During the Adaptation of Sweet Compounds. SUSAN S. SCHIFFMAN[1], ELIZABETH SATTELY-MILLER[1], BREVICK G. GRAHAM[1], ZOE S. WARWICK[1], SUZANNE D. PECORE[2], BARBARA J. BOOTH[2], and MICHAEL L. LOSEE[2] ([1]Duke University and [2]The NutraSweet Co.)

The purpose of this study was to determine the effect of two levels of tannic acid on the adaptation of sweet response for a variety of sweeteners. Sweetness intensity ratings were given by a trained panel for 14 sweeteners: 3 sugars (fructose, glucose, sucrose), 2 polyhydric alcohols (mannitol, sorbitol), 2 terpenoid glycosides (rebaudioside-A, stevioside), 2 dipeptide derivatives (alitame, aspartame), 1 sulfamate (sodium cyclamate), 1 protein (thaumatin), 2 n-sulfanylamides (acesulfame-K, sodium saccharin), and 1 dihydrochalcone (neohesperidin dihydrochalcone). Panelists were given 4 isointense concentrations of each sweetener by itself and in the presence of two concentrations of tannic acid. Each sweetener concentration was tasted and rated 4 consecutive times with a 30 second interval between each taste and a 2 minute interval between each concentration. When tannic acid was not in the sweetener solution, the greatest adaptation was found for acesulfame-K, rebaudioside-A, and stevioside; the least adaptation occurred with the sugars and the polyhydric alcohols. In the presence of tannic acid, adaptation was a function of both the chemical structure of the sweetener and the concentration. The highest tannic acid concentration caused greater adaptation in the natural sweeteners, but did not do so for some of the artificial sweeteners. Adaptation of sweet taste may result from the desensitization of sweet receptors analogous to the homologous desensitization found in other receptor systems such as the beta adrenergic, somatostatin, and prostoglandin E_1 systems.

Bitterness is Suppressed By Sodium Salts.
PAUL A.S. BRESLIN and GARY K. BEAUCHAMP (Monell Chemical Senses Center).

Interactions between compounds having different tastes are part of our daily experience with foods. We are investigating how combinations of different compounds give rise to specific changes (e.g., suppression, enhancement) of the individual taste sensations (e.g., saltiness, bitterness) elicited by each compound. Experiments were conducted to see how salts affected various compounds that elicit bitter sensations. Each experiment consisted of 12 binary combinations of 3 concentrations of one bitter compound with 4 concentrations (0, 0.1, 0.3, 0.5M) of one salt compound. Within a session, a subject received the 12 solutions twice in random order and rated each for its perceived bitter intensity and salt intensity using the method of magnitude estimation. As have others (Schifferstein & Frijters, 1992: *Chem. Sens.*, 17, 127-150), we found asymmetrical suppression of bitterness (up to 50-70%) when NaCl and quinineHCl were combined. We have further shown that several other bitter tasting compounds (urea, magnesium sulphate, caffeine) were affected differently by NaCl (and other salts) even when the bitter compounds were matched for intensity. For example, in most subjects sodium salts completely suppressed the bitterness of urea but only partially suppressed the bitterness of quinine. The active component of NaCl in bitter suppression, at least for some bitter compounds, appears to be the sodium rather than the chloride ion. Both sodium-salts and the lithium-salt had a suppressive effect on bitterness. The addition of KCl to urea, however, had no bitter suppressing effect. As with NaCl and quinineHCl, bitter suppression of the compounds was not accompanied by a reciprocal suppression of saltiness. In addition, there were marked individual differences in the extent of bitterness suppression. Taken together, these data suggest that table salt (NaCl) and other sodium containing compounds may alter the flavor of foods, in part, by suppressing bitterness. Although the mechanisms for differential suppression of the various compounds are not known, the effect of the sodium ion on bitterness appears to be independent of its perceived saltiness, since both NaCl and NaAcetate (which is perceived as substantially less salty than NaCl) reduced bitterness comparably. This suggests that there may be a major peripheral component to the suppression, but does not rule out the potential role of central effects (cf. Bartoshuk & Seibyl, 1982: *AChemS* abstract, 4th annual meeting).

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Pleasantness of Sweetened Juice Rated Individually and in a Social Setting.
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Raspberry/blueberry juice was sweetened with 3 and 9% sucrose to attain "low" and "high" sweetness, respectively. Two experiments were carried out, each with 24 young female subjects, to investigate social effects on pleasantness ratings. Subjects rated the samples on a 9-point hedonic scale before and following ad lib consumption in two conditions, alone and in the presence of another subject (randomized order). In the first experiment, ad lib consumption in the social situation was accompanied by a focused, tape-recorded discussion on the appropriateness of the samples to 16 listed use contexts, whereas in the second experiment the discussion was not confined to samples and no tape-recording was conducted. Time (sec) used in each session was recorded. After ratings in the social situation, subjects rated the pleasantness of the setting and their perceptions of the company on several evaluative scales.
The pleasantness ratings of juice were not affected by the social situation, but the overall intakes were higher in social than in individual sessions. Also, the intakes were positively correlated with the duration of each session. Ratings given initially and after ad lib consumption were generally well correlated. The sample with 9% sucrose was rated higher in all situations, and its consumption was to some extent correlated to pleasantness ratings. The samples were rated strikingly different in terms of appropriateness for listed contexts. "High" sweetness increased the appropriateness for children, for guests, in cold weather and for refreshment, whereas it decreased appropriateness for dieting, for quenching thirst, with sandwich and with hot lunch.

Effect of 2-(4-methoxy-phenoxy) Propanoic Acid (PMP-Sodium salt) on the Taste of Bitter-sweet Stimuli
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Substituted phenoxyalkanoic acids have been found to be potent inhibitors of the sweetness response of carbohydrate and non-carbohydrate sweeteners. There is also evidence that the bitter taste response is inhibited at higher levels of Na-PMP. In this study, time-intensity measurements were used to evaluate the effect of Na-PMP on the taste profile of bitter-sweet solutions of mannose, saccharin and a glucose/quinine mixture. Na-PMP was found to give a linear decrease in sweetness of glucose with increasing levels of inhibitor. The bitterness of quinine sulphate was unaffected by Na-PMP levels up to 100 ppm. Mannose was found to possess a very pronounced bitter after-taste, lasting up to one minute after expectoration. The sweetness response of stimuli was found to be much more transient, sweet taste disappearing after 15 sec. Na-PMP was found to influence both temporal and taste intensity properties of mannose. These results will help to elucidate the function of sweet and bitter receptors.

77

Saltiness ratings and preferences for salty foods of Japanese and Australians

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There has been little research on cross cultural taste preferences, especially using foods rather than simple solutions. As part of our ongoing program comparing Japanese and Australian taste preferences, saltiness intensity and .preference were evaluated in a wide range of Japanese and Australian foods by sensory panels in each country. Thirty four foods, both Japanese and Australian, were selected on the basis that they had a predominant salty taste and were popular brands in their respective markets. Foods were selected in the following categories: fish products, crackers, snack foods, nuts, soups, and peanut butter. All subjects evaluated both Japanese and Australian products. Across all product categories, Australians and Japanese were in agreement regarding the saltiness intensity of the foods with Japanese foods rated as less salty than Australian foods. Japanese and Australians showed a preference for the saltiness level of their own foods above that of the other culture, an effect most pronounced with the Japanese ratings of Australian foods. Interestingly, in terms of a *just right* (JR) rating of saltiness, both the Japanese and Australians rated Australian foods as closer to JR than Japanese foods. These results are discussed in terms of the food experiences and habits of the two cultures.

78

Modelling an Identification Experiment with Salty Stimuli

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It is important to separate sensory from non-sensory components of decision making if accurate chemical models of perception are to be developed. Identification experiments with feedback provide probabilities that a stimulus will be correctly identified or confused with another stimulus. From these probabilities, stimulus distributions (sensory component) and decision boundaries (non sensory component) can be estimated. Recent developments in multidimensional probabilistic modelling with decision boundaries allow these components to be separately identified. Nine deionized water-gum systems included one non-ionic gum level and two ionic gum levels, to which three levels of NaCl or NaCl+KCl were added. Twelve subjects identified ten replicates of randomly presented samples, judged the degrees of similarity between all possible pairs, and rated saltiness intensities. The Decision Boundary model accounted for 99.6% of the variance in the data, a significantly better fit than a deterministic multidimensional scaling model. Two sensory dimensions (due to salt and gum levels/types) were not perceptually correlated. Relationships among salt level/type, gum level/type and their perceptual effects were studied using canonical correlations.

79

Effects of Amiloride on Tracked Taste Intensity and on Taste Quality Descriptions of NaCl: Individual Differences and Dose-response Effects. B. P. HALPERN, J. S. MELTZER AND R. B. DARLINGTON (Cornell University, Ithaca NY 14853-7601).

We examined human time-intensity tracking (visually guided, single axis joystick, 100 msec resolution) and taste quality descriptor (end of each trial) effects of amiloride on NaCl in 6 practiced subjects over 8 data collection sessions. Solutions flowed for 4 sec thru a closed delivery system over 39.3 mm^2 of the anterodorsal tongue tip region, preceded by 10 sec H_2O and followed by 5 sec H_2O. Stimuli were 100, 250, 500mM NaCl in H_2O (pH ~6), in 10μM or 100μM amiloride, or in caffeine controls, or the caffeine or amiloride solutions in H_2O with no NaCl. Each subject selected caffeine solution concentrations to approximate amiloride tastes: 33μM-100μM caffeine for 10μM amiloride; 8.33mM-12.5mM caffeine for 100μM amiloride. We found that taste quality descriptions of 500mM NaCl did not change with 10μM, 50μM, or 100μM amiloride or their caffeine controls. Across all subjects, frequency of "saltiness" descriptions of 100mM and 250mM NaCl decreased by 15-30% when 100μM amiloride was in the solution; by 5-6%, for caffeine controls. Time-intensity (T-I) effects were complex, concentration dependent, and differed between subjects. For example, 10μM amiloride in 100mM NaCl T-I intensities were < 100mM NaCl alone in 3/6 subjects; =, in 3/6, while matched caffeine in 100mM NaCl T-I intensities were < NaCl alone in 2/6 subjects; > in 2/6, and unchanged in 2/6. In contrast, 100μM amiloride in 100mM NaCl gave T-I intensities < 100mM alone in 2/6, but > 100mM NaCl alone in 4/6 subjects; matched caffeine, < 100mM alone in 1/6, > 100mM NaCl alone, in 5/6.

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80

Perception of the Fat Content of Common Foods: A Comparison of Two Scaling Methods. BEVERLY J. TEPPER and SUSAN E. SHAFFER (Rutgers University).

The oral perception of fat involves the interaction of textural cues and volatile flavor sensations. A basic understanding of how these complex perceptions contribute to the judgement of the fat content of foods is currently lacking. This is due, in part, to the difficulty in designing taste stimuli that approximate real foods and are not markedly different in physical form and appearance across the stimulus range. The purpose of this study was to develop methods to examine the perception of fat content in 6 common foods. Milk-oil mixtures were made by substituting 0-30 g vegetable oil in 100 g reconstituted skim milk powder and sweetening with sucrose (6 g/100 ml). This produced 5 samples varying in fat content but relatively constant in flavor and appearance. Unsweetened milk-oil mixtures were added to an appropriate amount of food base to prepare instant mashed potatoes and pudding samples. Chicken spreads were made in a similar manner with fat-free mayonnaise-oil mixtures. Crunchy snacks and scrambled egg samples were prepared with graded levels of margarine and oil, respectively. Thirty-two young adults evaluated the fat content of the samples in two separate sessions using a ranking task and a 15 cm. line scale. Subjects successfully judged increasing fat content in the milk, pudding and potato samples using both scaling methods (p's $\leq$ 0.02-0.001) and correlations between the two methods were high (r=.32-.55; $p \leq 0.01$). Evaluation of the remaining samples was more difficult. Subjects reliably rank-ordered the snack samples ($p \leq 0.01$) but could not judge increasing fat content using the line scale. The egg and chicken samples were not correctly judged by either method. These data suggest that subjects can reliably evaluate increasing fat content in common foods but this ability varies by food type. Both ranking and line scale methods were effective in scaling these perceptions.

81

Gustatory Loss - A Taste of Malingering?

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B.M. MACKENZIE, Jr. (University of Illinois College of Medicine)

Head traumas often trigger chemosensory losses which may resolve in time. In some instances involving litigation seeking compensation, speculations may be difficult to prove regarding the falsity of claims of continuing deficits. In two separate cases, a man and a woman, both factory workers in their late 20s, complained of almost total loss of smell and taste following head trauma with loss of consciousness. Their losses had persisted, they said, for five years and four years, respectively, before they sought medical advice, yet neither the man nor the woman had changed food preferences or eating or smoking habits or lost weight since their accidents. Neurologic and psychiatric examinations showed abnormalities limited to cranial nerve I. On various standard olfactory tests, the woman was normal and the man ranged from anosmic on some to normal on others, refusing to answer some of the questions. On 40 scratch-and sniff identification questions (UPSIT) both patients rated anosmic. The man gave answer "A" to all questions. On Bartochek's gustatory spatial quadrant tests, the woman described substances tested on the palate, the entire tongue and the whole mouth as having no taste. The man noted only minimal taste on the tongue. On electrogustometry tests, both patients denied any detectable sensation even with maximum stimulation. Since cranial nerve V function tested normal, cranial nerves VII, IX, and X function tested otherwise normal, and no oral lesions were present, these electrogustometry data defy physiologic explanation and may prove to indicate malingering.

82

Clinical Application of the Smell Identification Test in Japan

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Clinical application of the standardized "scratch and sniff" olfactory test in Japanese is described. Over 300 subjects participated in three experiments. In experiment 1, 29 odorants used in the smell identification test were rated as to their experiences. The ratios of the experiences in each combination of odorants were compared. In experiments 2, the test was applied to normal subjects. Average test scores decreased as a function of age, with the greatest decline occurring between subjects with olfactory disorders and normal controls. This self-administered test now makes it possible to rapidly and accurately assess general olfactory function in the laboratory, clinic, or through the mail without complex equipment or spaceconsuming stores of chemicals.

83

Olfactory Discrimination of Carvone Enantiomers.

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It is now widely accepted that humans can discriminate the odor qualities of S-(+)- and R-(-)-carvone, although there is some suggestion in the literature of individual differences in this ability (e.g., Russell & Hills, 1971). Three studies were conducted to examine these possible differences further. The first revealed that: (1) in triangle tests in which stimulus intensity is intentionally confounded, approximately 50% of those tested failed to discriminate the two enantiomers; (2) R-carvone thresholds were significantly lower than S- thresholds regardless of discrimination ability and (3) among those capable of qualitative discrimination (D), but not among non-discriminators (ND), concentrated S- was rated more intense than R-. The second study tested whether the large number of ND's observed in Experiment 1 was an artifact of the discrimination paradigm by co-varying testing method and stimulus concentration in a within-subjects design. The results indicate that the ability to discriminate the isomeric forms of carvone is not a function of the type of discrimination test employed; however, the relative concentrations used in a given test do affect discrimination ability. Specifically, certain individuals (partial-discriminators [PD]) are able to discriminate the isomeric forms of carvone only when equal concentrations are presented. The third study employed a cross-adaptation paradigm to explore further the relationship between discrimination ability (i.e., D, PD, ND) and perceptual responsiveness to the carvone enantiomers. Significant self-adaptation by each isomer was observed among all groups. Among D's, adaptation to either enantiomeric form produced no significant cross-adaptation. ND's exhibited significant cross-adaptation of R- by S- at a number of concentrations, whereas PD's exhibited cross-adaptation at only the lowest concentration tested. Conversely, ND's exhibited no cross-adaptation of S- by R-, whereas PD's showed cross-adaptation at the lowest concentration tested and apparent facilitation at higher levels of stimulation. The implications of these findings will be discussed.

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84

Recency Re-Visited: Serial Position Effects in Olfactory Short-Term Memory.

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The term "serial position effect" refers to the function of errors across memory for a list of items. There are normally three parts to this function: Superior recall of the final portion of the list (*recency effect*), further good recall of the first portion of the list (*primacy effect*), and generally poor recall of the centre of the list. Serial position effects (SPE) have been shown to indicate the presence of short term memory processing in both visual and auditory sensory modalities. In a previous study, White (ACHEMS XIV) illustrated that the SPE could be found using olfactory stimuli. The present experiment replicates and extends that earlier finding. Two olfactory recognition tasks were used: One which examined item information, and one which examined order information. Altering the technique slightly from the earlier study allowed 2 new measures to be obtained: Level of verbalization, and the subject's estimate of an item's location in the list. Both the item and the order recognition tasks exhibited a recency effect without evidence of a primacy effect. Subjects showed higher levels of performance than the earlier study, primarily because the tasks were performed with better stimulus control. Comparison of levels of verbalization in subjects who had completed the recognition tasks, as opposed to a group of subjects who had not suggested that verbalization is not a large confounding factor in these olfactory recognition tasks. The implication of this data toward the existence of a separate olfactory short-term memory system which is qualitatively similar to other sensory memory systems is discussed, as well as possible methods of making judgements of order information.

85

Amyloid β-Peptide Toxicity in Organotypic Cultures of Rat Cortex as a Model for Alzheimer's Disease.

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Cortical olfactory structures have been shown to exhibit early histopathological changes associated with Alzheimer's disease (AD); *i.e.*, neuritic plaques and neurofibrillary tangles. A major component implicated in the pathogenesis of AD is the Amyloid β-protein (AβP). AβP neurotoxicity was tested on organotypic cultures of rat cerebral olfactory cortex using this 40 amino acid peptide (Bachem Co.) Slices were taken from 5-7 day old Long-Evans hooded rat pups and used after 2-3 weeks *in vitro*. Two concentrations of the AβP, 1.0 mM and 0.1 mM solubilized in 35% acetonitrile, 0.1% trifluoroacetic acid (ACT/TFA) diluted 1:100 in media, were applied to the surface of the cultures and examined after 2, 4, and 24 hours. Controls, consisting of application of the vehicle alone, were examined at the same time points. Adherence and diffusion of AβP were marked with an antibody to the AβP (anti-β-amyloid, Alzheimer, Boehringer-Mannheim) and a rhodamine conjugated secondary antibody. Toxicity was measured with a cell viability assay that used calcein-AM to label live cells and ethidium homodimer to label dead cells (Molecular Probes). Toxicity of the vehicle on the organotypic cultures was assessed both with the cell viability assay and *via* electrophysiological recording. At 2 hours there was some binding at both concentrations. At 4 hours, the peptide at both concentrations had diffused evenly throughout the culture. Toxicity as measured by increased cell death relative to controls was first apparent at approximately 8-12 hours and extensive after 24 hours, especially at the higher peptide concentration. Studies in progress include further anatomical and electrophysiological testing of the AβP's and control peptide's (AβP 1-28) effects at different time points.

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86

Taste and Smell Function in Persons at Risk for Alzheimer's Disease. SUSAN S. SCHIFFMAN, BREVICK G. GRAHAM, ELIZABETH SATTELY-MILLER, and KATHLEEN A. WELSH (Duke University)

The purpose of this longitudinal study is to assess taste and smell function in persons at risk for developing Alzheimer's Disease (AD) and to compare the performance with control subjects matched for age, sex, race, years of education, and handedness. The inclusion criterion for the experimental group was a strong family history of AD with one or more immediate family members affected. In addition to the chemosensory tests (which include measurements of detection and recognition thresholds as well as tasks to measure odor and taste memory, discrimination, and identification), a neuropsychological assessment is performed to determine cognitive status. The first phase of this experiment has involved the acquisition of base-line data from the experimental and control subjects. Analysis of these base-line data has revealed that the two groups differ on only one variable, odor memory performance. Persons at risk for AD perform more poorly than controls on this task which suggests that deficiencies in odor memory may be an early marker for AD. This finding suggests that odor memory tasks may be more effective than traditional cognitive tests as diagnostic tools in the early detection of AD. The tests will be repeated every 18 months to determine the degree of loss in chemosensory function and cognitive status over the lifespan in persons at risk for AD.

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87

Confidence Ratings for Odor and Visual Stimuli in Alzheimer's Patients and Normal Controls. DAYNA WILHITE, LETICIA ACOSTA, CARLO QUINONEZ (San Diego State University), STEVEN NORDIN (UCSD Medical Center and San Diego State University), and CLAIRE MURPHY (San Diego State University and UCSD Medical Center, San Diego, CA)*

The purpose of this study was to determine whether confidence ratings for odor and visual memory judgements differed between Probable Alzheimer's patients and normal control subjects. All subjects were participants in the UCSD Alzheimer's Disease Research Center (ADRC) on-going study of olfactory function and had been diagnosed either as having Probable Alzheimer's Disease or as normal by two different neurologists at the ADRC using the NINCDS-ADRDA diagnostic criteria. The average age of the Alzheimer's patients is 74 yrs. and the normal controls 73 yrs. The stimuli employed in the recognition memory task consisted of common odorants (e.g., Ivory soap and cloves), pictures of U.S. Presidents and Vice Presidents, engineering symbols, and colors. On a first trial, subjects were presented with ten stimuli from each of the above categories, in random order, and were asked to rate their familiarity with each stimulus. On a second trial, for testing recognitition, half of the stimuli from each category were presented again along with five distractors. Subjects were then asked to rate their confidence in responding "old" or "new" to the stimuli. Hits, misses, correct rejections and false alarms were calculated for each subject. Results showed that Probable Alzheimer's patients were less confident in their responses than the controls with respect to hits and correct rejections. The groups did not differ in confidence ratings for responses which were incorrect (misses and false alarms).

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88

Olfactory Functioning from Childhood to Old Age

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This study was directed to investigate olfactory functioning over human lifespan. Olfactory threshold (n-butanol; pairwise ascending staircase method), odor memory (20 everday odors, 10 old 10 new, 15 min. retention interval) and free odor identification (20 everyday odors were to be named) was assessed. 143 people participated in the study, 47 children (5 to 14 years), 54 young adults (18 to 19 years), 24 middleaged adults(30 to 59 years) and18 elderly people(60 years and older).Results are reported in the order children, young adults, middleaged adults and elderly people. Olfactory threshold was lowest for children, slowly rising for young adults and middleaged adults, althogh there were no significant differences. Elderly people had a significant rise. (TH: 6.95/6.77/6.50/5.50; high numbers mean low threshold). Odor memory, as measured by d', was significantly better for young adults than for children, and young adults were also significantly better than middleaged adults. There was a significant drop from middleaged adults to elderly people. (d': 2.00/2.74/2.14/0.98). Odor identification was significantly different for each group. (Ident: 16.8/26.4/21.4/10.4). Unexpectedly, no sex-differences concerning olfactory functioning was found. There seems to be a gradual reduction for odor detection threshold throughout life. Beginning from childhood, ability for odor memory and odor identification gets better, reaching a maximum at young adulthood. In later years, both odor memory and odor identification deteriorate gradually.

89

Relationships between Olfactory Identification Ability, Cacosmia, and Memory in Older Adults with and without Depression

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Previous studies have shown loss of central olfactory neurons and of olfactory sensory abilities in elderly with neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, suggesting decreased olfaction as a possible early marker of dementia. Geriatric depressives may be an important group in whom to evaluate this issue, as depression may itself be a precursor of dementia in certain individuals. In the present study, 31 elderly adults (74%F/26%M, mean age 77 $\pm$ 6 years) recruited by newspaper advertisement were examined for olfactory sensory identification ability (OLF)(Cain et al 1988), cacosmia self-ratings (CAC: before the current illness, a subjective sense of feeling ill from the odor of pesticide, car exhaust, paint, perfume, new carpet), depression on the Montgomery-Asberg Depression Rating Scale (MADRS), and verbal memory ability for immediate free recall and recognition memory (subscales of the Alzheimer Disease Assessment Scale, ADAS) and for delayed free recall (from the Mini-Mental State Examination, MMSE). Over the whole sample, median MADRS score was 15 (range 1-27); median MMSE score was 27 (range 14-30). Stepwise multiple regression analyses demonstrated that OLF accounted for 23% ($p<0.01$), CAC for 22% ($p<0.001$), and age for 14% ($p<0.01$) of the variance in delayed memory; MADRS, education, gender, and shyness (hyperreactivity to novelty) did not enter the equation. OLF accounted for 15% ($p<0.05$) and CAC for 33% ($p<0.0001$) of the variance in recognition memory score. As expected, MADRS score, but not olfactory/cacosmia or other measures, accounted for 28% of the variance in immediate memory. Poorer OLF correlated with older age ($r=-0.38$, $p<0.02$), poorer MMSE score ($r=0.43$, $p<0.01$), and less CAC ($r=0.27$, $p<0.07$), but not with MADRS depression ($r=0.05$, ns). These data support the hypothesis that current impairment of olfactory function as well as premorbid cacosmia may be predictors of memory deficits seen in early dementia, with a pattern distinct from that of geriatric depression.

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90

Lower Olfactory Functioning Associates with Nutritional Risk in Elderly Women.

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Seventy-six elderly women with moderate-to-high overall functioning (OMFAQ: Fillenbaum, 1988) were selected from a screening pool (n=102) to determine the nutritional risk of poorer olfactory functioning. All data was collected in four, home-based sessions. Olfaction was measured orthonasally (smell: Cain, 1988—butanol threshold, odor identification) and retronasally (flavor: Duffy, 1991—threshold in gelatin/sugar medium). A composite score for rated olfaction was obtained by adding the responses to rated smell, and rated change in both smell and flavor since age 30 (7-point scale, Cronbach's alpha=0.71). The nutrition variables included: food behaviors (Fey, 1983), yearly food group intake (National Cancer Institute Food Frequency, USDA grouping scheme), nutrient intake (5 non-consecutive, 24-hour food records), and measured height/weight. Significance criteria was $p<0.05$. Women showed a lower flavor than smell function. Lower flavor function was associated with both lower smell function and complete dentures (ANOVA). Women were accurate in rating their smell perception at the extremes of measured function, but less able to correctly rate their flavor function (χ-square analysis). In correlating measured function to nutrition, women with lower smell and flavor function had lower food interest (i.e. enjoying cooking, eating a variety of foods). Women with lower smell function consumed more sweetening products, fewer low-fat milk products, and had a higher cardiac-risk nutrient profile (i.e. higher % of saturated fatty acid and total fat calories). Lower flavor function was associated with a higher intake of high-fat desserts but less soups. In a 2x2 factorial treatment of measured by rated olfaction, women who rated dysfunction/measured normal function (n=11) were at a higher dietary risk (i.e. lower intake of A&C-rich fruits/vegetables, lower dietary diversity) than women with measured normal/rated normal function (n=20). Women with measured dysfunction/rated dysfunction had the highest intake of high-fat desserts. Women who had measured dysfunction/rated normal function (n=22) exhibited greater obesity risk (Body Mass Index>30) than those with measured dysfunction/ rated dysfunction (n=23) or measured normal/rated normal function. Conclusion: Elderly women with lower olfactory function are at increased nutritional risk. The additional information provided by rated olfaction clarified the nature of the risk.

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91

Effects of familiarity and odor pleasantness on food acceptance by the elderly.

MARCIA LEVIN PELCHAT (Monell Chemical Senses Center).

We define food neophobia as a reluctance to try or as a dislike for the flavor of unfamiliar foods. We previously reported (Pelchat & Stoess, AChemS XIII), that elderly subjects with poor olfaction were more willing to accept novel foods than were young subjects. We also found that elderly subjects with poor olfaction were more willing to accept foods with unpleasant odors than were young subjects. Unfortunately, there is often a confound between a food's odor pleasantness and its familiarity. Therefore, in the current study, we attempted to separate the effects of familiarity and odor pleasantness on food acceptance by the elderly. Four food stimuli were used. They included a novel food with a pleasant odor (NP), a novel food with an unpleasant odor (NU), a familiar food with a pleasant odor (FP), and a familiar food with an unpleasant odor (FU). Subjects were young (18-35 y.o.) or elderly ($\geq$ 65 y.o.) adults. The elderly subjects were divided into a good and a poor olfactory function group. Subjects were asked to sniff each of the foods and to indicate their willingness to try each food. They were led to believe that, if willing, they would be given a taste of the food at the end of the study. Overall subjects were less willing to try the novel foods than the familiar foods and were less willing to try the unpleasant-smelling foods than the pleasant-smelling foods. However, elderly subjects with poor olfaction showed less reluctance to try the unpleasant-smelling foods than did subjects in other groups. This was particularly apparent for the NU food. In contrast, there was no evidence for age/olfactory group differences in food neophobia. These results are consistent with the hypothesis that the increased willingness to try novel foods seen in our previous study was a function of food-odor pleasantness and not a function of food novelty per-se.

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92

Odor Threshold Sensitivity is Impaired in Patients With AIDS Dementia Complex

L. JILL RAZANI (San Diego State University), LISA CHARTIER (San Diego State University), TERENCE M. DAVIDSON (UCSD Medical Center), and *CLAIRE MURPHY (San Diego State University and UCSD Medical Center)

It is estimated that 25-40% of patients infected by the human immunodeficiency virus (HIV) will develop dementia some time during the course of their illness. This is referred to as AIDS Dementia Complex (ADC). In a recent study, measuring the olfactory function of patients with ADC using the University of Pennsylvania Smell Identification Test, Brody, et. al (American Journal of Psychiatry, 1991) found that ADC subjects scored significantly lower than normal controls. In the present study, subjects were given an ascending, forced-choice, two-alternative, odor threshold test for butanol. To control for task demand, subjects were also given a staircase, two-alternative, forced-choice, taste threshold test for sucrose, which is similar to the odor threshold task. Odor and taste thresholds for three different groups were compared. The groups were made up of ADC subjects, HIV positive subjects with no dementia, and HIV negative subjects. Subjects were divided into these groups based on the Heaton-Global neuropsychological test, which is a battery of tests including measures of memory, language, attention span and problem solving abilities. The score on this test ranges from 1 to 9 and those subjects who scored 5 or above were placed in to the demented group. The results indicate that the odor thresholds of the ADC patients are significantly higher than the thresholds of the two control groups. In addition, a significant correlation was found between the degree of dementia and odor sensitivity. No significant differences were found between the taste thresholds of the three groups. We suspect that these olfactory deficits found in the ADC subjects are primarily due to damage to the central nervous system. To investigate nasal infection as a contributing etiology, a second study will incorporate a complete nasal examination on all subjects.

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93

Direct Cortical Recording of EEG to Odors in Epilepsy Patients

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In some medically-refractory epileptics with a diagnosis of partial seizures in whom previous non-invasive long-term scalp EEG recordings have been unsuccessful for localization of the epileptogenic focus, subdural electrode strips are surgically placed on the left and right lateral frontal, and lateral and medial temporal cortex. 36 channels of subdural EEG are continuously recorded 24 hours a day at the University of Arizona Epilepsy Monitoring Unit in selected patients until 3 seizures have occurred. Research in progress is examining subdural EEG responses to suprathreshold and subthreshold concentrations of isoamyl acetate (IAA) and silicone solvent (no odor control). Prior to each trial, subjects are instructed to close their eyes and take two two second sniffs of a bottle placed in front of the subjects nose by the experimenter. Eight trials per odor are administered in a counterbalanced order. The subdural EEG is recorded on a BMSI system and is played back, off line, into a Gateway 386DX 33 mhz system running Rhythm 8.0 software for EEG spectral analysis. Analyses have been completed on a 36 year old male patient whose seizures were localized in the right temporal region. The subject reported a very slight odor in 25% of the solvent trials, 0% of the subthreshold IAA trials, and a moderate odor in 100% of the suprathreshold IAA. Widespread significant EEG decreases in alpha, theta and delta bands in medial and lateral temporal leads were observed to suprathreshold IAA, and more restricted significant EEG decreases to focal medial and lateral temporal leads were observed to subthreshold IAA. EEG olfaction effects were attentuated in right temporal regions, the area of seizure focus. Selective EEG decreases in focal lateral frontal regions were observed to suprathreshold IAA. The data are consistent with the hypothesis that the temporal region is involved with olfactory sensation, the frontal region is involved with olfaction perception, and that olfactory stimulation may find applications in epilepsy monitoring.

94

The EEG Response to Odour

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The use of EEG techniques to study olfaction seems to be on the increase. The relative availability and low cost of commercial mapping systems has encouraged this trend. However, after completing a large, well controlled study of the EEG response to odours it seems clear that major changes will be necessary in theoretical design, methodology and data handling if future studies are to achieve meaningful progress. The present study used a Neuroscience Series III Brain Imager to examine the EEG response of forty subjects to a variety of odorants. Diurnal and annual EEG variations were examined. Psychometric data were collected and when analysed were supportive of many findings in olfaction literature. However, the EEG data were analysed using Singular Value Decomposition (SVD), Principal Component Analysis (PCA) and Discriminant Analysis (DA) and the findings were inconclusive. No general comment or interpretation is possible about this complex data set and the relation of the EEG to odour processing. Largely because of these findings a complete examination of the theoretical underpinnings and practical design of such studies was undertaken. Briefly presented are suggestions both for those who may carry out such work in the future and for those who may wish to read the results of such studies with a critical eye. Possible avenues for advancement are identified.

Research supported by Quest International

95

Application of 3-channel Lissajous' trajectory to human olfactory evoked potentials: Consistency across subjects and stimulus intensities.

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The three channel Lissajous' trajectory (3-CLT) is a technique that reduces three time-voltage vectors to a 3-D voltage-voltage-voltage plot with time being inferred from the trajectory of the resultant figure. The voltages obtained from the three recording sites at a given time are represented by a single data point. Discrete generators result in a projection of the 3-CLT figure. A single generator, such as an axon will result in a 3-CLT figure that starts at the origin and returns along the same trajectory. Deviations from straight line imply the influence of additional factors on the trajectory. Thus, the complexity of the figure can reveal the number of generators. 3-CLT has been applied to electrophysiological recordings obtained in both humans and animals. These applications have included auditory and somatosensory evoked responses. Data for these analyses was obtained from 9 subjects who were presented with three levels of stimuli delivered intranasally by an olfactometer. Data were collected from Fz, Cz, and Pz referenced to linked mastoids for 2000 msec and the resulting evoked potential extracted for 3-CLT evaluation. The data indicate consistency within subjects regarding generator origin with less consistency being observed across subjects. This may imply anatomic variability or the differential evoking of other generators. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

96

Theophylline Treatment of Hyposmia

A. R. HIRSCH, M.D. (Smell & Taste Treatment and Research Foundation)
G. C. WECLAW (University of Illinois College of Medicine)

In order to assess the viability of theophylline treatment in olfactory loss we studied ten consecutive hyposmic patients of at least one year and one month duration (average eight years and one month). Six females and four males ranging in age from 24 to 76 years, with average age of 44.8 years, participated in the study. The etiologies of their olfactory loss included four idiopathic, three post-traumatic, two post-viral, and one of congenital origin. Olfactory function was assessed using Amoore's Unilateral Olfactory Thiophane Threshold Testing and the University of Pennsylvania Smell Inventory Test (UPSIT). Medical treatment was initiated with 100mg of theophylline and was increased as tolerated. Results: four subjects dropped out. Of the six remaining subjects, one reported olfactory improvement with 400mg of theophylline of 3 months duration. Dosages of theophylline in the nonresponder group ranged from 100mg to 500mg per day, with an average duration of treatment of four months. The responder compared to nonresponders had better baseline olfactory ability: bilateral Thiophane threshold of 10 decismells average versus right 33 left 30 decismells; UPSIT 38 versus average UPSIT 19.8. Despite our findings a study with higher doses, measured levels, objective follow-up testing and a larger sample size may yet reveal a role of theophylline for this condition.

97

Validation of the Chicago Smell Test (CST) in Patients with Subjective Olfactory Loss.
A.R. HIRSCH, M.D. (Smell and Taste Treatment and Research Foundation)
M.B. GOTWAY and A.T. HARRIS (University of Illinois College of Medicine)

The CST assesses detection and identification of isoamyl isovalerate, isoamyl acetate, and menthol valerate. In previous studies we validated the CST in undergraduate students as well as in neurologic patients without chemosensory complaints. To further evaluate the utility of this test, we compared the CST with pyridine threshold testing as described by Amoore in 79 consecutive patients presenting to a chemosensory clinic complaining of olfactory loss. The mean age of the study population was 45.1 years. Despite complaints of olfactory loss, 32.9% of patients had normal pyridine threshold tests (less than or equal to 25 decismels). A positive test (unable to detect any odors) predicted an abnormal pyridine threshold in 88.9% of patients; specificity in this group was 92.3%, whereas sensitivity was 30%. The odds ratio comparing the CST with pyridine threshold testing was 5.2 with 95% confidence intervals given by (1.51, 18.1). Of the whole group, 35.4% (28/79) correctly identified at least one odor (a negative test). The positive predictive value for identification was 72.5%, and the sensitivity was 69.8% with a specificity of 46%. The odds ratio was 1.98 with 95% confidence intervals given by (1.23, 3.19). Therefore, there is a strong association between the detection and identification aspects of the CST and pyridine threshold testing in the evaluation of chemosensory dysfunction.

98

Olfaction In A Cluster Headache Sufferer
A. R. HIRSCH, M.D. (Smell & Taste Treatment and Research Foundation)
N. THAKKAR (University of Illinois Medical School)

Olfactory ability has been reported to be reduced in 18% of migraineurs but it has never been assessed in those with cluster headaches. In order to evaluate this, a 32-year-old male cluster sufferer underwent objective unilateral olfactory testing with thiophane with the threshold method of Amoore over a two and a half hour period both during four headache and four pain-free intervals of a cluster cycle, as well as one month following resolution of the cluster attacks. This revealed hyperosmia isolated to the left nostril (greater than -30 decismels) restricted to the headache phase of the cluster cycle. This was independent of the cluster side. One month after resolution of the cluster attack, normosmia was documented (left -5 decismels, right -15 decismels). Heretofore, hyperosmia has only been found in Addison's disease. The explanations of the findings of hyperosmia in this cluster patient include limbic reticular activating system activation, cortical neurotransmitter changes, hormonal fluctuations, nasal mucus viscosity alterations, and change in air flow patterns. Since hyperosmia is not seen in any other headache type, if confirmed in others during the cluster attack, the finding of hyperosmia may be able to be used as a marker to objectively define this entity.

99

Validation of the Chicago Smell Test (CST) in Subjective Normosmic Neurologic Patients.
A.R. HIRSCH, M.D. (Smell & Taste Treatment and Research Foundation)
M.B. GOTWAY (University of Illinois Medical School)

The CST (three reuseable plastic pen-like devices impregnated with isoamyl isovalerate, isoamyl acetate, and menthol valerate) has been validated as a discriminator of anosmia in undergraduate students and in patients presenting to a chemosensory clinic complaining of olfactory loss. To further assess the utility of this test, 245 consecutive patients (average age 38.9 years) with neurologic disease who denied olfactory complaints underwent olfactory testing with both the pyridine threshold test as performed by the method of Amoore and detection and identification with the CST. Ninety-eight percent of subjects detected all three odors. With pyridine threshold of 25 or less decismels (normal), 98.9% detected all three odors. A negative test (detecting at least one odor) predicted normal pyridine threshold in 74% of subjects (180/244); specificity in this group was 100% (245/245). Of the whole group, 89.2% correctly identified at least one odor (a negative test); 10.8% identified none. The negative predictive value equals 74% (165/222); specificity was 91.7% (165/180). Of note, despite denial of olfactory complaints, only 72% of patients had normal pyridine threshold, whereas 28% were abnormal (2% were anosmic, 26% hyposmic). That neurologic patients are unaware of their olfactory loss underscores the need for the CST in this population.

100

Effect of an Ambient Odor on Slot-machine Usage in a Las Vegas Casino
A. R. HIRSCH, M.D. (Smell and Taste Treatment and Research Foundation)

Studies suggest that ambient aromas may impact consumers' behavior. To further investigate such effects, for one weekend, two sites in the Las Vegas Hilton casino were odorized with different aromas. A third area served as a nonodorized control. The amounts of money gambled in slot machines located in areas surrounding the odorized sites were measured for the weekend of the odorization and for the weekends before and after as well to control for any extraneous variables. The amount of money gambled in the slot machines surrounding one of the odorants increased by an average of 45.11% (p less than 0.0001) compared to the weeks before and after the odorant was used. There was no significant changes in the amounts of money gambled in the control area slot machines or in the machines surrounding the second odorized site. Further, the effect of odorant #1 appeared greater when the concentration was higher: mean increase 53.42% (p less than 0.0001) on the first day versus mean increase 33.66% (p less than 0.003) on the second day. These results suggest ambient odors impact upon leisure time gambling activity.

101

Lingering Time in a Museum in the Presence of Congruent and Incongruent Odors.
SUSAN C. KNASKO (Monell Chemical Senses Center).

This study explored the role of the congruency of pleasant odors on lingering time in a public setting. A room in an anthropology museum, displaying the crafts and clothing of the Alaskan Indians, was scented four days a week (Tues-Fri) for eight weeks. Scenting was accomplished with fan units containing scented blotters and with scented "air freshener" disks. There were four odor conditions: no odor, incense (rated in pilot testing as smelling pleasant and congruent with the display), bubblegum (rated as smelling pleasant but incongruent with the display) and leather (rated as unpleasant but congruent with the display). Leather was included to pilot test the effect of an unpleasant odor. The room was scented for one-half hour before video taping began and scenting continued during the four hours of video taping. A surveillance camera video taped visitors as they walked through the room. The tapes were scored to determine how long visitors lingered on the different odor days. Exit interviews were conducted for four weeks after the video taping was completed.

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

Susan C. Knasko

102

Can Anosmic Patients Separate Trigeminal and Nontrigeminal Stimulants?
DAVID E. HORNUNG (Biology Department, St. Lawrence University, Canton, N.Y.) DANIEL KURTZ AND STEVEN L. YOUNGENTOB (Physiology Department, Health Science Center, Syracuse, N.Y.)

A retrospective study was undertaken to determine if anosmic patients could separate trigeminal and nontrigeminal odorants. The Odorant Confusion Matrix (OCM) results from 145 anosmic patients were analyzed with a 2X2 Chi Square design to determine if these patients used odorant names associated with trigeminal stimulants when presented with ammonia and vinegar. Since ammonia and vinegar are the only two odorants in the OCM with strong trigeminal components, the use of names associated with trigeminal stimulants in response to these odorants would perhaps suggest an ability to detect trigeminal stimulants in general. The results of this analysis strongly suggested ($P<.00005$) that anosmic patients could separate ammonia and vinegar from the other 8 odorants of the OCM even though these odorants are usually not correctly identified. Likewise, these patients were apparently able to separate the other 8 OCM odorants from ammonia and vinegar again even though correct identification of specific odorants was usually not possible. The results of an ANOVA suggest that the ability to separate trigeminal from nontrigeminal odorants was not different for different diagnostic groups. So, at least using this test vehicle, and regardless of the etiology of their olfactory loss, anosmic patients were quite good at separating trigeminal and nontrigeminal odorants.

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103

Bilateral and Unilateral Assessment of Odor Memory Function.
STEVEN M. BROMLEY & RICHARD L. DOTY (Smell and Taste Center & Department of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA)

To date, no studies have examined the performance of normal subjects on odor memory tests administered to each side of the nose. Since the left hemisphere is typically specialized for verbal function in right-handed subjects, one might hypothesize, under the assumption that such subjects rely to some degree on verbal referents to remember odors, that scores on an odor memory test might be better on the left than on the right side of the nose. In this study, we administered two tests of odor memory both unilaterally (left and right) and bilaterally to the same set of subjects; in addition, neuropsychological tests of odor memory were also presented. The olfactory tests consisted of (i) a 9-item single target three-choice odor memory test with 10-, 30-, and 60-sec retention intervals and (ii) an odor memory test in which three target stimuli are chosen, after the aforementioned retention intervals, from larger sets containing both the target and three distractor stimuli. Preliminary analyses of the data suggest that (a) odor memory test scores obtained from bilateral testing are superior to those obtained from unilateral testing and (b) differences exist in the lateralization of odor memory function.

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104

Impairment of Odor Identification as a Function of Age and Disease State in Patients with Cystic Fibrosis.
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It has been somewhat controversial as to what, if any effect the disease Cystic Fibrosis (CF) has on odor perception. To further define the state of olfactory function as it exists in patients with Cystic Fibrosis, this study evaluates olfactory function in CF patients presenting to the Cystic Fibrosis/Pulmonary Clinic, University of California, San Diego, for annual examination. Age- and sex-matched normal controls were likewise evaluated. All CF patients were assigned Schwachman and Kulczycki clinical disease scores at the time of examination. Coincidentally, adult patients were given the odor identification portion of the CCCRC Test; children were given the Child Odor Identification Test (Anderson, Maxwell & Murphy, AChemS, 1992). Scores were reported as percent correct of the total in the respective test. The percent correct scores were then evaluated as a function of age and Schwachman/ Kulczycki scores. The results indicate a decrease in olfactory function in adults with CF in the fourth decade of life. This difference is statistically significant when compared to normal adults of similar age as well as CF patients in the first, second and third decades, $p < .05$. Older adults with CF whose Schwachman/ Kulczycki scores showed more severe clinical disease also had a greater degree of olfactory impairment, $p < .05$. These data seem to suggest that olfactory function and general state of health of individuals with CF may be more closely linked than previously thought. It follows, then that the nasal health of individuals with CF may deserve further evaluation.

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Temporal Irritant Perception

MARGARET CLIFF (University of Missouri-Columbia)
HILDEGARDE HEYMANN (University of Missouri-Columbia)

The temporal irritant responses for six concentrations of capsaicin, cinnamaldehyde, and piperine were evaluated by 12 subjects, using time-intensity (TI) methodology. TI curves were quantified using six TI parameters: maximum intensity (I_{max}), time-to-maximum (T_{max}), plateau time (T_{plat}), total time (T_{tot}), maximum rate of onset (Monset) and maximum rate of decay (M_{decay}) of perception. Maximum intensity was used to evaluate the appropriateness of the Beidler taste equation and calculate the degree of affinity of the stimuli for the receptor (K_b), while the other TI parameters were used quantify the TI responses. For cinnamaldehyde and capsaicin, correlations coefficients for the proposed Beidler model were 0.999, and 0.996, respectively. The association constants (K_b), for cinnamaldehyde and capsaicin, were 25 M^{-1} and 5.2 X 10^4 M^{-1} and consistent with their observed temporal responses. While the concentration dependencies of T_{max} for tastants, are typically linear, the concentration dependencies of T_{max} for irritants, were semi-hyperbolic. These responses were believed limited by diffusion of the irritants through the oral epithelium, and could be explained by the relative contribution of diffusion and adsorption processes.

The temporal perception of menthol

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HILDEGARDE HEYMANN (Department of Food Science & Human Nutrition, University of Missouri-Columbia)

Although menthol is a common ingredient in pharmaceutical and food products, its sensory properties have not been studied extensively. The objective of this study was to describe and compare the temporal properties of *l*- and *d*- menthol. The cooling, burning, and bitterness of two menthol isomers(*l*-, *d*-) each at 0.01, 0.02, 0.04, 0.08 %(w/v) were evaluated by 11 trained panelist using time-intensity methodology. Using a computerized system, the intensity of all three attributes were evaluated continuously from introduction of the sample into the mouth, through expectoration at 5 seconds, until the termination of the sensation. The *l*-menthol samples were shown to have a greater maximum intensity and longer total duration of cooling and burning sensations than the *d*-menthol samples. The total duration of cooling was not only dependent upon isomer type but also concentration. In contrast, the total duration of the burning sensation was dependent upon isomer type only. Increasing menthol concentration significantly increased maximum intensity and total duration of bitterness, whereas no differences between *l*- and *d*- isomers were detected. Differences and similarities between *l*- and *d*- menthol were successfully characterized by bitterness, burning, and cooling temporal properties.

A Portable Olfactometer for Human Psychophysics and Electrophysiology

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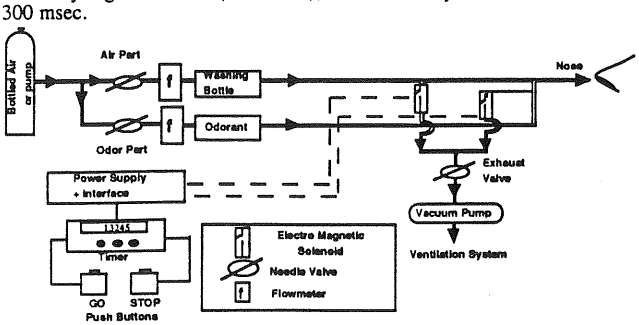
Olfactometers that afford precise control over stimulus onset, duration, temperature, and humidity tend to be large and expensive. We describe here a relatively inexpensive, portable olfactometer that delivers controlled monorhinic pulses of odorant in a constant stream of heated and humidified air. Air, coming either from a compressed air bottle or from a pump, is led through a flowmeter and humidified to 70-80% by leading it through a gas washing bottle filled with warm water. The duct of the outgoing airstream is surrounded by heating tape, coupled to a transformer, which assures that the stream that enters the subject's nostril is at body temperature. An odor stream is generated by leading a small stream of air through an "over the surface" saturator. Delivery of the odor stream into the larger air stream, is controlled by a vacuum pump and a pair of electromagnetic solenoids (20 msec response time), which replace part of the airstream by the odor stream for a controllable interval (typically set at 200 msec). Opening of the solenoid can trigger a timer or an evoked potential recording system through an interface. After 30 msec the odorant leaves the olfactometer and enters the subject's nostril. In the case of reaction time measurements, the timer is stopped by a pushbutton press of the subject. Preliminary results, using relatively high flowrates (8.5 L/min), show olfactory reaction times under 300 msec.



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Evaluating Nasal Obstruction by Video Morphometric Analysis of Expired Air Condensation Patterns.

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Changes in nasal patency can alter olfactory function in ways not yet fully evaluated. Increased nasal obstruction can lead to diminished sense of smell, while some increase in turbulant air flow may be beneficial. While sophisticated methods for measuring nasal airway flow exist, these methods are known to interfere with the nasal anatomy. The objective of this study was to evaluate the nasal mirror, an old and non-invasive method of measuring nasal flow, coupled with modern computerized video morphometric analysis. Subjects breathe in and out tidally through their noses and with their mouths closed onto a polished, chrome plated, stainless steel surface. The moisture in the warm expired air condenses on the cooler surface, producing patterns of condensation below each nares. These patterns are video taped and analyzed using morphometric software (American Innovision, San Diego, CA). Several parameters can be measured: surface area, perimeter, shapes and angles. In this study we wanted to make right (Rt) vs left (Lt) comparisons of nasal airflow, surface area of the patterns and olfactory scores in 10 patients attending UCSD Nasal Dysfunction Clinic, and in 10 subjects with no nasal symptoms as controls.

	Flow (L/sec)			Area (cm sq.)			Olf. Scores		
Groups	Rt	Lt	Ratio	Rt	Lt	Ratio	Rt	Lt	Ratio
Patients	187	238	0.8	6	8	0.7	68	66	1.03
Controls	318	247	1.3	7	6	1.1	100	100	1.0

A limitation of the cold mirror is that it measures expiratory airflow, while the symptoms of nasal obstruction are often inspiratory; however, during olfaction the expiratory phase may play a significant role. Expiratory nasal airflow and surface area correlated well. Olfactory scores were correlated with total surface area. This method is simple, quantitative, non-invasive and may prove useful after sampling a much larger population.

109

Repressive and Defensive Subjects show Selective Anosmia for Androstenone

JOHN P. KLINE, GARY E. SCHWARTZ and ZIYA V. DIKMAN
(University of Arizona)

Repressive and defensive coping styles have been associated with decreased responsivity to, and increased recognition thresholds for, sexual and unpleasant stimuli. We studied the relationship between repressive / defensive coping styles and anosmia to androstenone, a putative human sex pheromone, in 48 male and female undergraduates between the ages of 16 and 28. For many osmic subjects, androstenone has an unpleasant sweaty, urine odor. Subjects were designated as high defensive if they scored $\geq$ 7 on the Eysenck Personality Questionnaire (EPQ) L Scale, and low anxious if they scored < 9 on the EPQ N Scale. Subjects smelled pairs of bottles containing silicone (SIL, the solvent control) paired with high and low concentrations in silicone of isoamyl acetate (IAA), high and low concentrations of androstenone (AND) (5 α-andro-16-en-3-one), and paired with silicone only. Subjects were prescreened for detection of IAA and AND and were invited for testing if they consistently (1) detected IAA, and (2) either detected AND (osmic) or failed to detect AND (anosmic). Each of the five pairs was smelled eight times (order was counterbalanced with a modified Latin square) by each subject. Subjects told the experimenter which hand they believed the odor was in, and related the odor's intensity and their confidence in their response on a 0 to 10 scale. Multivariate Analysis of Variance (MANOVA) revealed that compared to low defensive subjects (LOW), high defensive subjects (HIGH) had significantly decreased detection rates (LOW = 83%, HIGH = 64%), rated intensity as less (LOW = 5.45, HIGH = 3.27), and had decreased confidence in their responses (LOW = 7.44, HIGH = 4.42) ($p < .02$). Groups did not differ on IAA. These results, along with Schwartz, Kline, Dikman, Wright and Polak (1992) suggest that anosmia for androstenone is centrally mediated. Specifically, selective anosmia for androstenone may be a specific instance of active repression of olfactory perceptual information.

