

Abstract Book

ACChemS-X

The Tenth Annual
Meeting of the
Association for
Chemoreception
Sciences

Hyatt Sarasota
Sarasota, Florida
April 27 — May 1, 1988

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AChemS-X Program Summary

Wednesday Evening April 27, 1988

7:00 PM Welcome and Opening Remarks
Hernando Desoto Ballroom

7:15 PM Talk Session
Chemical Signals: Detection and Processing

8:15 PM The Givaudan Lecture

9:00 PM Social Reception
Cash Bar
Prefunction Area

Thursday Morning April 28, 1988

7:30 AM Continental Breakfast
Prefunction Area

8:00 AM - 1:00 PM Poster Sessions
Sara Desoto Ballroom

Poster Session A
Human Taste and Stimulus Mixtures

Poster Session B
Sensory Evaluation and Consumer Research

8:00 - 11:00 AM Symposium
Hernando Desoto Ballroom
Insect Pheromone Systems: Behavior, Biochemistry,
Peripheral and Central Neurophysiology

9:15 - 9:45 AM Refreshment Break
Prefunction Area

11:30 AM - 12:45 PM Talk Session
Hernando Desoto Ballroom
Central Pathways of Smell

Thursday Afternoon April 28, 1988

Beach Transportation Departs from Hotel Front:
Hyatt to Lido Beach 1:30 PM and 2:00 PM
Lido Beach to Hyatt 4:00 PM and 4:30 PM

Discussion Session
Are There Parallel Pathways in Olfaction?
4:00 - 6:00 PM
Hernando Desoto Ballroom

Thursday Evening April 28, 1988

6:00 - 11:00 PM Poster Sessions
Sara Desoto Ballroom

Poster Session A
Central Pathways of Smell:
Anatomy, Physiology, Development and Behavior

Poster Session B
Chemical Signals: Detection and Processing

7:00 - 7:45 PM Talk Session
Hernando Desoto Ballroom
Sensory Evaluation and Consumer Research

8:00 - 10:00 PM Talk Session
Hernando Desoto Ballroom
Human Smell

9:00 - 10:00 PM Refreshments Available
Prefunction Area

Friday Morning April 29, 1988

7:30 AM Continental Breakfast
Prefunction Area

8:00 AM - 1:00 PM Poster Sessions
Sara Desoto Ballroom

Poster Session A
Structure and Function of Taste Receptors

Poster Session B
Olfactory Electrophysiology and Ionic Mechanisms

8:00 - 10:00 AM Talk Session
Hernando Desoto Ballroom
Central Pathways of Taste: Anatomy and Physiology

10:00 - 10:30 AM Refreshment Break
Prefunction Area

10:30 AM - 11:30 AM Talk Session
Hernando Desoto Ballroom
Human Taste

12:00 - 1:00 PM Talk Session
Hernando Desoto Ballroom
Clinical Studies of Chemosensory Dysfunction

**Friday Afternoon
April 29, 1988**

Beach Transportation Departs from Hotel Front:
Hyatt to Lido Beach 1:30 PM and 2:00 PM
Lido Beach to Hyatt 4:00 PM and 4:30 PM

Discussion Session
Clinical Chemosensory Trials: What, How and When?
4:00 - 6:00 PM
Hernando Desoto Ballroom

**Friday Evening
April 29, 1988**

6:00 - 11:00 PM Poster Sessions
Sara Desoto Ballroom

Poster Session A
Human Smell Perception and Clinical Studies

Poster Session B
Taste Receptors and Ionic Processes

7:00 - 10:30 PM Talk Session
Hernando Desoto Ballroom
Structure and Function of Chemosensory Tissues

8:30 - 9:30 PM Refreshments Available
Prefunction Area

**Saturday Morning
April 30, 1988**

7:30 AM Continental Breakfast
Prefunction Area

8:00 AM - 1:00 PM Poster Sessions
Sara Desoto Ballroom

Poster Session A1-A6
Central Pathways of Taste

Poster Session A7-A17
Chemosensory Behavior

Poster Session B
Structure and Function of Olfactory
Receptors and Other Structures

8:00 - 10:00 AM Talk Session
Hernando Desoto Ballroom
Taste Receptors: Ion Channels and Transduction

10:00 - 10:30 AM Refreshment Break
Prefunction Area

10:30 AM - 12:30 PM Talk Session
Hernando Desoto Ballroom
Olfactory Receptor Currents and Transduction

**Saturday Afternoon
April 30, 1988**

Beach Transportation Departs from Hotel Front:
Hyatt to Lido Beach 1:30 PM and 2:00 PM
Lido Beach to Hyatt 4:00 PM and 4:30 PM

**Saturday Evening
April 30, 1988**

AChemS Business Meeting
5:30 PM Sara Desoto Ballroom

Social Hour and Cash Bar
6:30 PM Prefunction Area

The Freeman Award Dinner
7:30 PM Hernando Desoto Ballroom

**Sunday Morning
May 1, 1988**

7:30 AM Continental Breakfast
Prefunction Area

8:00 - 10:00 AM Talk Session
Hernando Desoto Ballroom
Chemosensory Behavior

8:45 AM Invited Lecture
Edward M. Stricker
Hernando Desoto Ballroom

10:00 AM AChemS-X Adjournment

A Proposed Role of Adenine Nucleotides as Inducers of Feeding in Aquatic Animals. RICHARD K. ZIMMER-FAUST and LEE ANNE MARTINEZ (Marine Sciences Institute and Neurosciences Research Program, IES, University of California, Santa Barbara, CA 93106).

The purine nucleotide, adenosine 5'-triphosphate (ATP), occurs at highest concentrations in metabolically active tissues, including muscle. After an organism dies, ATP is rapidly dephosphorylated by enzymes in autolytic and bacterial degradations. AMP is produced during decomposition and can persist at highly elevated levels for several days after decomposition begins. We propose that the adenine nucleotides may be appropriate molecules to signal quality or "freshness" of prey tissues. Indeed, previous investigators found that AMP is chemoattractant to shrimps that scavenge dead animal materials. In this report, we summarize results from behavioral assays using the spiny lobster, *Panulirus interruptus*. This animal principally forages on live prey. Interestingly, we find that ATP is both chemoattractant and phagostimulatory to lobsters, causing oriented locomotory responses and ingestion of ATP-impregnated gels at concentrations occurring in flesh of live prey. ADP is only weakly stimulatory to locomotory and feeding responses, while AMP is non-stimulatory though it suppresses responses to ATP and ADP, when combined in mixtures. Given these results, we suggest that the adenine nucleotide responses of lobsters may increase the probability that only live and freshly killed prey will be eaten. However, we find a substantial reduction in AMP-suppression after lobsters have been starved for six to seven days. This result might mean that lobsters will modify their diet to include carrion when food is scarce. We propose that adenine nucleotides may act in conjunction with other chemical cues, and that behavioral assays of these nucleotides may thus be found generally useful in predicting foraging tactics.

Supported by NSF grant OCE-8801207, and a President's Postdoctoral Fellowship from University of California.

Effects of Cantharidin (Spanish Fly) on the Taste Nerve of a Frog. STEVEN T. KELLING, BRUCE P. HALPERN, THOMAS EISNER (Cornell University, Physiol./Psych./NBB, Ithaca, NY 14853-7601)

Cantharidin, a toxic vesicant, is a chemical defensive agent of meloid beetles. It is effective against insect predators, but some vertebrates ingest it. We were interested in whether cantharidin initiates a neural taste response in an insectivorous vertebrate.

METHODS Whole glossopharyngeal nerve recordings were made on 4 frogs (*Rana pipiens*) during stimulation of the tongue with 10 μ l of Frog Ringers, 10 mM CaCl₂ (CaCl), and 2 x 10⁻⁵ cantharidin

(Can5) and 2 x 10⁻⁶ cantharidin (Can6) in Ringers, using an automated pipette. Amplified nerve activity was digitally summated in 10 ms binwidths, and A/D converted at 1 kHz for 100 ms prior to stimulus onset and 2900 ms thereafter. **RESULTS** CaCl and Ringers responses were similar to those previously reported. Response magnitudes were: CaCl > Can5 > Ringers > Can6. Stimulation with CaCl and Can5 produced a sustained period of increased nerve activity in the first 500 ms, while Ringers and Can6 elicited no such response. Over the entire recorded duration the response to Ringers was significantly different (sig dif) (Wilcoxon Matched Pairs Sign Rank Test) from that to CaCl and Can6 (p<0.01), but not from that to Can5 (p>0.05). Paired comparisons of CaCl, Can5, and Can6 responses were sig dif for all cases (p<0.01). Responses relative to Ringers were > and sig dif for the first 500 ms (CaCl) or from 300-500 ms (Can5) (p<0.05) (Friedman's ANOVA). Responses to Can6 were sig dif (p<0.05) and < than that to Ringers between 1800-1900 ms, and from 2000-2200 ms. **CONCLUSIONS** Frog taste responses discriminate between Ringers, CaCl, and both concentrations of cantharidin. While cantharidin at the higher concentration (Can5) may elicit a positive response, cantharidin at the lower concentration (Can6) may induce late inhibition.

Behavior, Electrophysiology, and Pharmacology of the Response of Abalone Larvae to GABA. L. A. Barlow (Dept. of Zoology, Univ. of Washington).

The crustose coralline alga, *Lithothamnium*, induces metamorphically competent abalone larvae to settle and metamorphose. Morse (1979) has shown that a protein isolated from this alga is the cue for settlement, and that GABA mimics the effect of this natural metamorphic inducer. Abalone veliger larvae maintain themselves in the water column by beating the cilia of the velum in a metachronal fashion. When the velar cilia are arrested the animal ceases to swim and begins to sink. Veligers can be restrained and the velar cells impaled with microelectrodes without inhibiting the beating of the cilia. The cells show a resting potential of -55mV, and depolarization elicits an action potential which causes an arrest in the velar cilia and a cessation of larval swimming (Barlow, Neurosciences Abstract, 1987; Arkett and Mackie, ASZ Abstract, 1986). When *Halotis* velar cells were recorded from in flowing, artificial seawater, the background electrical and behavioral activity consisted of slight fluctuations in resting membrane potential and the velar cilia beat continuously. Exposure to GABA initiated a train of action potentials and arrested the velar cilia. This effect was removed upon washout of the neurotransmitter. The frequency of spikes elicited was dependent on GABA concentration (1mM - 10 uM), and the percentage of larvae able to respond to GABA increased with increasing GABA concentration. Larvae from 3 to 15 days post-fertilization could respond to the settlement cue in the manner described above, although only larvae older than 6 days could respond to GABA by initiating metamorphosis (Morse 1979). The velar cells themselves do not appear to respond directly to GABA since there was no change in velar cell input resistance in the presence of GABA. These data suggest that the velar arrest is a motor output in response to GABA reception elsewhere on the animal, and that this behavioral circuit is hooked up early in development, i.e., prior to metamorphic competency. Pharmacologically, this system behaves similarly to that described by Trapido-Rosenthal and Morse (1986). The GABA_A blockers, picrotoxin and bicuculline methiodide, do not block the electrophysiological response to GABA. Baclofen, a GABA_B agonist, mimics this effect of GABA. Trapido-Rosenthal and Morse (1986) have shown a similar pharmacological pattern for the induction of metamorphosis. These results provide support for the hypothesis that these two effects of GABA, induction of metamorphosis and triggering of ciliary arrest, may be related at the level of the receptor.

Properties and Functions of the Pectine Chemosensory System of Scorpions. PHILIP BROWNELL (Oregon State University, Corvallis, OR).

The pectines are large, comb-like sensory appendages extending ventrolaterally from the 9th body segment of all scorpions. These structures are evident in the earliest fossil records of aquatic (Silurian) and land-colonizing terrestrial (Carboniferous) species but their functions are unknown even in modern scorpions. Structurally, the pectines support an orderly array of peg sensilla, each with cuticular pores and accessory cells characteristic of arthropod chemoreceptors. Behavioral studies of sand scorpions (*Paruroctonus* and *Hadrurus*) indicate the pectines function in relocating the home burrow after hunting forays at night, and in directing solitary males to females prior to mating. Pectine tapping of the substrate is especially vigorous in males as they enter territory previously occupied by conspecific females, suggesting that the organs are sensing a sex pheromone deposited on the substrate.

The pectines are sexually dimorphic with males possessing the more elaborate structures. In both sexes sensory afferents from 28 (♀) to 40 (♂) pectine "teeth" project to the CNS in a topographically-ordered array and terminate in a sexually dimorphic and structured neuropil in the subesophageal ganglion. The afferent terminal fields are larger in males and contain at least 4 layers with synaptic cartridges as the structural unit. Biochemically, the sensillar portion of pectines synthesize unique, low-molecular weight (13-18 kd) polypeptides that appear to be species and gender-specific. These proteins are turned over at a high rate and otherwise possess properties of the pheromone binding proteins of insects. Thus, by their structural, behavioral and biochemical characteristics, the pectinal appendages of scorpions appear to be contact chemoreceptors involved in pheromone detection.

Supported by NSF BNS-8709890.

Structure, Solution Properties and Sensory Characteristics of Amino Acid Molecules. SARAH E. KEMP and GORDON G. BIRCH (Department of Food Science and Technology, PO Box 226, University of Reading, Whiteknights, Reading, Berks. RG6 2AP, U.K.)

Amino acids exhibit many sensory qualities but predominantly bitter and sweet tastes. Their tastes are not clearly predictable from their structure and chirality and even those that are "sweet" are judged by panellists to taste different from the sugars. Apparent specific volumes of the amino acids span a much greater range (0.50 - 0.82 cm³/g) than the sugars (0.58 - 0.62 cm³/g) in accordance with their greater range of structures and therefore corresponding range in hydrophobicity and polar character. Apparent specific volumes can aid prediction of taste quality and the tastes of the amino acids are in some measure predictable in this way. However, apparent specific volumes represent averages for the different molecular species of each amino acid in solution equilibrium and it is possible that these different species can each stimulate different receptors. In accordance with this idea all of the amino acids possess two, three or all four of the basic tastes in both of their enantiomeric forms and one of those usually predominated. Sensoric difference between (sweet) sucrose and L-hydroxy proline could therefore be eliminated if the sucrose was mixed with other appropriate basic tastants (N = 64, correct judgements = 34 in a duo/trio test). Partial specific volumes and other solution properties of amino acids can be explained by their molecular features (M.Wt., numbers of carbon atoms, chirality etc.) and the compatibility of the solute with water structure. As with the sugars, the hydrated solute is probably responsible for the mode of interaction with the receptor.

Supported by Ministry of Agriculture, Fisheries and Food (U.K.), The Society of Chemical Industry and the Research Board, University of Reading.

Caffeine Under Natural Conditions Does Not Alter Taste Intensity. D.J. MELA (Monell Chemical Senses Center, Philadelphia PA)

Schiffman et al.^{1,2} have reported that adaptation of the tongue to micromolar concentrations of caffeine (CAF) can markedly enhance the perceived intensity of many tastants, particularly non-carbohydrate sweeteners. We previously found no effect of acute caffeine exposure on detection thresholds for CAF itself or NaCl³. In the present work, human volunteers participated in a double-blind study to test the effect of acute CAF ingestion on perceptions of taste intensity. Subjects served as their own controls, receiving a placebo capsule in one of two test sessions and CAF (5.5 mg/kg) in the other. This dose of CAF raises salivary CAF levels to ≥10 μMolar in many subjects without inducing profound side effects. At 45 minutes post-dose, subjects evaluated the intensity of single solutions of 5 non-carbohydrate sweeteners, 2 carbohydrate sweeteners, 2 bitter tastants, NaCl, and Citric Acid. Each tastant solution was prepared in two ways, using either 1) deionized water or 2) 10 μM CAF as the medium, and were presented two times each in random order. Intensity was rated using both magnitude estimation and category scales. Preliminary analyses show no effects of condition (CAF or placebo) on any response measures. The results suggest that natural conditions of lingual adaptation to μMolar levels of CAF in plasma and saliva do not substantially alter taste intensity ratings.

¹Schiffman SS et al. 1985. Pharmacol Biochem Behav 22:195-203.

²Schiffman SS et al. 1986. Pharmacol Biochem Behav 24:429-432.

³Mela DJ et al. 1987. AChemS-IX Abstract #143. Supported by NSF Grant #BNS-8418953

The Influence of Gymnemic Acid Pretreatment on the Sweetness of Eight Natural and Artificial Sweeteners. S.J. MIZE & R.A. FRANK (Univ. of Cincinnati)

A recent series of experiments in our laboratory demonstrated synergistic interactions between some pairs of sweeteners, but not others (see Frank, Hubay, Carter & Mize, this volume). It was hypothesized that the synergism may have related to multiple sweetness transduction mechanisms. An attempt was made to evaluate this hypothesis by assessing the effects of gymnemic acid pretreatment on the eight sweeteners used in the previous research. If the gymnemic acid differentially influenced the sweetness of the substances that synergize, this could be taken as evidence that multiple transduction mechanisms are mediating the synergistic effects. Three concentrations of sucrose, fructose, glucose, aspartame, cyclamate, xylitol, acesulfame K and stevioside were used. The concentrations were chosen to approximately match the sweetness of 0.1, 0.3 and 0.5 M sucrose. Eighteen subjects judged the stimuli under three pretreatment conditions: gymnemic acid, commercial tea and distilled water. Prior to each test, the subjects were given samples of distilled water and 0.1, 0.3 and 0.5 M sucrose to anchor the 21 point category scale they used to rate the stimuli. Next, they were given one of the pretreatments. This consisted of 20 ml of distilled water, brewed tea or brewed *Gymnema Sylvestre* leaves. The subjects rinsed their mouths with the solution for 60 seconds, rinsed with distilled water and then judged the stimuli. A distilled water rinse and 30 second intertrial interval followed each sample. After each stimulus had been rated, the pretreatment was repeated and the stimuli were judged a second time. The sweeteners were run in two separate experiments: one with acesulfame K, cyclamate, sucrose and xylitol and the other with aspartame, fructose, glucose and stevioside. The same sample of 18 subjects participated in both experiments. It was found that gymnemic acid pretreatment uniformly reduced the sweetness of all eight substances. Therefore, the present study does not support the hypothesis that sweetener synergism is mediated through multiple sweetness transduction mechanisms.

This research was supported by an Ohio Board of Regents Research Challenge grant to R. Frank.

Perceived Bitterness and Mouth Dryness of an Astringent Stimulus: Effects of Repeated Exposure. BRIAN J. LYMAN and BARRY G. GREEN (Monell Chemical Senses Center)*

Sensations associated with ingesting astringent stimuli are complex and have so far escaped explanation. Astringency is most commonly characterized as a sensation of mouth dryness and most astringent stimuli are also perceived as being bitter. The present experiment was aimed at measuring the time course of perceived bitterness and mouth dryness in 0, 500, and 1000 mg/l solutions of tannic acid and deionized water. Subjects were presented with 10 ml samples of a 36 deg C. solution at one minute intervals for 20 minutes. On each trial, the subject swished a sample for 10 sec. expectorated, and then gave magnitude estimates of both bitterness and dryness at 15 sec intervals. Subjects did not rinse between trials. The results indicated that with tannic acid, (1) the mouth dryness component of astringency increases significantly over time, (2) this effect becomes more marked at higher concentrations, (3) although equal to dryness on early trials, bitterness increases only slightly over time, and (4) there is little effect of concentration on either magnitude or rate of growth of bitterness. We conclude that although bitterness and mouth dryness are both salient components of astringency at initial exposure, mouth dryness becomes increasingly dominant with repeated sips.

*supported in part by NIH grant # NS20577

Discrimination of Mixtures: Principal Components Analysis as a Tool for Elucidating the Nature of Differences in Composition of Mixtures. PETER C. DANIEL, JACQUELINE B. FINE-LEVY, CHARLES D. DERBY (Georgia State University)*

We have demonstrated that spiny lobsters can discriminate neurally and behaviorally between chemical mixtures representing crab, oyster, shrimp, and mullet extracts. The degree of discrimination exhibited biologically correlates well with statistical differences between mixtures based on the concentrations of each of the 41 chemical constituents. It is likely that not all of these chemicals are necessary to define statistical differences between the mixtures. Similarly, lobsters may identify a mixture based on a subgroup of the chemical constituents. We employ here several methods, using principal components analysis, to determine which chemicals most contribute to differences in the composition of mixtures. All methods involve the removal of the most contributory chemical followed by principal components analysis of the remaining chemicals. This process is repeated until differences have been minimized and no more contributory chemicals can be removed. The methods differ in the scope of the comparison examined (pairwise, pairwise with influence from the other two mixtures, and one mixture versus all others), in the quantitative method used to evaluate which chemical is most contributory, in the statistic used to define magnitude of differences, and in the economy of analysis. Comparison of these results with behavioral data on discrimination by lobsters demonstrate the potential of these statistical methods as analytical tools. Furthermore, given an appropriate empirical basis, the three methods may serve as models of chemosensory discrimination.