110

Perceived Odor Intensity in Subjects with Multiple Chemical Sensitivity

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Several subjects have been studied with symptoms of Multiple Chemical Sensitivity (MCS). The primary complaint by the subjects being one of heightened sensitivity to odors; nausea, vomiting, and headaches were attributed to this increased sensitivity. Doty showed no difference in olfactory thresholds between MCS and control subjects (Doty, et al., 1988). To examine the subjects' claim of heightened sensitivity, we gave the University of Pennsylvania Smell Identification Test (UPSIT) (Doty, et al., 1984) to a group of MCS subjects, and a group of age/sex matched controls. In addition to the identification task, both groups rated each UPSIT odor on a 10-point pleasantness scale, and a 10-point intensity scale. The MCS subjects averaged 39/40 correct whereas the normal controls averaged 35/40 correct on the UPSIT. MCS subjects rated the odors in the UPSIT as stronger and slightly less pleasant than the control subjects. Although preliminary, these data suggest that some forms of MCS may be controlled in part by mechsnisms which influence perceptual intensity.

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111

CSERPs to butanol: Correlations with olfactory performance

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(Washington and Lee University)

Chemosensory event-related potentials (CSERPs) hold great promise in the diagnosis of a number of disorders which produce olfactory pathology. To be a valuable diagnostic tool, the relationship between CSERPs and more traditional psychophysical measures must be examined.

Sixteen subjects participated in the study. CSERPs were collected to three concentrations of butyl alcohol (8%, 4%, and 2%) and a no odor control condition following individual testing with the Connecticut Clinical Chemosensory Research Center test (CCCRC). Chemosensory stimuli were presented in a constant air stream (5.6 l/min) which was warmed (35° C) and humidified (RH > 50%). Rise time of the chemosensory bolus was 60 msec to 70% of maximum and each bolus lasted 0.5 sec. ERP data were collected from nine scalp locations and above the right eye for 1.5 sec. Stimuli were administered in randomized blocks of 12 trials with a average inter-stimulus interval of 12 sec. Subjects played a brief video game between blocks of trials. Stimuli were presented through a nasal cannula placed just inside the nostril which had the best CCCRC score. Subjects mouth-breathed during stimulus presentation.

Single-trial ERP data were corrected for eye-movements and digitally filtered to a 0 - 15 Hz bandpass. Trials exceeding 40μV were excluded from the average. After construction of the grand average, separate temporal windows surrounding the negative and positive portions of the CSERP were searched for peak amplitude and latency. These peaks and latencies were submitted to analysis of variance. Results of these analysis indicated that topographical distribution of the peak amplitude of the positive component differed among the concentrations of butanol presented ($F(6,90)=2.839$, $p=.004$). More important to the present investigation, peak amplitude of the positive CSERP component correlated significantly with CCCRC scores at several scalp locations.

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112

CSERPs to Butanol: Signal Feature Extraction

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Chemosensory event-related potential (CSERP) data hold valuable information about the integrity of the olfactory system and the nature of olfactory information processing. Detailed signal processing of this waveform designed specifically to extract certain features has been limited. In the present investigation, signal dynamics of CSERPs to varying concentrations of butanol were evaluated with respect to subjects' scores on a standardized test of olfactory abilities (CCCRC).

Several problems inherent in the collection of CSERP data make these waveforms especially good candidates for advanced signal processing. In order to reduce habituation and adaptation to chemosensory stimuli, individual subject means are typically constructed with far fewer trials than are normally collected in other modalities. Additionally, individual differences in the nasal passage and the mucosa produce latency differences which degrade grand mean CSERP waveforms and may prolong cortical representation of some CSERP components.

In order to determine the frequency characteristics of the CSERP, separate spectral analyses of the temporal windows surrounding the N400 and P800 components were conducted on single-trial CSERPs from sixteen subjects. The spectra for individual trials were averaged within and across subjects and revealed that the majority of the power associated with the negative component peaked at 1.5 Hz. Power for the window associated with the P800 component extended from 1 to 5 Hz. Comparison of these data to visual ERPs indicated that the negative component of the CSERP is much slower than the visual ERP negativity which peaked at approximately 6 Hz. Based on these data, the raw CSERP data were re-analyzed using a 0-5 Hz bandpass. The peaks for the resulting waveforms were submitted to analysis of variance and correlated with subjects' CCCRC scores. Results of these analyses indicated a trend toward larger effect sizes and higher correlations suggesting improved signal-to-noise ratios for the heavily filtered CSERP data. Supported by NIH grant DC01323-02

113

Olfactory Impairment in Children Detected by the Children's Odor Identification Test. STACY MARKISON(San Diego State University), RANI NIJJAR (San Diego State University), CLAIRE MURPHY (San Diego State University and UCSD Medical Center)*

Past work with a child's version of an odor identification task has demonstrated validity and reliability as a clinical test of olfactory function (Anderson, Maxwell & Murphy, AChemS, 1992). The present study demonstrates the usefulness of the same task for clinical populations of children with olfactory impairments. A total of 60 subjects, including normal children, children being treated for allergy, children with cystic fibrosis, and children and adults with Down's syndrome were tested and compared. The original battery included 10 odorants. Two were removed because additional testing showed that they were identified correctly less than 80 percent of the time. The test now includes 8 natural odorants: playdough, cinnamon, bubblegum, coffee, chocolate, mustard, peanut butter, and baby powder. For testing, all subjects were blindfolded and individually presented with the battery of odorants in opaque odorless jars. On a test trial, the subject smelled the contents of one of the jars, removed the blindfold, and identified the odor by pointing to the correct picture on a picture cue sheet. The picture cue sheet consists of line drawings of the target odorants and twelve distractors. If unsure, the subject was required to guess. Regardless of incorrect or correct response, the tester provided positive feedback for participation and encouragement to continue. Each child was tested twice with a mean of 5.4 days apart. Scores were compared as an index of reliability. A subset of 27 subjects were also tested with a two alternative, forced-choice ascending detection threshold for butanol. Performance on the identification and detection tasks were comparable and test-retest reliability of the identification test was high. The results indicate that the odor identification test is an effective measure for testing olfactory deficits in children.

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114

Central and Peripheral Effects in Binary Mixtures: Importance of Side Taste Adjustments. CORINNE A. OSSEBAARD and JAN H.A. KROEZE (Psychological Laboratory, Utrecht University, The Netherlands)

In an experiment with 22 subjects, the interactions in two binary mixtures (NaCl-sucrose and NaCl-citric acid) were studied. Each tastant was presented either alone or in combination with the other tastant, in 5 different suprathreshold concentrations. We investigated whether the intensity of a quality of a tastant increased (enhancement) or decreased (suppression) when presented in a mixture, and, if so, whether these effects were located centrally or peripherally. In order to enable a separation between central and peripheral suppression/enhancement effects, the 'split-tongue'-technique was used. This technique enables independent stimulation of each tongue-halve, i.e., two tastants can be simultaneously applied to either side of the tongue, without physical contact between the solutions (bilateral stimulation). Any mixture suppression/enhancement in this condition must be located centrally. If two tastants are presented physically mixed on a tongue halve (unilateral stimulation), suppression/enhancement can both be central and/or peripheral. Subtracting the effect of bilateral suppression/enhancement from the effect of unilateral suppression/enhancement reveals the peripheral effect. In the present study, main taste estimates (e.g., saltiness of NaCl) as well as side taste estimates (e.g., saltiness of sucrose) were obtained. Traditionally, mixture quality estimates are often compared with quality estimates of one component (e.g., the saltiness of a NaCl-sucrose mixture as compared with the saltiness of unmixed NaCl). However, if a side taste is present, (e.g., if sucrose in a NaCl-sucrose mixture tastes salty), the mixture estimates should be compared with the combined estimates of both components in the mixture. In this experiment, effects of suppression/enhancement were calculated **with and without** side taste adjustments. The results showed that central enhancement disappeared and central suppression occurred in each mixture, if side tastes were taken into account. Furthermore, small peripheral effects were present with and without side taste adjustments.These effects may be slightly over-estimated without side taste adjustments. Peripheral saltiness suppression by sucrose occurred both with and without side taste adjustments. It is argued that in taste mixture experiments, side tastes should be taken into account. Without side taste adjustments, central suppression may be obscured, or even shift to enhancement.

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115

Pemenone Exposure Increases the Sensitivity of Human Subjects to Androstenone. DAVID A. STEVENS (Clark University, Worcester, MA 01610) and ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545).

The diastereoisomeric ketone, *cis*-4-(4'-t-Butylcyclo-hexyl)-4-methyl-2-pentanone (pemenone, PEM) shares with 5α-androst-16-en-3-one (androstenone, AND) a pronounced urine-sweaty type odor. We had earlier shown that specific anosmias to these compounds tend to coexist in samples of human subjects. Given the previously determined ability of regular androstenone exposure to influence the sensitivity of anosmic subjects to AND (Wysocki, et al. *Proc. Natl. Acad.* 86:7976-7978, 1989) we inquired into the ability of pemenone to alter human thresholds for PEM, AND, isovaleric acid (IVA), and phenylethyl alcohol (PEA). Thresholds for these substances were determined in duplicate as previously described (Stevens and O'Connell, *Chemical Senses* 16:57-67, 1991) and then the subjects began an exposure regimen in which they sniffed a mineral oil control or a PEM exposure swab twice a week for a minimum of 8 weeks. Following the exposure period thresholds for the 4 test compounds were again obtained in duplicate from the 41 volunteer subjects. A Kruskal-Wallis ANOVA by ranks was used to test the difference scores, computed by subtracting the pre- from the post-exposure average threshold dilution step for each odorant, for reliable changes between control (N=22) and exposure (N=19) groups. A statistically significant reduction in odor threshold was seen in the PEM exposure group for AND. The changes observed in the thresholds for the other three odorants were not statistically significant. Median changes in the pre- and post-exposure thresholds for each odorant are shown below.

MEDIAN REDUCTION IN THRESHOLD (BINARY STEPS)			
ODORANT	CONTROL GROUP	EXPOSURE GROUP	P LEVEL
PEA	-0.25	0.0	.59
IVA	0.0	0.0	.60
PEM	0.0	0.5	.39
AND	0.0	0.5	.03

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116

Cross-Adaptation of Androstanone by an Odorless Structural Analog.
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Cross-adaptation is the decrease in sensitivity to one odor following exposure to a different odor. Although a common sensory experience, many aspects of this phenomenon remain undescribed. For example, how similarity in olfactory perception or chemical structure affects cross-adaptation and whether odor perception is necessary for cross-adaptation are questions that remain unanswered. As part of an ongoing series of experiments designed to elucidate the role of similarity in determining cross-adaptation, the present study assessed cross-adaptation for androstenone (5α-androst-16-en-3-one), the structurally and perceptually similar androstanone (5α-androstan-3-one), and an essentially odorless structural analog of androstanone (3-Methylidene-5α-androstane). Twelve subjects, in each of four sessions, provided magnitude estimates for intensity-matched concentrations of androstenone and androstanone during and following adaptation to androstenone, androstanone, the odorless structural analog, and a blank (light, white, mineral oil). Significant self-adaptation and mutual cross-adaptation was noted in sessions where the odorous compounds were used as adapting stimuli. Significant, albeit weaker, cross-adaptation of androstanone, but not androstenone, was observed when the odorless structural analog was used as the adapting stimulus. No significant changes in perceived intensity occurred when the blank was the adapting stimulus. These results show that (1) structural similarity, in the absence of perceptual similarity, can result in cross-adaptation, and (2) a stimulus need not be odorous to produce cross-adaptation. These results have implications for structure-activity relationships in olfaction, as cross-adaptation may represent the degree to which odors share common sensory channels.

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Influence of Pleasantness and Concentration of Androstenone on Womens Assessment of Men

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In study I the influence of androstenol (1mg/ml), androstenone (0.05 ug) and a pleasant odor (phenylethylalcohol; 0.1%/v%) on womens assessment of photographed men was tested in a double blind study (control: aqua dest.; n=126; anosmics excluded from stat. analysis). Prior to testing one substance was applicated once on the upper lip of the subject. The women assessed 5 photographs of men (random order) concerning physical attractiveness and other attributes (7 stepped bipolar adjective scales). A standardized pre/posttest (EWL) was administered describing the subjects state of mind. A questionnaire for the morning after the experiment was handed out. Under the influence of androstenol the men were assessed as less masculine, more black (scale for positive/ negative measurements, used in group dynamics); under androstenone as less physical attractive, less good, more black; under phen. as less masculine. If pleasantness was rated positively: under androstenol men were assessed less masculine (negative pleasantness rating went with more masculine). Pleasantness being positive or negative (but not neutral): under androstenone men were assessed more masculine, under phen. less masculine. The pleasant odor influenced only 1 out of 25 assessments. In study II (same design; n=104; 27 items) the effects of two physiological concentrations of androstenone (cf.Gower, 1981; 0.014 and 0.022 ug in oil) on womens assessment of men (6) was tested (control:oil). Under the lower concentration the women assessed them men being more vigorous, stimulating, interesting, good, less physical attractive. During the 1st part of the menstrual cycle subjects assessed the men as more stimulating, interesting, sexy, but less masculine. The higher concentration was nearly ineffective. In none of our previous studies any assessment was influenced positively by androstenone alone.

Trimethylaminuria: A Metabolic Disorder Presenting with Primary Complaints of Dysosmia/Dysgeusia

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Trimethylaminuria (TMAU), also referred to as "fish-odor syndrome", is a metabolic disorder, likely of genetic origin, characterized by relatively high levels of trimethylamine (TMA) in body fluids. Elevated TMA levels may result from a functional problem in a microsomal, hepatic, flavin-containing monoxygenase. In the past three years, our Chemosensory Clinical Research Center has become a focal point for referred patients suffering from persistent oral malodor and/or bad taste. Fifty-two such patients have been evaluated since an initial case report was published. Seventeen (33%) have been found to be suffering from TMAU. Thirteen TMAU-affected patients underwent psychophysical evaluation in our Taste and Smell Clinic. All tested in the normal range in their odor and taste identification scores. Although several TMAU patients had levels of other odorous volatile organic compounds above those found in normal controls, no consistent pattern was found; however, initial analyses suggest that high levels of salivary TMA in the TMAU sufferers may be responsible for their persistent dysosmia/dysgeusia symptoms. In contrast patients presenting with persistent oral symptoms but not diagnosed as TMAU positive had significantly higher levels of malodorous volatile sulfur compounds than normal controls in their mouth and lung air suggesting these compounds as responsible, in part, for their oral complaints.

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Olfactory cues suppress newborn human infant crying

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Breast fed newborn human infants exhibit a preference for maternal odors. These breast fed infants exhibit head turns towards the maternal odor (Russell, 1976; Macfarlane, 1975; Porter, et al., 1988; Schaal, 1988) and decrease general activity when presented with the odor (Schaal, 1991). The present study assessed the responsiveness of both bottle fed and breast fed infants to maternal odors. The subjects were newborns from the fullterm nursery at the Oklahoma Memorial Hospital at the Health Science Center of the University of Oklahoma. The hospital gown was used as the odorant source and held approximately 5-10 cm from the baby's nose for 1 min. In isolation from the mother, infants were presented with one of the following odorants: 1) a hospital gown which has been worn by the infant's mother, 2) a gown worn by another newly postpartum mother, or 3) a clean gown. The odorant presentation was done while the infants were in one of three states: 1) quiet awake, 2) crying or 3) sleeping. All odorant presentations were videotaped and scored by an observer unaware of the experimental condition of the baby. The results suggest that in both bottle fed and breast fed newborns, crying is suppressed in the presence of maternal odor.

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Primary Structure of Olfactomedin: A Novel Olfactory Tissue-Specific Extracellular Matrix Protein.

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One of the most intriguing characteristics of olfactory neurons is their remarkable plasticity which allows their continuous replacement throughout the life of the adult animal. When newly emerging dendrites of olfactory neurons reach the luminal surface their apical regions differentiate into a dendritic knob with cilia that house the olfactory transduction machinery. The signals which trigger dendritic differentiation are unknown, but most likely reside in the extracellular layer of mucus which is in intimate contact with the chemosensory surface. Here we report the molecular cloning and primary structure of olfactomedin, a 57 kD glycoprotein previously identified as one of the major components of the extracellular mucous matrix of frog olfactory neuroepithelium. Olfactomedin is expressed exclusively in olfactory tissue, where it is produced by Bowman's glands and sustentacular cells and deposited into the deep mucus layer adjacent to the chemosensory surface. We extracted mRNA from olfactory tissue of *Rana catesbeiana* and constructed a cDNA library in Lambda ZAPII. A full length cDNA clone which encodes olfactomedin was identified and olfactomedin's amino acid sequence was deduced from the nucleotide sequence of its cDNA. Mature olfactomedin contains 448 amino acids preceeded by a 16 amino acid-long leader peptide. Olfactomedin's sequence is unique and does not show homologies to any known protein. It contains six consensus N-linked glycosylation sites and seven cysteines. We propose that four of these cysteines contribute to intramolecular loops and that the remaining three cysteines form intermolecular disulfide bridges with neighboring olfactomedin molecules. This leads, in agreement with experimental observations, to the formation of polymers which represent the principal building blocks of the olfactory extracellular matrix. By analogy to the functions of other extracellular matrix proteins in the nervous system, olfactomedin may influence the maintenance, growth or differentiation of chemosensory cilia on the apical dendrites of olfactory neurons.

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121

Expression of α-Gustducin in Human Taste Cells.

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The α-subunit of the taste-cell-specific G protein, α-gustducin, has been recently cloned and its mRNA has been localized in taste cells of lingual papillae of rats using *in situ* hybridization (McLaughlin et al., 1992, Nature, *357* : 563). Using a polyclonal antibody to α-gustducin generated in rabbits, we have demonstrated that this G protein is present in human taste cells. Lingual tissues including circumvallate and foliate papillae were collected at autopsy; taste buds from 4 patients ranging in age from 60 to 93 years were obtained; 3 of these had Alzheimer's disease. The tissues were immersion- fixed in Zamboni's for 2-6 hours, cryostat-sectioned at 15 μm and mounted on gelatinized glass slides. Taste buds were identified in Nissl-stained sections and standard immunofluorescence techniques were utilized to demonstrate α-gustducin localization. Sections were examined with fluorescence microscopy and confocal laser scanning microscopy (CLSM). Fluorescently-labeled immunoreactive taste cells were present in both papillae of all patients. Two patterns of immunofluorescence were observed. Most immunoreactive cells showed plasmalemmal immunfluorescence; intense fluorescence was observed especially in their apical region, near the taste pore. However, typically one or two taste cells per section of a taste bud contained intense fluorescence throughout their cytoplasm. Using CLSM on serial optical sections, the most intense label for the antibody to α-gustducin was localized in the apical region of the immunoreactive taste cells that showed the plasmalemmal pattern of immunofluorescence, and throughout the cytoplasm of the taste cells that showed the cytosolic pattern of immunofluorescence. Preabsorption of the primary antibody with the α-gustducin peptide completely abolished the immunofluorescence. We used rat taste cells as a positive control; the taste cells in the circumvallate papillae showed the same pattern as described in humans. Present results demonstrate that human taste cells contain the α-subunit of the taste-specific G protein, α-gustducin, and indicate conservation of its primary amino acid sequence across species.

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122

Developmental Expression of Rabbit Olfactory P450. XINXIN DING, HWEI-MING PENG, and MINOR J. COON (Department of Biological Chemistry, Medical School, The University of Michigan, Ann Arbor, MI 48109).

Mammalian olfactory mucosa has a high concentration of cytochrome P450 monooxygenases. The major olfactory P450 isoforms in adult rabbits include P450 NMa, which is found in both olfactory and respiratory mucosa, as well as in liver at a low level, P450 NMb (2G1), which is olfactory-specific, and P450 form 4 (1A2), which is found only in liver and olfactory mucosa. In the present study, we have found that the developmental expression of olfactory P450 in rabbits is not coordinated with the ontogenesis of hepatic P450. These three P450 isoforms were detected immunochemically and found to be at a relatively high level in olfactory but not hepatic microsomes in the first two weeks after birth. In the liver, NMb is not detectable at any age and NMa not until the 4th week. P450 1A2 is not detectable until the 3rd week, but its level increases rapidly in the 4th week. These P450 isoforms are also detectable in prenatal olfactory tissue at 2 days before birth, indicating that direct exposure to air is not a prerequisite for their early expression in this tissue and that the early appearance of these enzymes may be controlled by both endogenous and environmental factors. In addition, the developmental expression of 2E1, a minor olfactory P450 isoform, also occurs earlier in olfactory mucosa than in liver, and the same conclusion can be made about the expression of NADPH-cytochrome P450 reductase, which is detectable in olfactory microsomes but not in hepatic microsomes from prenatal rabbits. Thus, the regulatory mechanisms that control the basal prenatal expression in the olfactory tissue may be common for multiple P450 isoforms and perhaps also for other biotransformation enzymes. The tissue-specific early onset of expression of multiple forms of P450 in olfactory tissue suggests that these enzymes may play an important role in the neonatal period when olfactory ability is vital for the survival of the newborn. The presence of relatively high levels of biotransformation enzymes in the olfactory mucosa may also have important implications for neonatal inhalation toxicology.

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123

Identification of Novel Protein–Tyrosine Phosphatases Expressed by Sensory Neurons of the Olfactory Epithelium.

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Tyrosine phosphorylation by tyrosine kinases is known to be a fundamental regulatory mechanism for many biological processes such as cell proliferation and signal transduction. Recently, protein–tyrosine phosphatases (PTPases) have emerged as an integral element in the regulation of tyrosine phosphorylation. Instead of adding phosphoryl groups to tyrosine residues like tyrosine kinases, these enzymes cause dephosphorylation. The olfactory neuroepithelium serves as a useful model for the study of neurogenesis yet we know very little about the molecules endowing the olfactory neuroepithelium with its unique attributes of neural regeneration. Given the central role one can predict for PTPases in regulating various crucial cell processes, we hypothesized that specific PTPases, perhaps olfactory specific, might be involved in neural proliferation or other cell processes in the olfactory system such as axon extension. To identify PTPases (both known and novel) expressed by the olfactory neuroepithelium we undertook a molecular screen of olfactory mRNA for putative PTPase sequences. mRNA isolated from rat olfactory turbinates was used as a template to generate 1st strand cDNA. The cDNA was then utilized as template in a polymerase chain reaction (PCR) to amplify all sequences present in the cDNA that could be primed by a set of degenerate oligonucleotide primers designed to specifically hybridize to highly conserved sequences in PTPases. The resulting amplified products of appropriate size (≈ 300bp) were shotgun cloned and analyzed by DNA sequencing. Among the ≈ 150 initial clones analyzed, 13 unique clones were represented. Seven clones were previously identified PTPases (or their rat homolog): PTP1C, RPTP1, PTPepsilon, MLRPA, PTPS, PTPMEGA2, PTPgamma. The remaining 6 clones represented novel PTPase genes. The RNA expression pattern of these novel clones was predominantly neural. For one clone, OE41, we now have the full length cDNA sequence. Translation of putative coding sequence predicts a transmembrane protein with an extracellular domain of IgG–like repeats and two cytoplasmic phosphatase domains. OE41 shows a largely neural expression pattern with its greatest expression within the olfactory turbinates. Colorimetric *in situ* hybridization histochemistry reveals that OE41 is expressed by sensory neurons of the olfactory neuroepithelium. The expression pattern of OE41 and its predicted transmembrane structure with extracellular adhesion motifs suggest a role in signal transduction to the extending axon and/or migrating neural soma.

124

Expression of mucociliary-specific epitopes in the human olfactory mucosa

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We have examined the expression of olfactory (S10/8A6,S10/6C6) and respiratory (S9/1C3) mucosae-specific monoclonal antibodies (Mabs) in the human nasal mucosa that were identified previously in studies on rats (Strotmann & Breer, 1991). Only Mab S10/6C6 was cross-reactive with human tissue. Specimens were obtained at autopsy from eleven subjects that ranged in age from 16 weeks of gestation to 85 years old, including tissue from three subjects with Alzheimer's disease (AD). Olfactory mucosa (OM) was identified unequivocally by the presence of olfactory marker protein-immunoreactive receptor neurons in the epithelium. The mucociliary complex (MC) of the OM was intensely immunoreactive to Mab S10/6C6 and the MC of respiratory mucosa was negative at all time points studied. No differences in immunoreactivity were observed in tissue specimens obtained from AD subjects. There was transient expression of this epitope at the surfaces of the lumen and duct of Bowman's glands in the 16 week fetus that was not observed at other time points in the ontogenetic sequence. The results demonstrate the expression of a mucociliary-specific epitope in the tissue compartment associated with olfactory transduction in the human olfactory epithelium throughout ontogeny, indicate its phylogenetic conservation across species, and suggest that the use of this Mab may be of prognostic value in evaluating cases of human peripheral anosmia.

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125

Synaptic Organization of Tyrosine Hydroxylase Immunoreactive Processes in Rat Olfactory Bulb Glomeruli.

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Synaptic organization of the olfactory bulb glomerulus is not well understood. It is recognized that glomeruli include both axodendritic primary afferent synapses and dendrodendritic local circuit synapses. However, the extent to which these are homogeneously distributed among the several subpopulations of neurons contributing processes to the glomerulus is unknown. Moreover, the issue of whether single olfactory receptor cell axons can contact multiple populations of neurons remains controversial. This initial report begins to explore the glomerulus in detail by examining the synaptic organization of specific subpopulation of neuron immunoreactive (IR) for tyrosine hydroxylase (TH). Adult rats, 200-300g, were anesthetized and perfused with 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1M phosphate buffer. Tissue sections were incubated for 48 hrs. in primary TH Ab (Eugene Tech Int., 1:400) and then processed with Vector Labs *elite* ABC Kits. Following routine processing, 70nm sections were cut and examined with a JEOL 1200. IR cell bodies in the glomerular layer were morphologically similar to the periglomerular cells described by Pinching and Powell (Cell Sci., 1971, 9:305). Characteristics included a thin rim of cytoplasm surrounding an invaginated nucleus and asymmetrical synapses onto the cell body. Intraglomerular IR processes appeared dendritic, in part based upon their irregular outlines. These processes received asymmetrical synapses from terminals identified as olfactory receptor axons based on their flocculent electron dense appearance and high density of synaptic vesicles. Interestingly, cases were observed in which a single receptor cell axon synapsed onto both IR and non-IR processes. IR processes also received asymmetrical synapses from non-IR intraglomerular processes that were electron lucent and appeared equivalent to the mitral/tufted cell dendrites described by Pinching and Powell (*vide supra*). Finally, both IR and non-IR processes made symmetrical synapses onto similar electron lucent dendritic-like processes. Although no unequivocal instances were observed of either reciprocal synaptic contacts involving an IR process or of synaptic interactions between 2 IR processes, our data thus far do not eliminate these as possibilities. In addition to characterizing the glomerular synaptic organization of TH IR neurons, this report provides the first evidence of divergent synaptic contacts by single olfactory receptor cell axons.

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126

Localization of Immune System Markers in Adult Human and Rat Vomeronasal Organ.

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Cells of the immune system in the vomeronasal organ (VNO) of adult humans and rats were localized by immunofluorescence staining with fluorochrome-labeled antibodies to IgA, IgG, IgE, and β_2-microglobulin (β_2-m). Human VNOs obtained from 2 biopsies and 3 autopsies showed little evidence of active immune system involvement. No intraepithelial lymphocytes were observed. Several B lymphocytes of either the IgA or IgG isotype were localized in or near the VNO basement membranes. One intraepithelial IgE-immunoreactive cell and several in or near the VNO basement membranes in several patients were observed. VNO epithelial cells were moderately immunoreactive for β_2-m compared with intensely immunoreactive serous respiratory gland acinar cells. In virus-antibody-free (VAF) rats, previous studies (Getchell, Shih, and Getchell, 1992, Chem. Senses, *17*:628-9) demonstrated that respiratory and olfactory mucosae contained few lymphocytes, and epithelial cells exhibited very low levels of expression of β_2-m. In contrast, the VNO nonsensory epithelium (NE) contained large numbers of β_2-m-immunoreactive immune system cells; moderate β_2-m expression was evident on VNO cells within the sensory epithelium (VE) and on vomeronasal gland (VNG) acinar cells. Large numbers of IgE-immunoreactive cells and smaller numbers of IgG-immunoreactive B lymphocytes occurred primarily within the NE and its basement membrane. The major differences in VNOs of rats with positive serum titers for sialodacryoadenitis virus were increased expression of IgA by VNGs and increased intensity of β_2-m staining on VNO cells within both NE and VE.

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127

Vomeromodulin Gene Expression in the Nasal Mucosa of Rats During Postnatal Development.

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The purpose of this study was to investigate gene expression of the recently identified putative pheromone transporter, vomeromodulin (VM), at two postnatal stages (P2 and P27) of developing rats using *in situ* hybridization and Northern blot analyses. We generated antisense and control sense cRNA probes for VM labeled with ^{35}S for *in situ* hybridization and ^{32}P for Northern blots. At P2, an intense hybridization signal was associated with the acinar cells of the lateral nasal glands (LNG) that surround the maxillary sinus. At P27, there was an overall increase in the intensity of the hybridization signal in the LNG. In addition, discrete populations of posterior septal (PSG) and vomeronasal (VNG) glands located in the ventromedial nasal septum and at the junctional areas of sensory and non-sensory epithelia of vomeronasal organ now showed distinct hybridization signals. In these glands, the clusters of silver grains from *in situ* analysis were also associated with acinar cells. In Northern blot analysis, the hybridized 2.2 kb mRNA was more abundant at P27 than at P2. In controls, treatment of tissue sections with RNase prior to hybridization with the antisense probe or hybridization with sense probe resulted in a complete absence of signal. The results demonstrate that the expression of VM-mRNA is developmentally and differentially regulated in the LNG, PSG, and VNG. The earlier expression of VM-mRNA in LNG than in PSG and VNG may suggest a different role in these sites during development. VM is secreted directly into the vomeronasal organ from PSG and VNG. The results suggest that the function of VM is closely related to the developing chemosensory function of the vomeronasal organ in maturing animals.

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128

Axonal Abnormalities in the Mammalian Olfactory System. JAMES E. SCHWOB, KAREN E. MIELESZKO SZUMOWSKI, DONALD A. LEOPOLD AND STEVEN L. YOUNGENTOB (Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210)

In several human afflictions, including Alzheimer's disease, changes in the immunochemical phenotype and/or disposition of olfactory axons have been described by others in olfactory mucosa acquired at autopsy or by biopsy. The specificity of these changes has been called into question by previous observations on material from neurologically normal individuals. Here we describe axonal changes in humans with olfactory disease and compare them to the disordered axonal growth that occurs in several animal models. In biopsies of **human olfactory mucosa** analysed either by LM immunochemistry or EM, we see collections of axons superficial to the basal lamina in the olfactory epithelium and other aberrant axons that are loose in the lamina propria. These changes, which are often accompanied by a preponderance of immature neurons in the epithelium, can be observed in patients made anosmic by head trauma, in cases of congenital anosmia (including Kallmann's syndrome) and in patients that are either anosmic or hyposmic for unknown reasons. On rare occasion, we see similar changes in normosmic individuals. In **experimental animals**, olfactory bulbectomy induces the formation of large and numerous intraepithelial neuromas. The neuromas form as a consequence of the regrowth of axons back into the olfactory epithelium, as shown by the abundance of phosphorylated GAP-43 in the neuromas (which is absent from the proximal part of axons) and the continuity between neuromas and the underlying fascicles of the olfactory nerve. In addition, direct disruption of the epithelium by the combination of 3-methyl indole and methyl bromide also induces the formation of neuromas. However, with this type of lesion, the neuromas form because axons fail to exit the epithelium during the reconstitution of the epithelium; instead they grow superficial to the basal lamina and extend for only a short distance. Taken in combination our results indicate that the aberrant growth of olfactory axons is seen when the epithelium is unable to contact the bulb. The disconnection may occur because the bulb is absent or inaccessible, or it may be due to direct damage to the epithelium.

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Selective Association and Specific Bulbar Projections of Olfactory Receptor Neurons (ORNs) Reactive to an Anti-hsp70 Mab.

V.McM. CARR and A.I. FARBMAN (Northwestern Univ.)

During our immunohistochemical study of ORNs reactive with Mabs to the hsp70 family of stress proteins, we have found that axons of these ORNs are reactive to the Mab 2a4 (provided by Dr.R. Morimoto, N.U.). 2a4-reactive fibers appear to selectively aggregate within olfactory nerve bundles and follow predictable pathways to the olfactory bulb (OB) where they project densely to only a small number of glomeruli. Reactive fibers from the 1st and 2nd endo- and the 2nd and 3rd ectoturbinates project to 1 to 2 ventro-lateral glomeruli in the rostral OB. Fibers from the remaining turbinates and septum project more caudally, to 1, or possibly 2, glomeruli in the ventro-medial OB. Other glomeruli show only occasional single reactive fibers. After unilateral bulbectomy, reactive fibers from 2a4-positive ORNs in the reconstituting epithelium on the operated side follow similar centripetal routes as in control animals but then appear to aggregate into neuromas in the region of the missing OB. Contralaterally, these same routes are followed, and at early postoperative periods (6-8 days) 2a4-reactive glomeruli can still be seen. However, with increasing survival time, the reactive fiber density decreases. By 4-6 mos. after bulbectomy, reactive glomeruli can no longer be distinguished. Only scattered reactive fibers appear within glomeruli in the appropriate OB locales. These contralateral changes correlate with a possible post-bulbectomy decrease in the number of contralateral 2a4-positive ORNs, which is currently under investigation in the laboratory.

These observations suggest that the 2a4 labeling of a subpopulation of ORNs is probably not related to the level of physiological stress of these neurons but rather to some intrinsic factor, possibly related to specific connectivity or to their role in olfaction.

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Rapid Assessment of Interactions between Ligands and G-Protein Coupled Receptors.

MICHAEL R. LERNER, (Yale University School of Medicine); TIMOTHY S. MCCLINTOCK, MARC N. POTENZA, CHANNA JAYAWICKREME, GERARD F. GRAMINSKI AND SURESH KARNE.

Olfactory receptors are members of the large family of G-protein coupled receptors (GPCRs). As such, the odorant detection is part of the general problem of ligand/GPCR interactions. To address this question, a general means of analyzing functional interactions between chemicals and GPCRs has been developed. The technology is based on the ability of animals to rapidly change color by controlling the disposition of pigment within their chromatophores. cDNA coding for a receptor to be studied is expressed in immortalized frog melanophores. If the receptor normally functions to activate either adenyl cyclase or phospholipase C, exposing the cells to an agonist leads to centrifugal melanosome translocation and cell darkening. Conversely, application of an agonist to pigment cells expressing a receptor that operates to inhibit adenyl cyclase, induces centripetal pigment movement and cell lightening. Pigment translocation in either direction is complete within thirty minutes and the results can be quantitated on a large scale with a micro plate reader. Alternatively, the assay can be combined with video imaging microscopy and used to evaluate ligand receptor interactions at the level of individual cells.

Strategies of Comparison: Models, Opportunism, and Cladistics.

R. GLENN NORTHCUTT (University of California, San Diego)

Biological analyses are based on modeling, opportunistic, and phylogenetic (cladistic) strategies. Each approach has inherent limitations and is subject to potential errors that are related to the historical nature of the characters being analyzed. Analysis by modeling is an effective strategy when the character being modeled arose early in a radiation and is retained in a large number of descendants (a shared primitive or symplesiomorphic character), but modeling has limited predictive value when the character arose later within a radiation and has a more restricted distribution (a derived or apomorphic character). Opportunistic analysis involves the examination of highly developed characters whose complex nature facilitates recognition of their biological roles, but such characters are frequently derived or even unique to a few species, which severely limits the predictive value of such analyses. Phyletic analysis (cladistics) allows the identification of patterns of character distribution and the generation of hypotheses (scenarios) regarding this distribution. Whereas this strategy has considerable heuristic value, it also involves a high risk of supporting an invalid hypothesis regarding the homology of characters, their genealogy, and the mechanisms responsible for their evolution.

Neurochemical Conservatism in the Olfactory Bulb. HARRIET BAKER (Cornell Univ. Med. Coll. at Burke Med. Res. Inst.)

The vertebrate olfactory system exhibits a relatively conserved laminar structure. The axons of bipolar olfactory receptor cells coalesce to form fiber tracts that terminate within glomerular structures where contact is made with the dendrites of mitral cells. In most species, mitral cells also form a more or less distinct layer. Granule and, in some species, periglomerular cells constitute a large neuronal population that is thought to modify mitral cell activity. Recent data suggest that structural similarity is reflected biochemically in the expression of neurotransmitters and neuropeptides. For example, the neurotransmitters, dopamine and GABA, have been demonstrated in the olfactory bulb in a large number of vertebrate species. Olfactory marker protein, a peptide found in high concentrations in olfactory receptor neurons, exhibits expression in diverse species. However, disparities also exist such as species variation in the expression of substance P and differences in the laminar organization of cells containing dopamine. The distribution of these and other molecules will be described in diverse vertebrate species, such as teleosts, amphibia, reptiles, birds, and mammals including primates. Several questions will be addressed. Do the disparities in distribution of cells containing neurotransmitters suggest distinct species-specific functions for these molecules? Are these examples of convergent evolution at a biochemical level? Do these molecules subserve corresponding functions in non-vertebrate phyla such as in the arthropod olfactory system? Similarities across phyla could either suggest the early appearance of the components of the olfactory system or be an example of covergent structural as well as biochemical evolution.

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133

Development of Primary Olfactory Centers.
LESLIE P. TOLBERT (University of Arizona)

Primary olfactory centers in a wide variety of species ranging from insects to mammals share certain developmental features as well as some principles of organization. Most notably, virtually all primary olfactory centers that have been studied have revealed a critical dependence upon olfactory sensory axons for their normal development. In mammals and frogs, ingrowth of the sensory axons from the olfactory epithelium is necessary for the outpouching of the telencephalon that gives rise to the olfactory bulb. The earliest axons to reach the telencephalon probe deeply, perhaps even influencing cell division or early steps of determination in the ventricular zone. In invertebrates that have been studied, the influence of olfactory axons is less drastic: even in their absence, an olfactory lobe develops. In insects and perhaps crustacea, however, glomeruli, the knots of synaptic neuropil characteristic of most primary olfactory centers, fail to develop without ingrowth of olfactory axons. In the moth, we have shown that neurons that ordinarily would develop glomerular tufts instead branch diffusely, and glial cells, which ordinarily would surround glomeruli, form a thick rind around the perimeter of the entire neuropil.

Many laboratories are interested in the mechanisms underlying the more or less profound influence of olfactory axons on their developing targets. In my laboratory, we have found that not only olfactory axons but also glial cells are necessary for the formation of glomeruli in an insect. We are exploring the hypothesis that glial cells serve to constrain, either mechanically or via chemical signals, the branching of sensory axons and/or their target dendrites to developing glomeruli and have obtained evidence that a tenascin-like molecule may be involved in this process.

134

Comparative and Evolutionary Aspects of Olfactory Physiology.
ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545).

The response properties of olfactory receptor cells to both simple and complex odorants will be explored with the hope that a cross-phylum comparison of response capabilities may indicate general principals of olfactory function. Particular attention will be focused on the physiological mechanisms responsible for the detection and processing of odorants which are involved with the generation of important behavioral responses. General issues such as the role of context, experience and physiological status will be explored. The role of pheromone production and perception in the process of speciation will also be considered. Finally, the possibility that olfaction offers useful constraints and advantages when viewed as a communication channel will be evaluated.

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135

Towards a Common Strategy for Transducing Olfactory Information. Barry W. Ache (Whitney Laboratory and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine).

Notwithstanding species-dependent differences in mechanistic details, odors rapidly and transiently activate two second messenger cascades, one mediated by adenosine 3', 5'-cyclic monophosphate (cAMP) and the other by inositol 1,4,5-trisphosphate (IP_3), in the olfactory receptor cells of lobsters, catfish and rats. The presence of two odor-activated second messenger pathways in such phylogenetically diverse animals implies that having dual transduction pathways serves a fundamental role in olfaction. Differential odor sensitivity of the two pathways suggests this role relates to coding odor quality. In the lobster, the two second messengers activate separate conductances that, respectively, increase and decrease the probability that the cell will discharge action potentials. Activating opposing transduction processes effectively increases the range for coding olfactory information at the level of the receptor cell. By activating opposing processes, dual transduction pathways could be expected to serve the same or role in other organisms even though the detailed mechanisms could, and probably will, differ among species. Organisms, notably insects and amphibians, which to date appear to support only cAMP or IP_3-mediated transduction, may use some other additional transduction pathway. Alternately, some species may lack what could be a subset of receptor cells that express dual pathways, possibly as a subsequent adaptation to reduced demand for olfactory information. Cells possessing the inhibitory pathway in the lobster comprise only about 50% of the total receptor cell population.

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136

Androstenone Sensitivity in the Domestic Pig: Sex Difference and Role of the Vomeronasal Organ.
KATHLEEN M. DORRIES, MARK J. THOMAS, ELIZABETH ADKINS-REGAN, AND BRUCE P. HALPERN (Cornell University, Ithaca, NY 14853)

The steroid 5α-androst-16-en-3-one (androstenone) is emitted by adult male pigs (*Sus scrofa*) and facilitates proceptive and receptive behavior in estrous female pigs. We measured androstenone detection threshold in adult male, castrated male (castrated at 7-14 days of age) and female pigs to determine if olfactory sensitivity to androstenone is sexually dimorphic favoring females. We trained subjects (n=18) to perform an operant task in which they discriminated odor from blank, and tested them with androstenone and a control odorant (geraniol). Females' androstenone detection threshold – the concentration at which performance dropped to chance – was a five-fold dilution lower than that of males. Castrated males' androstenone threshold did not differ from those of either intact males or females. There was no sex difference in geraniol detection threshold. We are currently examining the role of the vomeronasal organ (VNO) in androstenone-mediated behavior. We blocked the VNO of 6 adult female pigs using veterinary surgical cement. VNO block does not significantly effect detection of a high concentration of androstenone (mean % correct = 89.17 (VNO blocked) and 88.3 (control); $t = -.217$; $p > .05$). These results suggest that the VNO is not necessary for detection of androstenone in pigs, but its role in expression of proceptive and receptive behavior in estrous female pigs remains to be determined.

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Added Significance of the First LHRH Injection in Facilitating Mating Behavior in Inexperienced Male Hamsters SANTIAGO MOLINA and MICHAEL MEREDITH. Program in Neuroscience, Florida State University, Tallahassee, Fl.

LHRH is known to facilitate mating behavior in several species and is also known to be released intracerebrally in response to opposite sex conspecifics. In previous work we have demonstrated that intracerebral injection of LHRH or related peptides substantially restores mating behavior deficits resulting from removal of vomeronasal organs (VNX) in inexperienced male hamsters. In experiments using repeated LHRH injections it appeared that the 1st LHRH injection might have a lasting facilitation on future performance. To test this possibility we devised an experiment where animals were either injected with LHRH "early" or "late" in a series of mating behavior tests. Sexually inexperienced male golden hamsters, 13 VNX and 12 intact (CON), were implanted stereotaxically with a 26-ga guide tube into the left lateral ventricle. All animals were tested for mating behavior once a week for 8 weeks. EARLY-VNX and EARLY-CON animals received 50ng LHRH in 2ul of saline through an intraventricular cannula 30 minutes before the 2nd mating test, while LATE-VNX and LATE-CON received saline. All animals were then tested for 3 weeks without injections. On week 6 all animals received 50ng LHRH and tested 30 min later, and then tested twice without injection over the next 2 weeks. All groups showed an increase in performance (Intromissions/minute in a 5 minute or 5 intromission test) over the 8 weeks of testing, as expected for initially inexperienced animals. EARLY-VNX animals (receiving LHRH) but not LATE-VNX animals (receiving saline) increased their performance on that test (week 2) - to the level of saline injected CON animals. Over the succeeding 3 weeks both groups of VNX animals increased their performance, as though repetition and time relieved deficits due to VNX, but LHRH accelerated the process. EARLY-CON animals (those injected with LHRH) but not LATE-CON animals also increased their performance in week 2 but, unlike VNX animals, neither group showed a substantial increase during the subsequent 3 weeks tests. The late LHRH injection (6th week) produced increases in all animals but greater increases in those receiving their 1st LHRH injection at this time. Following these late injections all animals showed a decrease in performance. Relative to their initial (wk. 1) performances, both EARLY groups had higher performance on their first LHRH test (wk. 2) than both LATE groups. Similarly both LATE groups had higher performance on their first LHRH test (wk. 6) than both EARLY groups- even though all animals recieved LHRH at that time. The differences between these and previous results, which showed a decrease and no increase (Meredith and Howard, '92; Fernandez and Meredith, '92) in performance of controls injected with LHRH, could be due to our use of single widely spaced LHRH injections here, or to the post-injection oscillation in performance that we have observed in several experiments using LHRH peptides. The timing between repeated mating tests varied from 3 days to 9 days in different experiments, possibly resulting in an enhancement or suppression of cumulative post injection effects. These effects should be explored further in future experiments. Supported by NIH Grant DC00906

Evidence for G Protein Coupled Chemoattractant Receptors in the Vomeronasal Epithelium of Garter Snakes

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The vomeronasal (VN) epithelium possesses high sensitivity to non-volatile odorants. The initial interaction of VN receptors with chemoattractant odorants has been demonstrated to take place on VN sensory neurons. We have previously isolated a 20 kDa protein, B7, from earthworm secretions that is detected by the VN system (Jiang et al., 1990). The mechanisms involved in transmembrane signaling are currently being addressed. With [^{3}H]-labeled B7 as ligand, the effect of GTPrS, a hydrolysis-resistant guanine nucleotide, on ligand-receptor binding was studied. The binding of [^{3}H]B7 to the VN membranes was inhibited in a concentration-dependent manner by GTPrS. The maximal inhibition of binding by GTPrS was 80%. The half-maximal inhibition of binding occurred at a concentration of 3.2×10^{-5} M GTPrS. The GTPrS inhibitory effect was more potent than that of GDP, or GMP. Adenosine nucleotide was without effect. Dissociation of presumed [^{3}H]B7-receptor complex was accelerated by the addition of GTPrS, indicating that affinity of chemoattractant receptors was reduced. Magnesium ions enhanced the [^{3}H]B7-receptor binding. These preliminary observations are in agreement with the properties of G-protein-coupled receptors as established in hormone or neurotransmitter systems. Furthermore, G proteins exist in VN primary sensory neurons as demonstrated by Western immunoblotting and immunocytochemistry. Based on molecular size and immunoblotting analysis, at least three G proteins, including Gi, Go and Gs, have been found in the VN epithelium. The immunocytochemistry studies on vomeronasal organ (VNO) sections verified the presence of $G_{i\partial}$ and $G_{o\partial}$. They all yielded strong positive immunostaining on the surface of the VN epithelium. Removal of the accessory olfactory bulb led to the disappearance of immunostaining for these G-proteins in the VNO. These results demonstrated that $G_{i\partial}$ and $G_{o\partial}$ are present in the VN primary receptor cells, especially in the microvillar area where the receptors could bind chemoattractants to trigger signal transduction.

Jiang, X-C., Inouchi, I., Wang, D. and Halpern, M. (1990) J. Biol. Chem. 265:8736-8744.

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Microscopical and Ultrastractural Anatomy of the Vomeronasal Organ and Vomeronasal Gland in the opossum *Monodelphis domestica*

NAOMIE S. PORAN (Zoology Dept., North Carolina State University, Raleigh, NC 27695-7617)

The opossum *Monodelphis domestica* is unique in that a very extensive tubulo-alveolar serous gland is associated with its Vomeronasal organ. The main excretory duct of the intercalated Vomeronasal gland is continuous with the lumen of the organ, beginning caudal to the sensory epithelium. The present work focuses on the different structural characteristics along the longitudinal axis of the neuroepithelium of the Vomeronasal organ and glandular epithelium. Special attention is placed on the distinctive intergradation zone between the two epithelia.

Preliminary Observations on the Morphology of the Vomeronasal Organ of a Newborn Asian Elephant.

L.E.L. RASMUSSEN (Oregon Graduate Institute), EDWARD W. JOHNSON, and BRUCE W. JAFEK (University of Colorado Health Sciences Center and The Rocky Mountain Taste and Smell Center).

Adult Asian elephants have an apparently typical mammalian vomeronasal organ (VNO) (Rasmussen and Hultgren, 1990). Presumably flehmen responses aid in the mechanics of presentation of bioactive molecules to vomeronasal neuroreceptors. Young Asian elephants do not exhibit flehmen responses until 6 to 17 weeks after birth. Histological studies of VNO in newborn elephants have not been available. Recently at the light microscopic level we have observed a structure that in gross appearance is similar to the VNO of other mammals; there is a lumen surrounded by a convex and a concave epithelial border; these borders join at both ends. Based on previous studies, we presume that the concave border would be the neuroepithelium with the receptor cells. The epithelia of both surfaces is pseudostratified. Within these epithelia are cells with different nuclear morphologies. Some of the nuclei are euchromatic and oval. Others appear heterochromatic. Round basal cells are also apparent. At the surfaces of the epithelia some ciliated cells can be seen. To our knowledge, ciliated cells have been identified in the VNO neuroepithelium of only one other mammalian species (Adams and Wiekamp, 1984). To further document the cell types found in the newborn elephant VNO and to attempt to identify receptor cells, we will do electron microscopy on representative regions.

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141

c-Fos expression during mating behavior in male hamsters: Contributions of vomeronasal sensory input and copulatory performance. GWEN FERNANDEZ and MICHAEL MEREDITH. (Program in Neuroscience., Florida State University. TLH FL)

Vomeronasal chemosensory input is important for mating behavior responses in male hamsters.The vomeronasal system, in contrast to the main olfactory system, has direct and restricted 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 previous work we have demonstrated a distinct difference in c-fos expression in vomeronasal pathways of sexually inexperienced male hamsters, exposed to females, compared to controls. Densely stained nuclei were evident in the AOB, MN, BNST and MPOA of intact stimulated animals. Unstimulated animals did not show this activation. Males with vomeronasal organs removed (VNX) exposed to females did not mate and had a dramatically reduced number of FOS positive nuclei in all these areas. From these data it was not possible to distinguish between activation by VN sensory input and activation as a consequence of mating. This study addresses the question of vomeronasal contributions to FOS activation. Intact and VNX (unilateral and bilateral VNX) male hamsters were exposed to hamster vaginal fluid (HVF) which contains known stimuli for the vomeronasal system (Clancy 1984) but which provides no opportunity for mating performance. Sexually inexperienced male hamsters from each group were placed in clean boxes with fresh bedding and exposed to HVF diluted 1:10 (with DW) and presented on deep well slides, for 45 mins. After an additional 45 mins they were perfused with 4% paraformaldehyde. Control animals from each group were put into clean boxes with fresh bedding and perfused 90 mins later. Fifty µm vibratome sections were processed for immunocytochemistry using a polyclonal fos antibody. (Cambridge Research). Using HVF to stimulate intact and VNX animals restricts the stimulus largely to vomeronasal and olfactory systems. FOS expression in vomeronasal areas of intact animals was similar to but somewhat less than that in mated animals. The MPOA/AH activation was dramatically reduced compared to animals that mated (performed intromissions) and it is possible that this activation is performance related. Compared to unstimulated animals, there was increased activation in the AOB, MN and posterior medial BNST of intact but not bilateral-VNX animals, suggesting that this activation is vomeronasal related. Unilateral VNX animals showed FOS activation in the AOB and MN on the intact side but no activation in the AOB and approximately half the activation in the MN, on the VNX side. FOS expression in main olfactory pathways, and paraventricular thalamus, was similar in all animals (stimulated and unstimulated). It is the VN pathways and their central connections that are differentially activated during mating and HVF stimulation. The activation in the MPOA/AH seems to be associated with copulatory performance rather than VN sensory input.