* Supported by NINCDS Grant No. NS22225 and by the Whitehall Foundation

Recognition of Components in Taste Mixtures: An Analytic Explanation. ROSEMARY SZCZESIUL, LINDA BARTOSHUK, (Pierce Foundation, New Haven, CT), and HARVEY GRILL, (University of Pennsylvania).

Fusion of taste components, considered by some as evidence of synthetic perception of taste mixtures, appears to be a less than robust phenomenon. Although Erickson and Covey (1980) found that subjects asked to taste two-component mixtures rated some mixtures as having a "singular" quality, closer inspection suggests that an analytic explanation of taste mixtures is more appropriate. We asked subjects to provide magnitude estimates of the intensities of saltiness, sweetness, sourness, and bitterness in the same mixtures. What previously appeared to be evidence supporting a synthetic view of taste perception of mixtures was really the loss of the salience of one of the two components. Upon close comparison of the evidence presented by Erickson and Covey with the current data, it is clear that some particular cases of "synthetic" taste are actually evidence of mixture suppression. For example, when quinine sulfate is mixed in a 0.0002 M solution with a 0.0045 M solution of hydrogen chloride it is not surprising that the bitterness of the former should overpower the sourness of the latter so that synthesis of taste may be incorrectly assumed while mixture suppression is actually occurring.

This work was supported by NIH Grant NS21600.

Erickson, R. & Covey, E. On the singularity of taste sensations: What is a taste primary?, *Physiology & Behavior* 25: 527-533, 1980.

The Ratings of Citral/Sucrose Mixtures are Affected by the Hedonics of the Components. RENEE J. MCCALL, MELVIN P. ENNS, DAVID E. HORNUNG. (St Lawrence Univ., Canton, NY 13617)

Evaluations of the overall pleasantness and intensity of three concentrations of citral, three concentrations of sucrose, and all combinations of citral and sucrose were made using the method of magnitude estimation. The solutions were presented via open-cups or The Two-Module Delivery System (Chemical Senses 1984, 9, 97-106). With open-cups, the intensity data were consistent with the published results of Murphy and Cain (Physiol. Behav. 1980, 24, 601-605). For the hedonic data, 12 of 32 subjects rated citral in the mouth as pleasant, whereas 28 of 32 subjects rated sucrose as pleasant. When sucrose was rated as pleasant, the mixtures were generally rated as pleasant regardless of the degree of like or dislike for citral. When sucrose was rated as unpleasant, the pleasantness rating of the mixture was more dependent on the rating of citral. The data suggest that for this stimulus pair a positive rating of sucrose can override the smell/taste dislike sometimes seen with citral alone. With the Two-Module Delivery System (i.e. citral presented to the external nares), many more subjects reported liking the smell of citral. In addition, most subjects rated the sucrose solutions as pleasant. Consistent with the observation seen with the open-cups, a liking of the smell and the taste almost always resulted in a liking of the mixtures. Thus, the results of this study demonstrate that the ratings of citral/sucrose mixtures are affected by the hedonics of the components and by the method of presentation.

Supported by a grant from General Foods Corp.

Perception of Odor Mixtures Under Environmentally Realistic Conditions. FRANK T. SCHIET and WILLIAM S. CAIN (John B. Pierce Foundation Laboratory & Yale University, New Haven, CT 06519).

The study explored the perception of odor intensity under environmentally realistic conditions in a climate-controlled chamber. Questions of interest included the following:

- What is the relation between the perceived intensity of a mixture and that of its unmixed components?
- Does the relation between the perceived intensity of a mixture and the intensities of unmixed components change when subjects inhale the stimulus continuously vs when they inhale it only periodically, i.e., under adapted vs unadapted conditions?
- Does degree of adaptation vary from simple to mixed stimuli?

Subjects (n=20) judged the odor intensity of five single odorants and four binary mixtures during 15 min exposures to increasing concentrations. Mixtures of both fixed and varying proportions were studied. Exposures were either continuous or periodic. As in previous experiments, the mixtures showed hypoadditivity of perceived intensity with respect to their components. The degree of hypoadditivity proved the same for the four pairs of odorants. Nevertheless, continuous exposure led to a closer approximation to simple additivity than did periodic exposure. This phenomenon seemed to derive from a tendency for mixtures to exhibit less adaptation than their components. The data suggest that what is lost in mixtures via hypoadditivity may be regained in a dynamic functional property of durability.

Supported by a grant from Unilever Research Laboratories, UK.

Opioid Blockade Decreases the Pleasantness of Food Flavor. GARY K. BEAUCHAMP, MARY BERTINO AND KARL ENGELMAN (Monell Chemical Senses Center and University of Pennsylvania).

Opioid blockade induced by naltrexone or naloxone decreases food intake in both animals and humans. One possible mechanism for the reduced food intake produced by opioid blockade is an alteration in taste. To test this hypothesis, eighteen fasted male college students rated the intensity and pleasantness of soup with varying concentrations of salt and Kool-Aid with varying concentrations of sucrose at hourly intervals during 2 separate sessions: Session 1, after receiving naltrexone; Session 2, after receiving a placebo. Mood state and hunger level were also assessed. Following the taste and mood tests, subjects ate a lunch during which food intake was recorded. After placebo administration, the pleasantness of the soup tended to increase over the 3 hrs of testing, independent of salt concentration. In contrast, after naltrexone, regardless of salt concentration, soup pleasantness remained stable or declined slightly over this period. Parallel changes were obtained for pleasantness ratings of Kool-Aid. Intensity ratings were influenced by the drug. One hr after naltrexone, the intensity of the soup and Kool-Aid was greater. Although naltrexone increased nausea ratings and decreased hunger ratings, these rating changes did not account for the drug effects on the pleasantness of the soup and Kool-Aid. Following naltrexone treatment, subjects consumed less food for lunch than after placebo. Statistically, this differential food intake could be explained by the decreased hunger ratings but not by the increased nausea ratings nor by the decrease in the pleasantness ratings obtained from the taste test. Thus, although naltrexone reduces the pleasure associated with tasting food, this reduced taste pleasure may not be the mechanism by which it reduces food intake. Furthermore, while these data support a role for endogenous opioids in controlling the pleasantness of food, they provide only indirect support for an effect on sweet or salty tastes.

Supported by NIH Grants RO1 HL-31736 and RR 00040.

Hedonic Ratings of Sucrose and Saline in Two Collegiate Athletic Teams. TIMOTHY J. McCROSSEN, SCOTT R. DEVANNY, MELVIN P. ENNS, and DAVID E. HORNUNG (St. Lawrence University, Canton, NY 13617).

This study examined the effects of exercise on the hedonic ratings of sucrose and saline solutions by members of two men's intercollegiate teams (cross-country and hockey). The training and performance requirements of these two sports are vastly different: cross-country running requires continuous effort over approximately 25 minutes duration, whereas hockey requires intermittent activity over a longer period of time. The subjects were asked to use the method of magnitude estimation to rate the pleasantness and unpleasantness of water, four concentrations of sucrose solutions, and four concentrations of sodium chloride solutions both before and after competition. For cross-country runners, estimates of the pleasantness of sucrose were higher following the competition. This effect was not seen with the hockey players. Hedonic responses to sodium chloride did not change with exercise. These results are consistent with the hypotheses that sucrose preference is related to nutritional state (Cabanac, 1971, *Science*, 173, 1103-1107) and that sucrose preference is increased following exercise (Like and Schupf, 1981, personal communication). The results of the present study further suggest that the type of exercise may be important in producing this effect.

Supported by a grant from the General Foods Corporation.

NaCl as an Oral Irritant. BARRY G. GREEN (Monell Chemical Senses Center)*

It is well known that in sufficiently high concentrations NaCl produces sensations of irritation as well as sensations of taste. Because oral irritation is thought to be mediated primarily (or exclusively) by the trigeminal nerve, the perception of "saltiness" may often be mediated jointly by the trigeminal and gustatory sensory systems. The study reported here explored the bimodal nature of saltiness by measuring the intensity of sensations of taste and irritation during repeated exposures to NaCl solutions. The use of repeated exposures enabled assessment of the relative strengths of the gustatory and trigeminal components of saltiness over time. Subjects sipped either a 0.4 or 0.8M solution of NaCl every 60 sec for 15 min. No water rinses were allowed between sips. On each trial separate magnitude estimates of irritation and salt taste were made during the sip and at two times following expectoration of the solution. The results showed (1) that irritation was significantly greater at 0.8M than at 0.4M, (2) that for both concentrations perceived irritation grew linearly over the 15-min exposure (more than doubling in magnitude at the 0.8M concentration), and (3) that the perceived intensity of the salt taste per se remained nearly constant after the first few sips (i.e., there was no adaptation). Irritation therefore becomes a more salient attribute of the saltiness of NaCl over time, perhaps because prolonged exposure to NaCl somehow "sensitizes" a subset of trigeminal nerve endings.

*Supported in part by NIH grant # NS20577.

Temporal Mouthburn From Capsaicin: What puts out the fire? Nasrawi, C. W. & Pangborn, R.M., (Nutrition Group, University of California, Davis).

Using a computerized time-intensity recording device, 22 subjects evaluated the efficacy of oral rinsing with six solutions on reduction of mouthburn induced by 3 ppm capsaicin solution. Mouthburn of the control sample rose sharply during the 15 seconds stimulation, continued to rise after expectoration and declined slowly to baseline (ca. 7 min). While the rinse sample was in the mouth, mouthburn decreased markedly, but gradually reappeared after expectoration. All rinses reduced mouthburn significantly in order of effectiveness: whole milk > skim milk > sucrose > 5°C water > 5% ethanol > 20°C water (p<0.05). There was large subject variation in maximum intensity, time to maximum intensity, total duration of mouthburn and in the shape of the time-intensity curve.

In a second experiment, 18 subjects rated reduction of mouthburn by solutions containing 5, 10, and 20% sucrose and with sweetened milk containing no fat, 10% fat (homogenized) and 10% fat (unhomogenized). Mouthburn was reduced significantly by sucrose: 20% = 10% > 5% (p<0.05). All milks produced a similar pronounced reduction of burn independent of fat content and fat globule size. Compared to eaters of chili pepper (n=9), noneaters (n=9) experienced greater mouthburn reduction by sucrose but comparable reduction by milks. Results suggest that trigeminal stimulation of capsaicin is reduced more by gustatory (sucrose) than by tactile (fat) stimuli.

The Effects of Pleasant Environmental Odor, Odor Variety, and Personality, on Cognitive Performance, Mood and Motivation. THERESA R. ROTTMAN (Texas Christian University), H. WAYNE LUDVIGSON (Texas Christian University)

The present project was aimed at providing basic information regarding effects of pleasant environmental odors (lavender and cloves) on fundamental psychological processes (cognitive performance, motivation, and mood). Subjects appeared at three sessions. A preliminary session was devoted exclusively to the assessment of individual differences (field dependence, locus of control, anxiety, mood, and motivation), whereas the first and second experimental sessions involved presentation of odorants and the experimental tasks. The experimental sessions were held in a small laboratory in which the odor was diffused. For the first experimental session subjects were divided into three groups: cloves, lavender, and "no odor." Subjects were administered a memory task and three cognitive tasks, followed by mood and motivational assessments. At the same time of day one week later each of the original groups was further divided into three subgroups in a factorial arrangement, with a subgroup receiving one of the three odor conditions; the same tasks were given. Cognitive functioning was adversely affected by the presence of lavender in the initial encounter with the odor. These detrimental effects were temporary, in that they did not appear in the final sessions. The effects of odor on motivation were complex. Lavender appeared to enhance affective motivation in the first experimental session. In addition, there was a suggestion that cloves fostered an environment in which persisting and general mood differences (such as anxiety) tended to be expressed. It appears from this study that the effects of contextual odors are primarily limited to motivational and emotional processes, with some disturbances of cognitive functioning at high concentrations, and that in general, learning and memory are quite independent of odorous context.

Single-point Versus Time-intensity Sensory Measurements: An Informational Entropy Analysis WILLIAM E. LEE III (College of Engineering, University of South Florida)

Single-point sensory response measurement techniques such as magnitude estimation and category scaling only contain a limited amount of information. Time-intensity (T-I) data collection techniques provide more details regarding the sensory response, addressing rate-related and duration parameters in addition to quantification of other aspects of the temporal intensity behavior. Single-point and T-I responses are compared from an informational (Shannon) entropy content viewpoint, including utilization of a series of single-point measurements attempting to approximate the T-I curve. Treating the T-I response of a hypothetical ten second sensory event as a series of discrete statistical events, calculations are performed based on assumptions such as equiprobable independent events (yields maximum entropy) and divergence from this maximum state. Results show that the T-I response contains a significantly greater amount of potential information storage capacity relative to the single-point measurement. The T-I response also displays greater information density. Finally, T-I techniques may also be more efficient in terms of rate of information acquisition.

Measurement of Psychophysical and Psychophysiological Responses to Sidestream Cigarette Smoke. JAMES C. WALKER, WALTER S. PRITCHARD, PATRICIA P. MANGAN, & DANIEL B. KURTZ (BGT; R. J. Reynolds Tobacco Company; Winston-Salem, NC 27102)

Four groups (male smokers, male nonsmokers, female smokers, female nonsmokers) in different sessions were exposed in a controlled manner to sidestream tobacco smoke produced by either a Kentucky reference cigarette or a leading commercial brand. In each session, four cigarettes were machine-smoked according to standard FTC parameters in pairs separated by a 12 minute interval. Subjects made ratings of Odor Strength (OS), Nasal Irritation (NI), Eye Irritation (EI), and Overall Acceptability (OA) of room air prior to, during, and after exposure to the smoke. Eyeblinks were also recorded by an IR emitter-detector unit mounted on a pair of glasses worn by the subjects. This unit recorded blinks as a drop in reflectance produced by the eyelid covering the sclera. For both products, nonsmokers rated OA significantly lower and NI, EI, and OS significantly higher than smokers. They also showed significant increases in blinking over time, within sessions, that were not evident in smokers. Few significant psychophysical or psychophysiological differences as a function of products or between males and females were obtained. For both smokers and nonsmokers, OS was a better predictor of OA than either NI or EI. Blinks/min was a poor predictor of OA.

Volume of Taste Solution Affects Judgments of Taste Intensity. CAROL M. CHRISTENSEN and SARA EPHROSS (Monsie Chemical Senses Center, Philadelphia, PA)

Preliminary investigations showed that individuals perceive 20 ml volumes of sucrose or acid solutions to be stronger tasting than smaller volumes (5 ml) of the same solutions. The effect is probably more cognitive than physiological because the same volumes of taste solution do not generally affect taste thresholds.

The objective of this study was to investigate this phenomenon by studying the perceived taste intensity of 5, 10 and 20 ml quantities of solution encompassing the 4 basic tastes: sweet (sucrose), sour (HCl), salty (NaCl) and bitter (quinine sulfate). Thirty subjects used magnitude estimation to judge the taste intensity of 5 concentrations of each tastant spanning a low to high intensity range. The time that solutions were held in the mouth was recorded surreptitiously and resting whole mouth salivary flow rate was determined.

The principal findings were: (1) tastant volume affected the perception of taste intensity for all taste qualities--20 ml quantities were perceived to be more intense than 5 ml quantities; (2) solution volume affected perception of sour more than other taste qualities--sourness increased 60% from 5 to 20 ml volumes whereas the increase was 30% for other tastants; (3) the volume effect was greatest for low concentrations of acid--an effect probably due to salivary changes in solution pH; (4) taste quality and volume did not affect how long solutions were held in the mouth but lower concentrations were held longer than higher concentrations; (5) high salivators perceived greater differences in intensity between the two volumes of acid but other tastants were not affected.

* Current address: The Pillsbury Company, Minneapolis, MN
** Research support by NSF (BNS 841953)

Tongue Dip vs. Swallow: Some Taste Responses May Predict Hedonic and Food Intake Behavior. ROBERT J. HYDE (San Jose State University, San Jose, CA).

At AChems IX (abs 176) I showed that sweet foods are judged to be sweeter and more liked during swallowing compared to tongue dipping. Presently, 20 caucasian (Group C) and 20 noncaucasian (Group NC) college women completed questionnaires that yielded dietary preference and intake scores for sweet foods (from Pangborn and Giovanni, *Appetite* 5:317, 1984). Subjects also judged perceived intensity and degree of liking on 100-mm visual analog scales for 10-ml volumes of chocolate milk (CM), flat Pepsi (FP) and fully-carbonated Pepsi (P). Each beverage was served in triplicate at room temperature (21°C). Subjects first gave responses while dipping anterior tongue into the beverage and then while swallowing. Sweetness was judged more intense for swallow (S) compared to tongue dip (TD) for CM and P ($p < 0.001$) and FP ($p < 0.05$). FP was judged to be sweeter than P only for TD ($p < 0.01$). Stronger stinging sensations judged for P during TD ($p < 0.001$) may have suppressed the sweetness perceived from anterior tongue and contributed to greater differences in sweetness between TD-S for P compared to FP. Unlike CM ($p < 0.01$) and P ($p < 0.05$), FP failed to differ for TD vs. S for hedonic responses. FP was liked less than P for TD ($p < 0.01$) and S ($p < 0.001$). Pearson product-moment correlations showed that sweetness could predict degree of liking ($p < 0.05-0.001$) for CM and P for Group C during TD or S, but only for CM during TD for Group NC. When the magnitude of difference between oral loci was considered, by subtracting S responses from TD, both Groups showed positive correlations between sweetness and liking for CM and P ($p < 0.001$), but no correlations for FP or with sweet preference and intake behavior from questionnaire scores. Sweet preference scores could predict sweet intake scores only for Group C ($p < 0.05$). For Group NC, degree of liking for CM during swallowing correlated positively with preference for sweet foods ($p < 0.05$). Unexpectedly for Group NC, sweetness intensity during S correlated negatively with intake of sweet foods ($p < 0.05$). For the 10 out of 40 Ss whose hedonic responses for P during swallowing exceeded those for TD by 10 mm on the rating scale, greater differences between TD-S for degree of liking predicted a diminishing preference for sweet foods ($p < 0.05$). The 15 Ss with such TD-S differences in hedonic response for CM failed to show significant correlations with preference or intake. Responses during swallowing and relative differences between oral loci seem to help explain food intake behavior only for subpopulations of subjects.

Age-related changes in hedonic and intensity ratings of dairy-sucrose and dairy-salt mixtures.

WARWICK, Z. S., GILL II, J. M., and SCHIFFMAN, S. S.
(Department of Psychiatry, Duke University, Durham NC, 27706)

Four groups of subjects (young normal-weight, young obese, elderly normal-weight, elderly obese) participated in this study. Each subject tasted a mixture and rated the sweetness/saltiness intensity and their preference. The mixtures consisted of increasing concentrations of sucrose (0, 5%, 10%, and 20% w/w) or NaCl (0, 0.146%, 0.292%, 0.584% w/w) dissolved in deionized H₂O, skim milk, whole milk, half and half, or heavy cream. These dairy products contain 0.5%, 3.25%, 10.5%, and 37% fat respectively. Intensity ratings for both the young and elderly subjects increased with increasing sugar and salt concentrations in deionized H₂O. For young normal-weight subjects, substitution of skim milk for water increased the perceived intensity of 5% sucrose. Further increases in fat concentration reduced this enhancement. In the 20% sucrose mixtures, an inverse relation between fat concentration and perceived intensity was observed. At low fat concentrations, low sucrose-concentration mixtures were most preferred. As fat concentration increased, higher sucrose-concentration mixtures were preferred. For all fat concentrations tested, 10% sucrose was preferred over 20% sucrose. These results confirm those of Drewnowski (1987). In the elderly normal weight subjects, increasing the magnitude of the fat content of the mixtures had no effect on either intensity or preference ratings. There was also no statistical difference between the obese and normal weight elderly subjects, unlike the differences found by Drewnowski between young obese and normal weight subjects. Similar results were obtained for NaCl. These data suggest that the sensitivity of the gustatory system to increasing fat concentrations may decline with age. This finding suggests that the elderly can significantly reduce their fat intake without sacrificing perceived pleasantness.