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142

Removal of the Vomeronasal Organ Produces Long-Term Deficits in Reproductive Responses of Female Prairie Voles. KIMBERLY V. TILLEY and JOHN J. LEPRI (Department of Biology, The University of North Carolina at Greensboro)

Female prairie voles, *Microtus ochrogaster*, require chemosensory and tactile stimulation from males to initiate ovarian activity. Previous work identified the vomeronasal organ (VNO) in the nasal cavity as an important mediator of male-induced activation of reproduction: females which had undergone the surgical removal of the VNO failed to respond with reproductive activation to male contact and odors, at least in tests lasting 3 days or less. We now report that this procedure produces similar impairments when measured in long-term tests (8 weeks). We used a surgical approach through the oral cavity to remove the VNO of adult females (VNX); other females were exposed to sham surgery (SHAM). The females were then paired with adult males for 8 weeks– only 1 of 9 VNX, but 7 of 11 SHAM, females produced pups in that interval. Behavioral interactions between our subjects and their male partners were recorded during the first 10 minutes of pairing. Our observations suggest that VNX females had a decreased interest in male voles: we found that VNX females took significantly more time to come into close contact with the perineal region of their male partners, and spent significantly less time in close proximity to the males' perineal region, than did SHAM females. However, in a number of other behavioral observations, such as naso-nasal contact, there were no significant differences between VNX and SHAM females. We conclude that the chemosensory stimulation of the female prairie vole vomeronasal system is an important component of both short- and long-term sexual responses. Conclusions stated here are subject to the results of histological verifications.

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143

Activation of Female Vole Reproduction: Species Specificity of the Stimulus and a Role for the Vomeronasal System. MAUREEN L. TUBBIOLA and CHARLES J. WYSOCKI (Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104)*

Female prairie voles (*Microtus ochrogaster*) require a chemical signal from a male vole and an intact vomeronasal system to induce reproductive activation. It is not known whether cues from heterospecific males will induce such activation. We compared the ability of urine from mice (*Mus musculus*) to stimulate female vole reproduction with that of urine from male voles. Results indicated that urine from male mice was not an effective cue for activation of reproduction in female voles: After 5 days of twice daily applications of urine or water (n=10 per group), voles exposed to male mouse urine or water had small uteri (each group mean ± sem = 48 ± 6 mg/40 gm body wt) whereas voles exposed to male vole urine had large uteri (mean ± sem = 78 ± 9 mg/40 gm body wt). In a follow-up experiment we determined that the initial response to male vole urine included neuronal activity in the vomeronasal system as indicated by FOS immunocytochemistry (FOS-icc; FOS is the protein product of the immediate early gene, *c-FOS*, and is used as a marker for neuronal activity). Stimuli were painted on noses of naive female voles. One hour later, the voles were killed and their brains were processed for FOS-icc. Female voles exposed to male vole urine had many FOS-immunoreactive cells in the accessory olfactory bulb. Voles exposed to mouse urine or to water had few cells staining for FOS-like proteins. We conclude that urine from male voles stimulates the vomeronasal pathway and induces uterine growth in female voles; that urine from male mice does not stimulate the vomeronasal pathway nor does it activate female vole reproduction. Functional specificity of the chemical cue is likely to be determined within the vomeronasal organ proper (perhaps in the expression of species-specific, molecular receptors) rather than via filtering within the central nervous system.

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144

Birth Rate and Pup Mortality Considerations in the Husbandry of Captive Prairie Voles: Role of "Dirty" Cages. Barbara Davidsohn and John J. Lepri (Department of Biology, The University of North Carolina at Greensboro)

Animal welfare considerations regarding husbandry practices seem to have emerged in the general absence of empirical data regarding their effects. Since social odors influence the development and expression of behavior in rodents, the disruption of the social-odor environment, as in lab practices of cage-cleaning, might have unknown effects. To formulate standards of optimal husbandry, we produced empirical measures of behavior and reproduction while manipulating the cage environment, using prairie voles, *Microtus ochrogaster*. Over 4 months, we examined reproductive performance of paired voles maintained in one of 4 treatment groups:

i) "small" cages [27Lx21Wx14H-cm] that were frequently cleaned;
ii) small cages that were infrequently cleaned;
iii) "large" cages [36Lx24Wx19H-cm] that were frequently cleaned;
iv) large cages that were infrequently cleaned.

The pine-shavings cage-substrate was changed every 2 weeks for the low-frequency treatment-groups and every 3rd day for the high-frequency groups. We found that pairs housed in large cages that were frequently cleaned produced the greatest number of pups, however, frequent cage cleaning was associated with higher levels of pup mortality. If maximizing the number of births is the highest priority, then large, frequently-changed cages should be used, but pup mortality is likely to increase. In behavioral tests conducted every 2 weeks, the males were observed for their responses to an olfactory stimulus consisting of a small aliquot of soiled bedding from other males, to determine whether their "social reactivity" underwent any changes due to cage size and/or frequency of cleaning. We found that male voles from infrequently-cleaned cages had higher levels of activity and various other behaviors.

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145

Gonadal-Hormone Dependence of the Sources of Sex-Specific Odors in Voles.
MICHAEL H. FERKIN (Cornell University)
ROBERT E. JOHNSTON (Cornell University)

Feces, urine, anogenital area, mouth, and posterolateral region are sources of sex-specific odor information in meadow voles, Microtus pennsylvanicus. We used a preference task to examine the influence of gonadal hormones on production of sexually-attractive scents by scent donors. The preferences of intact male and female voles for the scents of same- versus opposite-sex conspecifics were examined in a series of tests. The attractiveness of scents from feces, mouth, and postero-lateral region was eliminated by gonadectomy of scent donors and was restored by replacement with testosterone in male donors and estradiol-17β in female donors. Scents of urine and the anogenital area, of gonadectomized male and female voles, however, retained some attractiveness to the opposite sex, suggesting that the sexually attractive components of these scents do not depend solely on gonadal hormones. The present data suggest three major conclusions: First, the attractiveness of sex-specific scents differ in their responsiveness to gonadal hormones. Second, another factor, possibly other hormones maintain the sexual attractiveness of these scents. Third, the anogenital area and urine scents may provide sexually-distinctive information outside the breeding, when endogenous gonadal hormone concentrations are low.

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146

Individual Recognition: The Effects of Early Experience.
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Neonatal exposure to odors produces changes in the organization of the olfactory system and can influence behavioral responses to these odors. During development, exposures to littermates and parents may create odor-memory traces that may affect sensitivity to these chemical cues. We manipulated the odor environment of a group of developing mice and later tested each animal's sensitivity to the previously-exposed odor. During days 10-24 after birth, 8 C57BL/6J mice were exposed daily to the urine of specific donors from a heterogeneous group of outbred mice (in this experimental group, donors and young mice were paired throughout the exposure period). Eight C57BL/6J mice who received a daily exposure to water served as control subjects. Sensitivity testing in a Y-maze commenced at 1 month of age. The mice were fluid-deprived and trained to run to the arm of the Y-maze that contained the odors from a selected target urine. The target urine for each mouse of the experimental group was from the same mouse that provided the urine used for earlier exposures; the non-target urine in the other arm was obtained from another donor. For each mouse in the control group, outbred mice were arbitrarily selected as donors and provided both the target and non-target urines. Donors contributed urines that were used for both test groups. If choices in the Y-maze were correct, mice received sweetened water as a reward. Subsequent to reaching a repeated criterion of 80% or greater accuracy, the concentration of the target urine was serially diluted with water to establish a behavioral threshold. For members of the control group, performance reached chance levels at about 1-4% (v/v) of the undiluted urine. All mice that were exposed to the target urines during development were able to maintain 80% or greater accuracy at a dilution of 0.05% (v/v). These results suggest that early experience with individual odortypes alters sensitivity by about 100-fold. The mechanism(s) underlying changes in sensitivity are not yet known.

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147

Individual Recognition: Roles for the Olfactory and Vomeronasal Systems.
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Chemosensory cues provide information about their source, including that of individuality. The extent to which the olfactory or vomeronasal systems are involved in individual recognition remains equivocal. In a Y-maze, we trained mice to recognize cues from urines that were obtained from genetically heterogeneous individuals. The target urine was then mixed 1:1 with urine from various other donors and the trained mice were assessed for accuracy or continued learning; the non-rewarded choice was a 1:1 mixture of non-target urines. All mice performed at greater than 80% accuracy in these trials. Subsequently, mice underwent one of the following procedures: perfusion of the nasal cavity with zinc sulfate ($ZnSO_4$) to destroy the olfactory epithelium, which leaves the vomeronasal organ (VNO) intact (n=9); removal of the VNO (VNX), which leaves olfaction intact (n=8); a SHAM VNX procedure (n=8); or combined $ZnSO_4$ and VNX (n=8). The mice were again assessed for accuracy, or relearning; the target urine remained the same for each animal. SHAM VNX mice performed with 80% or greater accuracy in the first 20 trials following surgical intervention. By itself, VNX had no effect on performance. In the first 20 trials after $ZnSO_4$, performance was severely disrupted -- many animals performed at chance levels; however, with increasing numbers of trials (between 49-126), each of the mice re-established accuracy of choice. Importantly, all of the mice failed to find a buried morsel of preferred food in repeated tests, supporting the interpretation that olfaction was absent and success in the Y-maze resulted from inputs via an alternative chemosensory system. After the combined $ZnSO_4$/VNX, none of the mice were successful in the Y-maze. These results suggest that under normal conditions olfaction mediates chemosensory-based individual recognition, but in its absence, the vomeronasal system can participate in the task.

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148

The Vomeronasal Organ in Frog Functions as an Olfactory Organ for Odorants in Water.
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The nasal cavities in the frog, *Rana temporaria* are organized in two separate chemosensory organs; the olfactory organ proper and the vomeronasal organ. The vomeronasal organ is composed of three cavities; superior, middle and inferior. The superior cavity forms a vestibule and is found just underneath the external naris. The vestibule connects directly to the principal olfactory cavity containing the eminentia olfactoria. The middle and inferior cavities of the vomeronasal organ are separated from, and anterior to the principal olfactory cavity. The middle cavity connects the superior cavity to the inferior. The vomeronasal sensory epithelium is located in the medial region of the inferior cavity. All cavities are lined with cells equipped with kinocilia. The sensory epithelium of the vomeronasal organ contains ciliated cells, supporting cells and microvillous receptor cells. We allowed frogs to swim in water with a fluorescent colorant. Inspection of microscopic sections revealed fluorescence on the surface of the vomeronasal organ, but not of the olfactory organ. In vivo observations demonstrate that water pass from the external naris via two fissures, one on each side of the movable nasal lid. The two fissures unite and enter the middle cavity. At the medial/caudal part of the middle cavity there is an opening allowing water to pass to the inferior cavity. Water in the inferior cavity is led medially passing the sensory epithelium of the vomeronasal organ, forward and laterally to the lateral part of the principal olfactory cavity and into the mouth via the internal naris. Water is transported by ciliary action lining the fissures and epithelium of the middle and inferior cavities. The unique design of the frog nose makes it possible for this amphibious animal to sample the chemical composition of its environment; above water the frog can inhale air and expose its olfactory organ to the volatile substances; in water the construction of the vomeronasal organ and its specific connections to the external naris permits sampling of water borne substances.

Discrimination of Y-Chromosome-Dependent Urinary Odortypes within a Mouse Species.

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This study examined the ability of mice to discriminate between urines from two Y-chromosome-congenic strains (DBA1 and DBA1.C57BL10-Y). In previous research it was shown that the odors from these strains elicited a differential behavioral response for both urine marking and aggression. It was therefore hypothesized that these urines could be also discriminated in a Y-maze. Urines were collected by the bladder massage method from males of the above congenic strains, and were presented to three females trained to discriminate DBA1.C57BL10-Y urine or two females trained to discriminate DBA1 urine. Results showed that these C57BL6-H-2k/C57BL6 F1 females were capable of making this discrimination. These data thus lend additional support to the relative importance that mice place toward Y chromosomal recognition, as suggested by the behavioral assays previously described. Moreover, these data further extend the results obtained by Yamazaki et al. (*Proc. Nat. Acad. Sci.* , 83, 4438-4440, 1986) by indicating that mice can not only discriminate between Y-chromosomal odors of *Mus musculus vs. Mus domesticus*, but also between those of *Mus musculus vs. Mus musculus*.

Fetal H-2 Odortypes are Evident in the Urine of Pregnant Female Mice.

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The major histocompatibility complex of genes, called H-2 in the mouse, in addition to its well-known functions in regulating immune response, provides each mouse with a distinctive odor, called an H-2 odortype. Perception of H-2 odortypes provides a neurosensory basis for preferential mating among individuals of disparate H-2 types (limiting inbreeding and facilitating heterozygosity at this locus), and for other behavioral responses of reproductive significance. Previously, we demonstrated that urine of pups as young as one day of age expresses H-2-determined odor. Thus, H-2 odortypes could serve to regulate mother-infant and infant-infant interactions. This observation suggested that H-2 odortypes may even be expressed by the fetus, raising the question of whether a fetal odortype might appear in urine of the pregnant female, thereby altering the female odortype to become a mixture of female plus fetus odortype. To test this hypothesis, mice were trained to discriminate the odor of genetically identical pregnant females who had been mated to males differing only by H-2 type. Thus, these pregnant females were identical but carried fetuses of differing H-2 types. The results demonstrated that fetuses do express their own H-2 odortype as early as 9-12 days of gestation. This is the earliest period at which time H-2 genes become demonstrably expressed in the fetus. This striking correlation between the first evidence for production of H-2 gene products and the appearance of H-2 odortypes suggests that odor production is a fundamental aspect of H-2 genes. This is the first evidence in vertebrates that the genetic identity of a fetus can be signaled to the outside world via the scent of the fetus.

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Composition of an Aphrodisiac Pheromone

ALAN G. SINGER (Monell Chemical Senses Center)
FOTEOS MACRIDES (Worcester Foundation for Experimental Biology)

The protein aphrodisin was originally isolated from hamster vaginal discharge by the sequence: ion exchange chromatography, dialysis, gel permeation chromatography, dialysis, and lyophilization. This preparation had about 10% of the aphrodisiac activity of the whole discharge. Chemical and behavioral data displayed in this poster demonstrate that the protein occurs in the discharge with noncovalently bound, lipophilic cofactors that were 90% lost in the original purification and account for the missing pheromonal activity.

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Odor-cued Taste Avoidance: A Simple and Rapid Method for Assessing Olfaction in the Rat.

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(The American University)

As many behavioural studies incorporate the use of olfaction, it would be desirable to have a simple and unambiguous method to assess whether an animal can smell. To bypass the complex and time-consuming olfactometric methods developed for psychophysical analyses, we have devised a simple and very effective test for odour detection. The apparatus consists of a water-bottle and drinking spout.

Thirsty rats are 'trained' to lick tap water from the 4 mm opening of a water bottle spout in daily sessions until reliable drinking occurs (2-3 sessions). For conditioning, an aqueous mixture of 0.5% quinine and 0.1% amyl acetate is substituted for tap water. Rats briefly sample this mixture and then refrain from drinking. When next presented with the mixture, most rats carefully sniff at the spout but do not make contact with it. During randomly alternating 120-s presentations of the mixture and of the water, discriminative behaviour emerges. Rats respond within 1-10 s to the water solution but, after initial trials with the mixture, they sniff but do not lick the mixture. Acquisition occurs after the first or second 120-s conditioning trial but is somewhat slower if only one trial per day is run or if rats are excessively thirsty. The possibility that this simple method can be used to demonstrate odour discrimination, odour sensitivity and odour memory is currently being examined.

Generalization of a 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).

In previous work it was shown that an electrical pulse train, constructed to mimic the temporal pattern of the neural response to sucrose, could serve as a conditioned stimulus in a conditioned aversion paradigm. In the present study, the generalization of that aversion to natural tastants was measured. 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 10 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, animals received an injection of LiCl (.15 M, 1% b.w., i.p.). Training days were administered every third day until the number of licks paired with NTS stimulation fell below 200. On that day a series of 1 min exposures to representatives of the four basic taste qualities was presented. These stimuli were available in the following order: 0001 M quinineHCl, .03 M NaCl, .003 M HCl, .15 M sucrose and plain water. Results showed that all animals acquired an aversion to lick-paired NTS stimulation. Preliminary results suggest that those animals with NTS electrodes in the most rostral part of the NTS generalized the aversion to NTS stimulation to sucrose; those animals with more caudal electrode placements within the NTS generalized the aversion to quinine or HCl. These observations suggest 1) that there may exist a functional chemotopic map within the NTS and 2) the temporal pattern of NTS stimulation cannot by itself predict the taste quality that it evokes.

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Taste Aversion to NaCl Solutions as A Result of Exposure to NaCl Solutions in Rats. Jodi Rhinehart-Doty and James C. Smith (The Florida State University)

Devenport (1973) showed that a single 24-hr exposure to a 2% NaCl solution as the rat's only available fluid resulted in a total aversion to isotonic NaCl solution in a 2-bottle preference test with water. He also demonstrated a similar aversion to the isotonic salt solution when the single-day exposure was limited to .9% NaCl. In his experiments the rats drank large amounts of the salt solutions on the days where this was the only solution available, indicating that the NaCl solutions were not very good hydrators. The purpose of our experiments was to replicate the above work, showing the details of the pattern of drinking and eating during these conditioning tests and to determine how long the aversion to isotonic saline would last. In our first experiment, 16 Sprague-Dawley rats were housed in modified hanging cages which had infrared beams to detect licks on the drinking tubes and entries into the food compartment. When any of the three beams in a cage were broken, the signals were stored in consecutive 6-sec bins over 23-hr testing periods. After establishing a baseline isotonic NaCl preference, the rats were given only .34 M NaCl for 23 hours (conditioning day) and the preference for .9% NaCl was measured periodically for 47 days. A group of control rats were given only water on conditioning day. On conditioning day the average intake of the .34 M NaCl solution was 75 ml as compared to 47 ml water intake for the control group. This difference in intake apparently is the result of an increase in the length of the drinking bouts. Food intake for the EXP group dropped 25% on the conditioning day as a result of a significant decrease in bout length.. 64% of these shorter food bouts were followed immediately by drinking the hypertonic solution. On subsequent preference tests days, isotonic saline intake was significantly reduced for the next 47 days. In a second experiment, extensive salt preference tests were conducted following the single day exposure to hypo-, isotonic and hypertonic NaCl solutions. The details of the ingestion patterns and the longevity of the aversions are presented.

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The Saltiness Hypothesis and Taste Characteristics of Gluconate Salts in Mineral Replete Rats

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Sodium appetite has been characterized by Schulkin as representing a preference for saltiness and that other salts and minerals are sought in proportion to their degree of saltiness in sodium depleted animals. This theory assumes a similarity in taste quality across salts. We sought to determine the degree to which salts are similar in mineral replete rats by establishing a conditioned taste aversion (CTA) to Na^+, K^+, Ca^{++} or Mg^{++} gluconate (0.15 M) and then determining cross-generalization of the CTA to each of the other salts. Results show that only those rats aversively conditioned to Na^+ reduced their Na^+ intake and this group did not generalize the taste of Na^+ to any other salt. The K^+ CTA group significantly reduced their consumption of K^+, and generalized to Mg^{++} but not Ca^{++} or Na^+. Ca^{++} and Mg^{++} CTA reduced consumption of both Ca^{++} and Mg^{++}, but not Na^+. K^+ intake was significantly reduce in the Mg^{++} CTA, but not the Ca^{++} CTA. These results show that even though saltiness may be one component of salt selection, other taste qualities distinguish sodium from K^+, Ca^{++} and Mg^{++} in the mineral replete rat and that in the CTA paradigm these other taste qualities are more important in salt avoidance than saltiness. Sodium was not classified by the rats as similar to any of the other salts. In the deficient animal taste psychophysics may be altered.

Amiloride Reduces the Sham NaCl Intake of Sodium Deficient Rats. SANDRA P. FRANKMANN, JOHN H. DOKKO & ILENE BERNSTEIN. (Bourne Lab, NY Hospital/Cornell U. Med. Coll. & Dept. of Psychology, U. Washington)

The sodium channel blocker, amiloride, reduces the response of the chorda tympani nerve to NaCl by 60-70%. Behavioral studies support the notion that amiloride selectively alters the response to sodium containing solutions. Amiloride also acts at sodium channels in the kidney, giving it a natriuretic-diuretic property. Thus, in order to evaluate the behavioral response to amiloride, brief access tests which minimize postingestive effects have been used in previous studies. In the present study, rats were outfitted with gastric cannulae which, when open, allow gastric contents to drain from the stomach. By eliminating or minimizing postingestive delivery of ingested solutions the effect of amiloride on NaCl intake can be studied without concern about any actions of amiloride on the kidney. Ten, male, Sprague-Dawley rats were sodium depleted (10mg furosemide and sodium-deficient diet overnight) three times and then outfitted with stainless steel gastric cannulae. After recovery from the surgery, the rats were again sodium depleted, and their NaCl intake measured in a 1 hour salt appetite test. The gastric cannula was open for the duration of the test. Collection volume of the gastric drainage equaled or exceeded that of the intake volume for each rat. The rats received either 0.3M NaCl alone (n = 5) or a cocktail of 0.3M NaCl and 100 μM amiloride (n = 5). As can be seen in Table 1, the amiloride reduced the sham intake of NaCl, and this reduction was significant at 30 and 60 minutes.

Table 1. CUMULATIVE SHAM FLUID INTAKE, ml (Mean ± SEM)

Condition	5 min	15 min	30 min	60 min
NaCl	2.3±.07	7.4±1.0	12.8±1.8	18.4±3.3
NaCl + Amil.	1.8±0.7	4.5±1.6	6.0±2.4*	7.7±2.1*

* = $p < .05$ vs NaCl alone

These results strongly suggest that amiloride sensitive sodium channels are critical to the motivational properties of the taste of NaCl after sodium depletion. Chorda tympani nerve transection produces a similar blunting of NaCl intake (Sollars and Bernstein, 1992). The reduction of NaCl intake which follows chorda tympani nerve transection may be due to loss of information generated by taste buds which are activated by sodium transport through amiloride sensitive sodium channels.

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157

Effects of the Photoperiod on Saccharide Intake in Siberian Hamsters. JACQUELINE B. FINE AND TIMOTHY J. BARTNESS. (Departments of Psychology and of Biology, Georgia State University, Atlanta, GA 30303).

Siberian hamsters (*Phodopus sungorus*) exhibit a decrease in peak body weight (fat) when exposed to short 'winter-like' days (SD). This decrease in body weight is coincident with a decrease in food intake. We have found previously impressive differences in the composition of the diet selected by hamsters exposed to long days (LD) versus SDs. Most marked were the differences in the selection of carbohydrate-rich diets. The purpose of the present experiments was to begin to determine the relative contribution of gustatory versus post-ingestive metabolic factors in these diet self-selection patterns. The preferences of LD- and SD-exposed male hamsters were compared using 24h, two-bottle tests (saccharide vs water). Sucrose (SUC) or Polycose (POLY) at concentrations of 32, 16, 8, 4, 2, 1, .5, & .1 percent (weight/volume) were presented in a descending order, each for 5 consecutive days. Only photoresponsive SD-housed hamsters were tested (*i.e.*, animals showing decreases in body weight following 5wks of SD-exposure). There was no difference in preference for the two saccharides in the LD-housed hamsters; however, the degree of preference for POLY was less than that for SUC at the higher concentrations in SDs. Between the photoperiods, the preference for SUC was similar at all concentrations, but a marked decrease in the preference for POLY was observed in SD-housed hamsters at the two highest concentrations. The volume intake of both saccharides was similar for LD-housed hamsters, but the consumption of SUC in SD-housed hamsters was greater than that for POLY. Between the photoperiods, the volumetric intakes of the two highest concentrations of the saccharides were less in SDs than in LDs. Since the molecular weight of POLY is ~3 times that of SUC, the volume data translate into caloric intakes that were greater for POLY than for SUC within a photoperiod and that were less for both saccharides at the two highest concentrations in SDs vs LDs. Collectively, these data suggest that the photoperiod can affect saccharide preference, especially at high, more 'food-like' than 'water-like,' concentrations. To further resolve if these changes in saccharide preference are based primarily on gustatory or metabolic effects of the photoperiod, brief exposure tests are planned.

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158

Polycose Conditioning in Hamsters. C. KEARNS, B.K. FORMAKER & M.E. FRANK (The University of Connecticut Health Center, Farmington, CT.)

Hamsters show a preference for Polycose, a mixture of starch-derived glucose polymers, that is at least as strong as their preference for sucrose. However, using a single conditioning trial, taste aversions to Polycose are not as easily acquired as aversions to sucrose. Taste aversion studies in rats indicate that the taste of Polycose does not generalize to sucrose, NaCl, HCl or quinine. In order to examine behavioral taste responses towards Polycose in hamsters, we trained four groups of hamsters, using two conditioning trials, to the following stimuli: deionized water (controls), 0.05M sucrose, 0.1M Polycose, and a mixture of 0.05M sucrose and 0.1M Polycose. Polycose and sucrose concentrations elicited similar relative hamster chorda tympani (CT), steady-state response magnitudes. The mixture was used to examine the behavioral saliency of two equipotent CT stimuli. Generalization testing began following the second conditioning trial and consisted of the three conditioning stimuli plus 0.003M citric acid, 0.3M KCl, and 0.03M NaCl. Thus, four basic taste qualities in hamsters were represented among the generalization stimuli. Preliminary results showed that an aversion to Polycose generalized to the mixture, and that an aversion to the mixture generalized to Polycose. The magnitude of sucrose suppression in these two groups was not significantly different from controls. Neither Polycose nor the mixture generalized to NaCl, citric acid or KCl. Likewise, the aversion to sucrose was specific for sucrose. Mixture intake in the sucrose group was not significantly different from controls. These results indicate that hamsters can acquire an aversion to Polycose with two conditioning trials, and that at equipotent CT concentrations, Polycose is behaviorally more salient for hamsters than sucrose. Since hamsters were able to discriminate Polycose from sucrose, NaCl, citric acid and KCl, Polycose must contain a sensory component that is qualitatively different from the stimuli tested. However, whether this qualitative difference is based on an olfactory or gustatory discrimination remains to be determined.

159

Polysaccharide Taste in the Hamster (Mesocricetus auratus). BRUCE I. MACKINNON and BRADLEY G. REHNBERG. (University of Connecticut Health Center, Farmington, CT 06030)

Glycogen and Polycose are both mixtures of glucose polymers. Short chains of glucose, such as maltose, maltotriose and trehalose, are sweet tasting. What do longer glucose-based molecules taste like to the hamster? Syrian hamsters strongly prefer glycogen and Polycose in two-bottle preference tests. Glycogen dialyzed with 500 molecular weight cut off (MWCO) and 2000 MWCO tubing is preferred. Some preference is lost after 6000-8000 MWCO dialysis. The chorda tympani whole nerve responds strongly to 3.2% glycogen, but weakly to dialyzed glycogen. The chorda tympani is stimulated by sodium salts in the glycogen preparation, but sodium salt is not preferred by adult hamsters. A 3.2% glycogen conditioned taste aversion (CTA) does not generalize to 0.032 M Polycose, 0.1 M sucrose, 0.32 M maltose, 0.032 M Na^+nitrobenzenesulfonate, 0.0032 M Na^+saccharin, or 0.032 M Ca^{++}cyclamate. Hamsters prefer 0.032 M and 0.1 M Polycose to water. Dialysis of Polycose with a 500 MWCO membrane lowers preference. A single negative conditioning does not create an aversion against 0.032 M Polycose. But, hamsters can be moderately conditioned against 0.1 M Polycose and will generalize to 3.2% glycogen, although not to maltose or sucrose. In summary, after CTA against 0.1 M Polycose hamsters generalize to 3.2% glycogen, but CTA against 3.2% glycogen does not generalize to the lower 0.032 M Polycose concentration. CTA against glycogen and Polycose does not produce a generalization against sweet compounds. Dialysis does not totally remove preference to glycogen and Polycose. In spite of continued positive behavioral response, chorda tympani response is weak to both dialyzed glycogen and Polycose. We conclude that hamsters perceive Polycose and glycogen as similar to each other due to long glucose-chained molecules, but these polysaccharides are not sweet.

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160

The Effects of Gustatory Nerve Section on Concentration-Dependent Licking to Quinine in Rats. STEVEN ST. JOHN, MIRCEA GARCEA, and ALAN C. SPECTOR (Dept. of Psychology, Univ. of Florida).

Based on electrophysiological findings, the glossopharyngeal nerve (GL) appears to be more responsive to quinine than the chorda tympani nerve (CT). Combined section of the GL and CT in rats has been shown to produce marked decreases in responsiveness to quinine in a variety of behavioral experiments. In contrast, transection of either the GL alone or the CT alone has historically produced less profound and more equivocal effects. Very little work has examined the effect of gustatory nerve sections on spout licking behavior to quinine in brief exposure tests. This study examined the contribution of the GL and CT nerves to the rats' responsiveness to small volumes of quinine to quantify the suprathreshold concentration-response relationship. A wide range of concentrations was employed (.003 - 3.0 mM) and appetitive licking behavior was measured. In a specially designed gustometer, water-deprived rats licked a drinking spout to obtain 10 sec trials during which they had access to 7 quinine concentrations and water. These stimuli were randomly presented in three 40 min sessions. A 10 sec water rinse preceded each taste or water control trial. After this presurgical assessment, rats were deeply anesthetized and received bilateral CT section (CTx, n=9), GL section (GLx, n=9), combined nerve section (GLx+CTx, n=7), sham surgery (CON, n=9), or removal of the sublingual and submaxillary salivary glands (DSAL, n=8). After surgery rats were again water deprived and tested for three days under the same conditions as before surgery. Nerve cuts were histologically verified; one GLx rat was discarded from the analyses. Lick scores to quinine were standardized to licks during water trials as a ratio of quinine licks over water licks. A 2-way ANOVA of time (before vs. after surgery) x concentration revealed a significant effect of GLx+CTx ($p<.005$). No other group showed a significant effect. In a second analysis, sigmoidal curves were fit to the concentration-response data, and group mean curves were tested for a shift in the concentration of quinine that evoked the half-maximal response. The GLx+CTx group shifted 1.18 log units ($p<.01$) and the GLx group (n=8) shifted 0.18 log units ($p<.002$), both to the right. No other groups showed a significant shift. Elimination of both nerves severely impaired responsiveness to quinine, whereas only minor impairment (if any) was incurred if either nerve was left intact. These findings suggest that the quinine-generated signals from the GL and CT converge centrally. Moreover, in the absence of only GL or only CT input, the remaining nerves are sufficient to maintain relatively competent sensory function with respect to quinine.

161

Effects of Peripheral Injections of Bombesin on Feeding Patterns in the Rat

A. KURT THAW (Florida State University), JAMES C. SMITH (Florida State University) and JAMES GIBBS (Cornell University Medical College)

Peripheral injections of Bombesin (BBS), a tetradecapeptide, have been shown to reduce food intake in laboratory animals. Most studies involved restricting the food availability to only one access period daily. A single injection of BBS would be given immediately prior to this feeding opportunity and the effects of the BBS on feeding would be compared to baseline data. However, no data has been collected on the free-feeding subject's response to injections of BBS.

The present study investigated the effects of peripheral injections of Bombesin on the eating patterns of free feeding rats. Subjects were allowed to eat ad libitum for the entire study. After 10 days of baseline data the animals received a peripheral injection of BBS (8ug BBS/kg body weight) following the first night meal. Each subjects eating behavior was observed and compared to baseline data. Although each subject's results differed, there were some effects that proved to be generalized. These effects included lengthened inter-meal-intervals and decreased intake immediately following the BBS injection. The size of the first night meal was matched for the injection and baseline days as a control. Saline injections were also used as a control. Other concentrations of BBS were tested to determine if a dose dependent relationship was observable.

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162

Neural Mechanisms Regulating Gender-Specific Patterns of Behavioral Chemosensitivity During Foraging.

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Fiddler crabs are sexually dimorphic crustaceans that display sex-specific patterns of behavioral chemosensitivity to compounds eliciting feeding. Foraging behavior is regulated, at least partially, by chemosensory input from sensilla on the feeding claw. The number of feeding claws is sex-specific; females possess two feeding claws, while males have only a single claw. Both normal females and one-clawed females are more responsive than males to stimulatory compounds. This suggests sex-specific characteristics of the sensory system are responsible for the differences in behavioral chemosensitivity. Gender-specific differences in electrophysiological responses were assayed from extracellular recordings of single axons from chemosensory sensilla in the claw. Test chemicals were mixtures of sugars and amino acids known to excite feeding behavior. Initial results indicate that female sensilla may contain a sub-population of chemoreceptor cells with lower response thresholds than cells isolated from male sensilla. Anatomical organization in males and females was investigated by using TEM to obtain size profiles of axons innervating the claw. The initial analysis suggests females possess more small axons than males. Based on known relationships between axon diameter and type, the additional axons present in females may originate from chemosensory cells. Although preliminary, these results indicate several mechanisms potentially responsible for the regulation of behavioral chemosensitivity.

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163

Preference and aversion for "deterrent" chemicals in two species of *Peromyscus* mouse.

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Deterrent chemicals are generally considered to be aversive to mammals at all detectable concentrations. However, several species contain individuals that appear to prefer weak concentrations of deterrents. The present study examines this paradoxical preference in two species of mouse, *Peromyscus melanotis* and *P. aztecus*. Preliminary findings had suggested that whereas some *P. aztecus* prefer weak concentrations of quinine hydrochloride (QHCL), no *P. melanotis* prefer any concentration of QHCL. Experiment 1 tested the hypothesis that individual mice that prefer low concentrations of QHCL would respond similarly to four other deterrents (ouabain, hop extract, sucrose octaacetate, and tannic acid) in 48 h two-bottle preference tests. *Peromyscus aztecus* displayed a large amount of intraspecific variation in hedonic response to all five deterrents. Those *P. aztecus* with concentration-specific preferences for QHCL were significantly more likely to exhibit such preferences for the other deterrents. No *P. melanotis* displayed a preference for any of the deterrents. Experiment 2 examined the temporal stability of the hedonic response to 0.1 mM QHCL in *P. aztecus* over six consecutive preference tests. Mice were divided into three groups based on their initial hedonic response to the QHCL solution (preference, no response, or rejection), and then subjected to the 12 day test. The hedonic response of mice within each of the groups did not change significantly over time. Because the preference for the weak deterrent solutions is reminiscent of the preference many humans show for the taste of QHCL in Schweppes Tonic Water™, this phenomenon is referred to as the "Schweppes effect." Based on the results of this study and others, it appears that the Schweppes effect is (i) widespread in the animal kingdom, (ii) species-specific, (iii) present in some but not all individuals of particular species, (iv) apparent during first night of exposure, (v) gender nonspecific, and (vi) elicited by chemically unrelated deterrents. While the physiological mechanism(s) underlying the Schweppes effect and its ecological relevance remain obscure, several possibilities are discussed.

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164

The effect of minnow extract components on mudpuppy feeding behavior.

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Our previous studies have shown that minnow extract elicits feeding responses and reduces rejection of aversive compounds in the mudpuppy (*Necturus maculosus*). Sodium chloride at a concentration of 1M was also shown to stimulate feeding responses (Bowerman and Kinnamon, *Chemical Senses* 17:597, 1992). These results suggested that minnow extract and Na salts are stimulatory. The present studies are being conducted to determine what components in minnow extract cause the mudpuppy to feed. Minnow extract (.1 g/10 ml pond water) was centrifuged for 9 minutes at 800 rpm. Half of the remaining supernatant was dialyzed for 24 hours at 4°C using a 500 MW cut-off point. The undialyzed solution served as a comparison. Both undialyzed and dialyzed minnow extract were mixed with colored gelatin and placed in molds. Sets of mudpuppies were used in blind tests to determine the effects of minnow extract components on feeding behavior (cf., Bowerman and Kinnamon 1992). In addition, the amino acids l-proline (.1M, .5M), l-arginine (.1M), and l-alanine (.5M) were tested using the same procedure. Gelatin cubes containing pond water served as the control. Both undialyzed and dialyzed minnow extract cubes elicited a positive feeding response (retention of the cube), suggesting that large proteins stimulate feeding. Gelatin cubes containing either .1M proline or .1M arginine were rejected by approximately 50% of the animals tested, suggesting that they do not elicit a feeding response since the rejection levels were similar to that of the control. Cubes containing either .5M proline or .5M alanine were rejected more than 65% of the time. Taken together, these results suggest that several factors may play a role in feeding.

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165

Effects of Chemo-stimulation on Swim Path Patterns in Minnows.
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The effects of chemical stimulation (dilutions of food extract or skin mucus of a potential predator) on swim path patterns of minnow (Phoxinus phoxinus) and roach (Rutilus rutilus) were analized with a video-based motion analysis system (DOTFINDER 6cp, JVP). From color-marked fish in an arena tank, x-y coordinates were taken in dorsal view at a frequency of 2.5Hz. From these coordinates the following groups of swim path parameters were calculated: velocities, angles, spatial distributions and interindividual distances. Experiments with 12 individuals of roach have shown that stimulation with trout mucus results in significant changes of the swim paths as compared to blank controls, but stimulation with food extract does not. We presently repeat these experiments with minnow, including an increased sample size and a multivariate data analysis. In addition, swim paths of food and body mucus-stimulated minnows will be compared before and after removal of their olfactory epithelium. If swim paths of deprived fish still show a response to chemo-stimulation, chemorecepters other than the olfactory mucosa must be involved. We hope in that case, that differential swim path patterns may contribute clues, whether taste buds or solitary chemosensory cells were involved in the detection of the different odors.

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166

Intraoral Food Discrimination in Goldfish.
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The glossopharyngeal/vagal taste system of vertebrates is important for determining the palatability of substances within the oral cavity. This system is hypertrophied in particulate-feeding cyprinid fishes (e.g. carps and goldfish), which possess complex sorting behaviors to separate food from non-food items in the oral cavity. To determine how appetitive and aversive stimuli affect feeding behavior, goldfish were trained to feed on gelatin pellets (4x4x5 mm) and then tested with pellets containing different concentrations of quinine plus either L-alanine or L-proline. Behavioral patterns were characterized and rates of ingestion were recorded for each combination of stimulants. Quinine produced a dose-dependent rejection (spitting out) of pellets taken into the oral cavity, with behavioral threshold at approximately 0.01 mM and complete rejection at 10 mM. Pellets with low concentrations of quinine (none, 0.001, or 0.01 mM) were ingested within 5-10 seconds after being sucked into the oral cavity, while pellets with high concentrations (1-10 mM) were typically rejected immediately after the pellet reached the rear of the oral cavity. The addition of either L-alanine or L-proline (10 mM) to the quinine-flavored pellets markedly increased ingestion rates at both 0.1 mM (2x) and 1.0 mM (10x). Both L-alanine and L-proline stimulated food-sorting behaviors when mixed with quinine. With 0.1 mM quinine and either of the amino acids, pellet manipulation time increased to 20-30 seconds; the behavior was characterized by repeated open protrusion of the mouth. When the quinine concentration was 1.0 mM, the addition of amino acids produced both open and closed protrusion of the mouth, resulting in a robust pumping of water through the oral cavity for 45-60 seconds. Goldfish use these two protrusion behaviors, and the resulting orobranchial irrigation, to sample the gustatory qualities of food items in the oral cavity and to selectively hold those that are deemed palatable. The response to the combination of both appetitive and aversive stimulants illustrates that this sorting process is important for the determination of palatability.

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167

Apparent Novel Interactions Between Dennervated and Intact Taste Receptors in Dietary NaCl-Restricted Rats: Evidence for Local Circulating Factors?
D.L. HILL and L.M. PHILLIPS (Univ. of Virginia, Charlottesville, VA 22903)

Important determinants of functional responses seem to occur before or as the first generation of taste receptors form in rat (16 days gestation). The following study was originally designed to mimic the developmental events by exploiting the trophic influence of taste nerves on receptors. It has since taken on a much larger scope. Adult rats had the left chorda tympani nerve sugically cut; the right nerve remained intact. They were then placed on a NaCl-restricted diet (0.03% NaCl) for the duration of the experiment. Groups of control rats consisted of a) sham surgery and normal diet (1.0% NaCl; controls), b) chorda cut and normal diet (cut controls), and c) sham surgery and NaCl-restricted diet (diet controls). Data from all three control groups were similar for all measures; therefore, they will be referred collectively as controls. When neural recordings were obtained from the regenerated nerve in NaCl-restricted rats (40 - 120 days postsectioning), taste responses to concentration series of NaCl and sodium acetate were approximately 50-60% of controls, suggesting that responses from newly-formed receptors were abnormal. Responses to NH_4Cl and KCl were similar between groups. Surprisingly in the same rats, NaCl responses from the uncut chorda tympani were 30-40% greater than controls. A detailed examination of the time course of the changes in the uncut side revealed that responses to NaCl were initially very low following sectioning (about 20% of the normal response). During the following 50 days, responses increase monotonically to the hypersensitive response. Amiloride sensitivity increased concomitantly with increased sodium sensitivity. We have established further that this increasing responsivity with time occurs even if the cut chorda is prevented from innervating lingual epithelia. Although efferent neural effects cannot be ruled out, these results suggest that alterations in circulating factors and/or receptors following the initial cut may modulate response properties of intact, lingual taste receptors.

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168

Chorda Tympani Section has Profound Effects on Preference Behavior for NaCl and KCl in Golden Hamsters.
MICHAEL A. BARRY and DAVID C. LARSON (Department of BioStructure and Function, University of Connecticut Health Center).

The effects of denervation of fungiform taste buds on preference behavior were studied in golden Syrian hamsters. In normal hamsters, 0.01 to 0.4 M NaCl and 0.1 to 0.4 M KCl were strongly avoided relative to water as measured with 48 hour intake-based preference tests. After bilateral cuts of the chorda tympani nerve (CT) there was a dramatic decrease in the strength of aversions to NaCl and to a lesser extent to KCl. Only 0.4 M NaCl and KCl were strongly avoided after CT section, but even at these concentrations the strength of the aversions decreased significantly. After CT section, preferences for like concentrations of NaCl and KCL were similar. In addition, the animals did not distinguish between 0.1 or 0.2 M KCl and NaCl in preference tests between these two stimuli. The results are consistent with other studies which used short term behavioral tests to demonstrate the importance of the chorda tympani for sodium salt taste in rodents. In addition, our results suggest that the chorda tympani also has an important role in the mediation of KCl taste. In golden hamsters (unlike most rat strains), intake-based long-term preference behavior is significantly affected by selective gustatory deafferentation.

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169

Cross Adaptation to NaCl: Implications for the Coding of Saltiness.
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The taste qualities of salts have been shown to be concentration dependent and to vary with cation and anion composition. The present investigation employs direct scaling methods to investigate the psychophysical functions and taste quality profiles of both organic and inorganic salts and to examine the effects of NaCl adaptation and amiloride on their saltiness. Magnitude estimates were obtained of the total intensity of several concentrations 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). Subjects were instructed to divide each total estimate among the appropriate qualities (salty, sweet, sour, bitter or other). The stimuli were presented via dorsal flow to the anterior portion of the tongue at 34°C. The tastes of most of these salts were complex; most were salty with additional sour or bitter components. Based on the psychophysical functions, stimuli were matched for total intensity and single concentrations of these 18 stimuli were judged after adaptation to distilled H_2O or 0.1 M NaCl. After H_2O adaptation, the saltiness of these equally intense stimuli ranked from NaCl > Na-tartrate > K-acetate > KCl > Na_2SO_4 > NH_4Cl > Na-acetate > LiCl > MSG > Na-ascorbate > Na-citrate > other stimuli. Adaptation to 0.1 M NaCl for 60 sec produced a significant decrement in the magnitude of the saltiness of NaCl (> 90%); a similar decrease occurred in the saltiness of all the other salty stimuli. In another experiment, flowing 10 μM amiloride over the tongue produced only a 40-50% reduction in the saltiness of NaCl. Given that amiloride affects only apical ion channels and that adaptation occurs at the level of the whole cell, these data suggest that the degree to which a stimulus is salty is determined by how much it stimulates receptor cells that respond to NaCl; amiloride blocks only a portion of this message.
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170

Anions determine the taste intensity and perceived saltiness of three sodium salts. J. WEIFFENBACH and N. RYBA (National Institute Of Dental Research, NIH, Bethesda MD)

Saltiness is believed to depend upon the flux of sodium ions across the apical membrane of taste receptor cells. The mechanism by which anions modulate saltiness is less well defined. Recently Ye,Q., Heck,G.L. & DeSimone, J.A. (1991) suggested that anions effect saltiness by dissipating the electro-chemical gradient set up by sodium trans-location. They reported differences in electrophysiological response to three monovalent sodium salts (chloride, acetate and gluconate) that are consistent with anion permeability dependent modulation of saltiness in rats. We obtained judgments of intensity for the same three salts from human observers by cross-modal matching. Psychophysical measurements of total taste intensity and of the intensity of saltiness in humans parallel the permeability of the three anions across the epithelium of rats.

171

Ibotenic Acid Lesions of the Parabrachial Nucleus Disrupt Learned Taste and Odor Aversions, but Permit a Learned Capsaicin Aversion and a Learned Flavor Preference.
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Previous data indicate that the conditioned taste aversion (CTA) deficit found in rats with electrolytic lesions of the parabrachial nucleus (PBN) is not attributable to a failure either to detect or to process gustatory or visceral afferent activity. The present experiments extend these findings by using bilateral ibotenic acid (0.2ul;20ug/ul) lesions of the PBN and a higher dose of LiCl (0.3 M, 1.33ml/100g bw). In Experiment 1, acquisition of a CTA and a conditioned odor aversion (COA) was disrupted in rats with PBN lesions (PBNx) following two pairings of a gustatory stimulus or an olfactory stimulus with LiCl. SHAM rats consumed 0.06 ml and PBNx rats 18.6 ml of 0.3 M alanine. In the presence of the almond odor, SHAM rats consumed 0.12 ml and PBNx rats 11.6 ml of dH20. The results of Experiment 2 showed that this failure to acquire a CTA in PBNx rats was not due to an inability to detect or to process gustatory input because both SHAM and PBNx rats formed a taste-taste association, exhibiting a preference for a Kool-Aid flavor previously presented in a 0.15% saccharin solution (overall preference ratio = 0.67) relative to a Kool-Aid flavor presented in dH20 (overall preference ratio = 0.32). Finally, in Experiment 3, the same animals that failed to acquire a CTA or a COA were used to determine if a trigeminal, rather than a gustatory, cue could serve to predict the occurrence of illness. The results indicated that both groups learned to avoid 0.01mM capsaicin following two pairings with LiCl (0.15 M, 1.33ml/100g bw). Together, these data indicate that rats with lesions of the PBN suffer from a deficit that may be specific to associating taste or odor with illness.

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172

Memory for Brief, Widely-spaced Odor Presentations in the Rat
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BURTON M. SLOTNICK (The American University)

Staubli, Ivy and Lynch (Proc. Nat. Acad. Sci., 1984) and, more recently, Lu, Slotnick and Silberberg (Physiol. & Behav., in press) provide evidence that the accuracy of rats trained on odor discrimination tasks may be unaffected by the length of the intertrial interval. These results suggest that rats may have excellent short-term memory for odors. We now report that, after training on a standard olfactory learning-to-learn task, rats can show virtually perfect acquisition of novel odor discrimination tasks despite the use of a 10 min. or even 30 min. intertrial interval (ITI). Six rats were trained to sample 2 simultaneously available odors (S+ and S-) presented in separate ports and to respond for a water reward in the presence of the S+ odor. Thirty-one trials were given on each of 16 novel 2-odor discriminations and the ITI was 10 s. Problems were separated by 1 or 2 days. Four rats were then given 3 additional novel 2-odor tasks in which the ITI was 10 min. (2 problems) or 30 min. (1 problem). The remaining 2 rats were given 'no-cues' tests after the system was alcohol washed between problems to insure that residual odors were not available for discriminative responding. Rats rapidly acquired each of the short ITI problems and, in most of the last 4, made few (0-4) errors in learning. This same high level of performance was maintained when they were tested on new problems but using the longer ITIs. Indeed, the mean number of errors to criterion on the 30 min. ITI problem was 0.8 and, over all but 2 of the 12 memory tests, 13 - 15 correct responses were made in the first 15 trials. These results provide additional evidence for acquisition of an olfactory learning-set and, more important, confirm and extend the suggestion that rats have excellent short-term memory for odors. Clearly, rats can make virtually perfect use of information from widely spaced trials in learning an olfactory discrimination task.

173

Enhancement of Mucosal Inherent Activity Patterns in Rats Trained on an Odorant Identification Task. STEVEN L. YOUNGENTOB AND PAUL F. KENT. (Clinical Olfactory Research Center, SUNY HSC, Syracuse NY 13210.)

Youngentob et al. (Chem. Senses 17:725, 1992) have demonstrated that there are intrinsic spatial patterns of odorant sensitivity across the rat olfactory mucosa. The question of how these patterns are determined and whether they are modifiable with experience remains open. Therefore, the present study examined whether, and to what degree, the odorant-induced "inherent" activity patterns on the rat olfactory mucosa would be altered by experience. Using operant techniques and an odorant identification confusion matrix task (Youngentob et al. Physiol. Behav. 47:1053-1059; 1990) five Long-Evans Hooded rats were trained to differentially report (i.e., identify) the odorants propanol, carvone, citral, propyl acetate, and ethylacetoacetate. Following acquisition training, each animal was tested forty times using a standard 5x5 confusion matrix design. At the completion of testing, each animal was sacrificed and their mucosal activity patterns were recorded using a voltage-sensitive dye technique. Five additional animals served as food restricted age-matched controls. Using the dye, di-4-ANEPPS, we monitored the fluorescence changes at 100 contiguous sites in a 10x10 photodiode array on the olfactory mucosa of each rat's septum and medial surface of the turbinates in response to the same five odorants. The recorded spatial activity patterns of trained animals were compared to those of age matched controls. The results showed that for each of the odorants tested there was an increase in average response magnitude ranging from 5% to 40% for the trained animals, as well as, an apparent growth of an odorant's "hot spot".

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174

The Relationship between Odorant Quality Identification and Mucosal Inherent Activity Patterns. PAUL F. KENT AND STEVEN L. YOUNGENTOB. (Clinical Olfactory Research Center, SUNY HSC, Syracuse NY 13210.)

One goal of sensory physiology has been to understand the relationship between the neurophysiological activity of the receptors and the perception of the animal. Are various parameters of neural activity at the receptor level (e.g., spatial pattern, temporal pattern, and intensity) predictive of psychophysical data or is further processing through complex neural networks required? Therefore, the present study examined the relationship between odorant-induced "inherent" activity patterns and odorant quality identification in the rat. Using operant techniques and an odorant identification confusion matrix task (Youngentob et al. Physiol. Behav. 47:1053-1059; 1990) five Long-Evans Hooded rats were trained to differentially report (i.e., identify) the odorants propanol, carvone, citral, propyl acetate, and ethylacetoacetate. Following acquisition training, each animal was tested forty times using a standard 5x5 confusion matrix design. The results of the behavioral tests were subjected to an MDS analysis which established a two-dimensional perceptual odorant space. At the completion of testing, each animal was sacrificed and their mucosal activity patterns were recorded using a voltage-sensitive dye technique. Using the dye, di-4-ANEPPS, we monitored the fluorescence changes at 100 contiguous sites in a 10x10 photodiode array on the olfactory mucosa of each rat's septum and turbinate in response to the same five odorants. The relative position of the five electrophysiologically determined "hot spots", in two dimensions, were then compared to their relative position in the two-dimensional perceptual odorant space. The results of these comparisons were striking in their apparent similarity. These data suggest that the mucosal "inherent" activity patterns are preserved through further neural processing and serve as the substrate for the perception of odorant quality.

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175

Specific Anosmia: Practical Significance? HARRY LAWLESS, MARY JOHNSTON, CAROL CORRIGAN and MICHAEL ANTINONE (Cornell University).

Threshold and suprthreshold olfactory responses were measured in quadruplicate among groups of 50+ individuals to cineole, l-carvone and diacetyl. All three compounds showed wide individual differences in thresholds and multimodal distributions consistent with the existence of specific anosmia. Suprathreshold ratings made at the same time as threshold measurements showed moderate negative correlations with threshold measures. However, thresholds for cineole were poor predictors of suprathreshold ratings in a mixture experiment conducted several months later. Carvone thresholds taken several months apart showed considerable drift. Two groups of subjects were classified as good vs. poor discriminators of diacetyl in cottage cheese on the basis of their abilities to discern increasing levels of diacetyl in a forced-choice procedure. However, the two groups showed approximately equivalent performance on a subsequent re-test. Taken together, these results question the utility of threshold classification of individuals for olfactory sensitivity in predicting future threshold or suprathreshold performance. Drift in thresholds over time is worthy of future study before the phenomenon of specific anosmia can be assumed to have functional and predictive significance.

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176

Detection Thresholds of an Olfactory Mixture and its Three Components.
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JOSEPH C. STEVENS (John B. Pierce Laboratory)
WILLIAM S. CAIN (John B. Pierce Laboratory)
J. ENRIQUE COMETTO-MUÑIZ (John B. Pierce Laboratory)

Psychophysical measurements of olfactory mixtures generally have been concerned with ratings of suprathreshold levels. Such suprathreshold mixtures typically exhibit hypo-additivity. In contrast, this study, which took its departure from Laska and Hudson (Chemical Senses 1991, 16(6), 651-662), compared detection threshold of a mixture (1-butanol, butyl acetate, and 2-pentanone) with thresholds of the three compounds separately, revealing perfect stimulus additivity—the mixture's threshold was one-third of the components'. Twenty young (18-26 years) and 20 elderly (69-91 years) subjects underwent 2AFC tracking with Simpson's "step method" (Perception & Psychophysics 1989, 45(6), 572-576) using squeeze-bottle delivery. Essential to the additivity argument was the demonstration by gas chromatography that the bottles' headspace concentrations for all stimuli obeyed Raoult's Law during measurement. The elderly had an average threshold 20 times higher than the young, but stimulus additivity was equivalent for both.

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Common Chemical and Olfactory Responses to Homologous Alkylbenzenes.

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In the next step of our systematic investigation of nasal common chemical and olfactory thresholds for homologous series of nonreactive chemicals we studied the alkylbenzenes, from toluene to octyl benzene. Clinically diagnosed anosmics provided nasal pungency thresholds for these stimuli while age-, gender-, and smoking-status-matched normosmics provided odor thresholds. As found before with homologous alcohols (Physiol. Behav. 48(5): 719-725, 1990), acetates (Pharmacol. Biochem. Behav. 39(4): 983-989, 1991), and ketones, both thresholds decreased with carbon chain length. Anosmics failed to detect alkylbenzenes above propyl benzene, in contrast with the other series where they only failed to detect members having above seven or eight carbons in the chain. We found a strong linear correlation between pungency thresholds and saturated vapor concentration for all compounds tested so far. This suggests that nasal pungency from nonreactive substances might rely heavily on a broadly tuned physicochemical interaction with a susceptible biophase within the cell membrane. Further insight on the issue can be provided by experiments on the threshold perception of mixtures of these chemicals.

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A Semantically-Labelled Magnitude Scale of Oral Sensation with Apparent Ratio Properties.

BARRY G. GREEN, GREGORY S. SHAFFER and MAGDALENA M. GILMORE (Monell Chemical Senses Center)*

The desire to evaluate group and individual differences in the perception of chemical irritation and taste led us to develop a "category-ratio" (CR) scale. Theoretically, a CR scale can yield ratio-level data because category labels (e.g., "weak" and "strong") are placed at their "true" locations on the intensity continuum rather than at equal arithmetic intervals. Borg (In: Geissler & Petzold [Eds.] *Psychophysical judgment and the process of perception*, Amsterdam: North Holland, 1982) has argued that the CR scale he created, which was originally used to measure perceived exertion but was later modified and used in other sensory modalities, yields ratio-level data. We decided to develop a semantically-labelled scale of oral sensation empirically. The strategy was to have subjects give magnitude estimates to six verbal descriptors (barely detectable, weak, moderate, strong, very strong and strongest imaginable) as they appeared within a list of 24 familiar oral sensations (e.g., the sweetness of banana; the burn of cinnamon gum; the pain of biting the tongue) which the subjects also rated. The geometric means of the ratings given to the descriptors produced a scale that differed somewhat from Borg's most recent version of the CR scale. We then compared ratings of sweetness (sucrose), cold (chilled H_2O) and chemical irritation (ethanol) obtained within the same session using either the empirical scale or magnitude estimation. The two methods yielded virtually identical psychophysical functions. A second experiment compared ratings of sweetness and chemical irritation when subjects used either the empirically-labelled magnitude scale or a scale having the same semantic labels spaced at equal arithmetic intervals. As expected, the equal-interval scale yielded psychophysical functions with significantly different slopes and intercepts than those obtained with the empirical scale. The results of both experiments suggest that the labelled magnitude scale for oral sensation (1) yields ratio-level data, (2) enables direct evaluation of intermodal and interindividual differences, and (3) provides semantic information about sensation magnitude.

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Context and Attribute Response Restriction: Psychological Biases Influencing Time-Intensity Scaling.

COLLEEN CORNELIUS CLARK and HARRY T. LAWLESS (Cornell University)

Flavor perception is a time-dependent process in which the release of flavor and odor components over time contribute to taste. The temporal parameters of flavor perception have recently gained increased interest in sensory evaluation studies. However, better time-related measurements pose some challenges to sensory methodology, including the investigation of psychological biases which have been found in single-point scaling. Our research examined the effects of context and attribute response alternatives in time-intensity scaling using repeated category ratings over time. A common bias is the tendency to contrast sensory events with surrounding context. Thus, category ratings are sometimes incorrectly made on a relative basis, shifting the entire temporal pattern depending on the context of the samples. To study the contextual effect, panelists rated a .005M sample of tartaric acid solution in both high and low contexts over a 120 second period. The sourness rating of the common sample (.005M) was higher when presented in the lower context than in the higher context. The second bias studied was the dumping effect, in which panelist may "dump" quality ratings into other categories when the attribute response options are limited. Panelists rated a pseudo-beverage containing sweetener and flavor, and one with sweetener only over a 90 second period. The difference between the sweetness of the samples when panelists were given both flavor and sweetness response options was smaller than when the panelists were only given a sweetness scale. The flavor caused a marked increase in sweetness intensity when the panelists were limited to sweetness responses only. These studies show that context and response alternative restriction can influence results in time-intensity scaling shifting the temporal pattern.

Regeneration and Functional Response in the Chorda Tympani Nerve of the Golden Syrian Hamster (*Mesocricetus auratus*).

PETER CAIN and MICHAEL A. BARRY (Dept. of BioStructure and Function, University of Connecticut Health Center).

The recovery of physiological responses of the chorda tympani nerve (CT) after damage was examined in adult male hamsters. We were interested in the the time required for recovery of (1) responses to various taste stimuli and (2) amiloride sensitivity. Experimental animals had the CT exposed in the middle ear and crushed proximal to crossing behind the malleus. Intact and sham-operated controls had no damage to the CT. At intervals ranging from four to nine weeks after surgery, dilute solutions of sucrose, NaCl, sodium acetate, KCl and HCl were placed on the tongue. The whole nerve responses were recorded distal to the damage site. Amiloride effects on nerve responses to NaCl were also tested. In selected animals confirmation of nerve damage was determined histologically by examining the nucleus of the solitary tract for acetylcholinesterase (AChE) activity. AChE activity has previously been shown to decrease following CT damage. Preliminary results indicated that the nerve did not regenerate sufficiently at four weeks to respond to taste stimuli. At eight weeks the response of the nerve was stimulus dependent and was amiloride sensitive.

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181

Ultrastructure of Rat Fungiform Taste Buds After Chorda Tympani Denervation. MARNY BENJAMIN[1,2], TERRI A. SHERMAN-CROSBY[1,2], BRUCE OAKLEY[3] and JOHN C. KINNAMON[1,2]. (University of Denver, Denver, CO[1], the Rocky Mountain Taste and Smell Center, Denver, CO[2] and the University of Michigan, Ann Arbor, MI[3])

We are interested in the determinants of synaptic structure in taste buds. Previously we have provided evidence that synapses in fungiform and circumvallate taste buds are structurally different. Now we are examining taste buds from rats with denervated or cross-reinnervated fungiform and circumvallate papillae to determine whether synaptic structure in taste buds is determined primarily by the nature of the receptor cell or by the type of innervation.

In the present study we have used a combination of light microscopy and transmission electron microscopy to examine the fungiform taste buds of rats after denervation of the chorda tympani nerve for comparison with cross-reinnervated taste buds. Our preliminary results confirm the work of others that suggest that some fungiform taste buds survive in a degenerative state 3-4 weeks after denervation. We have classified these taste buds using previously described light microscopic criteria as "atrophic" or "remnant". Atrophic taste buds are approximately 50% smaller than normal. They retain the general shape of the taste bud and contain elongate cells. Remnant buds are considerably smaller and do not retain the characteristic onion shape of normal taste buds. Moreover, remnant buds have few elongate cells. Of 15 denervated taste buds examined thus far with the electron microscope, 7 were classified as atrophic, 5 as remnant, and 3 were judged to have no bud present.

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182

Chorda Tympani Section in Neonatal Rats Permanently Alters Taste Preference and Taste Bud Morphology. SUZANNE I. SOLLARS & ILENE L. BERNSTEIN (University of Washington).

Anatomical evidence suggests an inductive role of the primary gustatory nerves in the development of taste buds, with the sensitive period spanning the first 10 days postnatal. On the basis of evidence that taste bud development does not occur normally following perinatal denervation of the chorda tympani-lingual nerve complex, the current study examined the effect of chorda tympani nerve (CT) transection in 10-day-old rats on adult preference for salts. Wistar rat pups were given bilateral CT transection (CTX; N=13) or sham operations (SHAM; N=12) at 10 days-of-age. When 60 days-of-age, the animals were given two-bottle tests with various salt and non-salt solutions (NaCl, NH_4Cl, KCl, NaBr, $CaCl_2$, Quinine) and water. CTX animals displayed a significantly higher preference for NH_4Cl at all concentrations tested (.05M, .1M, .15M, .2M) while their preference for the other stimuli was not consistently altered. Animals were then tested for generalization of conditioned taste aversions (CTA) to salts. LiCl was used to condition a significant CTA to .1M NaCl. Generalization tests involved one-bottle access to NH_4Cl and KCl and a two-bottle test with NaCl and NH_4Cl. Generalization patterns were similar for CTX and SHAM animals and both groups were clearly able to discriminate between NH_4Cl and NaCl. Therefore, the enhanced NH_4Cl preference of adult rats that received neonatal CTX is not due to their inability to distinguish between NH_4Cl and NaCl. Histological analysis revealed an absence of fungiform papillae and no regeneration of the CT in transected animals. Since CTX in adult animals has little effect on preference for NaCl or NH_4Cl, we speculate that the development of the central gustatory system is altered by CTX at 10 days-of-age in the rat and that this change contributes to enhanced adult preference for NH_4Cl.

183

Expression of Human Blood Group Antigens by Rat Fungiform Papillae Following Cross-reinnervation by the Glossopharyngeal Nerve. MARK E. GERBER, RAISA KLEVITSKY, and DAVID V. SMITH (University of Cincinnati College of Medicine).

Cells in the gustatory and olfactory epithelia express a number of cell-surface carbohydrates, including several of the human blood group antigens, which may be indicators of cellular differentiation. The present investigation used immunocytochemical techniques to localize several blood group antigens in the fungiform papillae of normal rats and those with cross-regenerated chorda tympani (CT) and glossopharyngeal (IXth) nerves. The right IXth nerve was cut at the point where it enters the tongue musculature and its proximal segment was cross-anastomosed to the distal portion of the right CT nerve, which was avulsed from its exit from the petrotympanic fissure. Anastomosis was accomplished using a fibrin glue consisting of human pooled cryoprecipitate and rat thrombin. Control rats had their right CT nerve avulsed from the bulla and excised. Monoclonal antibodies 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 the tongue and examined by light microscopy. The $Lewis^b$ antigen was localized to a subset of cells in the taste buds of the vallate papilla; considerably fewer fungiform taste cells expressed this epitope. Similar differences were seen between vallate and fungiform taste cells in the expression of the A antigen. In contrast, the H and B antigens were expressed on a majority if not all of the cells within the taste buds of both vallate and fungiform papillae. Unilateral transection of the CT resulted in a loss of antigen expression. Following the cross-reinnervation of the fungiform papillae by the redirected IXth nerve, the fungiform taste bud expression of the H and B antigens was normal. Results to date indicate that there are increased numbers of fungiform taste buds with $Lewis^b$ and A antigen expression, which would indicate some influence of the innervating nerve on the target epithelium.

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184

Extracellular Matrix Molecules May Modulate Cell Adhesion during Papilla and Taste Bud Morphogenesis and Innervation. CHARLOTTE M. MISTRETTA and LINDA F. HAUS (Dept. of Biologic and Materials Sciences, School of Dentistry, Univ. of Michigan, Ann Arbor, MI 48109).

Laminin and tenascin are extracellular matrix molecules with multiple and sometimes contrasting roles in modulating morphogenetic processes of cell motility and adhesion. Laminin, a major component of basement membranes, promotes cell adhesion and also, is implicated in directing formation of peripheral nerve pathways. Tenascin, on the other hand, can stimulate disassembly of adhesive sites and may have neurite-inhibiting roles. We used immunohistochemistry to determine spatiotemporal distributions of laminin and tenascin during morphogenesis and innervation of sheep fungiform papillae and taste buds. To label nerve fibers, antibody to growth associated protein - 43 (GAP-43) was used. Fetal tongue tissue was examined at stages of: papilla and taste bud formation (~50 days of gestation); rapid papilla growth and beginning of taste bud multiplication (~100 days of gestation); and, stable papilla size and period of peak taste bud multiplication (term, or ~147 days if gestation). At 50 days of gestation, tenascin is absent or very weak in the papilla core, which is virtually filled with nerve fibers labeled with GAP-43 immunoreactivity. Laminin immunoreactivity is patchy within the papilla core. However, laminin immunoreactivity is intense throughout the tongue epithelial basement membrane, except for a large discontinuity in the apical papilla under presumptive taste buds. At 100 days of gestation, tenascin immunoreactivity is intense in the papilla core in apical regions only, under epithelium where taste buds are forming and proliferating. Whereas no tenascin is associated with papilla innervation, immunoreactivity for GAP-43 and laminin in the papilla core now virtually overlaps, labeling the central bundle of nerve fibers. Laminin discontinuity is still apparent in the basement membrane under developing taste buds. Near term in the fetus, tenascin immunoreactivity remains localized to apical portions of the papilla core under the taste buds, and outlines nerve bundles in the papilla but is absent from the fibers themselves. GAP-43 and laminin immunoreactivity continue to coincide and intensely label papilla nerve fibers. We suggest that the combination of tenascin in the papilla core under developing taste buds and breaks in laminin distribution in basement membrane under taste buds may provide a loose adhesion required by cells migrating into, and turning over within, the taste bud. With respect to innervation, the absence of tenascin in the early papilla may permit extensive innervation within the core; laminin patches could facilitate this innervation. In later papilla stages with an established nerve bundle, tenascin may promote fasciculation and branching.

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185

Innervation Patterns of Single Gustatory Papillae During Rat Development. ROBIN F. KRIMM and DAVID L. HILL. Department of Psychology, University of Virginia, Charlottesville, VA 22903.

During the development of most sensory systems there is a substantial neural rearrangement following the initial innervation of peripheral receptor organs. For example, in the gustatory system of the sheep a substantial pruning of fibers occurs during postnatal development concomitantly with increases in sodium sensitivity. Since the sensitivity to sodium increases in rat after postnatal day 10, it is possible that neural rearrangements in the peripheral taste system occur in the developing rat. The present study was designed to determine whether patterns of taste bud innervation change during postnatal development in the rat. The number of geniculate ganglion cells that innervate a single gustatory papilla was quantified for papillae on the tongue tip and on the middle portion of the tongue in 10 day old and adult rats. This was accomplished by iontophoretically injecting individual gustatory papillae with either the florescent tracer true blue or fluorogold. Serial 10 micron sections of the geniculate ganglion were reconstructed and the number of labeled geniculate ganglion cells was determined. Preliminary results indicate that papillae on both the mid-region and the tip of the adult tongue are innervated by an average of 13 geniculate ganglion cells. Analysis of the geniculate ganglion in 10 day old rats and the topography of labeled cells within the ganglion of both groups is currently in progress. Experiments in which single papilla are labeled at two different times during development are also in progress to examine possible rearrangements of innervation patterns for the same papilla. The techniques and findings derived from these studies will collectively be the basis for future studies that determine the effects of early dietary manipulations and the role of trophic factors on the development of peripheral innervation patterns, as well as detail events involved in the regeneration of taste afferents.

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186

Development of Nerve Supply to the Tongue of Rat Embryos. J. P. MBIENE and C.M. MISTRETTA (Dept. of Biologic and Materials Sciences, School of Dentistry, Univ. of Michigan, Ann Arbor, MI, 48109).

Although the requirement of innervation for maintenance and regeneration of taste receptors in mammals is well established, during development it is still unclear when and how innervation participates in the morphogenesis of taste organs. Therefore, we are investigating the development of the nerve supply to the tongue and gustatory papillae during the earliest stages of taste organ morphogenesis in the rat embryo. We used antibodies against growth associated protein-43 (GAP-43) as a marker for early nerve outgrowth in the tongue. Embryos were obtained from timed, pregnant Sprague Dawley rats and we designated the day of identifying a vaginal plug as E0. The entire head was obtained from embryos at 10 to 12 hour intervals from E13 to E14. E13 precedes the time of first, light microscopic identification of fungiform and circumvallate papillae and E14 marks the period of earliest papilla identification. Serial sagittal sections were cut at 20 um, fixed on slides and further processed for immunocytochemistry. Sections were traced by camera lucida to reconstruct labeled nerve pathways. Even in the early embryonic tongue at E13, three major nerve bundles are identified that are intensely immunoreactive for GAP-43. Serial reconstructions suggest that the bundles represent the sensory nerves, chorda-lingual and glossopharyngeal, and the motor nerve, the hypoglossal. The chorda-lingual bundle is located lateral to the glossopharyngeal and hypoglossal and at E13 has branches ramifying near the epithelium in the mid-region of the early tongue. The glossopharyngeal bundle is characteristically situated in the posterior tongue only, with a few small bundles coursing near the epithelium at E13. The hypoglossal nerve is located within deeper tongue tissue and does not have branches near the epithelium. The chorda-lingual and glossopharyngeal nerves undergo extensive ramification between E13 and early E14. At E14 numerous small branches of the chorda-lingual fan out under the epithelium, with neurites approaching very near the epithelium throughout the anterior tongue. Also, by E14 there is some overlap in more posterior tongue regions between chorda-lingual and glossopharyngeal nerves. Although the hypoglossal nerve also branches more extensively over the E13-E14 period, the nerve bundle remains relatively deep in the tongue. Identification of early tongue innervation with GAP-43 immunoreactivity indicates that the embryonic tongue receives extensive innervation just in advance of papilla formation and that sensory innervation ramifies extensively at the time of papilla morphogenesis. This temporal correlation suggests an important role for innervation in gustatory papilla morphogenesis.

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187

Effects of Early Postnatal Cross-Fostering Between Normal and Sodium Restricted Rats on Chorda Tympani Responses. L.M. PHILLIPS, R.E. STEWART and D.L. HILL (University of Virginia, Charlottesville, VA 22903)

Rats deprived of NaCl prenatally and thereafter exhibit abnormally low electrophysiological chorda tympani taste responses to NaCl as adults. Recovery of the chorda tympani response can be induced by ingestion of NaCl even at adulthood. Although the milk of sodium-restricted mothers contains a normal amount of sodium, we hypothesized that other substances present in milk may function in the normal development of the peripheral taste response. In order to examine the possibility that milk from sodium-replete mothers enables functional recovery in sodium-restricted rats, we recorded multi-fiber chorda tympani responses in adult animals that had been cross-fostered during the suckling period. Sodium-restricted litters were cross-fostered to sodium-replete mothers on the day after birth (day 1), and then reverse crossed to the original mother on postnatal day 14. Sodium-replete litters were cross fostered to sodium-restricted mothers according to the same protocol. Sodium-restricted rats cross-fostered to normal mothers during the first two postnatal weeks exhibited abnormally low sodium responses. Thus, they responded as if they had not been cross-fostered. Surprisingly, normal animals crossed to sodium-restricted mothers showed an exaggerated sodium response. That is, NaCl responses were greater than those in normally-reared, sodium replete rats. Alterations of chorda tympani responses in both groups were specific to sodium stimuli. While it is apparent that milk from normal mothers is not sufficient to restore gustatory function in sodium-restricted rats, it seems that changing the maternal environment during the early postnatal period in normal offspring enhances taste sensitivity to sodium.

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188

Terminal Field of Taste Afferents in the Nucleus of the Solitary Tract is unaffected by High Maternal NaCl Intake. ELIZABETH K. BASCO, ISKE L. VANDEVELDE, AND ROBERT J. CONTRERAS (Florida State University, Department of Psychology, Tallahassee, FL 32306-1051)

The chorda tympani nerve innervates the salt-sensitive taste receptors on the anterior tongue. Findings from several behavioral and electrophysiological studies indicate that the sensory signal transmitted by the chorda tympani is critical for the control of salt intake in the rat. The axons of the chorda tympani nerve terminate within the rostral region of the nucleus of the solitary tract (NST). King & Hill (*J. Comp. Neurol.* 303: 159-169, 1991) have recently shown that the terminal area of chorda tympani afferents in the NST can be modified by sodium deprivation during a vulnerable period of perinatal development. Using horseradish peroxidase (HRP) histochemistry, King and Hill found that the terminal area was larger in adult rats raised by mothers fed a sodium-deficient diet containing 0.03% NaCl from the third day of gestation to postnatal day 35 compared to rats raised on a control 1% NaCl diet. The functional significance of this alteration is unknown, but may underlie changes in NaCl intake due to early dietary experience. Our research has produced substantial evidence linking the NaCl intake of expectant and lactating rat mothers with the NaCl intakes of their adult offspring. Offspring from mother rats fed high NaCl preferred NaCl solutions more than offspring raised on either intermediate or basal NaCl. The purpose of the present study was to extend King and Hill's original observations. Adult female rats were fed diets containing either basal 0.15% or high 3% levels of NaCl from conception to postnatal day 30. The offspring were then fed an intermediate 1% NaCl diet for at least 1 month before anatomical study. Wheat germ HRP was applied to the central end of the chorda tympani in 4 basal and 7 high NaCl rats. Using camera lucida, the area of afferent labeling was determined by two independent observers blind to the animal's treatment condition. Contrary to our expectation, the area of afferent terminal labeling in the dorsal, intermediate, and ventral zones of the rostral NST was similar for basal and high NaCl rats. Together with King and Hill's findings, our results suggest that chorda tympani afferents in the NST develop normally despite a broad range of NaCl levels in the maternal diet. The maternal diet may have to exceed a minimal and/or maximal NaCl level to produce changes in neural development.

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189

The Role of Orochemical Stimulation in Postnatal Development of the Rostral Gustatory NST. PHILLIP S. LASITER (*Florida Atlantic University*), JAIME DIAZ (*University of Washington*).

Fungiform receptor damage or artificial rearing manipulations conducted during critical periods of postnatal development permanently alters the organization of primary gustatory afferent axons in the rostral nucleus of the solitary tract (NST). On the basis of these results we have examined the role of various orochemical stimulants in promoting 'recovery' of NST development during artificial rearing (AR) manipulations. Two groups of rats were used in all experiments; animals that received artificial rearing with a milk replacement diet between the postnatal ages of P4 and P10, and mother reared (MR) animals. Subgroups of these animals either received taste simulation (ARt/MRt), water stimulation (ARw/MRw), or no stimulation (AR/MR). Oral stimulation was conducted by applying 10 μl of NaCl, lactose (α-monohydrate), or rat milk to the anterior tongues of animals five times daily between the ages of P4 and P10. In adulthood fluorescent tracing studies (e.g., Lasiter & Diaz, *Brain Res. Bull.*, 29:407-410, 1992) were conducted to determine the organization of primary gustatory axons in the rostral NST. Water or taste stimulation in MR animals produced no significant alteration in N.VII terminal field development. In AR animals, however, water stimulation produced a slight recovery of N.VII terminal field volume, as compared to AR animals that received no stimulation. 30 mM, 150 mM, and 500 mM NaCl produced a concentration-dependent recovery of N.VII terminal field volume, with 500 mM NaCl promoting complete recovery of N.VII terminal field volume. 260 mM lactose (saturated solution) was similarly effective in promoting recovery of N.VII terminal fields, as was native rat milk. Results of groups receiving 80 mM lactose and rat milk subjected to dialysis (12 kD MWCO) will be presented. These results confirm that normal constituents of rat milk (e.g., NaCl and lactose) are important in inducing normal axonal development in the rostral gustatory NST.

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190

Establishment of the Early Solitary Tract and Boundaries of the Nucleus of the Solitary Tract in Developing Sheep Brainstem. CAMILLE TESSITORE KING and CHARLOTTE M. MISTRETTA (Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109).

Temporal and spatial patterns of the glial markers, glial fibrillary acidic protein (GFAP) and vimentin (VIM), and the extracellular matrix glycoprotein, tenascin (TN), suggest that these molecules have a role in the early establishment of functional subregions within the central gustatory system of sheep. Specifically, by 55 days of gestation (term = 147 days), GFAP, VIM, and TN immunoreactivities demarcate the boundary of the nucleus tractus solitarius (NTS), indicating that glia play an important role in formation of taste regions within the brainstem. In addition, these markers appear to delineate bundles of solitary tract fibers coursing towards and entering the NTS. To study possible interactions between these molecules and axon growth, it is necessary to determine the time of arrival of afferents in the solitary tract relative to the distribution of GFAP, VIM, and TN within the brainstem. Therefore, we are investigating immunolocalization of growth associated protein (GAP-43), a membrane phosphoprotein synthesized at high rates during axon development, to ascertain the time at which developing axons reach the NTS. Horizontal sections of sheep brainstem from fetuses aged 55 and 100 days of gestation were cut for immunocytochemistry. At 55 days of gestation, GAP-43 immunoreactivity is abundant within the pathway which is stereotypic for the solitary tract and is apparent within the fibers entering the NTS, indicating that at this early gestational age afferent fibers have innervated the NTS. Interestingly, GAP-43 immunoreactivity is also found within the border of the NTS. By 100 days of gestation, when immunoreactivity for GFAP and TN is still extensive within bundles of taste fibers, GAP-43 immunoreactivity is much reduced. This suggests that new fibers apparently are not entering the solitary tract, although the NTS is still structurally and functionally very immature at this age. The border of the NTS remains demarcated by GAP-43 at 100 days, suggesting a possible interaction between this marker and glia. In summary we have demonstrated that the solitary tract is well established and NTS borders are clearly demarcated at a very early developmental stage. Further experiments in fetuses at earlier and later timepoints will demonstrate whether patterns of GFAP, VIM and TN delineate NTS borders in advance of arrival of solitary tract fibers, and whether these boundaries exist only transiently during development.

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191

Are There CNS Consequences of Taste Cell Turnover? DAVID V. SMITH, MANTANA NORMAN, and MICHAEL T. SHIPLEY (University of Cincinnati College of Medicine).

A fundamental question in taste is how a code for gustatory quality can be maintained in the face of the continual turnover of taste receptor cells. Two extreme possibilities are: (1) peripheral axons reconnect with the same types of receptor cells or (2) the axons connect with different cell types but their central connections change. Although the peripheral consequences of taste bud deafferentation and regeneration are well-established, almost nothing is known about the central consequences of gustatory deafferentation. Injury to the CNS causes glial reactions within the brain, e.g., an increased expression of glial fibrillary acid protein (GFAP) in astrocytes. Here we have investigated whether there are similar changes in the medullary targets of taste fibers following axotomy. Further, we have examined the nucleus of the solitary tract (NST) of normal rats to determine if there is a higher level of glial activity associated with the ongoing process of taste receptor cell turnover. Rats were subjected to bilateral or unilateral axotomy of the glossopharyngeal (IXth) and/or the chorda tympani (CT) nerves. Following a survival time of 1 - 7 days, the brainstem was examined for the presence of a glial reaction using antibodies that specifically recognize astrocytes (GFAP) and microglia/macrophages (OX42). Significantly, the NST in *normal* brains was characterized by a higher level of constitutive expression of these antigens than surrounding areas of the medulla. Transection of peripheral gustatory axons caused a dramatic increase in both glial markers. These reactions were specific to the terminal projection zones of the IXth and CT nerves within the NST. Glial reactivity was detectable at the earliest time examined (24 hours) and was still present 7 days later. These results strongly suggest that there is central degeneration and/or synaptic reorganization following peripheral gustatory lesions. Moreover, the finding that NST glial cells have higher constitutive levels of these glial markers suggests that there is ongoing degeneration/synaptic reorganization associated with the normal turnover and replacement of taste receptor cells.

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192

THE EFFECTS OF DIETARY SODIUM CHLORIDE DEPRIVATION ON THE TERMINAL FIELD ORGANIZATION OF SECOND ORDER GUSTATORY AFFERENTS PROJECTING TO THE PARABRACHIAL NUCLEUS.

B.R. Walker and D.L. Hill (University of Virginia, Charlottesville, VA 22903)

Dietary sodium deprivation instituted early in prenatal development produces physiological, anatomical, and functional changes in the developing gustatory system. Neurophysiological recordings from the chorda tympani nerve (CT) demonstrate that whole nerve responses to NaCl are reduced in deprived rats while responses to non-salt stimuli are unaffected. Furthermore a rearrangement of the CT terminal fields within the nucleus of the solitary tract (NTS) is observed in deprived rats, while glossopharyngeal (IX) terminal fields remain similar to controls. Peripheral responses to NaCl "recover" to control levels when deprived rats are fed a NaCl replete diet; however, the altered pattern of CT innervation within the NTS remains. In addition, the size of the "recovered" CT terminal field is 3X the size of control and deprived fields. In light of these observations, the terminal fields of second order projections from the NTS to the parabrachial nucleus (PBn) were examined. In order to determine the possible rearrangement of the second order projections, the rostral pole of the NTS in both control and repleted rats was injected with the anterograde fluorescent tracer Fluoro-Ruby.

Preliminary results show a 16% increase of the PBn terminal field in the recovered animals. These results suggest that there is a rearrangement of the second order gustatory projections from the NTS to the PBn in recovered animals, but this rearrangement is not as dramatic as is seen at the first gustatory relay.

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193

The effect of neonatal capsaicin adminsitration on the morphology of the rat ethmoid nerve. W. L. SILVER (Wake Forest University, Winston-Salem, NC). T. E. FINGER (University. Colorado School of Medicine, Denver, CO))

Ethmoid nerve fibers innervating the nasal cavity respond to a variety of sensory stimuli, including mechanical, thermal, and chemical. Previously, we demonstrated that neonatal capsaicin administration eliminates ethmoid nerve responses to chemical stimuli, while leaving responses to mechanical stimuli intact (Silver et al., 1991, *Brain Res.* **561**: 212-216). In the present study, two-day old rat pups were injected with a 1% capsaicin solution (50 mg/kg) or the vehicle (controls). The ethmoid nerves of these rats were tested electrophysiologically for their response to acetic acid at approximately 100 days after the capsaicin injection. Following the recording session the ethmoid nerves were removed, placed in fix, and prepared for immunohistochemistry and electron microscopy. The ethmoid nerves of all of the control rats exhibited normal, vigorous responses to both acetic acid and mechanical probing of the outside of the nose. The ethmoid nerves of the experimental animals, however, did not respond to acetic acid, although they responded vigorously to mechanical stimulation. Examination of the ethmoid nerves revealed a reduction in the cross-sectional area of the nerve in the capsaicin-treated rats due to a decrease in the number of both myelinated and unmyelinated fibers. The area of the ethmoid nerve in the experimental animals was reduced by approximately 30%. The number of myelinated axons decreased by about 40% from the controls while the number of unmyelinated axons deceased by at least 80%. In addition, the number of CGRP-ir fibers in the ethmoid nerves of capsaicin treated rats decreased by 90% or more. Analyses of the fiber spectra of the nerves in both the experimental and control animals as well as the effect of capsaicin administration on the number and size of CGRP-containing fibers also will be reported. These results demonstrate that neonatal capsaicin administration has a dramatic affect on the morphology of the ethmoid nerve, correlating with the absence of chemical response from capsaicin desensitized animals.

194

Changes in Cell Proliferation in the Developing Olfactory Epithelium Following Neonatal Unilateral Naris Occlusion. DIANA M. CUMMINGS and PETER C. BRUNJES (University of Virginia)

Unilateral external naris occlusion results in profound changes in the ipsilateral olfactory bulb. For example, rat pups with one naris closed via surgery on the day after the day of birth (P1) exhibit a 25% reduction in the size of the ipsilateral olfactory bulb by P30. Along with this large change in bulb size there are attendent changes in bulb neurochemistry, anatomy and function. While considerable effort has been expended in describing these changes, relatively little is known about alterations in the olfactory epithelium. Nevertheless, Farbman et al. (*J. Neurosci.* **8**, 1988) demonstrated that by P30 the rate of neurogenesis on the deprived side of the olfactory epithelium decreases by 40%. The present study investigates the possibility that these changes occur even earlier in development. Pups underwent either external naris closure or sham surgery on P1 and were then injected with ^{3}H-thymidine on P10, P20, or P30 (Ns = 3-4/condition /age). After a 2 hour survival time, pups were perfused and the tissue was processed for semi-thin sectioning and autoradiography. Counts of cells covered with silver grains were made in three rostral-caudal locations in the olfactory mucosa. Data were expressed as the ratio of the number of labelled cells on the right vs. left side of the nasal cavity for both experimental and control pups. On P10 nearly equal numbers of labelled cells were found on the right and left sides of both experimental and control animals. However, by P20 decreased numbers of labelled cells in the deep quarter of the epithelium were observed on the occluded sides of the mucosa in septal (ratios = 0.835 vs. 1.086, experimental vs. control), dorsal (0.574 vs. 0.996), and lateral (0.615 vs. 1.053) areas. Preliminary results suggest, therefore, that a decrease in cell proliferation on the deprived side of the olfactory epithelium occurs between P10 and P20.

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195

Learning induced changes in metabolic activity in the adult rat olfactory system. WILLIAM D. HAMRICK, DONALD A. WILSON AND REGINA M. SULLIVAN (Developmental Psychobiology Lab, Dept. Psychology, University of Oklahoma).*

The olfactory bulb of newborn rats demonstrates marked plasticity to early experiences ranging from olfactory deprivation to associative learning. The mature olfactory bulb appears to be less plastic, however, demonstrating only minor functional changes following late onset deprivation and far fewer learning-associated changes than found in pups. The present experiment examined relative 2-DG uptake patterns in the olfactory bulb and several other olfactory system structures following a simple odor detection conditioning paradigm in adult rats. Mature Wistar rats (250-300 g) were trained in an operant task with peppermint odor serving as an S^+, S^- or S^o. After training to criterion, rats were injected with 40 μCi 2-DG and exposed to the peppermint odor. No change was detected in relative 2-DG uptake between conditioning groups in the olfactory bulb glomerular layer, the anterior or posterior pyriform cortex, or the dorsal or ventral hippocampus. However, relative 2-DG uptake was significantly enhanced in the dorsal anterior olfactory nucleus (AON) of both S^+ and S^- animals compared to S^o rats. Uptake in both the lateral and medial AON was also enhanced, though not significantly. These results suggest that the AON may play an important role in olfactory memory in adults.

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196

Plasticity of Olfactory Receptor Neurons

MORRISON, EDWARD E., PhD (College of Veterinary Medicine, Auburn University, AL)

One characteristic of olfactory neurons is their remarkable ability to be replaced normally and when experimentally damaged. This is due to a population of neuroblast that are capable of post natal neurogenesis. Previous studies have shown that fragments of olfactory mucosa can be transplanted into ectopic neural regions. Transplant neuroblast survive, produce new neurons that develop and mature. In the present study we have used electron microscopy to examine transplant neuron development and interaction with host brain tissue. Olfactory mucosa (septum) from P2-6 neonate was transplanted into littermates in the parietal cortex. Following survival time of 3 to 180 days animals (n=20) were deeply anesthetized and perfused with 0.6% paraformaldehyde and 2% glutaraldehyde in buffered sodium cacodylate. At early survival times (3-7 days) transplants were rapidly vascularized and consisted of degenerating mature olfactory neurons (axons severed), supporting and basal cells. At 2-6 weeks the transplant neuroepithelium had many morphologically mature neurons with dendrites having ciliated olfactory knobs. Mitotic activity was observed in the lower neuroepithelium region and also among migrating neurons in the lamia propria and also in the host brain. Transplant olfactory axons had similar structure as those observed in the nasal cavity. Olfactory axons fasciculated forming bundles containing many axons surrounded by sheath cells. These axons were unmyelinated, in direct contact with each other, contained microtubules and were uniform in size (0.1 - 0.3 μm). In some cases, we followed transplant axons arising from the neuroepithelium they formed large axon bundles which entered the host brain tissue. These transplant fascicles branched within the host cortex and some formed characteristic asymmetric synapses with host nerve tissue. Our results show that transplant olfactory neurons survive and develop neural processes, some capable of entering host brain tissue and forming new synaptic contacts.

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An Analysis of Olfactory Receptor Neuron Lineage Using a Replication Incompetent Retrovirus. MARY E. CAGGIANO, DALE D. HUNTER and JOHN S. KAUER. (Neuroscience Program, Tufts/New England Medical Center, Boston, MA. 02111).

Vertebrate olfactory receptor neurons have the unique ability to turn over throughout the life of an organism. This unusual characteristic provides a convenient system for analyzing olfactory receptor neuron cell lineage. Although two types of potential progenitor basal cells have been characterized based on their response to ablation of the olfactory bulb, the precise lineage of the receptor cell neurons still remains unclear. In order to understand how basal cells give rise to olfactory receptor neurons, we have employed a technique that permits progenitor cells to be labelled by a recombinant retrovirus containing the gene for a histological marker enzyme, alkaline phosphatase (DAP). This method relies on the replication incompetent retrovirus integrating into the genome of mitotic cells thereby labelling their progeny. Rat pups (8-10 days old) were infected by direct injection of 5ul of virus ($2\text{-}5x10^5$ infectious particles/ml) into the olfactory epithelium. Animals whose olfactory nerves were left intact, showed pairs of cytokeratin-positive cells near the basal lamina 5-10 days following infection. Pups whose olfactory nerves were lesioned showed both cytokeratin-positive pairs of cells near the basal lamina, and clusters of 8-10 cytokeratin-negative cells in the upper layers of the olfactory epithelium 5-10 days following infection. It thus appears that injury to the nerve caused an increase in mitotic activity of cytokeratin-negative basal cells. These data suggest that olfactory nerve lesion causes an increase in mitotic activity of globose basal cells and the progeny of these cells migrate toward the mucosal surface. Cytokeratin-positive horizontal basal cells are also mitotic; however, 5-10 days after infection they appear to divide a single time, giving rise to pairs of labelled cells. In order to analyze their progeny, we are examining longer time courses. We are also trying to determine the relationship and cell types present in the clusters of labelled cells.

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Pioneering Olfactory Axons May Influence Cell Cycle Kinetics in the Developing Olfactory Primordium

QIZHI GONG AND MICHAEL T. SHIPLEY (University of Cincinnati)

Pioneering olfactory axons reach the telencephalon and penetrate to the ventricular zone at the earliest stages of olfactory system development (E13 and E14). Subsequent to the arrival of these pioneering axons, the cellular architecture and morphology of this part of the telencephalon, the olfactory bulb primordium, undergo a dramatic alteration to form what we call the olfactory bulge at E15 (Gong et. al. Neurosci. Abstr. '92, 268.7). Since the stem cells for the olfactory bulb are located in the ventricular zone where the pioneering axons penetrate, we hypothesized that these pioneering olfactory axons may selectively influence the cell cycle of the stem cells and/or cause cells in the germinal zone to leave the mitotic cycle.

Here, we compared cytokinetics in the olfactory primordium and the adjacent neocortex in the telencephalon. Cumulative S-phase labeling with Bromodeoxyuridine (BrdU) was used to estimate the duration of the cell cycle (Tc), the duration of the S phase (Ts) and the percentage of the proliferating cells in these two telencephalic compartments. BrdU was administrated to E14 timed-preganant rats at 2 hr intervals up to 12 hrs; data were collected from E14 embryos from several litters. Cell cycle kinetics were calculated by the model of Nosakowski et. al. (J Neurocyto. '89, 18:311).

The average Tc in the olfactory primordium was 14.2 hr versus 11.7 hr in the rostrally adjacent neocortical compartment. The average Ts in the olfactory primordium was 7.7 hr versus 6.3 hr in adjacent neocortex. The results for neocortex are similar to those reported by Caviness et. al. (Neurosci Abstr. '91, 18.2) at comparable stages of mouse cortex development. These workers further demonstrated that Tc increases as the cortex matures from E12 to E15. Our finding that Tc is increased in the olfactory primordium compared to the neocortex indicates the olfactory primordium is at a more mature stage of development than the adjacent cortex. In addition, we found that the ratio of proliferating to total cells in the olfactory primordium was half (24.7%) that in the adjacent cortex (49.7%) 0.5 hr after the BrdU injection. This indicates that more cells have left the mitotic cycle in the olfactory primordium.

We suggest that the increased proportion of post-mitotic cells and the longer duration of the cell cycle in the olfactory primordium plays a key morphogenic role in the formation of the olfactory bulb from the primative telencephalon. These results are consistent with the hypothesis that pioneering olfactory axons regulate the mitotic cycle of the stem cells and cause a higher proportion of the daughter cells to exit the mitotic cycle. Thus, pioneering olfactory axons may induce the formation of the olfactory bulb.

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Some New Ultrastructural Aspects of Developing Olfactory Cilia and Supporting Cell Microvilli

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We found several new aspects on the morphology of developing olfactory cilia and microvilli not properly described (*MENCO & FARBMAN, J. Cell Sci., 78:283, 311 (1985)* and references therein). E16, E19 and E22 rat embryos (E1=day of conception, E23=P1=day of birth) and adults were rapidly frozen, freeze-substituted and embedded as described elsewhere (*MENCO et al., Neuron, 8: 441 (1992)*). Although most cytochemical characteristics were usually retained after paraformaldehyde fixation, several fine structural details were lost even in combination with freeze-fixation. Some of these details could only be seen because of the superior ultrastructural preservation obtained with rapid-freezing and freeze-substitution in the absence of chemical fixation. Most noticeably was the rather geometric parallel alignment of the supporting cell microvilli. At E16 the mucus layer tends to extend above the microvillar tips, whereas at E19, E22 and in adults the microvilli tend to reach the mucus boundary; their tips extend even quite often above the layer of cilia. Whether these microvilli play a role in establishing this boundary and/or whether they sense the environment of the cilia for homeostasis is unclear. Also, in embryos the microvilli are quite often aligned, whereas in adults they are more irregularly arranged and considerably more abundant. Concerning the receptor cells, dendritic and dendritic knob microtubules tend to organize near the periphery of developing olfactory neurons, whereas basal bodies tend to be positioned more centrally. Sometimes these microtubules seem to encapsulate the basal bodies rather similar to microtubule/organelle arrangements in nervous growth cones (*TSUI et al., J. Neurosci., 12:3002 (1984)*). Possibly most relevant to what we begin to know about olfactory sensory transduction is that occasionally we saw pockets of polyribosomes near the base of developing cilia similar to what has been described for CNS dendrites and their spines (see *STEWARD & BANKER, TINS, 15:180 (1992)* for review). We saw this in chemically as well as in freeze-fixed tissues. The suggests that some nuclear products can be specifically targeted to the cilia during ciliogenesis. With *STEWARD & BANKER* we remain in doubt why some proteins may be synthesized near their site of use rather than in a more central location and also about the putative nature of the site-specific synthesized proteins, and their possible role in olfactory transduction. *Supported by NSF grant IBN-9109851.*

L1, a Cell Surface Adhesion/Recognition Molecule, is Transiently Expressed on Immature Olfactory Receptor Neurons

MICHAEL T. SHIPLEY AND QIZHI GONG (University of Cincinnati)

Olfactory receptor neurons (ORNs) derive from basal cells located at the depth of the olfactory epithelium (OE). New ORNs differentiate into fully mature ORNs which express olfactory marker protein (OMP), signal transduction, and olfactory receptor molecules. This process of differentiation is poorly understood. Along their differentiation pathway, ORNs initiate axons which must navigate to the olfactory bulb, recognize appropriate postsynaptic target cells and form synapses. Each of these events requires appropriately timed expression of cell surface recognition/adhesion molecules. Cytoplasmic molecules such as TUJ1 and GAP43 are transiently expressed by immature ORNs but little is known of the identity or the time of expression of cell surface molecules in differentiating ORNS.

L1 is a cell surface molecule implicated in neuronal migration, neurite fasciculation and neuron-neuron recognition. It was recently reported that L1 is expressed by ORNs at early developmental ages but not in adult OE (Miragall, '88; Miragall, '89). Candidate recognition/adhesion molecules should be expressed by ORNs in adults, however, as there is continuous turnover and replacement of these cells. Therefore, we re-investigated L1 expression in the rat OE to determine when this molecule is down regulated in development.

L1 positive cells were found as early as at E14 and were located throughout the depth of the epithelium. Starting at E16, when we first observed OMP positive cells in OE, L1 cells became progressively restricted to the deeper parts of the OE. Contrary to earlier reports, we observed that ORNs in the adult OE robustly express L1. In adults, L1 is selectively expressed by ORNs restricted to the deepest part of OE. At all ages, L1 expression is very high on olfactory nerve axons but is much lower in their terminal arbors in olfactory bulb glomeruli. These observations suggested that L1 is transiently expressed by immature ORNs in animals of all ages. To assess this hypothesis, OMP and L1 double labeling experiments were performed. In adults, OMP and L1 are expressed by largely, if not exclusively, different populations of ORNs: OMP is expressed by ORNs in middle 35-50% of OE; L1 cells are in the deepest 20-25% of OE. Some cells at the interface of these two populations may express both molecules. BrdU pulse labeling will be used to estimate the onset and duration of L1 expression in postmitotic ORNs. Immature ORNs in culture tend to aggregate but mature ORNs do not (Pixley, '92). If L1 plays a role in the aggregation of ORNs, or fasciculation of their axons, then antibodies to L1 may block these functions. Experiments to test this hypothesis are planned.

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201

Induction of olfactory receptor sensitivity in mice. HAI-WEI WANG, CHARLES J. WYSOCKI and GEOFFREY H. GOLD (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104)*.

Exposure to androstenone can induce sensitivity to that odorant in humans with specific anosmia to androstenone (Wysocki et al., 1989). We have used EOG recordings in mice to determine if induction can occur at the receptor cell level in this species. Androstenone-insensitive (NZB/B1NJ) and -sensitive (CBA/J) strains were chosen as animal models for androstenone-insensitive and -sensitive individuals, respectively, based on their behavior in conditioned aversion assays. In NZB/B1NJ mice, 2-4 weeks of 16 hr daily exposure to androstenone caused up to a 3.6-fold increase in the EOG response amplitude to androstenone, but did not significantly affect responses to isoamyl acetate. However, in CBA/J mice androstenone exposure had no effect on response amplitudes to either odorant. These data demonstrate that induction of androstenone sensitivity in mice: 1) occurs at least in part at the receptor cell level, 2) occurs only in strains that initially have low sensitivity to androstenone, and 3) increases sensitivity specifically for androstenone. To test the generality of this phenomenon, induction of sensitivity to isovaleric acid was attempted in strains which are relatively insensitive (C57BL/6J) and sensitive (AKR/J) to isovaleric acid. Isovaleric acid exposure increased isovaleric acid response amplitude (3.4-fold) only in the C57BL/6J strain and had no effect on responses to isoamyl acetate in either strain. These data demonstrate that induction is not uniquely a property of androstenone receptors, and therefore may be a general property of olfactory receptors. In view of the prevalence of many specific anosmias in humans (e. g., 50% are anosmic to androstenone), our data suggest that a large fraction of the human population may experience changes in olfactory sensitivity and perception as a consequence of olfactory exposure.

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202

Adult Rat Nasal Mucosal Cultures Show Neurogenesis and Contain Functional Olfactory Receptor Neurons. R.J. GRILL, P. FARMER, ROBERT C. GESTELAND and S.K. PIXLEY (Univ. of Cincinnati, Cincinnati, OH 45267-0521).

The olfactory system of adult mammals differs from other neuronal systems in that extensive neurogenesis still occurs. By studying the mechanisms that regulate neurogenesis in the olfactory system, we may better understand why neurogenesis occurs so infrequently in the adult CNS of mammals. Recently, an in vitro system generated from newborn rat nasal mucosal cells has been described that demonstrates both neurogenesis and neuronal differentiation (Pixley, *Neuron* 1992, 8:1191). Since olfactory neurogenesis continues in adult mammals, a culture system derived from adult animals would permit study of the developmental properties not only of newborn, but adult animals. Here we describe an in vitro system derived from adult rat nasal mucosa that contains a neurogenic population of cells that produce functional olfactory receptor neurons (ORNs).

Dissociated nasal mucosal cells from adult Sprague-Dawley rats were grown on a CNS glial mat as recently described for newborn rat nasal mucosa (Pixley, *Neuron* 1992, 8:1191). Initially, there are large numbers of mature OMP^+ ORNs that disappear by days 4-6. Immature (OMP^-) ORNs that immunostain for neuron-specific tubulin (NST) are few in the first 2 days of culture, but after 3 days their number increases dramatically. The number of immature ORNs continues to increase until, after 10 days in vitro, they are difficult to count. Between days 15-20, ORNs appear that are OMP^+. Genesis of both OMP^+ and OMP^- neurons in culture was demonstrated with ^3H-thymidine.

Voltage-sensitive dyes were used to study the odorant responses of ORNs from adult rat nasal cultures. This technique has the advantage over other techniques such as patch clamping in that the odorant responses of a large number of ORNs can be studied at one time. Coverslips from 7-10 day adult cultures were loaded with the voltage-sensitive dye, di-4-ANEPPS, and showed responses to air-delivered odorants.

The study of these cultures will provide information on the processes that control neurogenesis and differentiation in the adult rat.

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203

Comparison of Expression of GFAP and S-100 in Glia of the Olfactory and Vomeronasal Systems of Postnatal and Adult Short-tailed Opossums, *Monodelphis domestica*. LENA SHNAYDER (SUNY-Downstate, Program in Neural and Behavioral Sciences), INNA SHCHUCHINSKY (Midwood High School at Brooklyn College), and MIMI HALPERN (SUNY-Downstate, Program in Neural and Behavioral Sciences)

Metatherian mammals are characterized by the extreme immaturity of their young at birth. We examined the development of opossum chemosensory systems using immunocytochemistry for two astrocytic markers: GFAP and S-100. There was a discrepancy in the onset of expression of these proteins: S-100 appeared earlier than GFAP. In 20-day-old animals, S-100 was present in the main olfactory nerve and in the olfactory nerve layer surrounding the main olfactory bulb (MOB). GFAP at this age was only present in structures caudal to the MOB. Two weeks later, at 34 days of age, both proteins were present in the main and accessory olfactory systems. GFAP was present in: 1) the olfactory nerve and glomerular layers of the MOB, 2) radial glial fibers of the MOB, 3) the accessory olfactory bulb (AOB) (glomerular layer primarily), and 4) rostral migratory stream. S-100, on the other hand, was never seen in the glomeruli of either the MOB or AOB, although it was clearly present in the nerve layers of both structures. Antibodies to S-100 were never seen to stain radial glial fibers, and the staining pattern of the cells in the rostral migratory stream appeared different from that seen with anti-GFAP. By 45 days of age, GFAP-positive radial glial fibers of the MOB had disappeared and the same pattern remained to adulthood. In the adult MOB, GFAP was present in the two most superficial layers of the MOB, appearing as a meshwork of immunostained processes inside each glomerulus, whereas S-100 was only present in the nerve layer and was never seen inside glomeruli. The same was evident in the AOB, where S-100 was seen only on the contours of glomeruli. The appearance of immunostained cells of the rostral migratory stream remained quite different for the two antibodies in the adult, where GFAP-positive cells were stellate in appearance, and S-100-positive cells formed a stream of small, round cells. The data from the present study strongly suggest that GFAP and S-100 are expressed in different types of glia in both chemosensory systems of developing and adult opossums.

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204

Developmental Expression of Carbohydrate Moieties in the Olfactory and Vomeronasal Systems of Postnatal Short-tailed Opossums, *Monodelphis domestica*. PEI-LEE EE (Midwood High School at Brooklyn College), LENA SHNAYDER and MIMI HALPERN (SUNY-Downstate, Program in Neural and Behavioral Sciences)

Metatherian mammals are characterized by the extreme immaturity of their young at birth. Using lectin histochemistry, the present study was designed to identify some of the sugars that are present in the opossum olfactory and vomeronasal systems, and to describe their onset of appearance and developmental and adult patterns of expression. Six peroxidase-labelled lectins were used (abbreviation and blocking sugar in parenthesis): 1) peanut agglutinin (PNA, galactose), 2) soybean agglutinin (SBA, N-acetylgalactosamine), 3) dolichos biflorus agglutinin (DBA, N-acetylgalactosamine), 4) vicia villosa agglutinin (VVA, N-acetylgalactosamine), 5) phosphocarpus tetragonolobus (PCT, fucose), and 6) helix aspersa (HA, galactose and sucrose). Only two of these lectins, VVA and DBA, were specific to the vomeronasal system of opossums, whereas the other four lectins bound to sugars in the main as well as accessory olfactory systems. However, PNA and SBA staining in the vomeronasal system consistently exceeded that seen in the main olfactory system at all ages. It is noteworthy that although SBA has previously been reported to be quite selective for the vomeronasal system (Key and Giorgi, *Neuroscience Letters, 69*, 1986), in the present experiment strong SBA staining was clearly localized to the main olfactory bulb throughout development. The intensity of staining for the last two lectins, PCT and HA, was about equivalent in the two chemosensory systems. Positive staining appeared at approximately the same age for each lectin (about 6 days of age for olfactory system, 10 days of age for vomeronasal system). The staining intensity of none of the lectins varied markedly as a function of age and no subclasses of positive cells were recorded within each system. The three lectins SBA, DBA, and VVA, which according to the literature bind to the same sugar (N-acetylgalactosamine), in the present study had different patterns of staining in the main and accessory olfactory systems. These results suggest that different carbohydrate residues, possibly components of glycosylated proteins, exist in the two chemosensory systems of developing and adult opossums. They may thus play different roles in these systems.