*Supported by NIA AG00443 and a grant from the Campbell Soup Company

Preferences for Sweet Taste During Weight Reduction. A. DREWNOWSKI, D. WIITALA and D. SCHTEINGART (The University of Michigan).

Hedonic preferences for sweet or high-calorie foods are thought to be elevated following successful dieting and weight reduction. However, many previous studies on dieting obese men and women were cross-sectional rather than longitudinal, and were based, moreover, on averaged group data. Individual taste preference profiles are known to be highly variable, and no consistent "obese" taste response has yet been observed. In the present study, 7 massively obese women (mean age: 44 years, mean wt: 141.7 kg) tasted sucrose solutions of increasing intensity (2 to 17% w/w) using the standard sip-and-spit technique and 5-point category scales. Two main types of preference response were observed: rise and decline (type I) and monotonic decline with increasing sweetness (type II). Individual response profiles of these subjects were stable even following substantial weight loss (mean loss: 34.1 kg) on a 600 kcal/day diet, and no elevation in preference for sweet taste was observed. In the second study, 9 massively obese men (mean age: 41 years; wt: 185.3 kg) tasted mixtures of milk, cream and sugar containing between 3.5 and 37.5% dairy fat and 2 to 17% sucrose w/w. Individual types of taste response to sweetness were again unchanged following sustained dieting and weight loss (mean loss: 44.3 kg). Taste responsiveness may be an enduring individual trait that is not directly related to short-term changes in body weight.

Supported by Grants AM37011, AM38073

Insect Pheromone Systems: Behavior, Biochemistry, Peripheral and Central Neurophysiology, Introduction. LINDA M. KENNEDY* and CHARLES E. LINN JR.** (*Clark University, Worcester MA 01610 and **Cornell University, NYS Agric. Expt. Station, Geneva NY 14456).

This symposium will present an overview of current research on insect sex pheromones, concentrating on specific areas from molecular to organismal levels, and focusing on information relevant to general problems in chemoreception. Continuity will be maintained throughout the various presentations by centering discussion on the moth pheromone system.

Beginning with definition of the relevant stimulus, presentations will progress from how components of the stimulus mixture control male behavior and how males orient to the pheromone plume and fly upwind, to the transport of stimulus components from the surface of the antenna to sites on receptor cell dendritic membranes and to subsequent transduction processes. Discussion of receptor cell responses to the various stimulus components and subsequent central nervous system processing of information about the stimulus mixture will follow. The symposium will end with a summary and discussion, including audience participation.

Supported by grants from NSF (BNS-8719518) and E.I. DuPont deNemours, Inc.

Chemical Characterization of Sex Pheromones and Their Biosynthetic Intermediates. LOUIS B. BJOSTAD (Colorado State University, Fort Collins CO 80523).

One of the major problems in studies on olfaction is that of defining a relevant stimulus. In the context of insect sex pheromones, this problem is that of defining a pheromone component. In recent years, a wealth of information has been obtained on the biosynthetic pathways by which sex pheromones are produced. Several rules have emerged that have allowed generalizations concerning the evolution of pheromone blends within taxonomic groups. This presentation will focus on these developments, with emphasis on advances in analytical techniques that have allowed precise and reliable identifications of sex pheromones. With the appropriate technology and the knowledge obtained from comparative studies between taxonomic groups, it now is possible to determine with a high degree of accuracy what the pheromone for a species is, and thus to precisely define and characterize the relevant stimulus for behavioral and electro-physiological studies.

Sensitivity of Male Moths to Multicomponent Pheromone Blends. CHARLES LINN (Department of Entomology, Cornell University, NYS Agric. Expt. Station, Geneva, NY 14456)

A fundamental problem in understanding the response of male moths to multicomponent pheromone blends relates to the factors that control the sensitivity of males to the signal. This problem involves two separate questions: 1) How sensitive are males to the quality and quantity of the pheromone? and 2) How is male sensitivity influenced by the periodicity of the response? Our studies have utilized the sustained-flight tunnel to study individual moth behavior. We have shown that males are very sensitive to both blend quality and release rate, and that specific changes in signal quality produce two unique changes in male flight behavior, allowing us to characterize the behavioral thresholds that control male response specificity. One of the most important principles to emerge from these studies is that males are most sensitive to the female released blend of components and that this signal functions as a unit to effect optimal sensitivity and peak response over the entire flight sequence. Most recently, we initiated studies to investigate the potential role of octopamine and serotonin as neuromodulators of male sensitivity and the periodicity of their response. We have shown that octopamine enhances male sensitivity and that serotonin alters the time during the photoperiod when peak response to pheromone occurs. Other pharmacological studies have supported our results and suggested that the activity is mediated by an adenylate cyclase system. Our studies have also demonstrated, however, that male response, as well as the action of the amines is critically dependent on appropriate photoperiodic cues. Based on these findings, we have proposed the hypothesis that the amines act as neuromodulators or neurohormones affecting CNS pathways involved in male perception and discrimination of the odor signal, and that the timing of their action is linked to other photosensitive pathways involved in the entrainment of the circadian based locomotor pathway.

Supported by NSF BNS-82167524 and BNS-8518855

Cessation of Pheromone-Mediated Flight in Moths is Correlated With Adaptation of Antennal Neurons. DR. THOMAS C. BAKER (University of California, Riverside)

A wind-borne plume of sex pheromone from a female moth or a synthetic source has a fine, filamentous structure that creates steep and rapid fluctuations in concentration for a male moth flying up the plume's axis. Recordings of the firing rates of single antennal neurons when antennae were placed 70 cm downwind of a pheromone source revealed that the cells adapted rapidly in a plume from a high-concentration source known to cause in-flight arrestment. No adaptation was found in lower-concentration plumes promoting high levels of source-location. When challenged by experimentally delivered pheromone pulses, chilled neurons became adapted at lower pulse frequencies than warm neurons, and this adaptation was also correlated with a higher percentage of in-flight arrestment exhibited by moths flying at cooler compared to warmer temperatures. These results indicate that the ability of antennal neurons to biochemically transduce and degrade rapidly-arriving, incoming pheromone filaments in a plume is a key, initial determinant of whether or not prolonged upwind flight and source location will occur.

The Molecular Basis of Pheromone Reception.
 RICHARD G. VOGT (Yale University, Section of Molecular Neurobiology)

In 1929 Phillip and Nellie Rau reported that a male silk moth was captured, attracted to a sex-pheromone releasing female that was 3 miles from the male's release site (*Trans. Acad. Sci. St. Louis* 26, 83-221). The Rauses had spent most of the nights of one May and June during the 1920s studying the photo-rhythmicity of sex-pheromone release by females and of responsiveness by males, all animals belonging to several species of native silkworm. Their approach was in part to set caged females in the third floor windows of their house, which was located in an urban community near St. Louis, Missouri. They would release marked males in nearby parks and stay up most of the night monitoring the arrival of males, both wild and released. The recapture rate dropped off decidedly with distance, but one moth did return from a release site ca. 4.5 km away. It is certain that this male did not follow a contiguous pheromone plume to the female. Nevertheless, it is remarkable that its behavioral strategies in response to external stimulation favored its return.

In my talk I will describe the current awareness of the biochemical and molecular biological events which occur during sex-pheromone reception. I will describe studies done by several groups who have identified pheromone binding proteins, pheromone degrading enzymes and pheromone receptor proteins. I will also describe studies of the mRNAs which code for the binding proteins. These macromolecules and their interactions with pheromone provide the molecular basis of pheromone processing within the sensory hairs. Thus their molecular properties partially encode that aspect of precopulatory behavior which is dependent on temporal and sensitivity aspects of pheromone processing.

Neurophysiological Responses to Pheromones from Insect Antennal Sensilla. ALAN J. GRANT,
 (The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545)

Female moths produce and release complex blends of pheromones which attract males moths of the same species, often from long distances, so that mating may occur. The specific composition of these chemical blends is thought to be involved in the maintenance of reproductive isolation among sympatric species. In a similar manner, male moths may release compounds which mediate short-range precopulatory behaviors. Detection of such behavior-modifying chemicals is accomplished by olfactory receptor neurons that are housed in specialized structures, sensilla, located on the antenna. However, the precise manner in which these species-specific chemical signals are encoded by the peripheral olfactory system is not completely understood. Consequently, a detailed description of the response properties of the pheromone-sensitive olfactory receptor neurons on the insect antenna may provide insight into how these chemical messages are processed. Morphological investigation of moth antennae reveal the existence of different classes of pheromone-sensitive sensilla. Extracellular recordings in response to stimulation with pheromones and related compounds show a high degree of sensitivity and specificity inherent in the response properties of the receptor neurons located within these different morphological classes of sensilla. Additionally, interesting similarities and differences were noted between the response properties of olfactory sensilla on the antennae of two closely-related sympatric species of moths.

Support is acknowledged to Grant NS 14453 from the NINCDS and from the Alden Trust awarded to Robert O'Connell (Worcester Foundation for Exp. Biology).

The Coding of Sex Pheromone Information in the Brain of the Male Moth. THOMAS A. CHRISTENSEN (Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, AZ 85721).

This talk will focus on what happens to olfactory information after it is passed on to neurons residing in the first-order olfactory processing centers - the bilateral antennal lobes (ALs). In addition, some of the remarkable similarities between the insect AL and the vertebrate olfactory bulb will be highlighted.

Not long ago it was thought that the ALs did little more than amplify incoming sensory signals and relay them to the protocerebrum. Through the use of intracellular recording and dye-marking techniques we now realize that many central olfactory neurons are considerably more complex. For example, information from sex pheromones converges on a sexually-dimorphic area of synaptic neuropil in the AL called the macroglomerular complex (MGC). In the sphinx moth, *Manduca sexta*, which utilizes two major pheromones in the female blend, some MGC neurons are "pheromone generalists", signalling only the presence of pheromones without regard to the chemical identity of the stimulus. Other cells demonstrate more advanced processing capabilities in that they are able to discriminate one pheromone from the other. These neurons, which link the MGC with the protocerebrum, are also capable of coding for changes in the temporal characteristics of the stimulus, changing their firing patterns to reflect changes in the duration and frequency of pheromone pulses. This specialized function is particularly relevant in light of recent behavioral evidence that an intermittent stimulus is an important factor in male orientation to pheromones.

Another level of complexity in MGC neurons is seen in two closely-related sympatric species, *Heliothis zea* and *H. virescens*. Responses to the conspecific blend of *H. zea* can be modulated by the addition of a minute amount of a pheromone from *H. virescens*. This synergism may be a physiological substrate for the repellent effect of *H. virescens* females on *H. zea* males.

Supported by grants from the USDA and NIH.

High-Speed Imaging of Olfactory Bulb Electrical Activity.

DAVID M. SENSEMAN, PAULUS B. MULJADI,
PATRICK L. NASH and MATTHEW J. WAYNER
(Brain Research Laboratory, UT San Antonio)

Information provided by global monitoring of electrical activity evoked in the olfactory bulb by selected odorants might be useful in elucidating the integrative mechanisms responsible for odor quality encoding within the vertebrate central nervous system. Kauer, Senseman & Cohen (*Brain Res.*, 418, 1987) have shown recently that such information can be obtained with optical recording techniques after vitally staining the bulb with a voltage-sensitive dye. In their preliminary report, Kauer *et al.* presented most of their data in a form known as a 'page display' in which optical signals are displayed as 124 individual analog traces arranged in a grid-like pattern corresponding to their detector origin within a 124-element photodiode array. While this display format is useful for assessing differences in signal waveforms between bulbar laminations, the format is less useful for visualizing the dynamic activation of bulbar regions following stimulation. We have therefore developed a family of software programs that use the analog voltage data recorded optically by our 124-element photodiode array to generate Pseudocolor Activity Maps (PAM's) of the bulb. In the PAM format, the normalized amplitude of the neuronal response recorded from 124 contiguous bulbar regions is translated into a 8-level color scale. Through rapid sequential display of a PAM series, an animated 'movie' of the evoked response can be observed. Because of the superior temporal resolution afforded by diode array-based imaging systems (1ms/frame), we are able to observe and study the rapid series of events that occur within the first 30 to 50 msec following electrical stimulation of the olfactory nerve. A movie showing these initial events will be presented.

Supported by: NSF Grants BNS-8507594, INT-8311534 and NIH Grant RR-08194-07

Thurs. Morn Talk 12:00 AM Abst. # 34

Functional Regeneration of Primary Olfactory Connections in Newborn Rabbits. R. HUDSON and H. DISTEL (Inst. Medizin. Psychol., Univ. München, Goethestr. 31, D-8000 München, FRG)

In 1-day-old rabbits (n=40) all nerve strands leading to one of the olfactory bulbs were transected intracranially. To test for functional recovery, the nares of either the intact or lesioned side were temporarily closed each day and the pups' response to the nipple-search pheromone recorded. In several cases the intact olfactory bulb was also removed before final testing. Animals were sacrificed 1, 3, 5, 10 and 15 days after lesioning and the olfactory bulbs and nasal septal epithelium examined histologically. Whereas no pup responded to pheromone with the lesioned side before day 8, all animals surviving to day 15 reacted vigorously to pheromone-producing females and showed normal suckling behavior - even after removal of the intact olfactory bulb. Histological examination of deafferented bulbs using silver impregnation methods showed only degenerating glomeruli to be present on days 3 or 5 but a clear reinnervation of ventromedial and/or ventrolateral areas by day 10. While most of the bulbar surface was reinnervated by day 15, the glomeruli were more widely spaced, smaller and irregular in shape compared to those of the control side. Interestingly, no correlation could be found between the pattern of reinnervation and the recovery of function. However, the question still remains to what extent this functional recovery depends on experience of the relevant odor stimuli postoperatively or on a transfer of information from the intact olfactory bulb via the anterior commissure.

Supported by the Deutsche Forschungsgemeinschaft.

Olfactory Dysfunction in Parkinsonism: A General Deficit Unrelated to Neurologic Signs, Disease Stage, Disease Duration, and Cognitive Factors. RICHARD L. DOTY, DANIEL A. DEEMS, MANUAL RIKLAN, AND STANLEY STELLAR (Smell and Taste Center, University of Pennsylvania, Philadelphia, and Neural Sciences Research Institute, St. Barnabas Medical Center, Livingston, NJ)*

To explore the nature of the olfactory dysfunction associated with Parkinson's disease (PD), 81 PD patients who scored well on the Picture Identification Test (PIT) were administered the 40-odorant University of Pennsylvania Smell Identification Test; 38 were additionally given a forced-choice phenyl ethyl alcohol odor detection threshold test. Clinical ratings of 11 neurologic symptoms (3 bilateral) were obtained at the time of testing, and odor identification was retested in 24 patients at intervals ranging from 5 to 39 months. 54 patients additionally received WAIS-R verbal and nonverbal memory subtests, the RANDT memory test, and other neuropsychological tests. Relative to matched controls, the PD patients exhibited consistent and marked decrements on both types of olfactory tests. The odor identification deficit was not restricted to any subset of odorants and did not evidence longitudinal change. A factor analysis of the intercorrelations among the variables yielded six easily interpretable factors: general motor, oral motor, olfactory function, cognitive function, tremor, and gender. Olfactory test scores were independent of all other measures, including disease stage and duration. 72% of the PD patients were unaware of a smell disorder before testing; those who were aware had significantly lower test scores. A statistical comparison of PD patients' olfactory test scores to those obtained earlier from a matched group of Alzheimer's disease patients found the olfactory disorders of these diseases to be indistinguishable. Overall, these data support the hypothesis that the olfactory deficit of PD is a general and stable one which is independent of both the cognitive and motor symptoms and which likely occurs early in the disease process.

*Supported by the NINCDS (NS 16365) and the Jennie S. Isermann Memorial Fund for Parkinson's Disease Research.

Thurs. Morn Talk 12:15 PM Abst. # 35

Memories of Odor Preference Learning are Accessed via Anterior Olfactory Nucleus in Infant Rats. W.G. HALL & DAVID KUCHARSKI (Dept. Psychology, Duke Univ., Durham, NC 27706)

Memories for an olfactory preference trained by pairing a novel odor with oral milk infusions is stored on one side of the brain in young rats when one naris is blocked during training. In 6-day-old pups the preference memory is only accessible by stimulation of the trained naris, but by 12 days of age it is accessible from both sides. Because odor preference shown by the untrained side was eliminated by transection of the anterior commissure (AC), the AC must subserve the new access to the contralateral memories in 12-day olds (Kucharski & Hall, *Science*, 1987, 238, 786-788). We have further assessed which components of the AC subserve the retrieval of contralateral odor memories. Twelve-day-old rats received pairings of a novel cedar odor and oral infusions of sucrose while one naris was blocked with a soft silastic plug. Knife-cut transections of separate limbs of the AC were made on the trained side and pups were tested for access to odor preference memories from the untrained side. Trained pups that received sham cuts and pups in which the posterior limb of the AC was sectioned showed conditioned preference for the novel odor. In contrast, section of the anterior limb of the AC disrupted preference. Cuts at distal/anterior levels of the anterior limb were as effective as those at the base. These findings indicate that memory retrieval does not involve the projections through the posterior limb to posterior piriform cortex. Neither are projections to anterior piriform cortex likely to be involved because section of the anterior limb at a level primarily carrying fibers to the anterior olfactory nucleus (AON) was as effective as more proximal cuts. We noted, in addition, that complete section of the olfactory peduncle distal to the AON did not disrupt memory retrieval, ruling out the necessity of presence of the "trained" bulb for preference memory. Thus AON is the likely target of AC fibers accessing olfactory preference memories.

(Supported by NICHD grant HD17458 to WGH and NIH fellowship MH09436 to DK.)

Unilateral Odor Deprivation: Rapid Effects on Cellular Regulatory Events. DONNA L. KOROL and PETER C. BRUNJES (University of Virginia)

Occlusion of a single external naris on postnatal Day 1 leads to a 25% decrease in the volume of the ipsilateral olfactory bulb when rats are examined on Day 30. Large alterations such as these undoubtedly represent secondary consequences of more basic, cellular regulatory events. For example, as early as 3 days following occlusion, metabolic changes are evident as reductions in the density of bulb succinate dehydrogenase staining (Dev. Brain Res. 35:35-42). We examined the possibility that deprivation has even earlier effects on metabolism (glucose uptake) and protein synthetic activity. Rat pups underwent either single naris occlusion or sham surgery on postnatal Day 1. Bulb glucose metabolism was measured with ^3H -2DG techniques. 2-DG was injected 1, 12, 24 and 48 hr after occlusion and the optical density of film autoradiographs was used to determine relative glucose uptake. To assess protein synthetic activity, ^3H -leucine was injected 12, 24 and 48 hr following occlusion and was quantified by grain counts from emulsion-coated slides. Deprived subjects exhibited rapid changes: left/right differences were evident for glucose utilization as early as 1 hr post-occlusion, and for protein synthesis as early as 24 hrs post-occlusion. Control subjects, however, demonstrated no laterality in glucose metabolism or protein synthesis. The data indicate that cellular activity in the olfactory bulb changes rapidly after nares occlusion, and suggest that examinations of the chain of events caused by deprivation might lend insights into the mechanisms by which experience controls brain growth processes. Supported by NS-35301, ONR N00014-86-K-0342, and the Whitehall Foundation.