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205

Retrovirus-Labeled Cells of the Olfactory Placode Migrate to the Brain of Embryonic Chick Along an NCAM Pathway. MIMI HALPERN (SUNY Health Science Center At Brooklyn), ANTHONY M. C. BROWN (Cornell University Medical College), MARLENE SCHWANZEL-FUKUDA (Rockefeller University), DREW NODEN (New York State College of Veterinary Medicine, Cornell University)

Shortly after its invagination, the olfactory placode gives rise to several types of mesenchymal cells, including precursors of glia (Couly & LeDourain, *Devel. Biol.* Vol. 110, 1985) and luteinizing hormone-releasing hormone-expressing cells (Schwanzel-Fukuda and Pfaff, *J. Steroid Biochem. Molec. Biol.*, Vol. 39, 1991 for review). The latter are associated with and appear to move towards the brain along a scaffolding of NCAM positive cells (Schwanzel-Fukuda et al., *J. Comp. Neurol.*, Vol. 321, 1992). In order to verify these descriptive observations, olfactory epithelial cells of stage 10-12 chick embryos were labeled with a replication defective retrovirus vector, derived from the avian spleen necrosis virus, carrying the marker gene *lacZ* (Mikawa et al., *Exp. Cell Res.*, Vol. 195, 1991). Embryos were killed at stages 30 to 35 and processed for the visualization of β-galactosidase using either an X-gal histochemical reaction procedure or anti-β-galactosidase immunocytochemistry. These tissues were also stained using anti-NCAM immunocytochemistry (antibody generously provided by Dr. G. M. Edelman). Retrovirus infected cells were observed in the olfactory epithelium, along the olfactory nerve and entering the brain medial and caudal to the developing olfactory bulb. NCAM immunoreactivity was observed in the olfactory epithelium, olfactory nerves and surrounding the olfactory bulb. The retrovirus-marked cells were always observed in close association with NCAM positive cell processes.

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206

The effect of unilateral, complete removal of the olfactory placode (OP) early in embryogenesis on the development of the olfactory bulb (OB) in chick embryos. EROL LALE AND ALBERT I. FARBMAN (Dept. of Neurobiology, Northwestern University, Evanston, IL 60208)

Burr (J. Exp. Zool., 20:27, 1916) reported that removal of the OP in amphibians (*Amblystoma* and *Xenopus*) results in a marked reduction in development of the anterior portion of the telencephalon mainly because of the absence of the OB. However, after metamorphosis the difference in telencephalon size between operated and unoperated sides was more obvious. More recently Graziadei and Monti Graziadei (Neuroscience, 46: 617, 1992) have shown that the OP in *Xenopus* may play an essential role in the development of the entire telencephalon even in premetamorphic stages; in one animal in which they removed the OP the ipsilateral telencephalon was virtually completely absent. We have chosen the chick embryo as an experimental animal in our ongoing studies on olfactory development. We removed the OP in the third embryonic day (E3) and examined the short term effect on formation of the OB at E10. We obtained lesions varying from complete to partial by means of electrocautery with a fine needle. Our results agree with those of Burr (1916) who concluded that when the OP is completely removed it does not regenerate or reform. Complete removal of the OP resulted in total absence of the ipsilateral nasal cavity, its walls and connective tissues, and a very small or absent OB. We observed no effect on the morphology of the developing telencephalon beyond the OB in the experimental period. On the other hand, partial lesions of the OP resulted in a diminutive nasal cavity, rudimentary olfactory nerve and an attenuated OB. This supports Burr's (1916) conclusion that an intact olfactory epithelium and nerve are required for normal development of the OB. Furthermore it appears that the removal of the OP in chick embryos does not influence the initial stages of telencephalon development beyond the OB as reported in studies in other species.

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207

Differentiation and Growth of the Olfactory Organ in the Zebrafish, *Brachydanio rerio*
ANNE HANSEN (Zool. Inst., Univ. Hamburg, Germany)
EKART ZEISKE (Zool. Inst., Univ. Hamburg, Germany)

The aim of our study was to investigate the development of the peripheral olfactory system from early placode to the olfactory rosette. The object of study was the zebrafish, *Brachydanio rerio*, reared in our institute at a water temperature of 26.5°C. We chose this fish since it has become a standard model animal in embryology. Scanning and transmission electron microscopy, immunocytochemistry and fluorescent tracers were used to visualize the development of the olfactory organ. Electron-microscopic pictures of the head of the embryonic zebrafish of 3 - 5 somites show a one-layered electron-lucent epidermis and electron-dense cells of the underlying nerve cord. Between these two structures lies a subepidermal layer of cells which differs considerably from either epidermis or nerve cord. The earliest sign of an olfactory placode is a thickening of this subepidermal layer when the embryo has about 6 - 8 somites. The cells of the subepidermal layer differentiate into all cell types of the olfactory epithelium, i.e. ciliated and microvillar receptor cells, basal cells, supporting cells and ciliated nonsensory cells. Axons of the receptor cells reach the forebrain (at about 20 somites) before the epidermis opens to allow the outgrowth of the olfactory receptor dendrites (about 30 somites). Within the placode, the cells in the rostromedial region differentiate first. The rostral and the caudal ends of the placode, which are still covered by the epidermis, contain a pool of roundish cells that are not yet differentiated. After hatching (3 - 4 days after fertilization), growth and differentiation processes of the olfactory organ become slow compared to the rapid development of the olfactory placode in the embryo. The first fold of the olfactory epithelium appears about 14 days after fertilization, and only after 50 days post-fertilization does the organ of the young zebrafish take on the shape of an olfactory rosette as in the adult.

208

The Ultrastructure of the Olfactory Organ in Embryonic Sea Lamprey. BARBARA ZIELINSKI, ELLA WONG (Department of Biological Sciences, University of Windsor, Windsor, Canada) and ROD MCDONALD (Sea Lamprey Control Centre, Department of Fisheries and Oceans (Canada), Sault Ste Marie, Canada).

Sea lamprey hatch from eggs in nests in gravel river beds, and move to feeding areas within two weeks of hatching. For this study, sea lamprey embryos were collected prior to abandoning their nests and were analyzed by light microscopy, and by scanning and transmission electron microscopy to characterize the development of the olfactory organ. At embryonic stage 14 (Piavis, 1971, in *The Biology of Lampreys* pp 361-400), the olfactory placode was a stratified epithelial structure at the rostromedial aspect of the head, and separated from the brain by a basement membrane. In later stages, a narrow lamina propria (10 μm) with nonmyelinated nerve fascicles and capillaries formed between the olfactory epithelium and the presumptive olfactory bulb. Morphologically mature ciliated olfactory receptor cells were observed in recently hatched embryos (stage 15) and were abundant by the time of downstream movement to feeding sites, at stage 17. Ciliated, as well as microvillar sustentacular cells were present after stage 15. Nerve fibers with large granular vesicles were located in the basal portion of the olfactory epithelium, in the lamina propria, and in the presumptive olfactory bulb, and fascicles of fibers with large granular vesicles were present lateral to the olfactory epithelium. The presence of olfactory receptor cells and the prominence of fibers with large granular vesicles suggests that the lamprey are capable of olfaction when still in their nests, and that peptidergic factors may modulate olfactory function or associated activity in these early stages.

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209

Protein Gene Product 9.5 in the Developing and Mature Rat Peripheral Olfactory and Vomeronasal Systems

EDWARD W. JOHNSON, PAMELA M. ELLER and BRUCE W. JAFEK, (University of Colorado Health Sciences Center and The Rocky Mountain Taste and Smell Center)

Protein gene product 9.5 (PGP 9.5) is a protein first isolated in the human central nervous system (Jackson and Thompson; J. Neurolog. Sci. 1981, 49:429-438). Immunolocalization studies have shown that PGP 9.5 is found within both neural and neuroendocrine tissues (Thompson et al.; Brain Res. 1983, 278:224-236). Since we have observed immunolabeling of the rat olfactory tissues with antisera directed against other proteins found in both systems, we tested the immunoreactivity to anti-PGP 9.5 of the olfactory and vomeronasal tissues during developmental stages and in the mature rat. As early as embryonic day E17 PGP 9.5-immunoreactivity was observed throughout the olfactory receptor neurons (ORNs), and their underlying nerve bundles, mimicking the immunoreactivity of this tissue to olfactory marker protein (OMP). Immunoreactivity was also seen within the presumptive septal organ of Masera. Preliminary work suggests the ORNs may sustain PGP 9.5 immunoreactivity through adulthood. PGP 9.5-immunoreactivity was also seen in virtually all E17 vomeronasal organ (VNO) receptor neurons, in contrast to the sparse number of OMP-immunoreactive neurons. The immunoreactivity to PGP 9.5 of the VNO neurons extended into adulthood. In the perinatal rats, but not adults, immunoreactivity was also seen within cells of portions of the respiratory epithelium, although not in its rostral regions. Some cells in the receptor free epithelium of the perinatal VNO were also labeled. These results suggest that from late embryonic stages through to adulthood both the ORNs and VNO neurons express PGP 9.5, a protein found in neural and neuroendocrine tissues. We will now try to distinguish immunoreactive cells, including those in the perinatal respiratory epithelium, with immuno-electron microscopy.

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210

Development of the Serotonergic System in the Olfactory Bulb of *Monodelphis domestica*. BENJAMIN D. PHILPOT and PETER C. BRUNJES (University of Virginia)

The offspring of *Monodelphis domestica* (grey short-tailed opossum) are born in a very immature state after a short (14-day) gestation period. As a result, they provide a useful mammalian model for examining quite early stages of brain development. In the present study we demonstrate, for example, that the onset and development of serotonergic (5-HT) projections to the *Monodelphis* olfactory bulb can be traced entirely after birth, without the need for embryonic manipulations. 5-HT was visualized in tissue sections from postnatal day 5 (P5), P10, P20, P30, and adult opossums using avidin/biotin peroxidase immunohistochemistry. 5-HT afferents were not apparent in the bulb until P10. By P20, the bulb contained a higher density of 5-HT terminals though the distribution was still relatively sparse and little laminar segregation was noted. Substantially more stained fibers were observed in P30 pups. For example, by this time networks of 5-HT fibers were found within many glomeruli. Although less robust, further developmental changes occurred between P30 and adult opossums, including denser and more well-defined glomerular innervation. General patterns of innervation observed in the adult opossum were quite similar to those reported for the rat. Due to the specificity and time course of 5-HT innervation, the *Monodelphis* bulb provides a model with which to study the regulation of serotonergic development.

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211

Neural Correlates of Growth Recovery from Early Hypothyroid Retardation in the Rat Olfactory Bulb. TIMOTHY J. SENDERA AND ESMAIL MEISAMI (Physiol. Dept., Univ. Illinois, Urbana, IL).

Recent studies from our laboratory have shown that rats made hypothyroid by administration of PTU (propylthiouracil) from birth develop, by day 25 (weaning), only half of the number of olfactory receptors, compared to controls (1) while total number of glomeruli remains unaltered (2). If hypothyroid rats are allowed to recover by withdrawal of PTU, body growth is markedly improved and total number of olfactory receptors neurons is restored (3) by 90 days. In this study we investigate the effects of recovery from hypothyroid neural retardation on olfactory bulb (OB) growth by determining the changes in volume of whole OB and its layers and number of mitral and granule cells as well as size and number of glomeruli. Male rats were kept hypothyroid for 25 days from birth by PTU administration. To initiate recovery, PTU was withdrawn at day 25 and recovery rats were observed at day 90. Preliminary results indicate significant reductions in volume of OB and its layers in hypothyroid rats and marked recovery of these parameters in recovery rats. Although these experimental manipulations significantly changed the size of glomeruli and mitral cell, the number of these remained unchanged (Per OB: glomeruli 2400, mitral cells 45000). The results indicate that OB shows considerable growth plasticity as evident by its ability to recover from marked growth retardation.

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212

Loss of somatic granule cell spines - effect of reduced information?

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It is well known that in the olfactory bulb (BO) granule cells bear spines on their dendrites. In mammals like the ferret, spines are also found on the soma of granule cells. During postnatal development the number of somatic spines shows age related differences; in subadult animals (postnatal days 60-150) numbers are higher than in adult conspecifics. Longterm exposure to 0.25 ppm formaldehyde gas (days 60-150) effects the olfactory epithelium in subadult ferrets severely. The percentage of olfactory receptor density is reduced, in addition olfactory knobs seem to dissolve. Likewise, only in subadult animals the number of spines per soma is significantly reduced after formaldehyde gas exposure. This effect is most obvious in animals 150 days old (4.75 spines/soma in experimental animals versus 5.75 spines/soma in controls; $p < 0.01$, Mann-Whitney-U-test).

This result supports the hypothesis that during ontogeny the structures involved in information processing in the BO depend on environmental stimuli for their development and wiring. If the olfactory epithelium is damaged and thus less information will reach the olfactory bulb, the reduced information will affect the development of the somatic spines of granule cells. Somatic spines, normally inhibiting, are no longer necessary when less information will enter the BO.

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213

Reinnervation and Behavioral Recovery Following Deafferentation of the Olfactory Bulbs.

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The olfactory system has the unique ability to replace damaged neurons and reestablish connections with the deafferented olfactory bulb. In this study, we examined the return of odor mediated behavior during reinnervation of the olfactory bulb. Adult hamsters, on restrictive feeding schedules, were trained and tested with three behavioral tasks. A "buried food pellet task" used latency measurements to assay a simple odor mediated behavior. In addition, activity level (time spent digging) was used to monitor general changes in postoperative behavior. "Odor detection" and "odor discrimination" tasks determined the capacity of the reinnervated bulb to process olfactory information. The "odor detection" task tested the ability to give a response to an odor stimulus (i.e., strawberry or cinnamon), and no response to an odorless (control) stimulus. The "odor discrimination" task tested the ability to respond to a reinforced odor stimulus (S+), and ignore a non-reinforced odor stimulus (S-). Hamsters were trained to perform tasks prior to surgery. Deafferentation was performed by bilateral transection of olfactory nerve fibers using a flexible teflon blade. Sham animals underwent identical surgical procedures except that the olfactory nerve fibers were not transected. Immediately after transection, hamsters were unable to find buried food pellets within a 3 minute test period. Sham animals continued to perform at preoperative levels. As early as 20 days after transection, latencies for finding buried food pellets began to decrease. To demonstrate that hamsters were using odor stimuli and not other sensory cues, a masking odor (pentyl acetate) was introduced to interfere with odor signals from buried food pellets. The masking odor blocked the ability to locate food pellets; and this effect was reversible. The ability to "detect" and "discriminate" odors was also lost after nerve transection and returned with recovery. These results demonstrate that following deafferentation in hamsters, newly reconnected olfactory axons reestablish functional connection with the bulb and that these connections can support "odor detection" and "odor discrimination" behaviors.

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214

Functional Recovery of the Deafferented Olfactory Bulb.

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The olfactory epithelium has a remarkable capacity to produce new neurons that can reestablish axonal connections to the deafferented olfactory bulb. This study used evoked potentials to assay functional recovery of the deafferented bulb of adult male hamsters. Deafferentation of the left bulb was accomplished by transecting nerve fibers projecting from the left nasal cavity. After recovery periods of up to 4 months, HRP was introduced into both nasal cavities to trace olfactory fibers from the nose to the bulb. Evoked potential measurements were done the following day. The left bulb of hamsters one day after transection showed little or no HRP fiber staining, indicating that the transection produced nearly complete or complete denervation of the olfactory bulb. To assay functional reconnection, we measured evoked potentials in the bulb in response to electrical stimulation (500 μA, 500μs) of the olfactory nerve fibers. Negative evoked potentials indicated activation of a population of bulb cells. In control animals, stimulating the medial fibers typically resulted in negative evoked potentials in the medial but not the lateral region of the ipsilateral bulb. Lateral fiber stimulation typically elicited negative evoked potentials in the lateral but not the medial bulb. This is consistent with the arrangement of fiber connections: most fibers from the medial epithelium project to the medial bulb, most lateral fibers project to the lateral bulb. In animals studied immediately after nerve transection (day 1), negative potentials could not be evoked. After 1 to 4 months recovery, negative potentials could be evoked. Medial stimulation evoked negative potentials in the medial bulb of most recovering animals. Lateral stimulation evoked negative potentials in the lateral bulb of most recovering animals. This suggests some aspects of the projection pattern in recovering animals is similar to that of control. However, in one animal, medial fiber stimulation resulted in negative evoked potentials in the lateral region of the bulb. This suggests that reinnervation can alter the pattern of projections from the epithelium to the bulb. Correlation of the negative evoked potential with the amount of HRP labeling may determine the minimum innervation required for the recovery of evoked potential function.

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215

Neural network processing of responses to odorants by a biological nose (mouse) and a bionic nose (chemical sensor array).

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A neural network is a processing device, either an algorithm or actual hardware, whose design was motivated by biological neural functions. It can be trained to operate as a classifier. Applications of neural networks to the study of olfactory processing *in vivo* and to identifying and classifying complex chemical mixtures from the outputs of chemical sensor arrays is the subject of this study. Data from 2-DG autoradiographic images derived from mouse noses after stimulation by odorants and voltage outputs from an array of Figaro chemical sensors were used as inputs to a feedforward neural network which was trained using a backpropagation algorithm. Results indicate that neural networks can be used to demonstrate that the mammalian nasal epithelium is responding in a complex way which nevertheless supports a classifier of chemical inputs. Neural network processing of outputs from the sensor array demonstrated that classification of odour mixtures can be achieved with minimal prior knowledge of the response capabilities of each sensory cell in the array. Neural networks should expedite development of devices for identification of complex chemical mixtures of interest to a number of industries.

216

Basic design of the cortico-cortical connections of the primate olfactory brain

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The precise extension and neural interrelationships of the olfactory association fields in the primate are largely unknown. In order to determine the configuration and projection sites of the olfactory cortices we used several anterograde and retrograde axonal tracing techniques. The intrapalaeocortical and archicortical connections between both the principle olfactory bulb (MOB) and several secondary olfactory fields, including the olfactory trigone (anterior olfactory nucleus, AON), the olfactory tubercle (OT), the prepiriform and the periamygdaloid cortex and the entorhinal area 28, were investigated in the new world monkey *Callithrix jacchus* using focal injections of WGA-HRP. This approach unraveled an intense bilateral involvement of the olfactory trigone in bulbar circuitry. Further olfactory recipient areas were the entire ipsilateral prepiriform cortex, the lateral component of the OT and lateral subdivisions of the entorhinal cortex, especially the prorhinal cortex (proRC). An analysis of the neo- and proisocortical fields showed that the area 13 of WALKER and the anteromedial field of the temporobasal perirhinal cortex (periRC, area 35/36 of BRODMANN) is monosynaptically linked to the MOB. Multiple fluorescent tracer applications into the left and right MOB, respectively, and into the proRC and the periRC resulted in dense retrograde labeling of a circumscript field within the transition area between the lateral retrobulbar (AON) and the lateroposterior orbitofrontal (LPOFC) cortex which receive input from the MOB. The arrangement of these connections suggests topographical organization of LPOFC-periRC and -proRC and AON-MOB projection neurons.

The present study indicates that the palaeocortices are involved in archicortical and proisocortical olfactory and limbic cortical circuitry and suggests the existence of separate channels connecting circumscript fields of medial OT to discrete subfields of the olfactory proisocortex (periRC and WALKER area 13). Together, our data fit the known scheme of olfactory circuitry, but extend the olfactory links to neo- and proisocortical association fields. However, in contrast to infra-primates, the primate olfactory OT (analogue to the ventral striatum to HEIMER) receives no substantial input from the MOB, pointing to species differences in the involvement of the OT in olfactory signal processing.

Information Flow Through and Between Glomeruli in the Mammalian Olfactory Bulb.

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Conventional optical recording techniques were employed to monitor neural activity caused by stimulation of the olfactory nerve layer (ONL) in 400 µm thick slices of rat olfactory bulb (OB). The voltage-sensitive dye RH155 was used. An improved light detector system allowed neural activity to be simultaneously monitored in 464 contiguous regions of the slice. When the slice was imaged with a 16X water immersion objective, each of these regions corresponded to a 42µ x 42µ x 400µ membrane volume. At the highest available sampling rate, a complete "frame" was recorded every 1.6 msec. To investigate lateral interactions between adjacent glomeruli, a pair of small diameter suction electrodes was placed against the olfactory nerve layer (ONL) with a separation of 0.5 mm. The ONL between the electrode tips was cut to prevent activity spread in the nerve layer. Various pulse paradigms were used to test for spatial and temporal interactions in the responses to the two stimulating electrodes. Following an ONL stimulation pulse, an action potential propagated across the ONL into the glomerular layer (GL). The ONL action potential triggered a large net depolarization that was confined to the GL and lasted about 120 ms. This GL depolarization was invariably followed by a prolonged (> 1.0 sec) net hyperpolarization in the GL. During this hyperpolarizing phase, the response to a second ONL shock was strongly suppressed. In most preparations, activity propagated through the GL into the external plexiform layer (EPL) and mitral body layer (MBL), although the amplitude of these signals was substantially lower than those observed in the GL. While some reduction in amplitude of the EPL and MBL signals would be anticipated because of transection of mitral cell dendrites in the slice preparation, the much larger size of the GL signals may indicate that these responses are not dominated by mitral cell activity, but primarily reflect activity of neuronal elements intrinsic to the GL, i.e., periglomerular and juxtaglomerular neurons. A movie showing the various patterns of evoked activity in the OB slice preparation as well as the results of the paired pulse experiments will be presented.

Descending innervation targets the somata of central olfactory neurons in decapod crustaceans.

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The CNS of arthropods consists of masses of neuropil surrounded by clusters of somata; somata are not integral as they are in the vertebrate CNS. Interactions between neurons are thought to be confined to the neuropil. Here immunocytochemical evidence is presented showing that in diverse decapod crustaceans the lateral soma cluster of the olfactory deutocerebrum is massively innervated by processes of descending neurons. Two different types of processes, both possessing numerous large varicosities, target the cell bodies of the lateral cluster: (1) processes with dopamine-like immunoreactivity originating from the eyestalk ganglia and (2) processes with substance P-like immunoreactivity belonging to a pair of somata in the median protocerebrum (see also Sandeman et al. 1990, J. comp.Neurol. 294: 569-582). Electron microscopical analysis of the lateral cluster of the spiny lobster revealed unusual profiles containing clear and numerous dense-core vesicles. These profiles, which presumably represent the dopamine-immunoreactive processes, are connected to somata by large zones of direct membrane contact and to neurites arising from the somata by classical synapses. The fact that descending neurons provide the innervation of the lateral cluster and specifically target the somata and neurites but not the processes of neurons, strongly indicates a type of neuronal interaction novel to the CNS of arthropods. Experiments are in progress to elucidate the functional role of this novel, descending input in processing olfactory information.

Some of the experiments were performed with support from NSF (88-10261) and DFG (Schm 738/1-1, 1-2).

Taste Representations in the Hamster Solitary Nucleus: Individual Differences and Effect of Stimulus Array.

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The taste representation in the solitary nucleus (SN) has an indistinct topography for taste fields and taste qualities. Projection patterns of the taste nerves overlap and there is convergence from several taste fields. Yet information channels established in the periphery are preserved in the medulla. We studied the taste representation for the anterior tongue in hamsters with multi-unit mapping and single-unit recordings. We stimulated the anterior tongues of anesthetized hamsters (Mesocricetus auratus) with (1) 0.1M sucrose, 0.03M NaCl and 0.1M KCl (stimuli that activate three taste systems of the chorda tympani nerve (CT)); and (2) 0.1M glycine, 0.03M NaOAc, 0.03M NH_4Cl, and 0.01M citric acid. Electrodes were micropipettes containing 4% HRP to mark recording sites. We completely mapped the anterior-tongue taste representation of the SN in 14 brains with array 1. The size of the taste representation, estimated as the ellipsoid encircling all taste-responsive sites, varied by a factor of 25. The amount of neural activity evoked by sucrose, measured as percent of total neural spikes recorded, varied from 15% to 42%. Response profiles of 104 single units to array 1 allowed us to predict responses of the 53 units that were also tested with array 2, except for citric acid. Citric acid was a very effective stimulus for units located lateral to the solitary tract, where there are few units responsive to array 1. Taste behavior of individual hamsters may reflect the varied representations in the SN. Brainstem neurons responding strongly to citric acid, not a strong CT stimulus, may receive trigeminal input.

Pruning of the cortical taste neurons by artificial neural network model.

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Taste quality is believed to be coded in the response patterns across a population of taste neurons, but we report that some neurons are not positively involved in quality coding. Recently we developed a novel method to analyze gustatory responses using artificial neural networks (Nagai et al., NeuroReport 3(9): 745-748, 1992). Three-layer neural networks were trained by the back-propagation learning algorithm, to classify the neural response patterns to four basic taste qualities (1.0 M sucrose, 0.03 M HCl, 0.01 M quinine hydrochloride, 0.03 M NaCl) recorded from the rat cortical taste neurons (n=47). The networks decoded quality information in the untrained input data (0.1, 0.3, 1.0 M NaCl). We hypothesized that the input neurons which have stronger connection weights to the hidden layer, play a more significant role in the taste discrimination than other input neurons in the networks. From the trained network 20 input neurons with weaker connection weights were 'pruned', but the network was still able to discriminate the taste qualities in the input data. The sensitivity of pruned neurons to the basic taste qualities was lower than 27 neurons which remained after pruning. The entropy value (H) indicating the breadth of tuning of neurons was not different between these neuron groups. By the 'pruning' technique we can evaluate a given taste neurons in terms of its relative contribution to the coding of taste qualities.

Whole Cell Patch Clamp Recording of Olfactory Bulb Mitral Cells from *in vitro* Slices: Membrane Properties and Synaptic Responses to Stimulation of the Olfactory Nerve

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The mitral cells of the main olfactory bulb (MOB) receive synaptic input directly from the terminals of primary olfactory neurons. Although the mitral cell and its excitation by the olfactory nerve have been the subject of numerous investigations, synaptic activation of mammalian mitral cells has not been previously studied *in vitro*.

We have recorded the responses of juvenile and young adult (65-250 g) rat OB mitral cells to stimulation of the olfactory nerve layer (ONL) *in vitro* using extracellular and whole cell patch techniques. Olfactory bulbs were cut into 400 µm thick slices in approximately the horizontal plane. Slices were submerged in a recording chamber. Patch clamp electrodes (5-10 MΩ) were guided into the mitral cell layer, which was visible in the field of a dissecting microscope. A bipolar stimulating electrode was placed onto the ONL just rostral to the location of the recording electrode.

Extracellular records of spontaneous and olfactory nerve evoked activity were obtained from 85 mitral cells. Mitral cells typically responded to ONL stimulation with a single spike followed by a 20-30 msec period of inhibition. This excitation-inhibition sequence was followed by a period of excitation lasting more than 1 second. These responses are similar to those recorded *in vivo*

Intracellular whole-cell patch recordings were obtained from 53 mitral cells. Membrane resistances (100 to 600; mean 199 ± 17 MΩ) were substantially higher than those reported in previous studies using sharp electrodes. This suggests that the electrotonic length of mitral cells is significantly less than previously thought.

At membrane potentials near -55 mV, ONL stimulation evoked prolonged depolarization and a train of action potentials in mitral cells. Hyperpolarization of the cell membrane to -65 mV blocked all but the first of these spikes. The first spike could be blocked only by hyperpolarization of the soma to much more negative potentials (-80 to -120mV). These results suggest that ONL stimulation produces an all-or-nothing spike in the apical dendrite and that these spikes an propagate through the soma even when the cell is strongly hyperpolarized.

The present findings indicate that the mitral cell is specialized to efficiently conduct olfactory signals from the apical dendrite to the axon. The insensitivity of the initial spike to hyprpolarization of the mitral cell soma suggests that some spikes generated in the glomerulus are propagated with high efficiency to olfactory cortex. (*Supported by DAMD17-91-C-1071 and NIDCD DC00347 & NS29218.*)

Electrophysiological Studies in Toad Olfactory Receptor Neurons.

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Olfactory neurons from the toad *Caudiverbera caudiverbera* were investigated using a variety of electrophysiological approaches. Isolated receptor neurons were stimulated with puffs of odorant mixture I (M-I: citralva, citronellal and geraniol) or mixture II (M-II: isovaleric acid, triethylamine and pyrazine) and their action potential firing patterns were examined. A fraction of the neurons exhibited an increase in firing rate to M-I and was unaffected by M-II; another fraction was inhibited by M-II and did not respond to M-I and a third fraction was both, excited by M-I and inhibited by M-II. An odorant-induced inward current was found to be associated to excitatory responses, whereas an current underlied the inhibitory responses. These results show that single olfactory neurons can elicit both, excitatory and inhibitory responses. Olfactory neurons were found to posses a same set of V-gated currents. Outward currents consisted of a delayed rectifier and a Ca^{2+}-dependent K^+component. Inward currents were made of a transient and a maintained component. The transient current was abolished in Na^+-free Ringer and was blocked by TTX (K_d = 25 nM). The maintained inward current was suppressed in Ca^{2+}-free Ringer, could be carried by Ba^{2+} and was selectively blocked by Cd^{2+} (K_d = 3 µM). Ion channels in olfactory cilia were investigated by fusing membrane fragments derived from the cilia into planar lipid bilayers. Fusion inserted a variety of ion channels, including a cyclic nucleotide activated, cation-selective channel, an odorant-activated, cation-selective channel, a Ca^{2+}-activated K^+-channel and a Cl^--selective, V-dependent channel.

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Molecular Cloning, Expression and Localization of Inhibitory G-Proteins in the Olfactory Epithelium of Channel Catfish.

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We have shown previously that pertussis toxin markedly inhibits stimulus-dependent IP3 formation in isolated cilia from the catfish and that two pertussis toxin substrates, Gil and Gi2, are expressed in olfactory rosettes. To further investigate the localization and functional role of these G-proteins in olfaction, a cDNA library was constructed from poly $(A)^+$ RNA isolated from olfactory rosettes. Size-fractionated cDNA was directionally cloned into a plasmid vector and propagated in DH5 cells. The library was screened using selective oligonucleotides labeled with digoxigenin-11-dUTP. Several clones that hybridized with each probe were obtained following the first round screening of about 10^5 colonies. Two clones that hybridized with each probe were selected for further study after a second round of screening. The insert sizes of these clones ranged from 2.1-3.0 kbp. To express the corresponding proteins, the inserts from the four clones were subcloned into a baculovirus transfer vector. Sf9 cells were cotransfected with the transfer vector and linearized virus. Recombinant plaques are now being purified for protein expression. In situ hybridization studies are also being per-formed using selective oligonucleotides to study the localization of inhibitory G-proteins in the olfactory epithelium.

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Second Messenger Production in Catfish Olfactory Cilia

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Olfactory transduction in vertebrates is mediated by stimulation of second messenger cascades resulting in depolarization of the olfactory neuron. Previous studies of second messenger production in catfish olfactory cilia have been performed for only a limited set of amino acid odorants, all of which result in increases in ciliary 1,4,5-inositol trisphosphate (IP_3) concentrations (Huque and Bruch, BBRC 137:36-42, 1986 and Restrepo et al., Am. J. Physiol. Cell Physiol. In press). Although these results suggest catfish may rely entirely on IP_3 as the secondary messenger during olfactory transduction, evidence for a cyclic AMP (cAMP) system in catfish olfactory tissue exists (Bruch and Teeter, Chemical Sens. 15:419-430, 1990 and Goulding, et al., Neuron 8:45-68,1992). Therefore, some odorants may activate the catfish cAMP cascade system. We examined odorant-stimulated production of cAMP and IP_3 in catfish olfactory cilia using rapid kinetic methods. Direct stimulation of G-proteins with 1 mM GTP or 20 µM GTPγS results in elevation of both cAMP and IP_3 levels in the millisecond time range (25 - 100 msec). Individual compounds and mixtures (e.g. amino acids, nucleotide, steroid hormones, etc.) were tested using electro-olfactograms (EOGs) to determine whether the catfish olfactory system responds to these compounds. Stimulatory compounds were tested for their ability to elicit cAMP or IP_3 formation using a rapid-quench methodology.

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Expression Of Inositol-1,4,5-Trisphosphate Receptor In Chemosensory Tissue of Channel Catfish and Rat

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In addition to cyclic AMP, inositol-1,4,5-trisphosphate (IP_3) has been implicated as a second messenger in chemosensory systems. A plasma membrane protein has recently been reported that serves as a ligand-gated receptor/channel for this molecule. Attempts are underway to isolate an olfactory-specific cDNA clone of this receptor. A rat cerebellar cDNA probe of the IP_3 receptor was kindly provided by Dr. Thomas Südhof. The membrane spanning region of this probe was used for both genomic Southern and northern analysis. This probe weakly recognized several catfish genes at low stringency. As a positive control, the membrane spanning region of the rat IP_3 receptor cDNA probe was hybridized to rat cerebellar RNA and exhibited a strong hybridization signal near 9.5 kb, the size of the rat cerebellar IP_3 mRNA, and a weaker lower molecular weight signal not due to hybridization of ribosomal RNA. Northern analysis of catfish taste and olfactory RNA yielded no hybridization signals even at low stringency. Similar negative results were obtained with the cerebellar IP_3 probe that corresponded to the soluble portion of the protein. An antisense oligonucleotide identical to the nucleotide sequence of the C-terminal thirteen amino acids of the rat cerebellar IP_3 receptor was kindly provided by Dr. Diego Restrepo. This probe consisted of the nucleotide sequence of the deduced peptide used to generate antibodies for recognition of expressed IP_3 receptor of rat cerebellum. This probe also failed to yield a hybridization signal with catfish olfactory or taste RNA. In contrast, total RNA from rat olfactory tissue exhibited a hybridization signal near 9.5 kb with the membrane spanning region of the rat cerebellar probe. The presence or absence of a lower molecular weight hybridization signal with rat olfactory RNA could not be determined. These results suggest sequence homology between the rat cerebellar IP_3 receptor and the rat olfactory IP_3 receptor, and further suggests that the IP_3 receptor of channel catfish exhibits little or no sequence homology to the rat cerebellar IP_3 receptor.

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Responses to Binary Mixtures of Amino Acids in the Facial Taste System of the Channel Catfish.

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Prior studies indicated that olfactory receptor responses to mixtures of amino acids in the channel catfish, *Ictalurus punctatus*, were enhanced only if the components bound to independent receptor sites (Caprio et al., *J. Gen. Physiol.* 93:245-262, 1989; Kang and Caprio, *J. Gen. Physiol.* 98:699-721, 1991). Here we report the responses of 32 multiunit preparations and 55 single facial taste fibers to binary mixtures of amino acids. Tested were (1) amino acids indicated to bind to independent taste receptor sites (Group I): L-alanine & L-arginine; L-alanine & L-proline; L-arginine & L-proline, L-arginine & L-glutamate, and (2) amino acids indicated to bind to the same or highly cross-reactive taste receptor sites (Group II): L-alanine & glycine; L-alanine & L-methionine; L-arginine and L-alpha-amino beta guanidino proprionic acid, L-alanine & L-glutamate. All component stimuli were adjusted in concentration to provide approximately equal response magnitude - height of integrated multiunit activity (IMA) or number of action potentials generated/3 secs of response time/single taste fiber (STF). The mixture discrimination index (MDI), defined as the response to the mixture divided by the average of the responses to the component stimuli, was calculated for each binary mixture. All Group I binary mixtures resulted in enhanced taste activity [MDI(IMA)=1.16±0.12(SD), n=44; MDI(STF)=1.17±0.20 (SD); n=116], whereas all Group II binary mixtures were without significant enhancement (MDI(IMA)=1.06±0.15 (SD), n=41; MDI(STF)= 1.03±0.14 (SD); n=34). There were no significant differences between the IMA and STF MDIs for Group 1 and Group 2 mixtures, respectively, nor were there differences between the MDIs of the two major fiber types, the alanine and arginine fibers (Kohbara et al. *J. Neurophysiol.* 68:1012-1026, 1992).

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Differential Localization of Putative Alanine and Arginine Receptors in Catfish Taste Buds.

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Whether individual taste buds or taste cells specifically detect single taste substances is important in order to understand the encoding of taste information in the nervous system. This experiment utilizes anatomical means to demonstrate the location of alanine and arginine receptor sites in catfish taste buds. As described previously (Kalinoski et al., Chem Senses, '92), the erythroagglutinin lectin from Phaseolus vulgaris (PhA-E) selectively inhibits the binding of arginine but not alanine to fraction P2 of catfish taste epithelium. In addition, this lectin binds to the apical region of taste buds in histological preparations of catfish barbels (Böttger & Finger, AChemS '92). Conversely, a monoclonal antibody, G-10, was developed (Goldstein and Cagan, '82) to detect alanine binding sites in the same epithelium. For the current experiments, mandibular barbels of channel catfish (*Ictalurus punctatus*) were immersion-fixed for 2 hours in 4% buffered paraformaldehyde and rinsed in buffer; non-specific reactivity was blocked by exposure for 1 hr. to serum albumin and horse serum. The intact barbels then were incubated overnight at 4°C in a mixture of rhodamine labeled PhA-E and antibody G-10, both diluted to 1:100. Following three rinses in buffer, the tissue was exposed to fluorescein-labeled goat anti-mouse secondary antibody to label the G-10 antibody. The barbel then was rinsed several times in buffer and examined under a standard epifluorescent or confocal microscope. The rhodamine and fluorescein binding sites were visualized independently and composite images were constructed. The PhA-E binding appeared as a relatively fine punctate pattern located within the central region of each taste pore; conversely, the G-10 binding appeared as coarser patches distributed around the outer edge of each taste pore. Virtually no overlap occurred between the PhA-E and G-10 binding sites. Accordingly, each taste bud appears to contain both alanine and arginine binding (presumed receptor) sites albeit located at the apices of different taste cells within the taste bud.

Taste Recognition at the L-Alanine Receptor in the Channel Catfish: A Preliminary Model.

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Molecular modeling techniques were used to determine the steric and electrostatic features of the L-alanine taste receptor of Ictalurus punctatus, the channel catfish. Semi-empirical and ab initio calculations were used to determine the energy-minimized conformations of the zwitterions of a series of analogs active at the receptor. A small number of molecular simulations were done to study the effect of solvent on the conformations of those analogs with polar side chains. By superimposing the α-amino and α-carboxylate groups of each of the analogs in their minimized conformations and by identifying the conformations of analogs with the lowest IC_{50}'s as the "optimum" conformations for binding, steric requirements for interaction with the receptor were determined. In addition, a possible electropositive binding site was identified. A molecular electrostatic potential study clarified the nature of this site. This site along with the sites accomodating the α-amino and α-carboxylate groups of the analogs comprise a preliminary model for activity at the L-alanine receptor.

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Structure/Activity Studies of L-glutamate Reception in the Olfactory System of the Channel Catfish, I. punctatus. BRUCE BRYANT, D. LYNN KALINOSKI, JOHN QUINN & RENEE LUCAS (Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104).

Electrophysiological studies have revealed an olfactory receptor for L-glutamic acid in I. punctatus, the channel catfish (Caprio and Byrd, 1984). We have pursued structure/activity studies using both electrophysiological and biochemical approaches to further characterize this receptor site and the requirements for its activation. EOG studies of a series of structural analogues (presented at 10^{-4}M) indicate that a free L-α amino group and a free ω carboxylic acid are both necessary for optimal activity. Substitution of a sulfonic (L-homocysteic acid) but not phosphonic acid (DL-2-amino-4-phosphonobutyric or DL-2-amino-3-phosphonopropionic acids) yields an active analog. Other structural analogues (L-aminoadipic acid, L-glutamic acid-γ-methyl ester and quisqualic acid) may derive their neural activity from activation of receptors other than the L-glutamate site. Studies examining the binding of L-[^{3}H]-glutamate to membranes obtained from olfactory cilia indicate that L-glutamate binding has a K_m of 1.3 μM and a B_{max} of 52 pmol/mg. Competition studies using structural analogues confirmed the above electrophysiological results. Agonists of the known classes of L-glutamate receptors were also used to characterize the catfish glutamate receptor. With the exception of the phosphonic acid analogues, which were only moderately good inhibitors (IC_{50} = 330 and 160 μM, respectively), all other compounds (AMPA, BMAA, NMDA, kynurenic acid, and quisqualate) failed to antagonize (IC_{50} > 1000 μM) the binding of L-[^{3}H]-glutamate. This suggests that the catfish olfactory receptors for L-glutamic acid are discrete from the known classes of CNS glutamate receptors.

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Nitric Oxide Synthetase Activity of the Taste Organ of the Channel Catfish, Ictalurus punctatus. TAUFIQUL HUQUE and JOSEPH G. BRAND, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104.

Recent research suggests that the molecule nitric oxide (NO) is a major regulatory factor that exerts its effects both intracellularly and intercellularly. NO is biosynthesized from L-arginine by the enzyme nitric oxide synthetase (NOS) in a reaction in which L-citrulline is also formed in equimolar amounts. When tissue extracts are incubated with labeled L-arginine, labeled citrulline is formed and is easily measured by cation-exchange chromatography. Using this method, and as a first step towards understanding the role of NO in taste tissue, we have characterized the NOS activity of the taste organ (barbel) of the channel catfish. Barbels were cut off and scraped in 25 mM MOPS (pH 7.1)/0.5 mM EDTA/0.5 mM EGTA. Scrapings were homogenized and then centrifuged at 16,000 g for 5 mins. The supernatant (cytosolic fraction) was passed through a column of AG50WX8 (Na^+ form) to remove endogenous arginine and cations, and then used as the source of NOS in all subsequent assays. Enzyme activity was monitored by measuring the conversion of [^{3}H]L-arginine (1 μCi/ml $\simeq$ 18 nM) to [^{3}H]L-citrulline in a standard assay medium (final volume 0.2 ml) containing 25 mM MOPS (pH 7.1), 1 mM NADPH, 1.8 mM $CaCl_2$ + 2 mM EGTA (yielding a calculated value for free Ca^{2+} of 1.1 μM). Assays were run for 10 mins at room temperature. Reactions were terminated by the addition of ice-cold 4 mM EGTA (0.2 ml) and the tubes placed on ice. The entire reaction mixture was then loaded onto a column of AG50WX8 (Na^+ form) which retained arginine but not citrulline. Citrulline was eluted with 4 ml water and the eluate counted for radioactivity. The amount of citrulline formed was calculated from the specific activity of the precursor arginine. NOS activity was found to be dependent on the presence of NADPH and calcium, omission of these two components reducing activity to non-detectable levels. NOS activity was also greatly inhibited by L-N^G-monomethyl arginine (NMMA) and L-N^G-nitroarginine (NA), both known to be competitive inhibitors of NOS. The enzyme had a K_m of 22 μM and a V_{max} of 25 pmoles/min/mg. When NOS activity was monitored in the presence of Ca^{2+}/EGTA buffers designed to yield calculated values for free Ca^{2+} of 0-1,000 μM, the Ca^{2+}-dependence displayed a bell-shaped profile with a maximum at 1.1 μM Ca^{2+}. The presence of neither calmodulin (0.5 μM) nor the calmodulin antagonist W-5 (100 μM) in the standard assay had any effect on NOS activity. These data suggest that the enzyme is calcium-dependent but calmodulin-independent. NOS activity was increased approximately two-fold in the presence of tetrahydrobiopterin (100 μM) but was not affected by FAD (5 μM). Activity was moderately enhanced, to the extent of 25-50% above basal, in the presence of 1-2 mM levels of kainic acid (KA) and GABA. Enhancement of NOS activity by KA and GABA was strongly reversed in the presence of the NOS inhibitor NMMA, suggesting that the effects of these two putative gustatory neurotransmitters (i.e., KA and GABA) may be mediated, at least partly, through their effect on NO. We conclude that a constitutive form of NOS, similar to that in other tissues, is present in catfish taste tissue and that, therefore, nitric oxide may have a functional role in taste tissue.

Human Olfactory Neurons Respond to Odor Stimuli with an Increase in Cytoplasmic Ca^{2+}. DIEGO RESTREPO[1,2], YUKIO OKADA[1,5], JOHN H. TEETER[1,2], LOUIS D. LOWRY[1,4] and JOSEPH G. BRAND[1,2,3]. [1]Monell Chemical Senses Center, Philadelphia, PA 19104, USA, [2]University of Pennsylvania, Philadelphia, PA, 19104, USA, [3]Veterans Affairs Medical Center, Philadelphia, PA, [4]Thomas Jefferson University, Philadelphia, PA, 19107, USA and [5]Nagasaki University School of Dentistry, Nagasaki 852, Japan.

Significant advances have been made in our understanding of olfactory transduction using animal models. However, relatively little is known about olfactory transduction at the cellular level in humans. We have developed a procedure that results in consistent isolation of viable human olfactory neurons. Cells were isolated from small (1-3 mm^2) biopsies obtained from the septum at the level of the high middle turbinate from patients undergoing elective endoscopic sinus surgery. Olfactory neurons could be easily discerned from sustentacular cells by their characteristic bipolar morphology, by the presence of cilia protruding from the olfactory knob, and occasionally, by the presence of a piece of axon. The isolated neurons maintained a resting potential of -50$\pm$12 mV (n=11) and displayed a membrane resistance of 3.4$\pm$1.7 GΩ (n=5). All neurons displayed sustained outward currents and a few displayed transient inward currents as well. The cells responded to addition of intracellular second messengers (IP_3 added to the pipette or 8-Br-cGMP added to the bath) by depolarizing under current clamp. The isolated olfactory neurons maintained a low resting Ca^{2+} concentration (ranging from nearly 0 nM to 90 nM), and responded to addition of olfactory stimuli (a mixture of 100μM each of citralva, hedione, geraniol, phenylethylalcohol, citronellal, eugenol and menthone) with a reversible increase in [Ca_i], which appears to be caused by influx of Ca^{2+}. This procedure can be used on a routine basis for the study of the cellular physiology of human olfaction.

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Olfactory Signal Transduction in Atlantic Salmon. DENNIS E. RHOADS, LEE-JU CHENG, RICHARD E. WOLKE (Univ. of Rhode Island) and PAUL C. STERNWEIS (Southwestern Medical Center, Univ. Texas, Dallas).

In Atlantic salmon, signal transduction for at least two classes of odors, L-amino acids and bile acids, appears to involve G protein dependent activation of phosphoinositide-specific phospholipase C (PLC). Ca2+ and protein kinase C (PKC) may be important regulators of olfactory sensitivity. In the present study, immunoblotting has been employed to investigate the expression of PKC, PLC and G proteins in the salmon olfactory epithelium. Using isozyme-specific monoclonal antibodies, immunoreactivity to both PKC and PLC-β1 has been identified in Western blots of a plasma membrane rich preparation from olfactory rosettes of Atlantic salmon (Salmo salar). At the light microscopy level, immunohistochemical staining of sections of the olfactory rosettes demonstrated the presence of PLC-β1 immunoreactivity in olfactory receptor cells. This immunoreactivity is unevenly distributed along secondary folds of the rosette, but it is not exclusive to receptor cells. Antibodies to PKC also stained receptor cells as well as non-receptor cells along the surface and interior of the olfactory epithelium. Of the well characterized PLC activities present in mammalian systems, PLC-β1 has been shown to be activated by the G_q family of G proteins. In Western blots of the olfactory plasma membrane rich preparation, specific reactivity was observed with antisera raised against either the specific G protein alpha subunit $G\alpha_{11}$ or the common carboxyl terminal peptide sequence of $G\alpha_{11}$ and $G\alpha_q$. There was no specific reactivity with antisera against $G\alpha_q$ itself. These results provide evidence for expression of specific signal transduction components in Atlantic salmon and may indicate a role for G_{11} or a salmonid homolog of G_{11} in olfactory signal transduction.
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233

Carbocyanine Dyes as Tracers in the Elasmobranch Olfactory System
LAURENCE DRYER (Florida State University).

Carbocyanine dyes are excellent tracers of neural pathways in living or fixed tissue. We have used DiI (1,1'-dioctadecyl-3, 3,3',3'-tetramethylindocarbocyanine perchlorate) and Fast DiI (1,1-dilinoleyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) to trace the primary and secondary olfactory projections in the fixed elasmobranch olfactory system. Although slow to migrate, the dyes provide discrete labeling and excellent resolution. Application of DiI onto the olfactory epithelium shows that the primary olfactory projections are arranged topographically onto the compartmentalized olfactory bulb, as suggested by previous *in vitro* experiments with biocytin. If applied onto the olfactory tract, the same dye migrates retrogradely within the cell membranes to reveal the morphology of the mitral cells. The mitral cells belong to the two types described previously from Golgi preparations. Both types lack basal dendrites. In some of these preparations, it was possible to observe labeled primary receptor neurons in the olfactory epithelium. These appear to belong to two morphologically distinct cell types. In other experiments, Fast DiI was injected into the olfactory tract and allowed to migrate anterogradely to the telencephalon. In both embryos and adults, the secondary projections are restricted to the lateral pallium, dorsal pallium, and striatum. In addition, embryos exhibit contralateral secondary olfactory projections. This aspect is still under investigation in adults.

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234

Sodium-Dependent Action Potentials in the Dendrites of Mudpuppy Olfactory Neurons. ADRIENNE E. DUBIN and VINCENT E. DIONNE (Department of Pharmacology, University of California San Diego)

Most accounts of neuronal membrane features suggest that dendrites have non-spiking electrical properties and that action potentials are generated by depolarization of the axon hillock. Using patch recording methods, we have examined the membrane properties of dendrites isolated from olfactory receptor neurons. Action potentials could be elicited in isolated dendrites as well as intact neurons by depolarizing current pulses. Isolated dendrites showed a fast inactivating inward current with a peak magnitude of -75 $\pm$ 29 pA/pF (m$\pm$SD, n=22) normalized to membrane capacitance, a value similar to that observed in the intact neuron. Greater than 80% of the dendritic inward current was blocked specifically and reversibly by tetrodotoxin (1 μM), a selective Na^+ channel blocker. The Na^+ current was activated by depolarization at a threshold of -47 $\pm$ 6 mV (n=20). Its peak-current/voltage curve showed a half-activation voltage of -27 $\pm$ 9mV (n=20) with maximum current at -5 mV, and it had a half-inactivation voltage of -60 $\pm$ 6 mV (n= 18). By comparison, in the intact neuron, $\geq$84% of the inward current was TTX-sensitive with an activation threshold of -48 $\pm$ 7 mV (n=28). The half-activation voltage was -30 $\pm$ 8 mV (n=19) with maximal current at -5 mV, and the half-inactivation voltage was -54 $\pm$ 8 mV (n=29). Thus activation and inactivation parameters of dendritic Na^+ currents are similar to those of the whole cell. The whole-cell Na^+ currents were insensitive to two intracellular second messengers. Neither the magnitude nor the voltage dependence of activation and inactivation were affected by the addition of cAMP (100-500 μM; n=3) or of IP_3 (10 μM; n=3) to the recording pipet. Exposure of isolated dendrites to a membrane-permeable analogue of cAMP (750 μM 8CPT-cAMP, 45-60 sec; n= 5) had no effect on the properties of the current. The presence of a large Na^+ conductance in the dendrites of olfactory neurons may be important for odor transduction since focal modulation of the apical membrane conductance by odors could alter dendritic excitability.

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235

Conductances in the Ciliated Dendrites of Mudpuppy Olfactory Receptor Neurons. ADRIENNE E. DUBIN and VINCENT E. DIONNE (Department of Pharmacology, University of California San Diego)

The short latency between exposure of olfactory neurons to odors and the initial neuronal response[1] suggests that odor-sensitive conductances must be located close to odor receptor sites, probably in ciliary and dendritic membrane. Previously, we showed that odorants can modulate at least 3 different conductances in mudpuppy olfactory neurons, a Cl^-, a K^+ and a non-selective cation conductance[2]. Using patch recording, we have now examined isolated, ciliated olfactory dendrites for the presence of Cl^- and K^+ conductances to determine if they are found close to the apical site of odorant action and to evaluate their effect on cell excitability. The majority (>80%) of the whole cell Cl^- conductance is located in the dendrites and is reversibly and specifically blocked by niflumic acid (IC_{50} $\approx$ 20 μM) and by DIDS (IC_{50} $\approx$ 30 μM). From ramp-induced currents measured on-cell in the presence and absence of Cl^- channel blockers, we estimate a normal Cl^- equilibrium potential of -46 $\pm$ 8 mV (m$\pm$SD, n=11). Odor activation of the dendritic Cl^- conductance should depolarize a resting cell, with a modest effect causing increased excitability and a large effect causing inhibition by inactivation of Na^+ channels. An odor-induced reduction of the Cl^- conductance would hyperpolarize the cell and cause inhibition. Olfactory dendrites also possess a Cs-sensitive K^+ conductance at a density similar to that seen in the soma. Odor-induced inhibition of the dendritic K^+ conductance should have little effect on membrane potential because much of the conductance in the rest of the cell would be unaffected; however, reducing the dendritic K^+ conductance should make the dendrite more excitable. Since dendrites have a large Na^+ channel density and can support action potentials, a focal depolarization of the dendrite could then activate spiking. This effect could underlie the "silent" modulation of excitability by odorants seen in these cells[3].

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236

Expression of Members of a Putative Olfactory Receptor Family in Salamander Olfactory Epithelium. ALEXANDER JESURUM, DONA M. CHIKARAISHI and JOHN S. KAUER (Neuroscience Program, Department of Neurosurgery, Tufts Medical School/New England Medical Center Hospitals, Boston, MA, 02111)

Odor recognition in vertebrates is mediated by the sensory neurons of the olfactory epithelium. It is here that odorants are believed to interact with highly specific receptors on the cilia of receptor cells. Recently, a large family of putative olfactory receptors has been cloned from the rat (Buck and Axel, 1991). We have used polymerase chain reaction (PCR) to amplify putative olfactory receptors from the tiger salamander (*Ambystoma tigrinum*). Sets of degenerate oligonucleotide primers based on conserved regions of Buck and Axel's sequences were synthesized and used for low stringency PCR. Products of predicted size were observed with reactions using mRNA derived from salamander olfactory epithelium. Similar reactions using mRNA derived from heart, brain and forelimb muscle did not yield discernible PCR products. Putative olfactory receptor sequence fragments were isolated from the PCR mixture by subcloning the PCR products into pBluescript vectors, followed by DNA sequencing of the subclones. Sequence analysis results show that seven of these clones are novel members of the seven-transmembrane spanning G-protein linked receptor family. The deduced amino acid sequences reveal conserved amino acid residues and presumed transmembrane domains found in these polypeptides. These preliminary data show that mRNA for several putative receptors are expressed in the salamander olfactory epithelium, supporting the suggestion that these polypeptides are involved in odor processing by the receptor neurons. *In situ* hybridization using ribonucleotide probes is currently being performed to characterize the distribution of these putative olfactory receptors in the epithelium.

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237

Imaging the Salamander Peripheral Olfactory System: Structure/Activity Relationships for Two Homologous Odorant Series. JOEL WHITE and JOHN KAUER (Neuroscience Program, Tufts/NEMC, Boston, MA 02111).

In the olfactory system, it is hypothesized that odorant quality information is carried in the pattern of activity distributed across cells at each level of the pathway. Exactly how different patterns relate to different odorants is currently unknown. In an initial investigation of odorant structure/activity relationships, we have studied olfactory responses in the tiger salamander (*Ambystoma tigrinum*) to two series of homologous odorant compounds: propyl, butyl, and amyl alcohol and propyl, butyl, and amyl acetate. We monitored these responses by video imaging voltage-sensitive dye signals from the olfactory epithelium and bulb after staining with the styryl dyes Di-4-ANEPEQ (gift of Dr. Leslie Lowe) and Di-4-ANEPPS, respectively. In the ventral epithelium, each odorant (10^{-1} dilution) elicited depolarizing optical signals with time courses similar to electro-olfactograms taken after optical recording. The alcohols elicited relatively small areas of activity, although the amyl alcohol signal was larger than that for propyl or butyl alcohol. The acetates elicited large areas of activity; for the three odorants, these areas overlapped but also showed discernable differences in spatial distribution. In the bulb, the alcohols elicited long-lasting hyperpolarizing signals. Responses to propyl and butyl alcohol were similar in that they were large and long-lasting, whereas the amyl alcohol response was small and brief. In contrast, the acetates elicited long-lasting depolarizing signals. The response to propyl acetate was small and brief, whereas responses to butyl and amyl acetate were similarly large and long-lasting. These responses were seen when odorants were diluted 10^{-1} or when odorants were diluted to the same molar concentration (approx. $3x10^{-5}$M). The dye fluorescence activity patterns evoked by the alcohol series and the acetate series were thus discriminable in both the epithelium and bulb. The three odorants within each series were also partially discriminable (i.e., the bulb response to propyl acetate was noticeably different from that to butyl and amyl acetate). The degree of bulbar discrimination corresponds to the degree of behavioral discrimination reported by Mason and Stevens (1981, *Physiol. Behav.* 26:647), suggesting that physiological correlates for these behavioral data are detectable in the olfactory bulb.

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238

Odor Responses of Olfactory Receptor Neurons Measured with with Voltage-Sensitive Dye Confocal Microscopy. ROBERT C. GESTELAND, JAN BROUWER, PEGGY FARMER, & BARBARA CINCUSH (University of Cincinnati College of Medicine, Cincinnati, OH 45267-0521).

Records of responses of olfactory receptor neurons (ORNs) acquired from single cell microelectrode studies are not adequate to determine how odor identity is coded. This is because each experimental preparation yields only a small amount of information. Measurements of odor-evoked voltage-sensitive dye fluorescence intensity changes acquired with a laser scanning confocal microscope overcome this limitation. The microscope allows each cell in an epithelium viewed *en face* to be distinguished and measured quantitatively. Odor evoked dendritic membrane depolarizations an order of magnitude above background noise are readily acquired. Odors and blank control stimuli are presented as vapors to excised amphibian epithelia. Odor durations are 1 sec. The epithelium is in an oxygenated Ringer bath for 5 minutes between stimulus presentations. Prior to each presentation the chamber Ringers solution is lowered so that liquid diffusion distance for the odor is minimized. Microscope magnification is chosen so that each field of view includes 150-200 ORN dendrite profiles. The majority of these are loaded to some extent with the dye. 20-30% of these are near the upper brightness limit and will produce responses well above noise. The image brightness is stretched so that changes in these brightly-illuminated cells are discriminable by the 8-bit pixel registers. To measure the responses, the brightnesses of 225 pixels within a square area enclosing the dendrite profile are summed before the stimulus presentation and subtracted from the summated pixel brightnesses in the same area at the response maximum. The results show that ORNs are differentially sensitive to different odors, that the number of cells responding to a particular odor are different for different odors, that increased odor concentration results in a shorter time to response peak, and that repeated presentations of an effective odor results in declining response amplitudes.

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239

IP_3 Receptors May Occur in Supporting Cell Microvilli of Rat Olfactory Epithelia. BERT Ph. M. MENCO (O.T. Hogan Hall, Northwestern University, Evanston, IL 60208, USA), CHRISTIAN DELLACORTE, D. LYNN KALINOSKI, DIEGO RESTREPO (Monell Chem. Senses Center, 3500 Market St., Phila., PA 19104).

There is physiological, biochemical (Restrepo et al., Am. J. Physiol. 32: C667, 1992; Ronnett and Snyder, TINS 15: 508, 1992), and ultrastructural evidence that suggests that IP_3 receptors (Ronnett and Snyder, TINS 15: 508, 1992) are involved in olfactory transduction. The receptor which has been identified in plasma membranes of olfactory cilia is thought to allow cations such as Ca^{2+} and Na^+ to enter the cell. Influx of Ca^{2+} affects various components of the transduction process. We examined the ultrastructural pattern of immunocytochemical binding with a polyclonal antibody generated against the 19 C-terminal amino acid residues of the rat cerebellar IP_3 receptor (Mignery et al., Nature 342: 192, 1989). Western blots using this antibody detected two polypeptides of 120 kD and 230 kD in membranes of olfactory cilia preparations. A 120 kD protein has previously been identified in such preparations with ^{125}ASA-IP_3 photoaffinity labeling (Restrepo et al., Am. J. Physiol. 32: C667, 1992). With light microscopy, the antibody bound to the olfactory epithelial surface. Ultrastructurally, we found no distinct pattern of labeling in unfixed rat olfactory epithelia processed with rapid freeze, freeze-substitution and Lowicryl K11M embedding (Menco et al., Neuron. 8: 441, 1992). However, sections of tissue obtained after transcardial perfusion, 4% paraformaldehyde and cryoprotection with glycerol (after Van Lookeren Campagne et al., J. Histochm. Cytochem. 39: 1267, 1991) displayed distinct labeling in supporting cell microvilli, suggesting that these organelles possess a protein with antigenicity resembling that of the C-terminus of the cerebellar IP_3 receptor. Therefore, these microvilli may perform a function that requires IP_3 receptors.

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240

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

The effect of intracellular application of inositol 1,4,5-trisphosphate (IP_3) from the patch pipette was analyzed in isolated rat olfactory neurons under whole-cell patch clamp. Intracellular dialysis of 10 μM 1,4,5-IP_3 in K^+-internal solution induced a sustained depolarization of 36 ± 11 mV (mean ± SD, n=16). The IP_3-induced response was observed in 75% of trials, but not when 10 μM ruthenium red was also included in the pipette. Lower concentrations (50-100 nM) of 2,4,5-IP_3 induced similar responses in 63% of trials. Steady-state I-V relationships of IP_3-gated currents with K^+-internal solution were classfied into two types: slightly outwardly rectifying and N-shaped. In Cs^+-internal solution linear and slightly outwardly rectifying patterns were observed. The IP_3-induced currents were inhibited by external 1 mM Cd^{2+}. In contrast, the Ca^{2+}-ionophore ionomycin (5 μM) hyperpolarized the olfactory neurons and greatly potentiated the outward currents at positive holding potential. It is concluded that IP_3 can depolarize rat olfactory neurons without mediation by intracellular Ca^{2+}.

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Carnosinase-Immunoreactivity in Human Nasal Mucosa

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Carnosinase is a peptidase that hydrolyzes carnosine into ß-alanine and L-histidine. Previous biochemical and immunohistochemical studies demonstrated that carnosine is abundant in the primary olfactory pathway of chickens, rats and humans (Margolis et al, 1980; Boldywev, 1990; Sakai et al, 1990) as well as in skeletal muscle and many histamine-rich tissues. The cellular localization of carnosinase in human nasal mucosa was investigated using a polyclonal antibody to mouse kidney carnosinase generously provided by Dr. Frank Margolis, and standard ABC immunohistochemical and simultaneous double-staining immunofluorescence techniques. Human nasal mucosa was obtained at autopsy from fourteen subjects, 5 of which had Alzheimer's disease. Olfactory mucosa was identified unequivocally by the presence of olfactory marker protein (OMP) immunoreactive receptor neurons within the epithelium. In the olfactory mucosa, carnosinase immunoreactivity was localized in the supranuclear region of sustentacular cells, and in the acinar cells and lumen of Bowman's glands. Carnosinase immunoreactivity was also identified in the perinuclear region of a small number of olfactory receptor neurons by double staining with antibodies to carnosinase and neuron-specific enolase. In respiratory mucosa, intense carnosinase immunoreactivity was localized in the secretory granules of the goblet cells in the epithelium and in the acinar cells and lumen of serous and mucous glands in the lamina propria as well as in the mucociliary complex. These results suggest that one of function of carnosinase in the nasal mucosa may be to provide amino acid precursors for protein synthesis in secretory cells and possibly in a small subset of olfactory receptor neurons.

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Expression of Members of the Trk Neurotrophin Receptor Family in Rat Olfactory Epithelium. MARK P. HATTON AND BARBARA R. TALAMO (Tufts Medical School , Boston, MA, 02111)

The neurotrophins are a family of related proteins which support the development and survival of various populations of neurons throughout the life of an animal. These proteins bind to at least two cell surface receptors which are distinguishable based on their binding affinity. The low affinity receptor ($Kd=10^{-9}$) binds each of the members of the neurotrophin family with similar affinity, while the high affinity receptors ($Kd=10^{-11}$) bind only one neurotrophin each. The olfactory receptor neurons possess a unique ability to undergo neurogenesis throughout the entire adult life of many animals. Relatively little is known about the regulation of neuronal turnover or about the molecules involved in the development of the olfactory epithelium. Although positive immunocytochemical reactions for nerve growth factor and the low affinity neurotrophin receptor in the olfactory epithelium have been reported, mRNA for these proteins have not been identified in this tissue. We have employed reverse transcriptase- polymerase chain reaction cloning to examine the expression of members of the trk family of neurotrophin receptors in the olfactory epithelium. Our preliminary data indicate that mRNA for both trkA and trkB is expressed in the olfactory epithelium, suggesting that their respective ligands, nerve growth factor and brain-derived neurotrophic factor, may contribute to the maintenance or turnover of receptor neurons in the olfactory epithelium. We are currently performing *in situ* hybridization to precisely localize the mRNAs for these receptors in an attempt to gain insight into their function in this tissue. The results from this work will contribute to our goal of identifying signals involved in the development and turnover of the olfactory receptor neurons.

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Interleukin-1 Expression in Normal and Traumatized Olfactory Epithelial Tissue. ANDREA DELKESCAMP and JOEL MARUNIAK (Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211).

Our lab has an ongoing effort aimed at elucidating the presence and function of growth factors in the mammalian olfactory system. The distribution of interleukin-1ß, (IL-1ß) was investigated in the olfactory epithelium of normal mice and mice which had undergone either bulbectomy or unilateral naris closure. Previous studies in our lab have shown that unilateral naris closure causes chronic low-level trauma to the olfactory epithelium on the open side of the nose, probably as a consequence of prolonged unilateral breathing. Using monoclonal and polyclonal antisera, we have found that in normal tissue, IL-1ß is present in caudal regions of deep Bowman's glands. Following bulbectomy, IL-1ß immunoreactivity appeared in the supporting cells as well as in the deep Bowman's glands all along the ipsilateral side. A similar pattern of immunoreactivity appeared on the open side following naris closure. These increases in immunoreactivity on the bulbectomized and open sides of the nose suggest that IL-1ß expression might be induced by trauma. It is known that the IL-1's are released by dying cells, appear in the brain after injury, and are important modulators of the immune system. Importantly, the interleukins appear to be involved in the attraction of polymorphonuclear leukocytes to sites of inflammation; thus, the enhanced secretion of IL-ß on the open side may function to mediate the immune system response to the trauma on that side of the nose. We are presently investigating the distribution of IL-1α in normal, bulbectomized and mice which have undergone naris closure.

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Comparative Analysis of Activity and Distribution of Nasal Carboxylesterases (CE) in Nasal Tissues and Olfactory Bulbs.

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Carboxylesterase (CE) activity is high in the nasal mucosae and can produce acid metabolites toxic to the olfactory epithelium. The regional distribution and activity of the CE enzymes have been studied in rodents, but no studies have compared this regional localization or activity across other species such as dogs or humans, nor examined the effect of aging. We determined the immunohistochemical distribution of CE in the respiratory and olfactory mucosae of Beagle dogs and the respiratory mucosa of the human nose and compared these distributions to those in the F344/N rat. In the dog the greatest CE immunoreactivity was in the subepithelial glands and surface epithelial cells of the respiratory mucosa. In the olfactory mucosa, immunoreactivity was observed in the apical portion of the sustentacular cells, and in duct cells and acinar cells of Bowman's glands. This distribution is similar to that found in rat except the subepithelial glands of the rat respiratory mucosa showed little to no immunoreactive CE. The human respiratory mucosa showed immunostaining in surface epithelial cells as well as glandular cells. Immunostaining in the human tissue samples was dramatically reduced in the presence of hyperplastic lesions and virtually eliminated in samples with squamous metaplasia. We have also compared metabolism of amyl acetate by CE in the olfactory mucosa and olfactory bulbs in both dogs and rats. In addition, we have compared activity in these tissues from 15-wk or 21-mo old rats. Our results indicate a two-site model best fits amyl acetate metabolism in olfactory mucosa for all groups. Similar levels of activity were observed across both species for all tissues, supporting the validity of interspecies extrapolation. However, the immunohistochemical differences observed in our human samples suggest mucosal damage may alter predicted metabolism. (This work supported by DOE Contract DE-AC04-76EV01013 and NIH Grant # RO1-DC-01714-01)

Altered Cellular Distribution of Mouse Olfactory Cytochrome P450 Immunoreactive Isozymes by Dichlobenil is Inhibited by Metyrapone.

ERIC WALTERS (Roche Institute for Molecular Biology, Nutley, NJ), KAY BUCHHEIT AND JOEL MARUNIAK (Biological Sciences, Univ. of Missouri, Columbia, MO).

We examined immunoreactivity obtained using antibodies to rabbit olfactory-specific cytochromes P450-NMa and P450NMb after treatment of mice with the herbicide, dichlobenil (2,6-dichlorobenzonitrile). Twenty-eight hours after a single dose (12 mg/kg) of dichlobenil, dorsomedial regions of the olfactory mucosa showed signs of necrosis. Accompanying this was a dramatic reduction in P450 immunoreactivity in Bowman's glands in the olfactory mucosa, and apparent redistribution of P450 immunoreactivity within sustentacular cells. Treatment of mice with metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone), a P450 inhibitor, at 10 minutes prior to and 2,4,6,and 8 hrs after a single dichlobenil injection, dramatically inhibited the damaging effects of dichlobenil. In addition, P450 immunoreactive patterns in metyrapone/dichlobenil treated mice were similar to controls. Western blot analysis of P-450 immunoreactivity after dichlobenil or metyrapone/dichlobenil treatment was consistent with immunohistochemical findings. These studies support previous reports that dichlobenil-induced olfactotoxicity is cytochrome P450 mediated.

Androgen Regulation of ß-glucuronidase Expression in Different Strains of Laboratory Mice: On the Enzyme Involvement in Pheromone Activation.

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The murine ß-glucuronidase (GUS) (ß-D-glucuronoside glucuronosohydrolase, EC 3.2.1.31) gene complex, located on chromosome 5, provides a unique model system for the study of the evolution of mammalian chemocommunication (Paigen, 1992). This enzyme appears to be crucially involved in the processing and regulation of mouse urinary pheromones (Novikov, 1988). We report here data comparing the effect of testosterone on GUS in male house mice of the CBA/Sto and C57BL/6Sto strains. Mature males of the latter strain are known to be pheromone deficient (Novikov, 1988). Levels of GUS in the urine, kidneys, and salivary and preputial glands were assessed. Distinct interstrain, sex, tissue and seasonal differences in GUS expression were observed. These differences may depend on specificity in regulation of GUS gene activity and/or on feedback inhibition by glucaro-1,4-lactone. Our results are discussed in terms of the possible role of murine GUS in the biotransformation of glucuronides into free and volatile physiologically active substances (pheromones).

Expression of Amyloid Precursor Protein Isoforms in Rat Olfactory Epithelium. NIKHAT ZAIDI AND BARBARA R. TALAMO (Tufts Medical School, Boston, MA, 02111)

Amyloid peptide precursor (APP) is a normal cell protein which is the source of the abnormally deposited peptide (ßA4) of amyloid plaques in Alzheimer's disease (AD) brain. Abnormal expression or processing of APP has been suggested to trigger the pathologic neuronal changes in AD. The normal biological function of APP is unknown. Olfactory receptor neurons provide a good model for examining both the normal role of APP in neuronal differentiation and neurite outgrowth and in pathological processes, since these neurons continue to be generated and grow axons throughout life and show pathological neurite outgrowth and expression of abnormal protein in humans with AD and certain other clinical conditions. In preparation for studying the regulation and role of APP expression and processing in olfactory receptor neurons during differentiation and during experimentally stimulated degeneration, regeneration and neurite outgrowth, we examined which isoforms of APP are expressed in rat olfactory epithelium. There are at least five different alternatively spliced forms of APP. Both soluble and membrane-associated forms of APP are known. APP 695 (695 aa) is the most abundant form in the brain, while 751 and 770aa forms predominate in peripheral tissues. We used reverse transcription -polymerase chain reaction (RT-PCR) to study the expression of mRNA for various isoforms of APP in olfactory epithelium. Our preliminary data indicate that three classes of APP mRNA are expressed in olfactory epithelium. Restriction digest patterns of these isoforms also confirm that these are 695, 751 and 770 forms of APP. The ratio of these various mRNAs appears to differ from that found in brain, where the 695 form predominates. Experiments using quantitative PCR are in progress to estimate the relative amounts of these isoforms. In-situ hybridizations also are being carried out to study the cellular localization of APP mRNAs in olfactory epithelium. Our next goal is to study the localization and regulation of APP isoforms during neuronal degeneration and regeneration following bulbectomy in preparation for future studies of APP expression in pathological states.

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A Ventrally-Projecting Subset of Olfactory Receptor Neuron Axons in Rats Displays Anti-Tau Protein Immunoreactivity. THOMAS A. SCHOENFELD, HENRY M. MELTSER AND AMY L. MAY (Depts. of Psychology and Biology and the Neuroscience Program, Clark University, Worcester, MA 01610)

Microtubule-associated protein 1B (MAP 1B) is ubiquitously expressed in olfactory receptor neurons and their axons in the olfactory nerve layer (ONL) of the olfactory bulb (Schoenfeld et al., 1989; Viereck et al., 1989; Talamo et al., 1989, 1991). On the other hand, another MAP, tau protein, does not show the same widespread pattern of distribution (Viereck et al., 1989; Talamo et al., 1991). In the present study, we have examined sections of adult rat olfactory bulb processed by immunocytochemistry with monoclonal antibody to tau protein (Tau-1). Consistent with the findings of Viereck et al., (1989), the vomeronasal nerve shows anti-tau immunoreactivity (IR). However, contrary to their report, the ONL is not devoid of tau IR but displays numerous, isolated immunoreactive olfactory nerve fascicles that are directed almost exclusively to the ventral olfactory bulb, both medially and laterally. Although the terminals of these axons in glomeruli are largely unstained, in rare cases immunoreactive axons extend into immunoreactive glomeruli. Since the pattern of anti-MAP 1B IR does not reveal negative fascicles in the ventral ONL that might correspond to tau-positive fascicles, we infer that the small number of tau-positive fascicles also express MAP 1B. The expression of tau in a small subset of ONL axons is consistent with the findings of Talamo et al. (1991) that anti-tau IR is localized to only a small subset of all olfactory receptor neurons in the primate. The distinguishable expression of MAP 1B and tau protein in olfactory receptor neurons and their axons provides a means to better understand the particular roles of these MAPs in regulating neuronal outgrowth and stability and to discover whether they mark olfactory receptor neurons of different age or developmental state.

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249

Sub-cellular localization of N-acetylglucosamine-containing glycoconjugates in the salamander olfactory mucosa.

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Ultrastructural localization of N-acetylglucosamine (GlcNAc) residues in the salamander olfactory mucosa was demonstrated by using a sugar-specific lectin as a molecular probe. Ultrathin sections of tissue embedded in Lowicryl K4M were incubated directly with the lectin *Datura stramonium*, which binds to GlcNAc residues, conjugated with 15nm gold particles (DSA-gold complex). Observations of the cellular compartment at the ultrastructural level showed that labeling was greatest in secretory granules (SG) of acinar cells of Bowman's glands (46.7 ± 6.8 gold particles/um^2). The density of gold particles in secretory vesicle (SV) of sustentacular cells (25.4 ± 2.9 particles/um^2) was about 1.8-fold less than that in SG. Observation of the mucosensory compartment showed that the mucus consisted of an electron-dense domain (hs) that lies superficial to an electron-lucent domain (hd). The mean density of gold particles was 25.1 ± 1.9 in hs and 11.3 ± 1.4 in hd. The electron density of the matrix and the distribution of gold particles in this compartment was not homogeneous. For example, hs was subdivided into an electron-dense matrix (hsD) and smaller-area, electron-lucent domains (hsL) that ranged in area from 0.02 to 1.9 um^2 and exhibited a 4.8-fold lower binding of the probe than hsD. In hd, there were trabeculae that projected from the epithelial surface and that bound a high density of gold particles. The olfactory cilia appeared to be surrounded by an electron-lucent sheath that was similar in appeared to hd. A comparative quantitative analysis of GlcNAc binding in SG of Bowman's glands and SV of sustentacular cells suggests that hs is derived from Bowman's glands and hd is derived from sustentacular cells. The sugar specificity of the DSA was confirmed with N,N',N"-triacetylchitotriose in a sugar inhibition control that resulted in 30-50% decrease in lectin binding. These results demonstrate the microchemical heterogeneity of cellular and mucosensory compartments.

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250

Retroviral labeling of clonally related cells in rat olfactory epithelium. JOSEE M.T. HUARD, MARLA B. LUSKIN, STEVEN L. YOUNGENTOB AND JAMES E. SCHWOB (Department of Anatomy and Cell Biology and Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210)

The permanent incorporation of replication-incompetent retrovirus into the genome of dividing cells has been applied with great success to the study of lineage relationships during neural development. We have been employing a similar approach to identify the progeny of indivivual dividing cells in the olfactory epithelium. *Lac Z*-containing, modified Moloney murine leukemia virus produced by the BAG64 cell line was used to generate clusters of labeled cells in two settings. First, we made direct injections of the retrovirus into the olfactory mucosa; with this method, we cannot say whether all the labeled cells in a cluster derive from a single founder cell, but the epithelium should be minimally perturbed. Second, we infused retrovirus into the nasal cavity of rats whose olfactory epithelium was lesioned 1 or 2 days previously by the inhalation of methyl bromide (MeBr) gas; in this case, application of relatively dilute retrovirus offers the opportunity to label individual dividing cells dispersed throughout the nose and thereby generate clonally-related progeny. In both cases animals were perfused and stained 10-14 days after virus. Direct intramucosal injection generates clusters of labeled cells that are smaller on average than ones seen after intranasal infusion in lesioned rats. For example, after virus infusion, a cluster may contain more than 100 labeled cells. With either type of labeling protocol, the clusters contain both neurons and basal cells (which are identified immunohistochemically). After MeBr lesion and retrovirus infusion, the clusters are few and widely separated, which indicates that the clusters are likely to be derived from a single virus-infected progenitor cell, i.e. clonal in nature. On the basis of the large size of some of the clones observed after lesion it is likely that individual basal cells can rapidly amplify the proliferating population during the acute recovery from injury; we would suggest that this amplification is a consequence of daughter cells remaining in the mitotic cycle through several rounds of mitosis.

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251

Cross-adaptation of response to odor and electrical stimulation in rat olfactory epithelium. J.W. SCOTT, P.I. EZEH, and B. MICHELL (Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia, 30322)

In another abstract (Ezeh and Scott), we showed local variations in the sensitivity of the electroolfactogram (EOG) to different odors. We also showed that population spikes with latencies of 10-20 msec. could be evoked in the epithelium by electrical stimulation of local regions of the olfactory bulb nerve layer. These population spikes were blocked during the peak of the EOG response evoked by strong odor stimulation and were diminished for a few hundred msec. after the peak EOG response. This indicates that the antidromic spike in the receptor cell axons collided with spikes produced by odor stimulation and that both the EOG and the population spike were produced by the same cells. We also tested cross-adaptation of responses to odor pairs. Presenting an adapting stimulus of amyl acetate abolished responses to a test stimulus of amyl acetate at the same concentration. Low concentrations of amyl acetate did not block responses to ethyl butyrate, cineole, or limonene at concentrations that produced responses equal in size to the adapting stimulus. These results indicate that it is possible to characterize the receptor inputs to local regions of the olfactory bulb and to give an approximate estimate of the interactions between test odor stimuli in the receptor populations projecting to those regions.

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252

Effects of the Herbicide Dichlobenil on Olfactory Function in the Rat. L. HASTINGS, A. ANDRINGA and M.L. MILLER (University of Cincinnati, Cincinnati, Ohio 45267-0056)

Dichlobenil (DCB)(2,6-Dichlorobenzonitrile), an herbicide, is a potent olfactotoxin. Following a single ip injection of 50 mg/kg DCB, extensive destruction of the olfactory epithelium (OE) and Bowman's glands was observed (Brandt et al, 1990). However, the functional status of the olfactory system was not evaluated. Many compounds severely damage the OE, but few have permanent effects. To investigate the structural and functional damage resulting from DCB exposure, adult Long Evans rats were trained on two go/no-go discrimination tasks, one employing a light cue, the other an odor cue. DCB (50 mg/kg, ip) produced no effect on either of the two discrimination tasks. Two weeks after the initial exposure, the rats were given a second injection (100 mg/kg). Again, no functional decrement was observed. After a single injection of 200 mg/kg DCB, performance on the visual discrimination task remained at pre-exposure levels (90%) while performance on the odor trials dropped to chance levels (50%). However, by day 5, performance on the odor task was nearly normal. Separate groups of rats exposed to 25 mg, 50 mg, or 50 mg followed by a second dose of 50 or 100 mg/kg DCB were sacrificed serially for histology. The lowest dose produced minor changes in globose basal, mature sensory bipolar, sustentacular and microvillar cells, and in glands in the lamina propria, whereas 50 mg/kg caused vacuolization and gross sloughing of the OE, and depletion of the Bowman's glands. The glands were reduced or eliminated at 4 days, but began to reappear at day 11, being within normal limits 25 days after exposure. The number of globose basal cells increased relative to the mature bipolar cells. The OE was fairly normal 25 days after treatment, though small intraepithelial nodules developed. Prominent alterations in the architecture accompanied regeneration.

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Localization of Presumptive Olfactory Neurons in Cultured Neurogenic Olfactory Epithelial Spheres (NOESs). S.K. PIXLEY, L. HASTINGS, D. MILLER, M.L. MILLER. (Univ. of Cincinnati, Cincinnati, OH 45267-0521).

In neurogenic cultures of newborn rat olfactory cells, olfactory receptor neurons (ORNs) are found in spheres that average 30 μm in diameter (Pixley, Neuron, 1992) after fifteen days in culture. These Neurogenic Olfactory Epithelial Spheres (NOESs) were fixed, embedded in plastic and sectioned. We found, first, that the NOESs were hollow spheres. Second, neurons were almost exclusively located in the external layer of the walls of the NOESs. Neurons were identified by immunostaining for Neuron-Specific Tubulin (NST), (all neurons), or for Olfactory Marker Protein (OMP), (mature ORNs) before sectioning.

NOES sections stained with toluidine blue were examined at high power in the light microscope. Presumptive olfactory neurons were identifiable by their bipolar nature and the presence of a nucleus with condensed chromatin. These often exhibited a cytoplasmic basophilia. Both nuclear and cytoplasmic characteristics were similar to those of ORNs in tissue sections of the adult olfactory epithelium. In some cavities, the lining cells included ciliated cells (resembling respiratory epithelial cells) and cells with mucus-type inclusions (resembling either respiratory or Bowman's gland cells). This suggests heterogeneity in the NOESs.

To further study the localization of the bipolar cells and the details of the cavity structure, one NOES was serially sectioned and stained with toluidine blue. All sections were viewed with an MTI 65 video camera and captured with the JAVA software (Jandel Scientific). After processing to remove background debris, without making any other changes, the images were imported into the Image 1 video imaging software (Universal Imaging Corp.) and used to create three dimensional, rotating movies. The 3D views allowed better visualization of external and internal structure. To determine placement of bipolar cells, camera lucida profiles of the serial sections were made from the computer images. Although not all NOESs contained cells identified as neurons, those found were organized around a lumen and were the most peripheral cell type.

Further study of the neuronal placement within the NOESs was done with confocal microscopy and double immunostaining with OMP and NST. Both OMP^+ and OMP^- neurons sent dendritic-like processes towards the cavities.

In summary, NOESs are complex miniature replicas of the actual nose, with internal cavities that appear to represent the nasal lumen.

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Morphometric Analysis of Mitochondria in Support and Receptor Cells in Human Olfactory Epithelium

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Rocky Mountain Taste and Smell Center)

Initial observations of the ultrastructure of olfactory epithelium from humans with Alzheimer's disease have led us to hypothesize that there are changes in the morphological characteristics of mitochondria in the diseased state. In order to test that hypothesis, we have begun a study to quantify the morphological features of mitochondria in receptor cells and support cells in normal olfactory epithelium. Control volunteers are tested for olfactory and gustatory function using a battery of tests currently employed at several Taste and Smell Centers. Biopsy specimens are processed and embedded for electron microscopy. A protocol for obtaining ultrathin sections and micrographs has been designed based on our previous experience. Sections are collected at 25 μm intervals through approximately 150 μm thickness. Cell boundaries and mitochondrial profiles are traced with a marking pen on the micrographs and the micrographs are scanned into a Macintosh computer. Measurements of cell area, mitochondrial area, and major and minor mitochondrial axes are made with the Image program. From these measurements, we are able to calculate density of mitochondria, average mitochondrial area, and mitochondrial size and eccentricity. We have applied a standard t test to our initial measurements and have determined that a significant difference exists in mitochondrial density between receptor cells and support cells. Receptor cells have more mitochondria than do support cells, but mitochondria in support cells are larger than those in receptor cells. This study will ultimately include data from 70 volunteers ranging in age from 21 to 90 and equally divided between males and females. The protocol also will allow us to determine a rate of positive biopsy for our technique and distribution of cell types within human olfactory epithelium. The same protocol will be applied to biopsies obtained from subjects with Alzheimer's disease to determine if there are significant changes in cell type occurrence and/or mitochondrial morphology in the disease state.

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Electroolfactogram recordings show regional differences in the rat olfactory epithelium. PATRICK I. EZEH AND JOHN W. SCOTT. (Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322)

The existence of different types of olfactory receptor cells in different regions of the olfactory epithelium has been suggested by reports employing the molecular biology approach. We investigated the distribution of odor responses by observing the electroolfactogam (EOG) at different parts of the olfactory epithelium after application of various odorants. The epithelium was exposed by drilling the nasal or frontal bones and was penetrated with micropipettes for recording the EOG without opening the nasal cavity. The size of the EOG response to different odors varied with recording positions. Amyl acetate and ethyl butyrate produced their largest responses from dorsal parts of the epithelium just lateral to the septum. Limonene and cineole produced their largest responses in the lateral recesses or more ventrally near the septum than in the dorsal epithelium. These results suggests functionally different receptors in these regions. Antidromic activation of a population spike from the receptors by stimulation of the olfactory bulb surface confirmed that the dorsal region and the lateral recesses of the olfactory epithelium project axons to different parts of the olfactory bulb. The responses from the lateral recesses of the epithelium always had longer latencies than those from medial sites (differences varied from 40 to 170 msec). This result suggests significant diffusional delays in odor access to these regions.

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Differential expression patterns of mouse odorant receptors in the olfactory epithelium

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The identification of the large, diverse family of genes encoding odorant receptors provides a powerful set of molecular tools with which basic questions regarding olfactory sensory coding can now be addressed. One such question is whether differential patterns of expression of receptor molecules within the olfactory epithelium constitute a spatial code for odor discrimination. Based on the expression patterns of 40 distinct receptor subfamilies, it is evident that odorant receptor genes are differentially expressed along the dorsal-ventral axis of the olfactory epithelium. The expression of each gene is confined to a specific sub-region of the nasal cavity. Furthermore, the observed expression patterns display bilateral symmetry in the two nasal cavities and are conserved among animals, indicating that the patterns are genetically specified. The study of receptor expression within the olfactory epithelium, combined ultimately with the fine mapping of olfactory epithelium to bulb neuronal projections, will provide insight into the organizational principles underlying olfactory information processing.

Spatial Segregation of Olfactory Neurons Expressing Identified Odorant Receptors. BREER, H., STROTMANN, J., WANNER, I. KRIEGER, J. and K. RAMING (Institute of Zoophysiology, University Stuttgart-Hohenheim), 7000 Stuttgart 70, FRG

The observation that different odorants elicit a characteristic spatial pattern of excitation in the olfactory epithelium has led to the concept that odor quality may be encoded by a spatial segregation of receptor cells with specific responsivity, which is supposed to be determined by the distinct odorant receptor subtypes expressed by the cells. Thus, the topographic pattern of responsivity suggests that subsets of olfactory neurons expressing a common receptor subtype may be segregated in certain regions of the epithelium.

Several cDNA-clones encoding putative odorant receptors have been isolated and characterized. Digoxigenin-labelled antisense RNA transcribed from various putative odorant receptor clones was used to probe coronal sections of the rat olfactory epithelium employing in situ hybridization techniques. Initial macroscopic analysis revealed that for most of the clones reactive cells seem to be relatively wide spread; more detailed studies showed that reactive cells are restricted to certain areas of the epithelium. Some of the clones are expressed in the same region, whereas others are expressed in non-overlapping, sometimes complementary regions. One of the receptor subtypes was expressed only in a clustered subset of cells segregated in two very restricted areas of the olfactory epithelium. The reactive cells appear symmetrically in both nasal cavities. A detailed mapping of the expression pattern for odorant receptors may contribute to unravel the chemotopy of the olfactory system.

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Second Messenger Signaling in Olfactory Receptor Cells. BREER, H., BOEKHOFF, I., SCHLEICHER, S., J. STROTMANN, E. TAREILUS (Institute of Zoophysiology, University Stuttgart-Hohenheim) 7000 Stuttgart 70, FRG

Olfactory neurons encode the strength, duration and qualitity of odorant stimuli into afferent neuronal signals. Recent advances in physiology and biochemistry of olfactory receptor cells implicate second messenger as critical link between initial odor recognition and the electrical response. Upon interaction of odorous molecules with specific receptors, second messenger cascades are activated in a G-protein dependent manner leading to a rapid and transient signal of either cAMP or IP_3. The second messenger 'pulses' are supposed to elicit the generator current via direct gating of cation channels. The characteristic phasic response of olfactory neurons is due to a rapid termination of the odor-induced primary reaction; a rapid termination of odor-induced second messenger signaling is accomplished by uncoupling the reaction cascades via a negative feedback reaction. This 'turn off' reaction is mediated by a sequential interplay of two types of kinases: a second messenger activated kinase and a ß-adrenergic receptor kinase-like enzyme. These kinases lead to a stimulus-dependent phosphorylation of olfactory ciliary proteins, probably the liganded odorant receptors.

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The Channel-Forming Properties of Novobiocin: A Compound that Enhances Salty Taste. A.M. FEIGIN (Monell Chemical Senses Center), S.M. BEZRUKOV, (NIH, LBM/NIDDK, Bethesda, MD), I. VODYANOY (ONR, Arlington, VA), J.H. TEETER & J.G. BRAND (Monell Chemical Senses Center & Univ. of PA, Philadelphia, PA).

Novobiocin, an aromatic nitrogen-containing antibiotic, selectively enhances the response of sodium-specific, amiloride-sensitive chorda tympani nerve fibers to sodium chloride. It is without effect on more broadly tuned cation fibers that are not amiloride sensitive (Feigin et al., AChemS XIV). Even though this selective activity at the nerve fiber level argues for a pharmacologic effect of novobiocin on the amiloride-sensitive sodium channel, we have found that novobiocin forms ion channels in pure lipid bilayers, suggesting that its activity as a salt enhancer may be due to its ability to form ion channels in taste cell membranes. Novobiocin increased the conductivity of pure lipid bilayers by forming cation-selective ion channels with low conductivity (~ 7 pS) and long mean opened and closed times of several seconds. These channels did not discriminate between Na^+ and K^+. Preliminary results demonstrate that conductivity of the novobiocin-channels reached half maximal saturation at 20 mM NaCl. Type of fatty acid in the lipid did not influence conductivity, but charge of the lipid did. Bilayers formed from negatively charged lipid (phosphatidylserine) allowed formation of higher conducting channels compared with those formed in neutral lipid. The structure of novobiocin suggests that the conductive unit (channel) is most probably formed from several molecules. The critical micellar concentration of novobiocin was determined in aqueous solution from a surface tension versus log novobiocin concentration curve. The value, 3 mM, is almost 2 orders of magnitude higher than the concentration of novobiocin needed to induce channel formation (0.05-0.2 mM). This result suggests that novobiocin transfers from the aqueous phase to the lipid phase in the form of free molecules and that the channels then assemble as molecular complexes within the lipid phase. Since the conductivity of the bilayer is directly proportional to the aqueous concentration of novobiocin, we propose that, within the lipid phase, novobiocin exists primarily in the associated state in complexes that may act like cation channels.

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The Electrochemical Concentration is the Complete Na^+-Salt Taste Intensity Dimension. QING YE, GERARD L. HECK, JOHN A. DeSIMONE (Department of Physiology, Virginia Commonwealth University, Richmond VA, 23298-0551)

Chorda tympani (CT) recordings were made from rat tongue under epithelial voltage clamp (VC). At submucosa negative VC, responses to a given concentration of Na^+-salt are enhanced relative to current clamp (CC) response. More generally CT vs. Na^+-salt concentration curves are shifted to the left on the concentration axis. At positive VC, CT responses are suppressed and shifted to the right. This indicates a membrane voltage dimension as well as a concentration dimension in the CT response function. If the actual stimulus for Na^+ receptor cells is an influx of depolarizing Na^+ current through apical membrane ion channels, then stimulus intensity should be a function of the Na^+ electrochemical potential difference across the transducing channel. This means that the actual Na^+ stimulus intensity dimension is the electrochemical concentration, C_e, where: $C_e = C \exp(-\delta\phi)$. Here ϕ is the dimensionless transepithelial potential ($\phi = F\Delta V/RT$), δ is the fraction of ϕ dropped across apical membranes, and C is concentration. The CT response of a low Na^+ salt concentration can be transformed into that of a higher concentration by recording it under negative VC. The transformation matches the entire time course of the CT response including that of adaptation. This is proof that C_e and not simply C is the full stimulus intensity. The dose-response curves are described by a modified Beidler equation parameterized by ϕ. However, the voltage-dependent dose-response curves can be collapsed to a single curve by introducing C_e as the new stimulus dimension. These results unify the "chemical" and "electrical" modes of taste stimulation, and are consistent with the hypothesis that apical membrane Na^+ channels are a major component in salt taste transduction.

261

Strain Differences in Amiloride-Suppression of Chorda Tympani Nerve Responses to NaCl and KCl. MICHELLE M. MINEAR and ROBERT J. CONTRERAS (Florida State University, Department of Psychology, Tallahassee, FL., 32306-1051)

When given a two-bottle drinking test between water and one of several NaCl solutions, Sprague-Dawley rats will consume a broad range of NaCl concentrations but most strongly prefer isotonic saline. In contrast, Fischer 344 rats do not prefer NaCl solutions at any concentration. Salt-sensitive taste receptor cells of the anterior tongue are innervated by the chorda tympani nerve (CTn) in rats. The mechanism for NaCl taste reception and transduction is thought to be mediated by the passive transport of Na^+ ions across the plasma membrane of salt-sensitive taste receptor cells. The purpose of the present study was to determine whether the strain difference in NaCl preference is associated with differences in the number of amiloride-sensitive Na^+ channels on the taste receptor cells. We predicted that amiloride would suppress the CTn responses to NaCl to a greater degree in Fischer 344 as compared to Sprague-Dawley rats. The magnitude of the tonic responses to various concentrations of NaCl mixed in 0.3, 1.0, or 10.0 μM amiloride were compared to the responses to the same NaCl concentrations used alone. Based upon preliminary analysis, amiloride appeared to suppress CTn responses to NaCl similarly in Sprague-Dawley and Fischer rats. Unlike prior reports in the taste literature, amiloride significantly suppressed CTn responses to KCl in both strains.

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262

Taste Pre-Stimulation Increases the Chorda Tympani Nerve Response to Menthol. ROBERT F. LUNDY JR. and ROBERT J. CONTRERAS (Florida State University, Department of Psychology, Tallahassee, FL., 32306-1051)

Electrophysiological recordings of the summated response of the chorda tympani nerve to menthol stimulation of the tongue were obtained from 15 adult male Sprague-Dawley rats. The chorda tympani nerve response to menthol was of short duration ending within 2.5 s after stimulus onset, leaving the receptors in a state of insensitivity to subsequent menthol stimulation. Rinse durations with deionized-distilled water up to 10 min failed to bring the receptors back to their original pre-stimulus state. Although stimulation with menthol prevented taste receptors from responding to subsequent presentations of menthol, the chorda tympani nerve would respond normally to NaCl, NH_4Cl, KCl, sodium acetate, glucose, citric acid, and quinine-HCL solutions. Prior stimulation with one of these taste solutions resulted in the recovery of the menthol response. The magnitude of the recovered menthol response depended on the magnitude of the phasic response elicited by the preceding taste stimulus. This release from inactivation may be voltage-dependent, because the magnitude of the pre-stimulus response is proportional to the magnitude of the recovered menthol response. Alternatively, recovery may be mediated by removal of the ligand from its receptor or by reactivating some locked transduction pathway.

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263

Apparent First-Order and Second-Order Binding Kinetics for a Disaccharide (Sucrose) and Its Constituent Monosaccharides (Glucose and Fructose) in the Hamster. HARRY WMS. HARPER (Duck Engineering Design, 500 E. 63rd St., New York, N.Y. 10021)

Concentration-response data using whole-nerve response magnitudes have been widely used to investigate the binding kinetics of sweet taste stimuli. In general such results have been obtained with "integrators" which rectify and average the nerve potential, which introduces a systematic quadratic error in the estimate of the nerve's population firing rate. When this error is eliminated by squaring and averaging the nerve potential, which accurately estimates the firing rate, a simple and striking result emerges: for sucrose the data agree well with a first-order Michaelis-Menten adsorption curve, while glucose and fructose agree well with a simple (a single dissociation constant) second-order curve. It is tempting to suggest that the monosaccharides bind two-at-a-time, in one step, to the same receptor which binds the disaccharide one-at-a-time. A property of these experiments is that the apparent dissociation constants vary somewhat from preparation to preparation (???). In analyzing such data frequent use has been made of "reciprocal plots" (for example, C/R against C, or R against R/C), which in appropriate cases yield linear relations. These procedures have been employed for both qualitative and quantitative ends: Does the data fit a Michaelis-Menten curve? What are the maximum response and dissociation constant? Unfortunately, these techniques have sometimes been misused, due to the fact that data which do not fit a Michaelis-Menten curve at all can yield linear plots, with the wrong maximum response and dissociation constant, leading to both qualitative and quantitative errors. (L. Kennedy has called attention to some of these problems at previous AChemS.) This can happen even with complete data sets, including responses from near threshold all the way up to saturation. (The author is a salty character and would appreciate comments from sweet people, especially regarding related experimental evidence.)

264

Structure-activity relationships of sweet taste suppression by hodulcin. LYNNE RUDNICK[1,2], LINDA M. KENNEDY[1,2] and MARK TURNBULL[3] (Dept. Biology[1], Neuroscience Program[2] and Dept. Chemistry[3], Clark Univ., Worcester, MA 01610).

Hodulcin, gymnemic acids and ziziphins selectively suppress sweetness perception in humans, post-synaptic afferent neural responses to sweet stimuli in some vertebrates, and behavioral and receptor cell responses to sucrose in flies (Kennedy et al., In: Sweet Taste Reception, Matlouthi et al., eds., Elsevier, 1993). For hodulcin, the structure consists of glycone (glucose: arabinose [3:1 ratio]) and aglycone (hodulcigenin) portions (Kennedy et al., Chem. Senses 13, 1988, 529). However, it is not known whether the glycone, aglycone, or overall intact molecule is required for the antisweet activity. To investigate these possibilities, we separated the glycone from the aglycone by acid hydrolysis (method of Sinsheimer & Rao, J. Pharmaceut. Sci. 59, 1970, 622) of a partially-purified extract (HDE) (Kennedy et al., 1988). Human psychophysical tests were conducted with the pre- and post- hydrolysates (0.05% w/v). Mean magnitude estimates of 80 mM sucrose (modulus of 10) were 1 and 7.5 after treatment with the pre- and post-hydrolysates, respectively. These preliminary results suggest that the overall intact molecule is required for the antisweet activity. Structural confirmation of pre- and post-hydrolysate materials, and the specific hodulcigenin structure, is in progress by correlated proton/^{13}C nuclear magnetic resonance spectra. Future work will include comparative structural studies with gymnemic acids and ziziphins.

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Brazzein, A Natural Thermostable Sweet Protein From *Pantadiplandra brazzeana*

DING-MING & GORAN HELLEKANT(Department of Animal Health & Biomedical Sciences, University of Wisconsin-Madison)

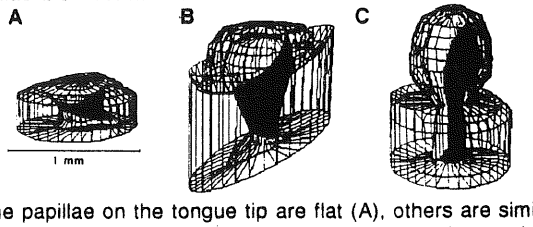
A novel protein sweetener from fruits of *Pantadiplandra brazzeana* has been isolated, characterized and sequenced. We named this protein BRAZZEIN. Brazzein is the smallest(~6,000 Daltons) in the sweet protein family and has 52 amino acid residues. It is about 2,000X as sweet in 2% concentration as sucrose solution in the same concentration. This protein is exceptionally thermostable(tasting sweet after incubation under 98°C for two hours). It is rich in lysine. As an outstanding thermostable protein sweetener, this protein has great potential in academic research of protein biochemistry and taste physiology, as well as in the food industry.

Synthesis and Evaluation of Fluorescent Guanidine Sweeteners as Probes for Binding Sites. MICHELLE SULIKOWSKI, GARY SULIKOWSKI, P. R. DROUPADI, JERRY ANCHIN,and D. SCOTT LINTHICUM (Dept. Chemistry, Center for Macromolecular Design and Dept. Vet. Pathobiology, Texas A&M University, College Station, TX 77843)

The series of N-(carboxymethyl)guanidines with di-and tri-substitutions constitute some of the most potent sweeteners known to man. We have a library of monoclonal antibodies that bind the guanidino based sweeteners and in order to study the molecular interactions in the binding sites we synthesized several fluorescent N,N'-di-substituted guanidino acetic acids following a modification of the procedure of Muller and Walters. We have synthesized N'-α-aminonapthalene-, N'-β-nathylamine- and N'-dansyl-guanidino acetic acid as probes for the study of the monoclonal antibodies that bind the high potency N-aryl-N'-aryl-N"-carboxymethyl-trisubstituted sweeteners (which is 200,000 times sweeter than sucrose in taste threshold experiments). Using fluorescent energy-transfer spectroscopy techniques, these probes allow the examination of the natural fluorigenic residues, such as tryptophan, that are involved in ligand recognition. These probes may also permit the study of supramolecular motifs which might be present in the biological receptor. The potency of these compounds is currently being evaluated. These and other fluorescent probes should prove useful for the study of the taste receptor binding sites and may help elucidate the geometry of the ligand-receptor interaction. We thank Drs. S. Nagarajan and Jeff Carter for suggestions regarding the chemical reactions. This work was supported by NIH/NIGM 46535

Anatomical Features of Human Fungiform Papillae. J MILLER, JR, DF BLACK & DW SINK (Dept. Neurobiol. and Anat., Bowman Gray Sch. of Med., Wake Forest Univ., W-S, NC 27103)

There is increasing evidence that some variations in human taste perception are associated with anatomical differences on the tongue. The number, size and form of "fungiform" papillae, and the number of taste pores on them, are related to differences among subjects in taste sensitivity for 6-n-propylthiouracil (Reedy, et al, this vol). In order to compare anatomical features to relative taste sensitivity in living human subjects, we have attempted to devise methods for quantification and characterization of the taste receptor organs. Specifically, we want to perform studies of taste perception on single fungiform papillae in living humans and to compare the results with anatomical features of the same papillae without destroying them. To do this, we have used microcomputer-based image processing with videomicroscopy to obtain digitized images of fungiform papillae. Critical measurements were taken from the images and applied to commercial CAD software to simulate the papillae. Three examples of human fungiform papillae are illustrated below.



Some papillae on the tongue tip are flat (A), others are similar to the short, wide (B) papillae of rodents, while those with the classical "mushroom-shape" (C) are also present. We are trying to characterize these shapes by mathematical definitions which involve ratios of critical measurements like height and diameter.

These experiments were supported by NIH Grant DC 00230.