Regional and Intensity Patterns of Afferent Terminal Degeneration in the Glomeruli of the Rat Olfactory Bulb. ESMAIL MEISAMI (Physiol Dept, Univ Illinois, Urbana, IL) & JOACHIM R. WOLFF (Anat Dept, Göttingen Univ, Göttingen, FRG)

Acid phosphatase (AP) is a lysosomal enzyme commonly used as a histochemical marker for this organelle. Gallyas et al (1) developed a histochemical method for localizing sites of degenerating terminals (DT) of axons based on lysosomal activity. Olfactory receptor neurons innervating the olfactory bulb (OB) are known to undergo continuous degeneration & regeneration. We utilized a combination of the above histochemical methods to study patterns of degeneration occurring in glomeruli (GL) of normal rat OB. Serial frozen sagittal sections of OB from young adult rats were stained alternately for AP (2) and for DT (1). AP staining in the OB was largely limited to the GL neuropil and mitral cells soma. The individual GL varied markedly in intensity of staining: on the average about 5% of the GL appearing in each section stained very dark, 25% dark, 35% light and 35% showed no staining. The very dark and darkly stained GL occurred in all regions and in any combination with other less darkly stained GL. Thus a single very dark GL could be found surrounded by two unstained GL or by one light and one dark GL. To determine whether the very dark GL of AP sections are sites of massive DT, several examples of large very dark GL, surrounded by light ones, were matched with the neighboring DT stained sections that contained the same GL. A clear correspondence was found between the intensity of AP staining and number of black granules representing lysosomes of DT. The results indicate that afferent degeneration occurs in the normal rat OB and it shows a patchy pattern. The patches vary in intensity and size, may involve from one to several GL and may occur anywhere in the OB. Further, the results indicate that this combination of AP/DT staining offers a useful and quantitative method for studying normal and abnormal afferent degeneration in the OB.

1) Gallyas, Wolff, Bottcher, Zaborszky, Stain Tech 55:299, '80. 2) Gomori, Stain Tech, 25: 81, '50. The able assistance of Frau H. Bottcher & Ms. Lisa Oldson is acknowledged.

Thursday Evening

Video Imaging of Odor Responses in the Salamander Olfactory Bulb using a Voltage-sensitive dye. J.S. KAUER (Tufts-New England Medical Center, Boston, MA.)

Video-rate imaging of voltage-sensitive dye fluorescence permits observation of global electrical events with high spatial and temporal resolution. This method has shown that electrical stimulation of the olfactory nerve gives rise to signals distributed across and within the layers of the salamander olfactory bulb in a sequence consistent with electrophysiological recordings (Kauer, Nature, 1988). One of the strengths of the method is its ability to characterize distributed patterns of activity with substantial temporal resolution and, in the olfactory system, it provides a way to test the hypothesis that odors are encoded by parallel, differential activation of many parts of the system. In the present study, the distribution of activity in the olfactory bulb elicited by odor stimulation of the olfactory epithelium has been observed. Square, concentration-controlled odor pulses (Kauer and Shepherd, Bx. Res., 1975) were delivered to the exposed olfactory epithelium at a defined time point during the acquisition of 64 sequential video frames (64 pixels x 64 pixels x 8 bits) of the changes in voltage-sensitive dye fluorescence from the olfactory bulb. Various odorants and concentrations were tested. Distinctively different patterns of depolarization and hyperpolarization were observed within the bulbar layers after single odor pulse stimulations lasting one second, which is within the time course of normal sniffing for this animal. Examination of relationships among patterns generated by different odors and the manipulation of these patterns with pharmacological treatments of the bulb should provide data important for understanding how parallel processing of information occurs in the nervous system.

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Pharmacological Manipulations of Voltage-sensitive Dye Signals Elicited by Electrical Stimulation of the Salamander Olfactory Bulb. S. NEFF and J.S. KAUER. (Tufts-New England Medical Center, Boston, MA)

Video-rate imaging of voltage-sensitive dye fluorescence has permitted observation of distributed activity in the salamander olfactory bulb after electrical (Kauer, Nature, 1988) and odor (Kauer, Achews-X, 1988) stimulation. The spatial and temporal resolution of the method are adequate for allowing correlations to be made between the dye responses and the bulbar layers and, by inference, between the responses and the various cell types in the bulb. In the present study we have pursued this correlation further by observing the changes in the distribution of electrically elicited dye responses after treating the bulb with pharmacological compounds chosen to interact with putative transmitter systems in the salamander bulb (see Hamilton et al., Chem. Senses, 1987). Sequences of 16 (128 x 128 x 8 bit) video frames of the olfactory bulb acquired at 30 frames/sec were generated after shocks to the intracranial portion of the olfactory nerve. Fluorescence patterns generated before and after the application of the drugs were compared. DL-2-AP7 at 40-200 μM and picrotoxin at 30-100 μM were tested. DL-2-AP7 (competitive antagonist for NMDA receptors, which may be post synaptic at mitral/tufted(M/T) - granule synapses), causes a decrease in the magnitude of the fluorescence we identify as arising from granule cells. We interpret this as a blockade of depolarization of granule cells by M/T's. Picrotoxin, a competitive antagonist for GABA-A receptors, causes an increase in the granule cell fluorescence signal. We interpret this as a blockade of the feedback inhibition from granule cells onto M/T cells, thus permitting depolarization of M/T cells to be more clearly seen, as well as potentiating depolarization of the granule cells themselves. We expect that additional experiments of this type will allow us to dissect the neurochemical events underlying activity generated by odors.

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In Vitro Pharmacological Studies on the Elasmobranch Nervus Terminalis: Evidence for Cholinergic and Catecholaminergic Suppression of Ganglion Cell Activity.
JOEL WHITE and MICHAEL MEREDITH (Department of Biological Science, Florida State University, Tallahassee, FL 32306).

Investigations in this laboratory and others have indicated the presence of synapses in the nervus terminalis (NT) ganglion of various species. Furthermore, histochemical studies by Wirsig & Leonard (1986) suggest that some NT ganglion cells in the hamster may receive cholinergic synaptic input. Immunocytochemical studies by Fernald & Finger (1984) suggest that the goldfish NT ganglion receives catecholaminergic input, perhaps from the locus ceruleus. To investigate the possible action of these neurotransmitters on NT ganglion cells, we are currently conducting *in vitro* pharmacological investigations on the NT of the bonnethead shark (*Sphyrna tiburo*). In these experiments, multi-unit activity arising from NT ganglion cells was recorded extracellularly from the central trunks of nerves with glass suction electrodes. As we have shown previously, electrical stimulation of the peripheral trunk elicited suppression of this ganglion cell activity. Bath application of acetylcholine (ACh), norepinephrine, dopamine and epinephrine (EPI) at 1 to 100 μ M also suppressed ganglion cell activity. These agonists suppressed the activity when applied in calcium-free conditions to block synaptic transmitter release. This finding suggests that both the ACh and the catecholamine agonists were acting directly on the NT ganglion output cells and not through interneurons or presynaptic mechanisms. Preliminary experiments further suggest that ACh may be acting via muscarinic receptors and EPI via alpha-adrenergic receptors. At concentrations of 500 nM to 50 μ M, the muscarinic antagonist homatropine reduced or eliminated suppression of multi-unit activity elicited by electrical stimulation or by ACh application, while 10 μ M curare (a nicotinic antagonist) had no apparent effect. At 10 and 100 μ M, the alpha antagonist tolazoline reduced suppression elicited by 1 μ M EPI application, while no effect was seen with 100 μ M isoproterenol (a beta antagonist).

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Neuropeptide Immunoreactivity in Nerve Fibers of the Nose of Mice and Rats. VAR L. ST. JEOR^{1,2}, JOHN C. KINNAMON^{1,2}
(¹Univ. of Colorado, Boulder, CO 80309) and THOMAS E. FINGER²
(²Rocky Mountain Taste and Smell Center, Denver, CO 80262)

Chemosensitive trigeminal nerve fibers that innervate the nasal cavity are capsaicin sensitive and therefore are likely to contain neuropeptides such as substance P. The distribution and ultrastructure of peptidergic nerve fibers in the nasal cavity is being studied in an attempt to elucidate the morphological substrates for nasal trigeminal chemoreception. Antisera directed against substance P or calcitonin-gene-related-peptide were utilized for light and electron microscopic studies of the nasal epithelium including both olfactory and non-olfactory regions. Dual label studies revealed that the vast majority of peptidergic fibers were immunoreactive for both substances. Numerous peptide-immunoreactive fibers were found in the submucosal layer. These fibers usually were grouped into fascicles which often followed the larger submucosal blood vessels. Branches of the immunoreactive nerve fibers often could be seen ramifying to innervate the blood vessel walls. At various regions of the nasal epithelium, single peptide-immunoreactive fibers turned outward from the submucosa, penetrated the basal lamina and extended toward the epithelial surface. In some cases a small immunoreactive varicosity could be seen at the distal tip of these processes. Similar varicosities were found along the length of most superficial peptide-immunoreactive nerve fibers. The superficial peptide-immunoreactive fibers were not distributed homogeneously throughout the nasal cavity, but occurred in patches interspersed among larger regions devoid of such peptidergic innervation. Superficial immunoreactive fibers were present in the olfactory epithelium but were rare in comparison to the innervation of the respiratory epithelium. Preliminary ultrastructural observations indicate that the superficial peptidergic fibers are approximately 0.1 μ m in diameter as they pass through the basal lamina. Varicosities along the length of these fibers measure 0.2-0.3 μ m. Further ultrastructural studies are underway to determine whether the peptidergic fibers reach the epithelial surface and if so whether any ultrastructural specializations can be observed in this area.

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Contribution of Cell Types to the Coding of Odorant Quality by Spiny Lobsters. MARIE-NADIA GIRARDOT and CHARLES D. DERBY (Georgia State University, Atlanta, Georgia)

Spiny lobsters can behaviorally discriminate the quality of various odorants (Fine-Levy, et al., *Chemical Senses*, in press). Each odorant is identified by the pattern of activity generated across the neurons (ANP) of the primary olfactory tract (Girardot and Derby, *Chemical Senses*, in press). The following question is now addressed: Is the entire ANP necessary to determine an odorant's identity, or does the code reside predominantly in restricted groups of neurons, such as those responding "best" to the odorant, as seems to be the case in the gustatory system of the hamster? (Smith et al., *J. Neurophysiol.*, 50:541-556, 1983) We have recently demonstrated that the "best" and the "least" responders to an odorant are required to establish its identity; neither group alone is necessary (Girardot and Derby, *Neuroscience Abstracts*, #388.85, 1987). To further assess the functional role of these and other groups of cells in the neural identification of olfactory stimuli, two simplified models will be proposed. In the first model, the neurons respond selectively to only one or two stimuli, as seen with individual compounds. In the second model, the neurons respond to a broad range of stimuli, as observed with complex mixtures of compounds (i.e., extracts of tissue of potential prey to lobsters, and reconstructed artificial mixtures of these extracts). The two sets of hypothetical stimuli will then be distributed spatially using multidimensional scaling; their relative location as well as the distance between them will be related to their ANPs to determine the contribution of "best", "least", "both best and least", and "neither best nor least" groups of cells to the identity of each stimulus. The conclusions derived from these two models will then be tested using 1) responses to single compounds of receptor cells, 2) responses to extracts of receptor cells, 3) responses to single compounds of output neurons with synapses in olfactory brain centers, 4) responses to extracts of neurons located in the circumesophageal connectives. Generalization of findings throughout types of odorants (single vs. complex) and throughout the nervous system (from primary receptor cells to the circumesophageal connectives) may provide a unified model by which the olfactory nervous system identifies, recognizes and discriminates chemicals.