Response properties of lingual trigeminal nerves to acid stimuli. PAUL A. MOORE and BRUCE BRYANT (Monell Chemical Senses Center, 3500 Market St. Philadelphia, PA 19104)

The lingual trigeminal nerve signals the presence of a variety of noxious and innocuous stimuli in the oral cavity. In order to determine how potentially irritating acidic stimuli are encoded by trigeminal neurons, we studied the response properties of lingual trigeminal neurons using both single- and multiunit extracellular recordings from anesthetized rats. Stimuli were presented at 30° C to the dorsal surface of the tongue for 5-10 s and were rinsed with deionized water at the same temperature. Chemical stimuli consisted of saturated fatty acids (carbon chain length 1 to 8) at three different concentrations: 4.7 mM (C8 to C6), 86 mM (C6 to C1), and 300 mM (C5 to C1). These concentrations were determined by the solubilities of octanoic acid (4.7 mM), hexanoic acid (86 mM), and pentanoic acid (300 mM). Other acids found in food and beverages were also tested. These included l-malic, l-tartaric, citric, oxalic, quinic acids (all tested at 300 mM) and CO_2 (5-6,000 ppm). Thermal stimuli consisted of hot (40-50° C), cool (16-20° C), and cold (4-8° C) deionized water. Initial integrated multiunit recordings indicated that hot (>45° C) sensitive nerve bundles were not sensitive to any of the acids and were inhibited by cool and cold water. Conversely, cool and cold sensitive bundles were excited by acids and were inhibited by hot water. Single units that responded to cool and cold water also responded to at least some of the acids tested, while a high-threshold cold nociceptor responded to none. These results show that acid and cool/cold stimuli are encoded by some of the same neurons and therefore these classes of stimuli may have sensory interactions. The mean of the responses of single units to acid stimuli increased with increasing carbon chain length. The majority of individual neurons followed this trend, although some neurons had bell-shaped response curves with respect to carbon chain length. Differences in latency of response onset and adaptation time course were also observed which, similar to response amplitude, may be attributable to the physico-chemical characteristics of the organic acids (pKa, octanol:H_2O partition coefficients, *etc.*).

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269

Capsaicin Eliminates Peptidergic, But Not Synaptophysin Immunoreactive Fibers From Rat Circumvallate Taste Buds. GINA M. NELSON (Mountain and PlainsRTC for Chemosensory Disorders, University of Colorado Health Sciences Center, Denver, Co.)

Two types of gustatory nerve fibers are associated with rat taste buds. One type is peptidergic fibers which are primarily perigemmal, but also ramify within the taste bud. The other type of intragemmal fiber is immunoreactive for synaptophysin and other synaptic vesicle proteins, and include those postsynaptic to taste cells. A few fibers within the taste bud are immunoreactive for both peptides and the synaptic vesicle proteins. To test whether capsaicin eliminates any of the intragemmal populations of nerve fibers in the rat circumvallate papillae, rat pups were injected neonatally with 1% capsaicin (50 mg/kg), and were perfused 100 days following injection. Antibodies directed against Substance P, CGRP, and Synaptophysin were visualized by immunofluorescence. Peptides and synaptophysin were visualized by utilizing different fluorochromes. The results indicate that capsaicin eliminates all peptidergic fibers in the rat circumvallate taste buds. Peptidergic basal plexus fibers remain, but are reduced in number. In contrast, synaptophysin immunoreactive intragemmal fibers are not obviously different from those in control animals. Elimination of peptidergic fibers is consistent with the absence of the double labeled peptide-synaptophysin immunoreactive pattern. These results indicate capsaicin does not affect synaptophysin-immunoreactive fibers. This correlates well with previous evidence which indicates that synaptophysin-immunoreactive fibers include fibers which are post-synaptic to taste cells. This suggests that antibodies directed against synaptophysin are useful markers for the intragemmal gustatory nerve fibers.

270

Interpretation of the Cryoprotectant Effect of α,α–Trehalose through its Solution Properties and its Time/Intensity Profile. M.O. PORTMANN, G.G. BIRCH, I. HUNT (Department of Food Science and Technology, University of Reading, Whiteknights,P.O. Box 226, Reading RG62AP, UK.)

α,α–trehalose is a natural occurring non-reducing disaccharide of glucose well known as the most efficient sugar for protecting the structure and function of food components from damage caused during the freeze-drying process. α,α-trehalose is followed in order of efficiency by maltose, sucrose and lactose. All these sugars interact strongly with water and participate in the water lattice through hydrogen bonds. Consequently, we focussed our interest on the interactions between α,α-trehalose and water, collecting as much information as possible about hydration using the investigation of solution properties: intrinsic viscosity[η] and apparent specific volume $\overline{V}_{\omega_2}$. α,α–trehalose undergoes a better packing among water molecules than the other cryoprotectant sugars: {($[\eta]_t = 2.58$ cm^3 g^{-1}, $[\eta]_m = 2.55$ cm^3 g^{-1}, $[\eta]_l = 2.50$ cm^3 g^{-1}, $[\eta]_s = 2.45$ cm^3 g^{-1}) and ($\overline{V}^0_{2t} = 0.608$ ml g^{-1}, $\overline{V}^0_{2l} = 0.610$ ml g^{-1}, $\overline{V}^0_{2s} = 0.615$ ml g^{-1})}.We can suppose α,α–trehalose increases the structuring of water molecules to a certain degree which partially inhibits ice formation during the freeze-drying process. Parameters monitoring water/solution interactions will be prime determinants of taste qualities. We have undertaken an interpretation of the evolution of sweetness intensity and persistence of α,α-trehalose as functions of concentration based on its solution properties and its effect on water structure. α,α-trehalose is found to be less sweet but more persistent than sucrose, fructose, glucose or maltose. Due to its high compatibility with water structure, α,α-trehalose can pack efficiently at the receptor site and the persistence is high. Because α,α-trehalose has good prospects of use in freeze-drying industries, we compared the differences of texture, colour and sweetness of fruit pulps freeze-dried with maltose, sucrose and α,α–trehalose. In every case, the sample freeze-dried with α,α-trehalose presents the charateristics closest to those of fresh pulps.

271

Maintenance of Isolated Rat Taste Buds. C.J. RUIZ, M.MCPHEETERS, R.S. LASHER & S.C. KINNAMON (Colorado State University, University of Colorado Health Sciences Center and the Rocky Mountain Taste and Smell Center.)

The long-term goal of this study is to maintain taste buds in culture. The applications of culture are numerous, including: 1) taste cells may recover receptors and ion channels lost during the dissociation process, 2) factors controlling taste cell development and survival can be identified, and 3) fewer animals will be needed for physiological studies. As a preliminary step toward this goal, we have defined conditions for maintenance of rat taste buds following dissociation. Taste buds were isolated according to the procedure of Behe et al. (J. Gen Physiol 96:1061, 1990). The variables tested included substrate, medium, and temperature. Trypan blue exclusion and gigohm seal whole cell recording were used to determine taste cell viability. The following media supported maintenance of taste cells: 1) Pixley (Pixley, S.K., Neuron 8:1191, 1992), 2) Pixley + substance P (10^{-8}M), 3) Pixley + K^+ (20 mM), 4) Pixley + db-cAMP (1mM), and 5) L-15. The media DMEM + Nu serum (8%) + FBS (4%) + ITS, DMEM + ESGRO, and DMEM + substance P (10^{-8}M) induced fibroblasts to grow underneath the taste buds, causing them to float away. The morphology of taste buds varied with the substrates tested: laminin, fibronectin, gelatin, collagen I & IV, Matrigel, polylysine and Cell-Tak. Taste buds survived equally well at 37° C and 23° C. Photographs and electrophysical recordings were made on days 2, 4, 6, 8, and 10. Voltage-dependent Na^+ and K^+ currents were recorded on days 2 and 4 from taste cells maintained with Pixley medium on Cell Tak at 37° C, and with L-15 on laminin at 23° C. These data suggest that taste cells can maintain electrical excitability for at least 4 days following isolation from the tongue under appropriate conditions.

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272

Rapid Kinetic Measurements of Bitter Stimulus-Induced IP3 in Mouse Taste Tissue. ANDREW I. SPIELMAN (New York Univ., College of Dent., NY), TAUFIQUL HUQUE (Monell Chemical Senses Center, Philadelphia, PA), INGRID BOEKHOFF and HEINZ BREER (University Stuttgart, Hohenheim), GLAYDE WHITNEY (Florida State Univ., Tallahassee, FL) & JOSEPH G. BRAND (Monell Chemical Senses Center, & Veterans Affairs Med. Ctr., Phila., PA).

Recent evidence suggests that one of the second messengers involved in bitter taste transduction is inositol 1,4,5-trisphosphate (IP3). These initial observations measuring IP3 formation were performed at 30 secs after stimulation. We have now extended these studies into the subsecond time frame. Using a rapid kinetic technique and taste tissue from circumvallate and folliate regions of B6.SW and SWR strains of mice, we found that in response to 100 μM denatonium or 100 μM sucrose octaacetate (SOA), IP3 levels began to rise at 50 msec and returned toward baseline at 500 msec, with a peak (two-fold increase over basal levels) at around 150 msec. At 100 msec, caffeine (10 mM), strychnine (100 μM), SOA (100 μM) and denatonium (100 μM), but not sucrose (100 mM), induced an increase in IP3 levels between 110% and 135% over basal levels. At 10 and 100 μM, SOA and denatonium produced a concentration-dependent increase in IP3 production, at both 50 msec and 100 msec. The present data demonstrate that several bitter tasting compounds use IP3 as a second messenger, and that this second messenger is produced within a physiological time frame.

This study was supported by funds from NIH, the Dept. of Vet. Affairs and by the Deutsche Forschungsgemeinschaft.

273

Effect of modulators of the adenylate cyclase system on sweet electrophysiological taste responses in gerbil. SUSAN S. SCHIFFMAN (Duke University), MARK S. SUGGS (Duke University) and MICHAEL L. LOSEE (The Nutrasweet Co.).

The adenylate cyclase system has been implicated in both sweet and bitter taste transduction. The purpose of this study was to determine whether application of modulators of the adenylate cyclase system to the tongue alters sweet taste responses. Integrated chorda tympani (CT) recordings were made in gerbils to sweet tastants before and after a four minute application of four types of modulators of the adenylate cyclase system. The sweet compounds tested were: sucrose (30 mM and 100 mM), glucose (300 mM), fructose (300 mM), maltitol (150 mM and 300 mM), mannitol (300 mM and 500 mM), sodium saccharin (10 mM), D-tryptophan (6.5 mM), dulcin (0.88 mM, 1.75 mM and 3.5 mM) and stevioside (0.55 mM and 1.1 mM). NaCl (30 mM and 100 mM) and KCl (300 mM and 500 mM) were used as control stimuli. Four types of modulators tested were: 1) NaF, a compound which promotes dissociation of GTP binding protein; 2) forskolin, a powerful stimulant of adenylate cyclase; 3) 8-bromoadenosine 3':5'-cyclic monophosphate sodium salt (8BrcAMP) and N^6,2'-O-dibutyryladenosine 3':5'-cyclic monophosphate sodium salt (DBcAMP), two membrane permeable forms of cAMP; and 4) 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine dihydrochloride (H-7) and N-(2-[methylamino]ethyl)-5-isoquinolinesulfonamide dihydrochloride) (H-8), which are protein kinase inhibitors. The main findings were as follows. NaF (20 mM) significantly enhanced all of the sweet compounds by at least 23%, except for 10 mM sodium saccharin and 6.5 mM D-tryptophan, while all control compounds were suppressed. NaCl (20 mM), which was used as a control for NaF, did not significantly enhance any of the responses. 8BrcAMP (1.16mM) enhanced 30 mM sucrose by 16%, 300 mM glucose by 36%, 300 mM maltitol by 18% and 6.5 mM D-tryptophan by 24%. DBcAMP had a minimal effect on most of the compounds tested with a 26% enhancement of 300 mM mannitol and a 17% blockage of 300 mM KCl. H-7 (300 μM) had little effect on any of the tastants except for 30 mM sucrose and 1.75 mM dulcin which were enhanced by 21% and 28% respectively. H-8 (147 μM) was run during one trial with no significant results. These data indicate that modulation of the adenylate cyclase system can modify the intensity of some sweet and bitter taste responses.

274

Solvation Studies of Amiloride. R.A. BUONO, C.A. VENANZI (Chemistry Div., New Jersey Institute of Technology, Newark, NJ 07102), T.J. VENANZI (Chemistry Dept., College of New Rochelle, New Rochelle, NY 10805), R.J. ZAUHAR (Biotechnology Institute, Pennsylvania State University, University Park, PA 16802)

The GROMOS molecular mechanics and dynamics force field was extended and modified in order to investigate the static and dynamic conformational properties of amiloride. The effect of solvent on the enthalpies and intramolecular hydrogen bonding pattern of the free base (A1, A4) and protonated (F1) forms of amiloride was examined. Molecular dynamic simulations of 30 psec in length were carried out for each of the species solvated by approximately 400 water molecules. The large torsional barrier to interconversion of the A1 and A4 conformers, as well as the F1 and F4 conformers, constrained the average structure of each species to a near planar conformation. This has important implications to the potential modes of binding of amiloride and its analogues to ion transport channels. Hydration enthalpies calculated from the above molecular dynamics simulation and from an induced polarization charge continuum solvent model predicted the A1 conformer to be lower in energy than the A4. This clarifies the NMR studies of Smith, et al [1] which could not distinguish between the two conformers in solution.

1. R.L. Smith, D.W. Cochran, P. Gund, E.J. Cragoe, Jr., J. Am. Chem. Soc. 101, 191-201, 1979.

275

A Hair-like Structure in the Vallate Papilla of the Balb Mouse. R. XIAO & I. MILLER, JR., (Dept. Neurobiol. and Anat., Bowman Gray Sch. of Med., Wake Forest Univ., W-S, NC 27103)

A hair-like structure (HLS) has been observed to emerge from the troughs of vallate papillae on the tongues of Balb mice. The objective of this report is to characterize this HLS and compare it with typical hair from the facial skin of the mouse's lip. The significance of this aberrant structure in association with gustatory papillae and taste buds (tb) may indicate common features of their developmental origins. The animals were from 3 groups of N=104, 5-week-old, male Balb/cByJ mice obtained from Jackson Labs, in September, November, 1990 and September, 1992. The vallate papillae of 89 mice were embedded in paraffin, prepared by serial section and stained with H & E. HLS were present in 58%, 52/89 of them. The number of taste buds on the vallate papillae in which the HLS was present (212 ± 33 tb / pap, SD, n=52) or absent (211 ± 31tb / pap, n=37) did not differ. Vallate papillae and the hair on the lip were sampled from 15 additional mice. Material was prepared for scanning electron microscopy (SEM), immunofluorescent microscopy and semi-thin plastic sections for light microscopy. SEM showed that the HLS was polarized with sharp end pointed out. The surface pattern differed from the hair on the lip and the HLS. The HLS originated below the epithelium from deep among the acini of von Ebner's gland. The HLS were approximately 300 μm in length with diameters of 20 μm compared with 400 μm of length and 20 μm in diameters for the down hairs of the lip. Both structures appeared to be hollow. Immunohistochemistry with primary antibodies against keratin 19 and keratin 5/6 was inconclusive because of strong autofluorescence of both the HLS and hair from the lip. In conclusion: 1. the HLS found on vallate papilla seems to grow there; 2. It has many features common to hair; but 3. It shows some different structural characteristics.

These experiments were supported by NIH Grant DC 00230.

276

A Computerized Gustatory Stimulator That Delivers Taste Solutions Through a Single Nozzle Without Contamination EVEY L.A., and NORGREN R. (Pennsylvania State University, Hershey, PA 17033)

Experiments with taste require delivery of a fluid stimulus to the receptor surface. Presenting fluids through separate nozzles increases variability. A single nozzle reduces this variability. Requirements of this nozzle are a precise aim, a constant flow rate, and no contamination. We developed a computerized gustatory stimulator that meets these requirements. A key feature is a vacuum drain that diverts fluid away from the receptor surface. This drain makes it possible to flush the system between trials. There is a concentric vacuum chamber surrounding the stimulus nozzle. At one end there is an opening that receives a vacuum tube. At the other end there is a small funnel shaped opening for the tip of a fluid nozzle. Fluid reaches the receptor surface when the tip is outside the chamber. Fluid reaches the vacuum drain when the tip is inside the chamber. A miniature pneumatic cylinder extends and retracts the nozzle through a linear movement of about 1 cm. The extended nozzle fits tightly into the funnel shaped opening. This shape forms a seal and precisely aims the nozzle toward the stimulation site. The seal prevents vapors within the chamber from reaching the receptor surface. The tip of a water rinse nozzle extends out of the chamber when the stimulus nozzle is inside. A 386DX or faster computer is necessary for the software to run in *Microsoft Windows 3.1*. The computer language chosen for the stimulator software was *Microsoft C70*. Operation of the stimulator is under menu control. The stimulator program runs as a "windowed" DOS application in *Microsoft Windows 3.1*. Our data acquisition program runs simultaneously, also as a "windowed" DOS application. Fluid delivery and data acquisition are under the control of one computer. The gustatory stimulator has an added advantage for neural recording because automation makes it unnecessary to disturb the preparation, thus, enhancing stability, eliminating vibration, and reducing electrical noise.

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277

Specific Anosmia and Olfactory Sensitivity to 3-Methyl-2-Hexenoic Acid: A Major Component of Human Axillary Odor.
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Recent studies have determined the identity of the compounds that make up the characteristic odor of the human axillae. A major component of the odor is (E)-3-methyl-2-hexenoic acid. After synthesis of 3-methyl-2-hexenoic acid (3M2H) we noted that one member of the group could not perceive an odor from 3M2H. This prompted us to separate the (E)- and (Z)-isomers of 3M2H, to determine olfactory thresholds for (E)-3M2H, and to examine the possibility that specific anosmias existed for the isomers. Micro-preparative gas chromatography and column chromatography were employed to separate the isomers for use in psychophysical assessments of thresholds for (E)-3M2H and to screen for specific anosmias. Average threshold for (E)-3M2H in 10 subjects was 1×10^{-4}% (1.00 microgram/ml) in light, white, mineral oil. Concentrations of 0.1% (1 mg/ml) (E)-3M2H and 0.1% (1 mg/ml) (Z)-3M2H were used to screen for specific anosmias. Among 100 subjects, we determined that 7.3% of the males and 13.6% of the females could not smell (E)-3M2H and 12.2% of the males and 11.9% of the females could not smell (Z)-3M2H. Two males (4.9%) and four females (6.8%) were anosmic to both isomers. Thus, we conclude that specific anosmias exist for both of the isomers of 3M2H.

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278

Congenital anosmia: MR volumetric analysis
DAVID M. YOUSEM, CHENG LI, & RICHARD L. DOTY (Smell and Taste Center & Departments of Radiology and Otorhinolaryngology, University of Pennsylvania, Philadelphia, PA)

Five patients with congenital anosmia (two with Kallmann's syndrome) and eight normal controls were evaluated with high resolution MR scans. A surface coil was used to study the olfactory bulbs and tracts and a head coil was used for evaluation of the temporal lobe. Volumetric analyses of the olfactory bulbs and tracts, hippocampus, and temporal lobe were performed with three dimensional display of MR scans taken at 3 millimeter intervals through the representative anatomy. The patients with Kallmann's syndrome had complete absence of the olfactory bulbs and tracts [mean, (standard deviations)] [0.00 cc, (0.00)]. The three patients with congenital anosmia, but without Kallmann's syndrome, had mean bulb-tract volumes of 0.024 cc (0.008). These values differ greatly ($p < .001$) from the normal control values [0.19 cc, (0.05)]. However, the mean temporal lobe volume values for all patients with congenital anosmia (Kallmann's and non-Kallmann's [133 cc, (11.4)] were similar to control values [141 cc, (34.07)]. No significant difference in amygdaloid-hippocampal complex volumes was noted in patients with congenital anosmia [15.0 cc, (0.35)] or normal controls [16.3 cc, (2.7)]. The signal intensities of the bulbs, tracts, hippocampi, and temporal lobes were normal. These data indicate that high resolution surface coil MR is able to identify agenesis or extreme hypoplasia of olfactory bulbs and tracts in patients with congenital anosmia.

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279

EEG Registration of Conscious and Unconscious Concentrations of Isoamyl Acetate and Androstenone
GARY E. SCHWARTZ, JOHN P. KLINE, ZIYA V. DIKMAN and ERNEST H. POLAK (University of Arizona)

Nineteen channels of EEG were recorded from 52 college students while they smelled pairs of bottles. One bottle per pair contained isoamyl acetate (IAA) or androstenone (AND) (5-α-andro-16-en-3-one) dissolved in silicone. The other bottle contained silicone (the control). Subjects were prescreened for IAA and AND detection and were invited for EEG testing if they consistently detected IAA and either detected AND (osmic) or failed to detect AND (anosmic). EEG was collected during two sniff periods per bottle for 8 trials per odor at two concentrations, one suprathreshold and one subthreshold (subthreshold AND determined by AND osmic subjects). The order of odors, concentrations, experimental and control bottles, and hand, was counterbalanced within subjects. After each trial, subjects indicated which bottle contained the odor (detection), rated their confidence of detection and the odor's intensity. EEG was amplified using the NeuroSearch-24. EEG was sampled at 256 Hz, spectral analyses were performed on 8 sec of EEG per trial, and relative alpha power was displayed in topographic maps. Correct odor detections for the suprathreshold concentrations were IAA (99%) and AND (99% for osmics, 41% for anosmics), and for the subthreshold concentrations were IAA (48%) and AND (57%) (chance = 50%). When subjects smelled suprathreshold IAA, significant alpha decreases were observed in anterior, central and posterior regions, whereas when subjects smelled subthreshold IAA, significant alpha decreases were observed primarily in the central region to sniff two. Significant alpha decreases were also observed to suprathreshold AND in both osmic and anosmic subjects, whereas significant EEG alpha decreases were observed to subthreshold AND in both groups to sniff one. These data support the hypothesis that humans can register odors at subthreshold levels, and AND anosmia may involve cortical inhibition of olfactory perception.

280

Stimulus and Recording Parameters of the Olfactory Evoked Potential in Humans.
W. JAMES EVANS and ARNOLD STARR (Department of Neurology, University of California, Irvine)

Olfactory evoked potentials were recorded from the scalp of normal human subjects in response to odorants presented monorhinally through a nasal cannula (3/16" internal diameter) in a continuous flow of humidified air heated to body temperature. Stimulus duration was 100 ms with a stimulus rise time of 20 ms. The subjects breathed through the mouth during stimulation and a thermistor was used to record respiratory activity. No behavioral response was required of the subjects and they did not perform velopharyngeal closure. The sound of solenoid activation was masked by white noise played through earphones. Evoked potentials were recorded from multiple electrode sites, referenced to the inion, filtered using a bandpass of 0.1-100 Hz and digitized at a rate of 500 Hz.

In response to a 10% amyl acetate stimulus at a volume flow rate of 10 L/min and interstimulus interval of 15 s, the potentials recorded from the nasion consisted of a triphasic waveform with a negative peak component at 270 ms latency (N270), a positive component at 390 ms (P390) and a second negative component at 560 ms (N560). The amplitudes of the P390 and N560 components were greatest over the anterior regions of the head (Nose, Nasion, Fpz, and Fz). An earlier negative component (N160) was observed to phase reverse at the vertex (Cz) on a bipolar electrode montage. Topographic mapping of the olfactory evoked potentials suggested an ipsilateral centrotemporal origin for the P390 component and a posterior central origin for the N270 and N560 components. The evoked potential component latencies decreased and component amplitudes increased with increasing stimulus flow rates from 2 to 10 L/min. The N270 peak amplitude decreased significantly with stimulus repetition whereas, the P390 and N560 component amplitudes appeared to increase.

Supported by NIH Grant DC-00033.

Does Allergic Rhinitis Contribute to Olfactory Loss? **ANDREA APTER, APRIL MOTT, MARION FRANK, and JON CLIVE. (Univ. Conn. Health Ctr, Farmington, CT)**

Allergic rhinitis is recognized as a cause of olfactory loss, but experience is limited to case reports. Patients with diminished smell function frequently have nasal polyps or sinusitis, making it difficult to separate the impact of allergic rhinitis from the effects of these other problems. To assess the importance of allergic rhinitis in olfactory loss, 69 consecutive patients (36 males, 33 females, mean age 46, age range 17 to 71) with olfactory complaints and rhinitis were examined. A history, physical examination, skin testing, endoscopic rhinoscopy, olfactory testing, and, if indicated, CAT scan of the paranasal sinuses were performed. Skin testing explored sensitivity to birch, elm, maple, oak, timothy grass, June grass, short and giant ragweed, Alternaria, Aspergillus, Cladosporium, Penicillium, Dermatophagoides pteronyssinus, Dermatophagoides farinae, cat, and dog allergens. 49 patients (71%) had at least one positive skin test, 47 (68%) to a perennial allergen, and 39 (57%) to mite. There was no significant difference between the group with positive skin tests and the group with negative tests in terms of mean age, smoking history, incidence of nasal polyps, or possible precipitating viral respiratory tract infection. Repeat olfactory testing after treatment was available for 30 patients, 43% of those with positive and 45% of those with negative skin tests. Improvement occurred more often in those with negative skin tests (7 out of 20) than in those with positive tests (6 of 49, Fisher's exact, $p<.05$). These findings suggest the role of perennial allergic rhinitis in olfactory loss requires further investigation and positive skin tests may be a marker for a less satisfactory outcome in patients with nasal sinus disease.

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Inhalation of 2-Acetylpyridine for Weight Reduction

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Everyday experience tells us that odors influence appetite and weight. Anatomic connections of the olfactory bulb to the ventromedial nucleus of the hypothalamus and the observation that acute anosmic patients often gain significant weight lends credence to this observation. In order to assess this, 105 volunteers who wished to lose at least 10 lb, between 18-64 years of age, not pregnant, nursing or with asthma, were enrolled randomly in a four-week, double-blind, crossover trial of inhalational 2-acetylpyridine. Subjects were instructed to inhale this three times alternating in each nostril whenever hungry. No other diet or behavior modification instructions were provided. Weight was obtained on initiation, when crossover occurred and at completion of the study. All underwent the carbinol olfactory threshold test of Amoore and the Chicago Smell Test. Significant findings (p less than 0.05) based on comparisons of individual subjects (46 completers) weight loss in the active vs. placebo phase included those with both carbinol threshold $\leq$ 25 decismels as well as positive Frito hedonics. Between group comparisons of active phase obese (baseline BMI greater than 27.5) subjects revealed significantly greater weight loss in those with carbinol thresholds $\leq$ 25 versus those with greater than 25 decismels. Conclusion: Data is suggestive of the possibility of 2-acetylpyridine inhalation as a weight reducing agent in those with normal olfaction.

Further studies on the effect of semiochemicals and olfactants on the human vomeronasal organ (VNO). L.MONTI-BLOCH*^, D. DOLBERG*, C. JENNINGS-WHITE* and D.L. BERLINER*. (^Univ. of Utah, Salt Lake City, UT), (*Pherin Corporation, Menlo Park, CA).

Exposure of the human VNO to naturally occurring human pheromones, induces a local receptor potential, electro-vomeronasogram (EVG), followed by reflex activity, likely integrated within the hypothalamus [1]. We now report the effect of certain semiochemicals, Ph-15, 94A, 94B, and 84 (Pherin Corp.), and the primary olfactants, tonalid, cineole and carvone, on EVGs recorded from the VNO of 80 human subjects of both sexes (20-48 y.o.). All substances were locally applied to the VNO in equal quantities, using a multi-functional miniprobe described elsewhere [1]. Also monitored were body temperature (BT), galvanic skin reflex (GSR), cortical evoked activity (CEA) and pupilary diameter (PD). Femtomolar quantities of Ph-94B produced significant stimulation of the female VNO ($p<0.01$), increased BT (Δ_{BT}=0.4±0.2 °C), decreased GSR (Δ_{GSR}=-46.2± 22 KΩ) and PD (ΔPD=-1.6±0.7mm), and induced cortical evoked activity from Fz-A1 with 300 ms latency. Activity was less or absent when stimulating the female VNO with similar concentrations of the stereoisomer Ph-94A. Similar quantities of Ph-15 and Ph-84 significantly stimulated the male VNO ($p<0.02$), Ph-84 being more potent. They also decreased GSR ($p<0.01$) and PD ($p<0.01$), and induced CEA from Cz-A1. Ph-15 increased BT (Δ_{BT}=0.28±0.12 °C). None of the Pherin substances induced a significant local potential when applied to minifields of olfactory epithelium, except for Ph-94A. Olfactants did not induce significant EVGs in the female and male VNO, but were effective stimulants of the olfactory epithelium. These results are discussed in terms of receptor sites for specific ligands in the human VNO.

1 J. Steroid Biochem. Molec. Biol., 39(4B): 545-680, 1991.

Applying chemosensory science: CSIRO's Japan Project.

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The Japan Project is a programme of cross-cultural taste psychophysics and consumer sensory behaviour analysis being conducted in Japan and Australia. It is currently being expanded into Korea, Taiwan, Singapore and Malaysia. The Japan Project has found that Japanese taste panelists are no more or less sensitive to variations in tastants than their Australian counterparts and both groups rate the same sweet and salty foods as equally intense, and express similar liking-intensity functions for tastants (except umami and sourness at high concentrations). However, the Japanese subjects usually rate the Japanese product as preferred over the import, even when tested "blind". Preference is usually determined by a combination of sensory attributes. Although familiarity with sensory qualities may also be important in determining Japanese preferences, nevertheless spectacular successes have been achieved by exporters with novel formulations. Food product formulation for the Japanese market requires fine-tuning of products to their most preferred settings on several sensory attributes. While attention to sensory quality is essential, so are other factors, such as appropriate packaging, presentation and an understanding of the product's position in both the culture and the complex Japanese distribution system.

285

The National Geographic Smell Survey and Gas Warning Odor: Replication and Extension. WILLIAM S. CAIN, JANNEANE F. GENT, & J. ENRIQUE COMETTO-MUÑIZ (John B. Pierce Laboratory and Yale University, New Haven, CT)

The National Geographic Smell Survey offers a unique view of risk factors for impaired perception of natural gas warning odor. The survey included questions regarding the perception of six microencapsulated scents, including a gas warning agent. In an effort to address the issue that respondents inevitably constituted a select group (a self-selected 10 % of the readers of the magazine), we used random dialing in a large midwestern area to line up 1,000 persons over 18 who would agree to return the survey; 720 did so. We sent copies of the original form with the additional question: Which sample, if any, smells like natural gas? The ethnicity, gender, age, and medical history of the random sample rather closely resembled the 20,000 people from the same area who had sent in the original survey, though proportionally half-again as many participants in the random sample smoked. The groups corresponded very well with respect to their ratings of intensity and pleasantness, but the random sample exhibited a little more vulnerability to aging. For example, whereas 7 % of persons over 70 in the original sample rated the warning agent as zero, 20 % of those in the random sample did so. In both samples, both male gender and smoking were associated with lower perceived intensity. Ability to choose the patch that resembled gas fell off dramatically with age, from a high of only three-quarters correct among persons in their 20's and 30's to a low of only about one-third among persons 70 and above. Those who failed to identify the correct patch found all odors, but particularly gas, less intense. Smoking and male gender again constituted risk factors for failure to choose the patch, with each variable beginning to show an effect for persons in their 40's. Differences became quite pronounced for persons 50 and above. Our results imply that the original survey reached sound conclusions despite the selectivity of the respondents and that the ratings respondents assigned to gas warning patch reflected themselves in how well they could in fact identify it.

286

The olfactory system as a model for the assessment of neurotoxicology

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There are still major difficulties in predicting sensory effects and effects on the nervous system of chemicals at commonly encountered indoor concentrations. To close this gap we investigated the sensory ability (establishing of thresholds for different odors and odor discrimination ability) of Wistar rats and ferrets after longterm exposure to different concentrations of formaldehyde gas. In addition we quantitatively investigated the olfactory epithelium (Septum nasi) for possible noxious effects due to the exposure experiments.
Animals were exposed to formaldehyde gas for a minimum of 1 month and a maximum of 12 months. Exposure concentrations were 0.25 ppm and 0.5 ppm. To analyse the sensory abilities in the rat we used an olfactometer (Slotnick and Schonoover, Chem. Senses 9, 1984, 325), for the ferret we deviced a computerized y-maze. We found a significant increase in the olfactory threshold already after one month exposure time (0.25 ppm); also olfactory learning and discrimination abilities were significantly decreased. In the ferret neuroanatomical evaluations of the olfactory epithelium indicated exposure time depending noxious effects which were not reversible within one month after terminating the exposure experiments. The here reported animal model seems to be a promising approach for the evaluation of possibly noxious airborn chemicals at low concentrations.

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287

Averaging of sensory Time-Intensity curves with Principal Component Analysis. S. BONNANS (University of North Carolina, Chapel Hill, NC 27514) and A.C. NOBLE (University of California, Davis, CA 95616).

Sweetness of four lemon flavored aqueous solutions was continuously rated by eighteen trained subjects. These time-Intensity data were analysed by Principal Component analysis (PCA) to investigate in detail the potential of the method proposed by Van Buuren (Food Tech., Feb 92, 101). Because the reported temporal patterns of taste perception vary differently across subjects, although each subject has a consistent pattern, simple averaging across individuals is influenced by outliers. Principal Component analysis can efficiently address this problem because it extracts as the first factor the latent variable common to all individual responses, regardless of their differences. This variable corresponds to signal or the time-intensity characteristic of the sample. The method also allows mapping the subjects in the PC space to visualizally classify groups of individuals who report similar types of temporal patterns. The practical drawback of the method is that it is extremely labor intensive. The principal components, as standardized variables, do not have any meaningful units and need to be "destandardized" to be interpretable on the original intensity scale. Furthermore the temporal responses of the subjects need to be of approximately the same duration and therefore normalized to a common duration prior to the analysis. The results of these PCAs will be presented and discussed.

288

Keratins in Taste Buds. BRUCE OAKLEY, ANNE LAWTON, LIANNA WONG[1] CHUNXIAO ZHANG, Department of Biology, University of Michigan, Ann Arbor and [1]Department of Molecular and Cell Biology, University of California, Berkeley.*

Immunocytochemical evaluations of gustatory epithelia were made in rat, gerbil and rabbit with 21 monoclonal antibodies (MAb) specific for one or more of the soft keratin polypeptides 1-19. The most useful immunocytochemical procedure treated cryostat sections of acid-alcohol fixed tissue with an MAb followed by a biotinylated secondary antibody and an avidin-biotin-peroxidase complex and DAB. An MAb against human keratin 20 (MAb 20.5) reacted specifically with human fungiform taste buds (tissue courtesy of Inglis Miller), but not with rodent taste buds. Within the gustatory epithelium of rabbit, rat or gerbil, monoclonal antibodies reactive with keratin 8 (MAbs M20, LE-41 and DK80.20), keratin 18 (MAb LE-65) and keratin 19 (MAbs 4.62, 170.2.14 and LP2K) only the fusiform cells of taste buds were immunoreactive. Denervation eliminated this immunoreactivity. Most fusiform taste cells appeared to be immunoreactive to MAbs for keratins 8 and 19 and 20, whereas keratin 18-like immunoreactivity often occurred in less than half of the fusiform cells in rat taste buds. Comparison of adjacent sections and double staining with polyclonal antisera (TPA) revealed that the basal cells, including those adjacent to and within the base of fungiform taste buds were immunoreactive for keratin 14 (MAb CKB1) but not for keratins 8, 18, or 19. Transitional keratin expression may reveal which suprabasal cells are destined to contribute to the taste bud. We conclude that fusiform, but not basal, cells of taste buds probably contain keratins 8, 18, 19 and 20 and that available antibodies against keratin 8 or 19 could serve as useful markers of differentiated taste cells in development, regeneration or tissue culture.

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A High-Resolution Casting Method for Visualizing Human Taste Papillae In Vivo. WALTER E. MYERS, THOMAS P. HETTINGER, JOSEPH A. D'AMBROSIO, STANLEY L. WENDT, CHRISTINE B. PEARSON and MARION E. FRANK (University of Connecticut Health Center, Farmington, CT)

We have developed a method using polyvinylsiloxane (PVS), a high-resolution dental impression material, to obtain negative images of the lingual surface in humans. From this mold, a replica of the tongue is made with epoxy resin that reveals structural details of the human tongue when visualized by scanning electron microscopy (SEM). This method has been developed for the purpose of studying the changes in the lingual surface anatomy that may occur with time in living human beings. Because identification of human fungiform papillae is not routine, the method was calibrated with hamster tongues, which have well-defined fungiform papillae, each containing a single taste bud and taste pore. Replicas made from PVS impressions of tongues of living hamsters were compared with the same tongues after fixation. A high degree of detail was retained in the replicas as compared to the fixed tongues. Individual filiform and fungiform papillae, identified on the fixed tongues by SEM, were also found on the tongue replicas. In addition, depressions in the centers of papillae in the replicas agreed with the size and location of taste pores in hamster fungiform papillae. We are currently correlating lingual papillae on photographs of methylene blue-stained tongues of human subjects with structures on replicas of the same tongues. This non-invasive casting technique makes it possible to obtain an accurate record of papillary details for tongues of living subjects. Temporal changes in lingual surface features due to aging, disease or trauma may be studied with this method.

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Locus Coeruleus Increases Perithreshold Sensory-Evoked Excitation of Mitral Cells

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A major modulatory input to the main olfactory bulb (MOB) is the noradrenergic (NE) system which arises exclusively from the pontine nucleus locus coeruleus (LC) and terminates densely in the internal plexiform layer (IPL) and granule cell layer (GCL) and to a lesser degree in the external plexiform layer (EPL). Reports of the neurophysiological actions of *exogenous* application of NE are contradictory: iontophoretic application of NE decreased the activity of mitral cells; this reduction was blocked by bicuculline suggesting that NE excites granule cells causing release of GABA which inhibits mitral cells. In the turtle MOB (*in vitro*) NE increased mitral cell firing, an effect blocked by GABA. Thus it was argued that NE inhibits granule cells. In dissociated MOB cell cultures NE was recently reported to inhibit synaptic transmission between mitral and granule cells and calcium currents via a-2 receptors.

Here, we have characterized the actions of *endogenously* released NE on mitral cell discharge. Mitral discharge was recorded before, during and after selective, confirmed activation of LC in methoxyflurane-anesthetized rats. A dual pipette assembly was advanced into LC; one pipette was used to record LC discharge and microinjection of acetylcholine (ACh) from the second pipette was used to reversibly activate LC neurons. Another pipette was inserted into the mitral cell layer to record spontaneous and epithelium-evoked activity; mitral cells were identified by field potential profile, antidromic activation and histological localization.

Microinjection of nanoliter quantities of ACh produced a sustained (5 min), 4-7X increase in LC discharge rate and a simultaneous desynchronization of the EEG. Activation of LC had no consistent effect on mitral cell spontaneous activity. However, there was a doubling of mitral cell excitatory responses to peri-threshold, electrical stimulation of the epithelium; the mean magnitude of evoked-excitation was increased from 42 ± 8.3 to 86 ± 19.8 spikes/100 epithelium shocks ($n = 6$; $p < 0.02$). This potentiation lasted for 5 to 30 min. Supra-threshold excitatory responses to epithelium stimulation were not changed.

Olfactory cues evoke NE release in the olfactory bulb and NE release is increased during reproductive/maternal behaviors. NE plays an important role in olfactory learning and pheromonal regulation of pregnancy. The present results suggest that *endogenous* NE release may preferentially increase the sensitivity of mitral cells to weak olfactory stimuli. Future experiments will determine which class(es) of adrenergic receptors mediate NE's actions, and if increased sensory-evoked mitral responses are mediated by NE actions on GABAergic granule cells.

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Mitral/Tufted Cell Late Depolarizations in Salamander Olfactory Bulb Slices. A.R. CINELLI and J.S. KAUER (Tufts Med.School and New England Med. Ctr., Boston, MA 02111).

Although mitral/tufted cells (M/Ts) have been the focus of numerous studies, the sources of long-lasting excitability changes following odor and electrical stimulation remain unclear. Using standard intracellular techniques in salamander olfactory bulb slices (400-600 um thick), we have observed late depolarizing responses in M/Ts related to some of these excitability changes. Following olfactory nerve stimulation, salamander M/T responses are basically similar to those found in other species. Olfactory nerve volleys applied at moderate intensities first evoke a brief period of depolarization which often generates a single action potential, followed by a prolonged period of hyperpolarization. This late hyperpolarization is thought to arise predominantly from dendrodendritic inhibitory feedback via granule and periglomerular cells. As observed in other species, this hyperpolarization shows three different components; an initial sharp onset, a second short component, and, finally, a longer-lasting phase. Each component has a different reversal potential with hyperpolarizing current injections; the period between the first and second components reverses at less negative membrane potentials than either of these components or of the third component. This complex reversal pattern may reflect the widespread distribution of granule-to-mitral inhibitory synapses or it could be due to a previously undescribed late excitatory event. In addition to the results described above obtained under standard conditions, we have found that olfactory nerve stimulation applied just above threshold can evoke a second period of late depolarization. Under these conditions, M/T responses consist of an initial depolarization often with an action potential, a subsequent brief period of hyperpolarization, and then a second period of depolarization. This second depolarizing phase resembles a similar component that has been observed in the turtle after blocking GABA-a receptors with bicuculline. Using low stimulation intensities in slice preparations, inhibitory activity is apparently reduced, thus facilitating the appearance of this late depolarization. As stimulus intensity is increased, this component is progressively masked by the late hyperpolarization. Late depolarizing responses have also been found in voltage sensitive dye and field potential records, and associated with late calcium transients in M/Ts at low bath temperatures. These data indicate that the complex patterns of M/T responses are critically dependent on stimulus conditions. Specifically, these late depolarizations may have implications for the generation of intrinsic oscillatory events in the olfactory bulb and for understanding the complexities observed in M/T odor responses.

Supported by PHS Grant NS-20003 and the Dept. of Neurosurgery, Tufts-New England Medical Center.

The Intrabulbar Association System is Formed by CCK-containing Tufted Cells in the Rat

WEILIN LIU AND MICHAEL T. SHIPLEY (University of Cincinnati)

Mitral and tufted cells are the output neurons of the main olfactory bulb (MOB). In addition, a subpopulation of tufted cells give rise to the intrabulbar association system (IAS). These tufted cells project massively to the internal plexiform layer (IPL) on the opposite side of the same bulb. The transmitter(s) released by IAS terminals is unknown, however. Here we show that the IAS is comprised of tufted cells that contain the excitatory neuropeptide, CCK.

WGAapoHRP-Au was injected into the internal plexiform layer (IPL) on either the lateral or medial side of the olfactory bulb. Such injections labeled a *discrete* population of tufted cells specifically located in the superficial part of the external plexiform layer (EPL) and the deep part of the glomerular layer (GL) on the same and *opposite* sides of the bulb as the injection site. Lateral injections labeled tufted cells on the medial side of the bulb in a discrete region *ventral* and *caudal* to the level of the injection site. Medial injections labeled tufted cells in a discrete region on the lateral side of the bulb, *rostral* and *dorsal* to the level of injection site. Thus, the IAS derives from superficial tufted cells.

Biocytin injections into the superficial part of the EPL on one side of the bulb, anterogradely labeled a dense, terminal projection in the IPL both on the same and the *opposite* side of the olfactory bulb as the injection site. The axons of superficial tufted cells coursed directly through the EPL and MCL to the IPL where they turned abruptly to run ventrally and dorsally. These axons gave rise to two discrete terminal fields in the IPL: one on the same side of the bulb as the injection site and a second on the opposite side of the bulb. Axons from lateral injections formed a dense, focal terminal field in a region of IPL on the medial side, *ventral* and *caudal* to the injection site. Axons from medial injections terminate in a focal region of the IPL on the lateral side, *rostral* and *dorsal* to the injection site.

Antibodies to CCK labeled numerous tufted cells and a dense terminal field throughout the IPL. This suggested that the IAS may be formed by axons of CCK-containing tufted cells. To test this hypothesis, CCK immunocytochemistry was combined with WGAapoHRP-Au retrograde labeling. The results demonstrated that all tufted cells retrogradely labeled by WGAapoHRP-Au injections in IPL were immunoreactive for CCK.

These results show that the IAS is discretely and topographically organized. The IAS is composed exclusively of CCK containing axon terminals originating from the superficial tufted cells. The strict localization of IAS terminals to the IPL suggests that this pathway synaptically targets neural elements present in the IPL. The most likely target would be the dendrites of granule cells coursing through the IPL towards the EPL. In other neural systems, CCK consistently produces postsynaptic depolarizing responses. If CCK has a similar action in the IAS, then it is reasonable to hypothesize that IAS functions to depolarize granule cell dendrites. This could increase GABA release from granule cell synapses thus inhibiting a discrete population of mitral/tufted cells on the same and opposite sides of the bulb. Alternatively, CCK could shunt conduction in the granule cell dendrites causing a decrease in GABA release, thus exciting discrete populations of mitral/tufted cells. Experiments to test these alternatives are in progress. *(Supported by: NIH DC00347 & NS 29218)*

293

Olfactory Bulb Input to the Rat Olfactory Tubercle is Limited to Specific Tubercular Regions.

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The rodent olfactory tubercle (OT) is the site of converging input from the olfactory bulb (OB), limbic cortex, mesencephalon, amygdala and thalamus. A multipart study is in progress that will examine how all of these disparate systems interact in the OT. In this part of the study, wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was injected into defined regions of the OB to determine the precise pattern of OB input to the OT. Following a survival of 2 or 10 days, the OB and OT were processed and examined by light microscopy. Regardless of where the injection was placed in the bulb, the pattern of terminal labeling in the OT was the same. At the most rostral level, label extended across the width of the OT layer IA. However, with each subsequent section the medial edge of the label moved laterally until, at the caudal sections, only the central to lateral part of layer IA was labeled. Layer II somata were not labeled. The exception to this pattern was consistent labeling of the neuropil just medial to the characteristic "hook" formed by the most caudal and medial layer II OT neurons. In rats that survived only 48 hours a scant amount of label was occasionally apparent just under the pia of the medial part of layer IA. However, the label was not present after the 10 day survival. This suggests that the superficial label noted after a 48 hour survival time was not in terminals but rather still en route through the fibers known to cross the ventral surface of the OT in an oblique lateral-to-medial direction. The conclusion to be drawn from this preliminary study is that the OB does not project terminals to the medial OT except at its most rostral and caudal ends.

294

A morphometric view of the mink olfactory bulb - a comparative analysis of three different races

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The American mink (*Mustela vison f. dom.*) is kept and bred in captivity for about 100 generations. During that time different races were developed which vary in coloration and in some cases in body size. Some of these races are rather old while others are comparatively young. An example for an old representative is the race "Standard", while the races "Silverblue" and "Pastel" are bred in captivity for only about 50 generations. The present study was therefore undertaken to elucidate possible differences in the olfactory bulb between these three races.
There are no differences in the mean body weight between the three races. However, we found differences in the mean brain weight and volume, as well as in the volume of the olfactory bulb (BO). Lowest values were found in the old race "Standard" (brain weight 8.42 g, BO volume 108 mm^3) while the highest values were present in the race "Pastel" (brain weight 8.89 g, BO volume 129 mm^3). The volume covered by the glomeruli and external plexiform layer (EPL) also differs. For "Standard" the data are for the glomeruli 15.3 mm^3 and for the EPL 24.4 mm^3; in "Pastel" we found for the glomeruli 20.6 mm^3 and 27.3 mm^3 for the EPL. Whether the here reported differences are due to differences in the time the races were bred in captivity remains to be explored.

295

Evidence for Columnar Organization in the Rat Olfactory Bulb: Intracellular Injection of Lucifer Yellow into Mitral Cells.

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Evidence from numerous investigators suggests that the olfactory bulb is organized into anatomical and functional columns. By intracellularly injecting Lucifer yellow (LY) into lightly fixed bulb cells, we add further anatomical support for this notion. LY completely fills injected cells, demonstrating their dendritic and axonal morphologies, and passes through gap junctions, filling any coupled cells. Horizontal, 300μm thick sections from lightly fixed bulbs of postnatal Day 10 (P10), P20 and P30 old rats (n = 6/age) were stained with a blue fluorescent nuclear stain. Under an inverted scope, mitral cell (MC) somas were located and iontophoretically injected with LY. After the injection, the cells were photo-oxidized with DAB to form a permanent stain. When a single MC was injected, both proximal MCs and deeper granule cells (GC) filled with dye. After single cell injection (n = 17) in P10 rats a mean of 2-3 dye-coupled MCs and 27 GCs were observed. Most stained GC were found within 100μm of the MC layer. Mean length of the column of dye-coupled GCs was 170μm while the width was 85μm. These values were unchanged at P20 (n = 12 cell injections). By P30 the mean number of GCs per column increased to 42 (n = 12 cell injections). The addition of GCs in the P30 rats occurred in the area proximal to the MC layer while overall column length and width remained unchanged. The existence of dye-coupling between mitral cells and between mitral and granule cells suggests cell subpopulations which have specific information processing domains. The fact that the labelled cells were always found in vertical bands of similar length and width provides further evidence for the existence of a columnar organization in the bulb.

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296

Morphological and Physiological Characterization of Salamander Olfactory Bulb Granule Cells.

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Olfactory bulb granule cells are GABAergic interneurons that inhibit bulbar output neurons. Few studies have examined directly the synaptic inputs to granule cells in an intact circuit. In order to understand better the synaptic circuitry underlying olfactory processing, we have developed an *in vitro* hemibrain preparation that contains the olfactory bulb and central structures. To date, 74 salamander olfactory bulb granule cells have been recorded with the whole cell patch clamp technique. Biocytin-filled granule cells show spiny dendrites both perpendicular and parallel to the bulbar layers, often extending over 50% of the width of the bulb. Granule cells that are particularly well-filled show a thin, axon-like non-spiny process that projects into the external plexiform layer. Granule cells exhibit normal resting potentials (-61 ± 12 mV), high input resistances (2.1 ± 0.3 GΩ), long charging time constants (123 ± 11 msec), and more sustained voltage-gated K^+ currents as compared to output cells recorded under similar conditions. Granule cells fire only one or two spikes to strong intracellular depolarizing pulses and electrical stimulation of the olfactory nerve (ON) or medial olfactory tract (MOT). Following ON/MOT stimulation, the spike(s) ride on the rising phase of a several hundred msec EPSP, which is sometimes followed by a long, 1-4 mV hyperpolarization. The synaptic current underlying the ON or MOT-evoked EPSP has a rapid rise and slow decay. In the presence of bicuculline methiodide (BMI), a $GABA_A$ receptor antagonist, spontaneous and electrically-driven excitatory synaptic input to granule cells is enhanced. This is observed by an increase in synaptic currents, in the size and duration of the evoked EPSP, and in the number of action potentials evoked. Spontaneous and ON/MOT-initiated periodic depolarizations are also often observed in these conditions. The excitatory drive present in BMI is blocked by combined application of glutamate receptor antagonists CNQX and AP5, indicating that granule cells receive glutamatergic inputs. Recordings of granule cells in conditions that enhance GABA-mediated chloride currents further indicate that granule cells receive GABAergic synaptic input, which might prevent repetitive firing during strong excitation. Finally, the time course and pharmacological sensitivity of granule cell responses correlate well with voltage-sensitive dye recordings, supporting the hypothesis that bulbar dye signals arise mainly from granule cell activity.

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GABA and Taurine in the Frog Olfactory Bulb: Possible Colocalization in the Granule Cells.

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Granule cells, which are inhibitory interneurons in the olfactory bulb and the target of intrinsic and many centrifugal inputs, play an important role in processing of olfactory signals. There is strong evidence that the synaptic effects of granule cells are mediated by GABA, and GABA-immunoreactivity has been observed in somata and dendrites of granule cells in different species. In this study, we compared the distribution of immunoreactivities for GABA and taurine, another potent inhibitory amino acid, in the granule cell layer of the frog olfactory bulb using PAP-immunohistochemistry and postembedding immunogold technique. Taurine-immunopositive neurons, identified with light and electron microscopy, exhibited higher intensity of immunostaining than GABA-immunopositive granule cells, and their processes could be traced at longer distances than those of GABA-positive cells. Taurine-immunopositive somata, were found to localize only in a caudal dorsolateral portion of the granule cell layer. Unlike these cells, GABA-immunopositive neurons composed all of the cell population in the granule cell layer, including the area occupied by taurine-immunopositive cell bodies. These observations suggest an existence of a limited subpopulation of granule cells in which GABA and taurine may be colocalized.

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Multiple Amino Acid Receptors Mediate Inhibition in the Olfactory Bulb.

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The inhibitory amino acids (IAA) glycine and GABA, acting at glycine and GABAa receptors respectively, dominate inhibition in the mammalian CNS. In the olfactory bulb (OB), GABA localization to periglomerular and granule cells supports the notion that GABA contributes to the inhibition mediated by these cells. Immunohistochemical experiments have also demonstrated localization of glycine and glycine receptors in the OB. Recently, however, a complex picture of IAA receptor diversity in the brain has evolved. Molecular biological evidence has demonstrated the expression of many isoforms of both glycine and GABA receptors each with unique properties dependent on subunit composition. These results have led us to explore the diversity of glycine and GABA receptors that contribute to synaptic inhibition in the OB. We examined the responses of OB neurons to glycine and GABA using primary cell culture and whole cell recording techniques combined with a rapid flow drug application system. OB cultures from E18-P2 rat pups contained both mitral/tufted cells and periglomerular/granule cells. All neurons tested (n=40) responded to glycine (EC50=125 μM) and GABA (EC50=42 μM). When intracellular [Cl^-] was altered, the membrane current reversed at the Nernst value for Cl^-, consistent with activation of a ligand gated Cl^- channel. Both the glycine and the GABA evoked currents showed nonclassical antagonist pharmacology; both were blocked by strychnine (1-30 μM) or picrotoxin (30-100 μM) whereas bicuculline (1-10 μM) was selective for GABA. GABA but not glycine evoked currents were inhibited by zinc (10-100 μM). In most neurons GABA desensitized the response to glycine; in a smaller proportion of neurons glycine desensitized the response to GABA. In some cells there was no cross desensitization. GABA and glycine evoked currents were additive in cells which did not show cross desensitization but were not additive in cells which cross desensitized in either direction. Interestingly, variations in cross desensitization did not affect antagonist pharmacology. These results suggest that both GABA and glycine contribute to inhibition in the OB. The effects of cross desensitization and antagonist pharmacology demonstrate the expression of multiple isoforms of IAA receptors which could influence synaptic inhibition through selective modulation.

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NMDA receptor modulation of olfactory bulb inhibitory circuits.

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Olfactory bulb mitral/tufted cell excitability is controlled through extensive dendrodendritic synaptic interactions with granule cells. This circuit is believed to involve glutaminergic excitation of granule cells followed by a reciprocal GABAergic inhibition of mitral/tufted cells. The majority of these mitral/tufted - granule cell reciprocal synapses are located in the external plexiform layer, which also contains a high density of the NMDA subclass of glutamate receptors. Previous work has demonstrated that blockade of NMDA receptors in the olfactory bulb with APV reduces presumed granule cell-mediated inhibition (Jacobson, et al., 1986). The present study further examined the role of NMDA receptors in mitral/tufted - granule cell interactions using evoked potential and single-unit recordings and hybridization of c-fos cRNA as a marker for cell activity. Wistar rats were anesthetized with urethane (1.5 g/kg). After baseline measurements of LOT paired-pulse inhibition and LOT single-pulse suppression of spontaneous mitral/tufted cell single-unit activity, animals were injected with the NMDA antagonist MK-801 (1 mg/kg, i.p). Measurements of inhibition were repeated 90 min post-injection. Additional animals were anesthetized, injected with MK-801, sacrificed 90 min later, and the bulb tissue processed for in situ hybridization with c-fos cRNA. The results demonstrate that MK-801 reduces excitation of granule cells which in turn, disinhibits mitral/tufted cells. These results suggest that NMDA receptors serve an important role in modulating olfactory bulb excitability.

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Secretions from Olfactory Bulb Influence Migration of Ensheathing Cells.

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Ensheathing cells (ECs) are the glial cells in the primary olfactory pathway which share some common characteristics with both Schwann cells and astrocytes. However, little is known about their functions in olfactory axonal development. In rat and mouse embryos, ECs are observed migrating with the pioneering olfactory axons towards the presumptive olfactory bulb. This suggests that ECs may play a role in guiding the olfactory axons to their appropriate target. Previous studies have shown that ECs express molecules such as N-CAM, L1 and laminin which are neurite promoting *in vitro*. As such, they provide a permissive substrate on which axons can grow. However the directional cues for guiding olfactory axons towards their target remain to be elucidated. In this study, we use a blind-well chemotactic chamber to show that the olfactory bulb may perform an instructive role in EC migration. The chemotactic chamber is composed of two compartments, an upper and a lower, separated by a fibronectin coated nuclepore filter (12 μm pore size). Ensheathing cells, purified from the olfactory nerve layer of neonatal rat olfactory bulbs, are seeded onto the upper surface of the filter. The lower chamber contains medium conditioned by neonatal olfactory bulbs. After 6 hours of incubation at 37°C, the filter is removed, fixed and processed for scanning electron microscopy. The cells that have migrated through the pores and are now found on the underside of the filter are counted. The number of cells is significantly greater in the experimental group in which the conditioned medium is present. Therefore, the results indicate that ECs can respond to diffusible signals from the olfactory bulb by migrating towards the source of the signals.

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301

Ultrastructure of the Elasmobranch Olfactory Glomerulus.

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The elasmobranch olfactory bulb shares many similarities with the olfactory bulb of higher vertebrates. However, it also presents several differences, including the lack of an external plexiform layer, lack of mitral cell basal dendrites, absence of periglomerular cells, and innervation of the glomeruli by granule cells. In order to understand the nature of the connections resulting from these features, we have examined the ultrastructure of the elasmobranch olfactory circuitry in bonnethead sharks. The glomeruli exhibit a peculiar arrangement in bouquets, in which several glomeruli originate from a stem of sensory fibers coming from the olfactory epithelium. The mitral cells are immediately adjacent to the glomeruli, resembling mammalian tufted cells in their position. Semi-thin sections show the distinct boundaries of the glomeruli (25-300 μm diameter). The mitral cells, lacking basal dendrites, send thick primary dendrites to the glomeruli. The sensory terminals are filled with vesicles (30-50 nm) embedded in a dark matrix, conferring a dense appearance to the glomeruli. In addition to regular dense olfactory vesicles, a few sensory terminals contain another type of large (100-200 nm) dense core vesicle. The sensory terminals synapse onto small dendritic profiles within the glomeruli as well as onto the large dendrites that enter the glomeruli. We are currently investigating the possibility of reciprocal and serial synapses, as well as the synaptic arrangement of the granule cells.

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302

NADPH-Diaphorase Localization in the Olfactory System.

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Nitric Oxide (NO) has recently been identified as a neuronal messenger. NO synthase is abundantly distributed in olfactory bulb (Bredt et al., Neuron 7: 615, 1991). NO synthase and NADPH-diaphorase have been shown to have identical localization (Dawson et al., PNAS 88: 7797, 1991; Hope et al., PNAS 88: 2811, 1991). To test the hypothesis that NO may be involved in odor processing (Breer and Shepherd, TINS 16: 5, 1993), we have investigated NADPH-diaphorase localization in olfactory epithelium and bulb of salamander and rat using NADPH-diaphorase histochemistry. In the olfactory epithelium we found the most intense staining of NADPH-diaphorase concentrated in the cilia layer. The respiratory epithelium in rat was only weakly stained and showed no evidence of lamination of the staining pattern. In the olfactory bulb of both salamander and rat the olfactory nerve and glomeruli were stained. However, the glomerular staining in salamander appeared homogeneous while in rat individual glomeruli were discretely stained. To test if NADPH-diaphorase was localizing to olfactory cells we employed transection of the olfactory nerve or total bulbectomies. Transection of the olfactory nerve in salamander or bulbectomy in rat resulted in decreased staining in the cilia layer of the olfactory epithelia. Transection of the olfactory nerve in salamander also caused the disappearance of staining in the olfactory nerve and glomerular layer of the olfactory bulb. These results support the idea that NADPH-diaphorase localizes to the cilia of olfactory receptor cells at the level of the epithelium and to the axonal terminals of the receptor cells within the olfactory bulb glomeruli. This further suggests a functional role for NO in modulating olfactory transduction and perhaps a mechanism for interaction between receptor cells.

303

Comparison between OMP-immunoreactive and LHRH-positive fibers in the brain.

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It has been previously reported that, in rats, numerous OMP-positive fibers extend past the olfactory bulb to the olfactory peduncle parenkyma where they intermingle with neurons of the anterior olfactory nuclei (AChemS 1992). For their immunoreactivity to anti-OMP (olfactory marker protein) serum, it was postulated that these fibers originate from primary olfactory neurons. From those preliminary observations it appeared that the OMP-immunoreactive fibers were following the route of others (LHRH-positive) belonging to the terminal nerve. As a forward step in this investigation, it seemed appropriate to study the topographical relationship of the two sets of fibers and, in addition, to extend the observation to other areas of the brain associated with the terminal nerve. Under deep anesthesia, the animals were perfused with various fixatives: 4% paraformaldehyde, Bouin's, periodate-lysine-paraformaldehyde or a mixture of aldehydes. The brains, removed from the skull, were sectioned with a vibratome in the frontal or sagittal plane. Alternate adjacent sections, 100μm thick, were immunohistochemically stained for OMP and LHRH (1:60,000 and 1:100,000 respectively) using the Vectastain ABC staining procedure. OMP-immunoreactive fibers were seen to extend from the olfactory bulb to the diagonal band. They were more numerous than the LHRH-positive fibers of the terminal nerve and their distribution, although partially coinciding with that of LHRH-positive fibers, involved larger areas. These results raise new questions about the relationship of the olfactory sensory organ with the brain and ,possibly, about the role that OMP plays in this interaction. (Supported by NIH grant NS 20699. Anti-OMP serum was a gift of F.E. Margolis; anti-LHRH serum a gift from R. Benoit).

304

Single Trigeminal Ganglion Cells have Collateral Branches in the Nasal Epithelium and the Olfactory Bulb.

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Our previous studies have shown that peptidergic, capsaicin-sensitive nerve fibers of the trigeminal nerve innervate the nasal epithelium and glomerular layer of the olfactory bulb. The termination of presumed trigeminal sensory axons in the neuropil of the olfactory bulb is peculiar since peripheral sensory fibers are not believed to convey information arising in the central nervous system. One possible role for these bulbar trigeminal sensory fibers may be to convey information about nasal irritants to the olfactory bulb via an axon-reflex type of mechanism (i.e. conduction into collateral branches). For this to be the case, however, a single trigeminal ganglion cell must extend collateral branches to both the epithelium and the bulb. In order to test this hypothesis, different fluorescent retrograde tracers were injected into the olfactory bulb and nasal epithelium in the same rat. For most experiments, nuclear yellow was injected into the epithelium (10 μl in at least 4 sites) while true blue was injected into the olfactory bulb (1 μl in 3-4 sites in the rostral half of the bulb). Following a 2-day post-surgical survival period, the rats were reanesthetized and perfused with 4% buffered paraformaldehyde. The trigeminal ganglia were sectioned on a cryostat at 15-20 μm and examined on a fluorescence microscope. In most cases double-labeled, as well as more numerous single-labeled ganglion cells were found in the ipsilateral trigeminal ganglion. The single-labeled true-blue cells were a variety of sizes, ranging from large to small. The single labeled nuclear yellow cells were sparser and tended to be small or medium-sized. The double-labeled cells always were small cells and were situated in a relatively small area of the ganglion. These results indicate that single trigeminal ganglion cells have a sensory arborization with branches in both the nasal epithelium and olfactory bulb. Thus an avenue exists whereby nasal irritants that stimulate trigeminal nerve endings might act via an axon reflex to release neuroactive peptides into the bulbar neuropil thereby affecting processing of simultaneous or subsequent olfactory stimuli.

305

Optical Recording of Cortical Activity Evoked by Chorda Tympani Stimulation in the Hamster. J. ZEIGER and J.A. LONDON (Dept. BioStructure and Function, Center for Neurological Sciences, Univ. of CT Health Center, Farmington, CT)

Electrical stimulation of the chorda tympani in the middle ear of the hamster evoked cortical activity in the area of the gustatory cortex, and in discreet areas posterior and medial to this cortical region. Male golden Syrian hamsters were anesthetized with pentobarbital (80 mg/kg body weight, i.p.), and maintained with urethane (425 mg/kg body weight, i.p.) throughout the experiment. A 50 mm^2 area of cortex was exposed over the gustatory cortex and surrounding area. The cortex was then stained with the voltage-sensitive, fluorescent styryl dye, RH795 (Molecular Probes) for 60 to 90 minutes followed by a 30 minute wash with physiological saline. The animal was paralyzed with gallamine triethiodide (50 mg/kg body weight, i.p.) and artificially respirated. Optical recordings were made with a fluorescent upright microscope and photodiode array as described previously (London, 1990). The stimulating electrodes were two $Ag/AgCl_2$ wires spaced about 0.8 mm apart, inserted through the tympanic membrane, and positioned against its dorsal margin in the vicinity of the chorda tympani. A constant-current stimulus isolation unit was used to deliver 100 ms DC current pulses of from 0.5 to 1.5 mA through the stimulating electrodes. At threshold currents between 0.5 and 0.8 mA, optical signals were recorded in an area of cortex just anterior and medial to the base of the zygomatic arch, with clusters of smaller signals of opposite sign occurring several millimeters posterior and medial to these. Reversing the polarity of the nerve stimulation blocked this activity. Increased stimulating current resulted in signals of increased amplitude, as well as recruitment of additional areas of cortical activity around the sites of activity seen near threshold. These preliminary results suggests the possibility of determining the extent of the gustatory cortex by stimulating peripheral taste nerves.

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306

Optical Recordings with Voltage-Sensitive Dyes in Organotypic Cultures of Rat Agranular Insular Cortex.

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

Fluorescent optical recording techniques using voltage-sensitive dyes and a photodiode array were used to record neural population activity in organotypic cultures of rat brain containing agranular insular cortex (gustatory cortex) and granular cortex. Tissue was taken from 5-7 day old Long-Evans rat pups and maintained *in vitro* for up to two months. Transverse slices, 300μ thick, were taken from the area of the brain between the genu of the corpus callosum, and caudally to the crossing of the anterior commissure. The styryl dye, RH795 (Molecular Probes), was applied to the tissue at concentrations of 0.2 mg/ml and 0.5 mg/ml. Staining times of 30 minutes to 2.5 hours were tested. Slices were placed in a recording chamber mounted on the stage of an upright microscope fitted for fluorescence. An excitation filter of 530nm BP50 (Omega) and an emission filter of 610nm (Schott) were used. A 2.5X or a 25X long-working distance objective were used to collect dye signals. The toxicity of the dye on the cultures was assessed by evaluating changes in neural activity recorded using glass microelectrodes, and was verified with a two-stain viability test using calcein-AM to stain live cells and ethidium homodimer to stain dead cells (Molecular Probes). The higher concentration of the dye, 0.5 mg/ml, was toxic after 1 hour of staining. The largest signals were obtained with the lower concentration of the dye, 0.2 mg/ml, applied for 1.5-2.5 hours. The dye bleached after several trials, but signal was restored after re-application of the dye for 45 minutes to 1 hour. In order to study the generation of epileptic activity in the gustatory cortex, 0.1 mM of bicuculline methiodide was applied to the bathing medium. Seizure-like activity was recorded both optically and with glass microelectrodes, and occurred in both the agranular and granular cortices.

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307

Secondary Facial Lobe Connections in a Specialized Cod, the Rockling (Ciliata mustela, Gadidae, Teleostei)

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In the rockling, the primary sensory facial lobe consists of distinct dorsal (DFL) and ventral (VFL) subdivisions. It was previously shown that the DFL receives its input exclusively from nerve fibers innervating solitary chemosensory cells (SCC) of the anterior dorsal fin, whereas the VFL receives fibers from the remaining body surface, mainly from taste buds (Kotrschal & Whitear, 1988). As the two lobes may be involved in different functional roles (predator avoidance in case of the DFL, food search in case of the VFL, Kotrschal, 1991), their higher order connections were expected to differ significantly from each other. The carbocyanine dye DiI was used to investigate their higher order brain connections. Both lobes send fibers into the contralateral facial lobes. Ascending fibers were traced into the secondary gustatory nucleus and into a small area in the dorsolateral brain stem directly adjacent to the caudal cerebellar crest. Additionally, the VFL had fiber connections with a dorsal reticular area directly rostral to the VFL. Both, DFL and VFL were found to have descending fibers into two dorsal brain stem nuclear areas, the VFL connections being considerably more extended. With VFL dye application, labelled perikarya were found in the two small areas rostral to the gustatory brain stem as well as in the two caudal brain stem areas, indicating reciprocal connectivity. With DFL dye application, labelled perikarya only appeared in one rostral and one caudal area. Contrary to our expectations, the secondary connections of either lobes were not distinct. The more specialized function of the DFL as compared to the VFL may account for its more restricted secondary connections.

Kotrschal, K. (1991) Rev. Fish Biol. Fisheries, **1**, 3-22.
Kotrschal, K. and Whitear, M. J. (1988) Comp. Neurol., **286**, 109-120.
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308

Innervation of the Nares Constrictor Muscle by the Nervus Terminalis and Palatine Ganglion in the Tiger Salamander.

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The nares constrictor muscle (NCM) is a small smooth muscle located along the ventral border of the external nares in salamanders[1]. Constriction of this muscle closes the nares, and may also press on the external nasal glands causing mucus secretion. These external nasal glands are situated just rostral to Jacobson's organ, a strategic location for controlling chemical access to this organ. No information has been available regarding the innervation of the NCM. We have recently demonstrated that the NCM is innervated by luteinizing hormone-releasing hormone-immunoreactive (LHRH-ir) processes (probably of nervus terminalis origin[2]) which travel along a myelinated nerve bundle of unknown origin.

In the present study, we have used biocytin-tract-tracing and immunocytochemical procedures to elucidate the origins of neural innervation of the NCM. We now report that the NCM is innervated by parasympathetic fibers (which form the myelinated nerve bundle) from the palatine ganglion which is located just posterior to the nasal capsule. The LHRH-ir fibers innervating the NCM originate from nervus terminalis cell bodies located within the autonomic nerve bundle. In addition, LHRH-ir fibers and cell bodies are found in the palatine ganglion and may contribute to the projection of LHRH-ir fibers to the NCM. We hypothesize that the significance of LHRH-ir fibers within the NCM and palatine ganglion lies in controlling the access of pheromones to Jacobson's organ during courtship activities in salamanders.

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2. Wirsig-Wiechmann, C.R. 1993 *Cell Tiss. Res., In press.*
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309

In Vitro Patch Clamp Analysis of Postsynaptic Potentials Mediated by Excitatory and Inhibitory Amino Acids on Neurons in the Gustatory Zone of the Nucleus Tractus Solitarius. LIMEI WANG, MICHAEL S. KING and ROBERT M. BRADLEY (Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan).

To define synaptic input from afferent fibers onto neurons in the rostral nucleus tractus solitarius (rNTS) we electrically stimulated the solitary tract while recording intracellularly from second order neurons. Sixty neurons in coronal slices of rat rNTS 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. The solitary tract was stimulated with pulses (0.05-0.1 ms duration, ≤ 10 mA) using a bipolar stimulating electrode. Neurons in the rNTS have a complex pattern of postsynaptic potentials, the majority of which are excitatory (EPSP), but inhibitory (IPSP) and EPSP-IPSP complexes were also recorded. EPSPs are mediated by glutamate acting at both the NMDA and non-NMDA glutamate receptors. Both APV, an NMDA receptor antagonist, and CNQX, a non-NMDA receptor antagonist, reduce or abolish the amplitude of the EPSPs. When superfused with a Mg^{2+}-free solution containing CNQX and APV, the EPSPs were totally blocked. Because the IPSPs were reduced or blocked by the $GABA_A$ antagonist bicuculline and the $GABA_B$ antagonist phaclofen, GABA apparently mediates IPSPs in rNTS. Many rNTS neurons have both excitatory and inhibitory inputs because the amplitude of the EPSPs increased, or IPSPs became EPSPs, once the GABA input was blocked by GABA antagonists. In addition, application of glutamate receptor antagonists resulted in the abolition of the excitatory component of the postsynaptic potential leaving the inhibitory component. Seventeen neurons were filled with biocytin permitting subsequent correlation of morphology and postsynaptic potentials. Multipolar, elongate and ovoid neurons all receive both excitatory and inhibitory input. These results indicate that rNTS neurons receive multiple inputs that could exert powerful control over sensory processing by rNTS neurons.

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310

Fos Protein Expression in NST Neurons Following Electrical Stimulation of Taste Nerves. THERESA A. HARRISON and NANCY W. MILLER (Cellular Biology & Anatomy, Medical College of Georgia)*

Fos protein immunocytochemistry was used to study the localization of populations of neurons in the nucleus of the solitary tract (NST) which are synaptically activated by different taste nerves. Either the lingual-tonsilar branch of the IX^{th} nerve, or the chorda tympani nerve, was stimulated electrically with high frequency pulse trains for three hours in anesthetized rats. Frozen sections of 4% paraformaldehyde-fixed brains were reacted with an affinity-purified anti-Fos peptide antibody (Santa Cruz Biotechnology, Inc.) at a final concentration of 0.05 μg/ml. Fos immunoreactivity was visualized with the avidin-biotin-HRP procedure and DAB histochemistry. After IX^{th} nerve stimulation, labeled cells were located unilaterally in the NST, extending from 500 μm anterior to the obex for a distance of over 1.0 mm, to within 250 μm of the anterior pole of the nucleus. Posteriorly, the cells formed two groups, one clustered around the solitary tract, the other dispersed within the medial subdivision. More anteriorly, the cells were concentrated medially in the nucleus. Chorda tympani-activated cells were found unilaterally beginning about 1.3 mm anterior to the obex, and extending no more than 500 μm to the anterior pole. While a few labeled cells were found in the NST posterior to the obex when either nerve was stimulated, these cells were also found in sham-operated control animals. These studies provide important information about the distribution of neurons activated from different nerves and complements previous studies of afferent termination zones and electrophysiological data.

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311

Spatiotemporal coding of taste quality in the nucleus of the solitary tract of the rat. S. MONROE & P.M. DI LORENZO (Dept. of Psychology, SUNY at Binghamton, Binghamton, N.Y. 13901-6000)

An appropriate model of taste neural coding should adequately predict the abilities of the rat to discriminate taste qualities. Three possible mechanisms were investigated as candidates for the neural code for taste quality: spatial, temporal and spatiotemporal models. To compare these models electrophysiological responses were recorded from 67 taste-responsive units from the NTS of the rat anesthetized with urethane (1.5 gm/kg). Taste stimuli consisted of NaCl (.1 M), HCl (.01 M), quinineHCl (.01 M), sucrose (.5 M), and saccharin (.004 M). Multidimensional scaling analyses (MDS) were used to visualize the relationships among the stimulus evoked response patterns for the different models. Pearson correlation coefficients were used to compare the similarity between spatial patterns of response. A new approach to compare the similarity between temporal and spatiotemporal patterns of response was used. With this method, the time course of response was represented by a vector in a 60-dimensional space, where 60 is the number of 50 ms time bins during the first 3 sec of response. The angle between two vectors was used to describe the similarity between two time courses of response. The length between the endpoints of the two vectors was used to describe the similarity between two spatiotemporal patterns of response. Results of the MDS analysis were then used to compare the ability of the three models to predict taste discrimination in the rat. Comparisons among the results of the three models, showed that the results based on the spatiotemporal model more closely approximated the ability of the rat to discriminate taste qualities. In addition, only the spatiotemporal model was able to predict the ability of the rat to discriminate taste qualities within the first 1000 ms of response, and may as early as 200 ms. This corresponds to the time that the rat uses to make these discriminations (Halpern, 1985).

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312

Morphology and Physiology of Neurons in the Gustatory Zone of the Nucleus Tractus Solitarius. MICHAEL S. KING and ROBERT M. BRADLEY (Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan).

To evaluate the relationship between the structure and function of neurons in the rostral nucleus tractus solitarius (rNTS) we studied the morphology and physiology of single rNTS neurons in rat medullary slices. Electrophysiological properties and responses were determined using whole-cell recordings. Morphological characteristics were determined by filling the neurons with 1% biocytin during recording, and fixing, sectioning and then processing the slices with avidin-HRP and DAB. 62 neurons were reconstructed using the Eutectic Neuron Tracing System. Reconstructed neurons were separated into three morphological groups by visual inspection, based on previously defined criteria (Lasiter and Kachele, 1988). Most neurons (48%) were classified as ovoid, suggesting an abundance of presumed interneurons, while 34% were multipolar and only 18% were elongate. Ovoid neurons have the shortest and thinnest dendrites which extend over the smallest area. Multipolar neurons have the most dendrites which possess the greatest number of spines. Elongate neurons have oblong somas and two relatively long dendrites which span a large area of the rNTS. The passive membrane properties of the three groups were similar, except that the input resistance of the ovoid neurons was largest. While a high percentage (43-58%) of all three types of neurons responded to a 1200 ms, 100 pA depolarizing current pulse with a repetitive spike train, 32% of the ovoid neurons responded with a short burst of action potentials and 55% of the elongate neurons showed a delay in the onset of the spike train following a hyperpolarizing prepulse. Only 11% of the multipolar neurons demonstrated either of these firing characteristics. The responses of the three types of neurons to substance P, GABA and NMDA were also tested. A high percentage (57-100%) of each type responded to one or more of these neurotransmitters. These results indicate that the three morphological types of rNTS neurons have several different electrophysiological properties, which are most apparent following current injection, and which may be the basis for functional differences.

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GABA Inhibition of Taste Responses in the Hamster Solitary Nucleus. HONGYANG LIU, MICHAEL BEHBEHANI and DAVID V. SMITH (University of Cincinnati College of Medicine).

Immunohistochemical studies have demonstrated that cells in the rostral portion of the NST contain GABA or its principal degradative enzymes. Previously, we have shown in an *in vitro* slice preparation that cells in the rostral hamster NST can be inhibited by application of GABA. In order to verify the effects of GABA on the responses of taste neurons in the NST, we have recorded the activity of taste-responsive cells *in vivo* before and after GABA administration. The sensitivities of 19 single neurons in the hamster NST were determined to three basic taste stimuli presented to the anterior tongue: 0.032 M NaCl; 0.1 M KCl and 0.1 M sucrose. Almost all cells were sensitive to more than one of these stimuli and the neurons responding best to sucrose, KCl or NaCl were determined. The response profiles of these cells were compared before and after pressure injection of 3 mM GABA. All neurons were spontaneously active with firing rates that ranged between 0.15 and 19.2 Hz; the mean spontaneous frequency was 3.5 ± 2.1 (sd) Hz. The spontaneous activity of 10 neurons was inhibited by GABA in a dose-dependent fashion; the other cells were not affected by GABA. BICM blocked the GABA inhibition in 4 of 6 cells tested. Of the 19 recorded cells, 7 were KCl-best neurons, 8 were NaCl-best and 4 were sucrose-best. GABA induced a statistically significant inhibition in the activity elicited by gustatory stimuli in 8 of these 19 cells. The response to sucrose was significantly inhibited by GABA in all 8 cells, with an average decrease of 56% in impulse frequency. Inhibition of the KCl response occurred in 5 of these 8 neurons; the mean decrease in firing rate was 39%. GABA produced an inhibition in the response to NaCl in only 3 cells, with an average decrease of 17%. These data strongly suggest that a GABAergic inhibitory network within the hamster NST serves to modulate taste activity. The greater effect of GABA on the response to sucrose parallels the suppression of sucrose seen in mixtures of sucrose with QHCl or citric acid.

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Chemosensitive Neurons in the Globus Pallidus. I. Endogenous Chemosensory Characteristics

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The globus pallidus (GP), in addition to its motor functions, is known to be involved in feeding-associated perceptual-motivational and metabolic regulation. To analyze firing characteristics and to reveal specific neurochemical attributes of local cells, extracellular single neuron activity was recorded and the multibarreled micro-electrophoretic technique was applied in the GP of anesthetized rats and anesthetized and alert rhesus monkeys. In both species, almost 8% of all cells tested displayed inhibitory activity changes to glucose administered electrophoretically. A majority of these glucose-sensitive (GS) neurons were suppressed by GABA and both the GS and the glucose-insensitive (GIS) cells exhibited either excitation or inhibition (or biphasic discharge rate changes) in response to glutamate or the NMDA-enantiomeres. The pallidal neurons showed distinct sensitivities to acetylcholine and to noradrenaline and dopamine as well. These results, along with previous observations, demonstrate the existence of glucose-detecting cells in the GP. The specific neurotransmitter modulation of these and other pallidal neurons may be of particular importance in the central control of feeding.

Chemosensitive Neurons in the Globus Pallidus. II. Exogenous Chemosensory Characteristics

KARÁDI, Z., FALUDI, B., CZURKÓ, A., HAJNAL, A., SÁNDOR, P., VIDA, I. and LÉNÁRD, L. (Institute of Physiology, Pécs University, Medical School, Pécs, H-7643 Hungary)

Despite the data on involvement of the globus pallidus (GP) in various aspects of feeding, little is known yet about the functional significance and neurochemical attributes of pallidal neurons. Our parallel studies showed that there exist GP cells whose activity is specifically suppressed by microelectrophoretically applied glucose. To elucidate feeding-related exogenous chemical properties of these chemo(glucose-)sensitive (GS) and the glucose-insensitive (GIS) pallidal cells, their extracellular single neuron activity was recorded in rats and rhesus monkeys during 1) micro-electrophoretic administration of chemicals, 2) gustatory and 3) olfactory stimulations. Approximately 5% of all GP neurons tested responded, at least to one, tastant. Mainly the GS cells exhibited gustatory responsiveness and many of the taste-responsive GS units changed in firing rate in repsonse to odorants as well. The taste- and odor-responsive GS and GIS pallidal cells displayed distinct sensitivities when noradrenaline, dopamine, acetylcholine, GABA and NMDA-enantiomeres were applied microelectrophoretically. These results indicate that the GP neurons are involved in central processing of feeding-associated endogenous and exogenous chemosensory information.

Effects of Chorda Tympani Section on NaCl taste-induced expression of C-*fos* in the parabrachial nucleus of Golden Hamsters. DAVID C. LARSON and MICHAEL A. BARRY (Department of BioStructure and Function, University of Connecticut Health Center).

We have been examining the use of the immediate early gene, c-*fos*, as a marker for gustatory activity in the central nervous system. A purpose of these studies is to determine central deficits that result from selective gustatory deafferentation, and ultimately to reveal the extent of recovery after nerve regeneration. Our previous behavioral studies have shown that the chorda tympani nerve (CT) is critical for sodium salt taste. In this study we examined the effects of CT section on NaCl induced expression of c-*fos* in the central nervous system. The CT was sectioned unilaterally in the middle ear of hamsters. Two-three weeks following surgery, the animals were accustomed to a 23 hour water deprivation schedule. On the test day, 0.15 M NaCl was substituted for water. Two hours after the NaCl drinking bout the animals were perfused with fixative, and the brains were removed. Transverse sections of the brain were processed for immunohistochemistry with a monoclonal antibody to c-*fos* protein. Preliminary evidence indicates that in the parabrachial nucleus the number of cells expressing c-*fos* were much greater on the control side than the chorda tympani lesioned side. Within the parabrachial nucleus, the labeled cells were located primarily in the central medial subnucleus and in the adjacent waist area (scattered among the fiber tracts of the superior cerebellar peduncle). Thus the behavioral deficits following CT section are accompanied by measurable changes in gustatory-evoked metabolic activity in the brain.

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Responses of Single Hamster Parabrachial Neurons to Binary Taste Mixtures: Sucrose + Citric Acid and NaCl + Citric Acid. MARK B. VOGT and DAVID V. SMITH (University of Cincinnati College of Medicine).

Although taste experience typically arises from a mixture of sapid stimuli, most neurophysiological research has focused on individual chemical components. Recently we have investigated the responses of hamster PbN neurons to anterior tongue stimulation with binary mixtures of sucrose + citric acid and NaCl + citric acid. Preliminary results are based on 69 response-concentration functions for mixtures and individual stimuli recorded from 24 neurons. Overall, mixture suppression (mixture response at least 5 imps/s < the response to the more effective component, MEC) was apparent in 29% of mixture responses, 65% did not differ from the MEC, and only 6% were greater. Sucrose suppression was displayed almost exclusively by sucrose-best neurons to the mixtures that contained the strongest sucrose and citric acid concentrations (mean mixture response < mean sucrose response, p's < 0.01). Significant NaCl suppression and citric acid suppression were also observed, though both were smaller in magnitude. The across-neuron patterns (ANPs) of activity evoked by the mixtures were generally very similar to those of the *more* stimulatory component. However, for the sucrose mixtures which evoked robust suppression, the ANPs were similar to those of the *less* stimulatory component. These data, in conjunction with our previous study of sucrose + QHCl mixtures, indicate that suppression is characteristic of the responses of third-order neurons to heterogenous mixtures. The forms of mixture suppression we have observed are analogous to those demonstrated previously in human psychophysical studies that employed similar mixtures.

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Physiological Mechanisms in Differential Context Effects.
KRYSTYNA M. RANKIN (John B. Pierce Laboratory and Yale University, and Psychology Department, Stockholm University)
LAWRENCE E. MARKS. (John B. Pierce Laboratory and Yale University)

This study investigated possible mechanisms underlying differential effects of context and how similarity affects the magnitude of the effect. Earlier studies have shown that (a) context effects arise when the stimuli are mediated by different receptors, and (b) stimuli judged as qualitatively similar are not subject to differential context effects. If stimuli are perceived as similar but processed via different receptors, does the information mediated into the CNS override the perceived qualitative similarity between them? In a series of experiments subjects judged the intensity of sucrose and one other taste or smell stimulus on one sensory scale, using the method of magnitude estimation. The taste stimulus was NaCl (perceived as different from sucrose) and the odours were vanillin and orange (perceived as similar to sucrose). In one set of experiments subjects tasted the sucrose and the odourants and in another they tasted the sucrose and sniffed the odourants Intensity judgements for all pairs of stimuli showed a significant effect of context, thereby lending no support to the view that these effects depend on perceived similarity per se.

Chimpanzee Single Taste Fibers

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YUZO NINOMIYA, (Asahi University, Gifu, Gifu Prefecture, Japan).
GRANT DUBOIS, (Corporate Research and Development, The Coca Cola Company, Atlanta GA, USA.)

These experiments have several purposes. More specifically: How similar is the sense of taste in humans and chimpanzees? How good of an animal model in taste is the chimpanzee? Are taste fibers in the chimpanzee more specific than in other mammals? Has the specificity of the taste fiber increased or decreased with evolution of primates? Is their specificity high enough so that their identity can code for a taste quality? If so, what is the contribution to the sensation of a taste fiber that responds somewhat to many taste qualities?

To study this we used 31 different compounds and the sweet taste modifiers, gymnemic acid and miraculin. We recorded the summated responses and responses in some 50 single nerve fibers from the chorda tympani nerve of 15 chimpanzees to sweet, sour, salty, bitter and umami tastants. About 50% of these tastants taste sweet to humans. Some of these had been chemically modified so that their sweetness was changed,

The recordings show that the sense of taste probably is very similar in humans and chimpanzees; that the taste fiber specificity in the chimpanzee is higher than in any other mammal studied; that a fiber that responds to many taste qualities probably adds noise to the taste sensation, but that a non-sweet fiber, responding to a sweetener, probably contributes with a non-sweet taste quality.

Chromosomal Location of Genes Affecting Sweet and Bitter Tastes in the BXD Recombinant-inbred Strains.
DAVID A. BLIZARD (Pennsylvania State University)
APRIL CHANG (Mayo Clinic)

A brief access test was developed in order to screen inbred mouse strains for variation in sweet, bitter, sour and salty tastes. Using this test, C57Bl/6J (B) had greater preference for sucrose and saccharin (sweet), greater aversion for quinine (bitter) and sour (HCl), and less aversion for salty (NaCl) solutions than DBA/2J (D) male mice. Based on these strain differences, concentrations were selected to screen the BXD recombinant-inbred (RI) strains, derived from the B and D progenitor strains. In the case of sweet and bitter solutions, the distribution of RI strain means was discontinuous, suggestive of the influence of genes with large effect size. In the case of sour and salty tastes, the distribution of RI strain means was continuous suggesting the influence of many genes of smaller effect size influencing these characters. Using Manly's RI-Manager (a computer program developed for the Mackintosh) the strain-distribution patterns (SDP's) for sucrose was mapped to mouse chr. 4, and confirming previous assignments, the SDP for quinine was mapped to mouse chr.6. The significance of these results to the neurobiology of taste will be discussed.

Amino Acid Preference in Rats Controlled by Activin A Release in Plasma as a Neurotrophic Factor and Neural Plasticity in Brain under Protein or Lysine Deficiency

K. TORII[1,2], K. OOSAWA[2], M. FUNABA[3], T. MURATA[2], M. TAKAHASHI[4] and T. ONO[5] ([1]Ajinomoto Co. Inc., Yokohama, Japan; [2]ERATO, JRDC, Yokohama, Japan; [3]Azabu Univ., Sagamihara, Japan; [4]Tokyo Univ., Tokyo, Japan; [5]Toyama Med. Pharm. Univ., Toyama, Japan).

Each L-amino acid(AA) in plasma and brain of Sprague-Dawley strain male rats remains unchanged all day long while normal diet is available. Once L-lysine(Lys) deficient diet offered to rats, Lys in plasma and brain declined. When solutions of AAs were offered, they selected the Lys solution and their food intake and growth normalized. The recording of single neuron activity in the lateral hypothalamic area (LHA) of these rats suggested that the neural plasticity occurred, specifically responding to deficient nutrient, Lys, centrally and during ingestion of AA. This neural plasticity in the LHA highly responding to speific AA previously ingested under protein or Lys deficiency were long lasted while normal diet was available. Also the release of possible neurotrophic factors, activin A (β_A-β_A) or inhibin (α-β_A), in plasma of rat with or without deficiency both protein and Lys were determined respectively by *Hydra Japonica* assay. The localization of immunochemically positive area for antibodies against α-subunit in the brain were seen in the arcuate nucleus (AN), the ventromedial hypothalamus, CA1 layer of the hippocampus and the nucleus of tractus solitarius (NTS). In the case of antibodies against β_A-subunit, the AN, the amygdala and the NTS were also positive. The degrees of positive area were quite comparable to each other nutritional treatment. These results suggest that the neural plasticity is not merely happen in the lateral hypothalamic area induced by Lys deficiency, and that activin A and/or inhibin may be involved in the induction of plasticity in the brain to maintain AA homeostasis, coupling with changes of preference and appetite for deficient AA.

On the Relativity of Chemosensory Perception.

LAWRENCE E. MARKS (John B. Pierce Laboratory and Yale University)*

Research in human chemosensory perception, as well as other perceptual realms, commonly rests on a set of assumptions, often tacit, about the relative contributions of early and late processes: sensory reception, perceptual encoding, judgment, and overt response. In particular, a common view - which essentially dates from the structuralism of Titchener and his contemporaries in psychology and physiology - holds that certain "simple" perceptual attributes, such as quality and intensity, largely or wholly reflect the outcome of relatively early sensory processing; higher-order (e.g., perceptual and cognitive) processing may later overlay sensory processing in the microgenesis of perception, but does not change it. In brief, this viewpoint entails an implicit position: that perception = sensation + cognition. An alternative view denies that sensation can be strictly dissociated or isolated .from perception in most psychophysical tasks. Evidence for deep contextual effects in perception implies that neither chemosensory perception nor any other kind of perception automatically provides a direct window on early sensory processes.

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Time-Intensity Measurements of Astringent Subqualities in Selected Organic and Inorganic Acids

CAROL CORRIGAN and HARRY T. LAWLESS (Cornell University)

Many foods and drink produce astringent sensations, most commonly those substances associated with acids. Four experiments different in scope and design were devised to measure the time-intensity of astringent subqualities and other taste qualities in various organic and inorganic acids. In the first experiment, focus groups were held to determine suitable terms for the succeeding experiments. The complexity of astringent perceptions may correlate with other taste qualities, which was investigated in the second experiment by measuring the intensities of six acids and alum, a reference for astringency. In addition, the volumes of the oral cavities of the subjects were measured under two conditions to determine if volume affected astringent perceptions. The third experiment measured the time-intensity of four acids at three concentration levels. The fourth experiment measured acid and acid mixtures at two concentration levels. Saliva samples were collected to determine the extent of correlations between astringency and saliva flow.

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Interindividual Differences in Temporal Perception of Sweet and/or Bitter Stimuli and Implications at the Receptor Level

JEAN-XAVIER GUINARD (The Pennsylvania State University)
DOREEN Y. HONG (The Pennsylvania State University)

Interindividual differences in temporal sensitivity to 23 sweet and/or bitter stimuli were measured and analyzed using multivariate statistics to investigate the number and specificity of peripheral receptor mechanisms for sweetness and bitterness. For each stimulus, the concentration producing a taste intensity equal to that of a 200 mM NaCl solution was determined by a panel of 17 trained subjects using the method of constant stimulus. The overall taste intensity of the equi-intense stimuli was then rated over time in triplicate by 25 subjects using a computerized system for time-intensity measurements. The dimension of the gustatory space represented by the stimuli was investigated by principal component (PCA) and cluster (CA) analyses of the interindividual differences for a number of TI parameters, including maximum perceived intensity, time to maximum intensity and total duration of the sensation. In the gustatory space produced from intensity-related TI parameters, stimuli were clustered based on their suprathreshold quality. Results confirm the existence of at least two distinct receptor mechanisms for bitterness, suggest the existence of distinct receptor mechanisms for sweet and bitter tastes, and indicate that the duration of the sensation produced by a stimulus is independent of its taste quality.

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325

A Temporal Study of Bite and Burn Perception in Carbonated Water

STEVEN J. HARPER (Oregon State University)
MINA R. MCDANIEL (Oregon State University)

Perceptual responses to bite and burn were recorded using a computerized time-intensity system. Five subjects evaluated bite and burn in carbonated water at four CO_2 levels (non-carbonated, 1.7 vol., 2.8 vol., and 4.6 vol.), two temperatures (3°C and 10°C) and two ingestion conditions (one swallow and four swallows). A number of parameters were derived from the intensity/time curves produced during the evaluations. These included maximum intensity, time of maximum intensity, total duration, duration of the maximum intensity plateau, area under the curve, time to onset of perception, and slopes of the onset and decay curves. The level of carbonation, temperature, and ingestion condition all had a pronounced effect on most of the parameters derived from the curves for bite and burn. Maximum intensity, total duration, duration of the maximum intensity plateau, area under the curve, and slopes generally increased as the level of carbonation increased. Values for these same parameters were generally higher for the 3°C temperature compared to the 10°C temperature. Ingestion of the samples using the "four swallow" condition also affected most of the parameters of the curve, usually accentuating the results in comparison to the "one swallow" condition. Major differences in the perception of bite and burn were found. These included much later times to onset of perception, longer total duration, and shallower onset and decay curves for burn. Subjects were able to replicate satisfactorily, but significant differences in individual perceptual patterns were found among the subjects.

326

Lateral Inhibition of Citric Acid on the Anterior Tongue in Humans

J. ZUNIGA (University of North Carolina)
S. DAVIS, N. CHEN, C. PHILLIPS (University of North Carolina)

Sensitivity to stimuli tasted within localized areas of the tongue may be enhanced following the anesthetization of extensive areas of the oral cavity, suggesting lateral inhibition from the anesthetized area. The purpose of this study was to determine if anesthesia of localized and regional areas of the anterior tongue modified the detection threshold of citric acid. Serial dilutions of citric acid (3 to 3×10^{-7} M) were used to measure detection threshold in 14 paid volunteers. Whole mouth threshold was determined using the sip and spit method. Localized threshold was determined by delivering stimuli to isolated, spatially-matched areas of the right anterior tongue in a low rate (10cc/min) flow chamber. Lidocaine (50mg) was applied before testing under 3 conditions: a, into the chamber; b, around the chamber; c, via contra-lateral chorda-lingual nerve block. Distilled water was applied in place of lidocaine to serve as control. Localized and regional anesthesia did not modify whole mouth threshold values ($p=.31$) but altered spatially-matched threshold values ($p=.01$). In contrast to control ($.8\pm.5$M), threshold increased following anesthesia of the chamber (1.8 ± 1M) and decreased following contra-lateral nerve block ($.3\pm.4$M). These findings suggest that anesthesia of the tongue effects taste function only within localized areas. This data also suggests that unilateral anesthesia of the anterior tongue results in enhanced sensitivity of the contralateral side. This may be due, in part, to lateral inhibition by the anesthetized chorda-lingual nerve.

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327

Measuring odor plumes which direct chemo-orientation in turbulent flow.

PENTCHEFF, N.D., and ZIMMER-FAUST, R.K. (University of South Carolina, Columbia, SC 29208).

Many animals use olfaction to find resources such as food or mates. However, the mechanisms by which odor plumes direct search activity remain unclear. Unlike molecular diffusion (which is effective only at very small scale), turbulent dispersal in a flowing fluid results in an odor plume which is irregular in both time and space. Although the time-averaged concentrations of odors downstream from an odor source can be predicted, the instantaneous concentrations vary as a complex function of the turbulence characteristics.

Simultaneous sampling at the level of the organism and at the level of the plume as a whole is necessary to understand both the signal received by the organism's sensors and the processes generating that signal. We have recently developed methods involving the parallel application of micro-electrochemistry and video imaging of laser-induced fluorescence to study the dispersal of waterborne odors in marine environments. Micro-electrochemical sensors provide excellent spatial (30 μm) and temporal (200 Hz) resolution of odor concentration sampled at a point. This measurement can be used to simulate chemoreception at a single olfactory sensillum. Video imaging of a laser-sheet illuminated dye/odor mixture provides a 30 Hz sample of plume dynamics in a plane. This provides a way to measure the entire plume structure through time.

We are combining these techniques with velocimetry to describe and predict odor transport in turbulent plumes for application to problems as varied as larval settlement responses to waterborne chemical cues and crab navigation to prey items.

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328

The Olfactory System of Sea Lamprey is Highly Sensitive and Specific to Bile Acids Naturally Produced by Fish

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DANIEL D. GALLAHER (Dept. of Food Science and Nutrition, University of Minnesota, St Paul, MN 55108)

Bile acids are a class of potent odorants for salmonid fish and have been hypothesized to function as migratory cues for anadromous fishes. The sea lamprey (*Petromyzon marinus*) serves as a good model for comparative studies of the olfactory potency and function of bile acids because it is a modern representative of jawless vertebrates and is anadromous. This study examined the olfactory responsiveness of sea lamprey to bile acids produced by sea lamprey and other fish. Our electro-olfactogram (EOG) recordings indicate that bile acids are potent odorants for adult sea lamprey. Allocholic acid and sulfotaurolithocholic acid are most potent and are detected at 10^{-13} Molar. The presence and orientation of several specific groups at several key positions are critical to the potency of bile acids. For example, replacing the 3α-OH of taurolithocholic acid with 3α-sulfate, or switching the 5β-H of cholic acid to 5α-H decreases the detection thresholds of lamprey to these compounds 10,000 times. Cross-adaptation and mixture experiments indicate that bile acids are detected by three separate receptor mechanisms. Finally, using high performance liquid chromatography (HPLC) we have confirmed that larval sea lamprey produce and release several unique bile acids which are highly potent and specific odorants for migratory adults. These results support the hypothesis that bile acids may act as conspecific cues for migratory adults.

Supported by Minnesota Sea Grant and the Great Lakes Fishery Commission.

Environmental Correlates to Chemically Stimulated Behavior in Hermit Crabs. D. RITTSCHOF and J. SARRICA. (Duke University Marine Laboratory, Beaufort NC 28516)

Work over the last 30 years shows shells are central to hermit crab behavioral ecology. The striped legged hermit crab *Clibinarius vitatus* is assisted in shell location by odors from predation events in which prey are gastropods of certain species or conspecifics. Hermit crabs respond to the odors by 1) withdrawing; 2) fleeing; or 3) attraction and shell investigation behavior. Each crab displays only one of the behviors. The fit of the shell determines crab responses to odors. Crabs that withdraw into their shells are in the largest shells relative to their body size. Crabs that flee are in intermediate sized shells relative to their body size. Crabs that show attraction and shell investigation behaviors are in relatively small shells. We chemically stimulated crabs from different microenvironments and at different times of the year and tabulated responses. Responses depended upon microenvironment and time of the year. Investigators were usually in *Litorina irrorata* (small) shells and occurred in the water on sand at low tide. Fleeing crabs werealso usually in small shells (*Urosalpinx cinerea and Pisania tincta*) and in the water at low tide. Withdrawing crabs were usually large crabs in *Busycon carika* shells exposed on oyster reefs. Investigators were 5% of the popoulation in the spring and fall and 60% of the population in late summer. Shell fit is correlated with microenvironment. Shell availability changes dramatically with season.

Epidermal growth factor-receptor (EGF-R) and EGF and/or Transforming Growth Factor-α (TGF-α) may play a role in regulating olfactory cell proliferation. ALBERT I. FARBMAN, RICHARD C. BRUCH AND JUDITH A. BUCHHOLZ (Dept. of Neurobiology, Northwestern University, Evanston, IL 60208)

Vertebrate olfactory sensory cells are continually replaced throughout the lifetime of the animal. We have shown previously that the *rate* of cell division in the olfactory epithelium can be up-regulated by ablation of the ipsilateral olfactory bulb, or down-regulated by unilateral naris occlusion. Further, we showed in an *in vitro* system, that EGF (100 ng/ml culture medium) increased the rate of cell division in olfactory epithelium by about twofold. We used an organotypic culture of olfactory mucosa from 19 day rat fetuses and grew the tissue in a serum-free medium containing various doses of growth factor. Cultures were grown for 3 days and then exposed for 1 hr to [^{3}H]-thymidine or bromo-deoxyuridine (BrdU). Cultures were then fixed and prepared for autoradiography if [^{3}H]thymidine was used or for immunohistochemistry if BrdU was used. Using the same organ culture system we have now found that TGF-α (1 ng/ml culture medium) is 100 x more potent as a mitogen than EGF in eliciting a 2-3 fold increase in the number of dividing cells. Both EGF and TGF-α bind to the EGF-receptor. We have assayed adult rat olfactory mucosa for the presence of EGF-receptor by Western blotting and found it is abundantly present. To determine where in the mucosa the receptor was localized we used immunohistochemistry and found the receptor is present in (globose) basal cells and in supporting cells. The *in vitro* data, showing that the two growth factors enhance mitotic rate, and the *in vivo* data, showing the presence of EGF-R in globose basal cells, support the notion that TGF-α and/or EGF participate in the mechanism that regulates mitosis in the olfactory epithelium.

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Early Events in Development of Olfactory Bulb Glomeruli: A Cytochrome Oxidase Study in the Rat ESMAIL MEISAMI AND TIMOTHY J. SENDERA (Physiol. Dept., Univ. Illinois, Urbana, IL 61801).

Cytochrome oxidase (CO) is a histochemical marker for neural regions with high metabolic activity. Olfactory bulb (OB) glomeruli stain intensely for CO and CO is a reliable marker for delineation of neuropil of glomeruli proper. Using CO stained OB serial sections we have found that total glomerular number is established during the early neonatal period (1). Now we report on observations pertaining to early developmental changes in the pattern of staining of glomeruli and adjacent external plexiform layer (EPL) for CO. Serial frontal frozen sections of OB were prepared from rats ranging in age from 1-7 d PN and were stained for CO. Glomerular counts indicated that total adult number (2400/OB) was established by 3-5 d PN. Although numerous glomeruli could be located in the OB of 1-3 d rats, a tight packing of round glomeruli were noted only in some regions; in other regions, the presumptive glomeruli appeared as long diffuse bands, with little evidence of globular parcelation, characteristic of more mature glomeruli. Interestingly, wherever glomeruli were round and parcelated, the adjacent EPL also showed a distinct banding pattern, possible evidence for differentiation of sublayers of EPL. Our results suggest that OB glomeruli initially form as long diffuse bands and later segregate into parcelated globules and this stage of development is linked with differentiation of the EPL.

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Metabolic Activity in Mouse Septal Tissue following Odour Stimulation JUNNI ZHAN[1], GRAHAM A. BELL[2] and MALCOM K. HORNE[1] ([1]Department of Anatomy, Monash University, Clayton, Australia 3168; [2]CSIRO Division of Food Processing, North Ryde, Australia 2113)

A previous study(1) has demonstrated the existence of odour-specific patterns of metabolic activity in the glomerular layer of the rat main olfactory bulb following the stimulation of the olfactory system with controlled dilutions of pure chemical odorants. In addition, suppression of one odour by a second odour in a mixture could be observed by suppression of features of the metabolic activity pattern of the first by the more dominant pattern of the second. This study aims to determine whether similar metabolic effects can be observed in the olfactory mucosa of a mammal. As an earlier attempt to reconstruct a sufficient number of [^{14}C]2-deoxyglucose autoradiographs from rats' noses proved logistically impracticable(2), this study has now tried to make sense of the mouse epithelial activity by making autoradiographs of flat-mounted septal tissue, thereby removing the need for 3-dimensional reconstruction and allowing more robust data sets. Results so far have shown that there are some differences in the spatial characteristics of metabolic activity under different odour stimulation. Further analysis will be required to corroborate whether these activity distributions are odour-specific and consistent, so that mixture suppression in olfactory epithelium might be demonstrated.

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Name Index to Slides and Posters