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Lectin-binding to a Unique, Membrane-bound Glycoprotein in the Adult Vomeronasal Nerve.
CHARLES J. WYSOCKI, JOHN J. LEPRI, LINDA M. WYSOCKI and RICHARD BRUCH (Monell Chemical Senses Center, Philadelphia, PA)*

A peroxidase conjugated lectin from soybean binds the vomeronasal nerves of cat, mouse, prairie vole, guinea pig, musk shrew and opossum. Histologically, binding was evident from the vomeronasal epithelium to the accessory olfactory bulb. In adults, binding in the main olfactory bulb was not observed. Furthermore, no binding was detected in the olfactory bulbs of catfish or starling, species that lack a vomeronasal nerve. Surprisingly, binding was also absent from the vomeronasal nerves of garter snakes. Unilateral removal of vomeronasal receptors eliminated binding of the lectin ipsilaterally, but not contralaterally. Polyacrylamide gel separation and Western blotting of membrane preparations of vomeronasal epithelium from mice followed by incubation with the lectin indicated a single band of approximately 200 kDa. Soybean lectin binds N-acetyl galactosamine (Gal-Nac) and galactose. Peanut lectin binds galactose, but peanut lectin did not bind to the vomeronasal nerves of prairie voles. Preincubation of tissue sections with GalNac eliminated binding of the lectin. We conclude that the soybean lectin is binding to GalNac. Both the olfactory and vomeronasal neuroepithelia experience neuronal turn-over, yet the sites of axonal termination (the respective bulbs) are contiguous. Hence, the turn-over process should demand a high resolution projection system. The membrane-bound glycoprotein in the vomeronasal nerve that is recognized by the soybean lectin may serve as a cell surface marker, effectively segregating vomeronasal from olfactory nerves.

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Pharmacology Of Inhibition In The Glomerular Layer Of The Olfactory Bulb. W.T. NICKELL and M.T. SHIPLEY (Dept. of Anatomy and Cell Biology University of Cincinnati College of Medicine, Cincinnati, Ohio).

There are two different central GABA receptors: GABA_A receptors are activated by muscimol, and blocked by picrotoxin and bicuculline. GABA_B receptors are activated by baclofen, and are not blocked by picrotoxin or bicuculline. GABA_A receptors mediate classic postsynaptic inhibition, while GABA_B receptors may be the source of GABA-mediated presynaptic inhibition.

A recent autoradiographic study (N.G. Bowery, *et al.*, 1987) demonstrated a striking segregation of these receptors in the olfactory bulb of the rat: GABA_A receptors are present in all layers of the bulb; GABA_B receptors are almost exclusively located in the glomerular layer. An earlier study (A.A. Potapov and V.V. Trepakov, 1986) showed that, in the frog, baclofen decreases the response to olfactory nerve (PON) stimulation, but muscimol does not. This suggests that, in the frog, inhibition in the glomerular layer is mediated by presynaptic GABA_B receptors on the terminals of the PON. We have studied the effects of GABA_A and GABA_B agonists in the mammalian olfactory bulb.

The lateral surface of the bulb of anaesthetized rats was exposed, and a recording electrode was inserted into the glomerular layer. Orthodromic responses in the olfactory bulb were elicited by electrical stimulation of the olfactory epithelium using double pulses with interpulse intervals of 25 to 200 msec. The second response was always inhibited. With the gas anaesthetic methoxyflurane, inhibition (1/2 amplitude) lasted about 50 msec; with pentobarbital, which is known to prolong the duration of GABAergic inhibition, the inhibitory response lasted more than 100 msec. Either baclofen, muscimol, or picrotoxin were applied to the exposed surface of the bulb. Unlike the frog, in the rat both baclofen and muscimol inhibited the response to both pulses. Inhibition of the second response was not blocked by picrotoxin.

The persistence of inhibition after blockade of GABA_A receptors by picrotoxin suggests that, as in the frog, inhibition is mediated by GABA_B presynaptic receptors on PON terminals. In the rat, however, functional GABA_A receptors are also present in the glomerular layer.

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Organization of Astrocytes in the Rat Olfactory Bulb. M.S. BAILEY, M.T. SHIPLEY (Dept. of Anatomy and Cell Biology) and R. AKESON (Division of Basic Research Children's Hospital Research Foundation) Univ. of Cincinnati Med. Ctr., Cincinnati, Ohio

Two monoclonal antibodies, 2C8, 2F5.1 and an anti-GFAP polyclonal antibody (Eng, 1978) have been used to characterize the organization of astrocytes in the rat olfactory bulb using immunocytochemistry.

Anti-GFAP labeled typical stellate shaped fibrous astrocytes (FAs). Astrocytes were differentially concentrated in layers of the bulb. Highest densities were in the glomerular (GL) and in the external plexiform layers (EPL). The internal plexiform (IPL) and granule cell layers (GCL) had much lower densities of FAs, but there was a high concentration in the subependymal zone.

The morphology of cells stained with 2C8 and 2F5.1 were indistinguishable from those seen with anti-GFAP, and the laminar patterns were similar although the overall density of labeled cells was lower. While many long processes are observed coursing in a radial fashion in the GL and EPL, astrocytes immediately deep to the mitral cells preferentially send processes parallel to the layer. Surprisingly, even with the generally lower staining levels of 2C8 and 2F5.1, the nerve layer was very strongly labeled in contrast to anti-GFAP bulb sections.

Preliminary Western blot analysis indicated that 2F5.1 is reactive with a protein band of similar molecular weight to that recognized by anti-GFAP. 2F5.1 also binds to a higher molecular weight band.

The large number of astrocytes in GL and EPL which are zones of high synaptic activity may relate to astrocytes' role in regulating ionic milieu and neuronal metabolism. The intense label in the nerve layer may correspond to ensheathing cells, modified astrocytes found surrounding entering olfactory neuron axons as described by Doucette (1986). Studies are in progress to determine how astrocytes respond to the lesioning and regrowth of primary olfactory neurons from the epithelium and during normal development. (Supported by: NIH 23348, 20643, 22053 and U.S. Army DAMD 17-86-C-6005)

Medial Anterior Olfactory Nucleus: Interface for Olfactory-Limbic Coordination. J.H. McLEAN¹, M.T. SHIPLEY¹, W.T. NICKELL¹ and C.K.H. REYHER². ¹University of Cincinnati Medical Center and ²Freie University of Berlin

At last year's AChemS we reported a dense, focal projection from the dorsomedial anterior olfactory nucleus (AON) to the medial septal (MS)-vertical limb of the diagonal band region (VLDB) in the rat. This projection may be involved in the coordination of hippocampal rhythm and sniffing during exploratory behavior (Macrides *et al.*, *J. Neurosci.* 2:1705-1717, 1982). Here, we report new neurophysiological and anatomical evidence in favor of this possibility.

Single unit recordings in the dorsomedial AON revealed numerous neurons that could be antidromically activated by a stimulating electrode in the MS-VLDB region. Antidromic activation was confirmed by collision test and by high frequency following. Most of the neurons which were antidromically activated from MS-VLDB could also be antidromically activated from MOB; this suggests that many neurons in medial AOB send projections to both MOB and MS-VLDB.

These electrophysiological findings were anatomically confirmed in retrograde double fluorescent tracer experiments. Fluorogold was injected into MS-VLDB and rhodabeads were injected into MOB. Preliminary results indicate that there are numerous intermingled neurons labelled with one or the other tracer and some labelled with both. Thus, the medial AON contains populations of neurons that project either to MOB or the MS-VLDB and some neurons that project to both structures. These results further support the suggestion that the medial AON may function to coordinate limbic and sniffing rhythms.

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Termination of Primary Olfactory Neurons in the Frog. H.J. DUNCAN, W.T. NICKELL, M.M. DASTON, R.C. GESTELAND and M.T. SHIPLEY (Department of Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267).

Despite its extensive use in olfactory research, little is known about the anatomical organization of the olfactory bulb in the frog (*Rana pipiens*). We are using a variety of anterograde and retrograde labelling paradigms to examine the axonal projections from the olfactory epithelium to the olfactory bulb in this species.

The projection from the entire epithelium has been labelled by gelfoam implants of WGA-HRP. Dense axonal and terminal labelling was found in the glomerular layer of the main and accessory olfactory bulbs. In all cases, the projection extended beyond the midline into the glomerular layer of the contralateral bulb. The labelling was organized into circular or ellipsoidal aggregates. In Nissl counterstained sections neurons in the glomerular layer were preferentially located at the periphery of terminal aggregates. Thus, although juxtaglomerular neurons in the frog do not form the glomerular walls typical of mammals, they do appear to outline olfactory nerve terminal clusters.

In other frogs the projection from the ventral epithelium alone was labelled, after excision of the dorsal epithelium. The projection from the ventral epithelium appears to terminate more densely in the lateral half of the bulb.

Most preparations were cut in the horizontal plane with several millimeters of the olfactory nerve in the same 30-50µ section as the bulb. Examination of such sections with polarized light microscopy revealed a curious but consistent feature of the anatomy of the olfactory nerve. From the epithelium to the bulb nerve fascicles appear to be fairly straight and parallel to each other. At the junction with the bulb, many fascicles abruptly change direction and/or break into smaller fascicles. Some fascicles in the medial part of the nerve bend and project towards the lateral side of the bulb and *vice versa*. The overall impression is that fibers from all parts of the nerve intermingle considerably at the junction with the bulb.

Experiments with focal injections of WGA-HRP are in progress in an effort to delineate the spatial organization of projections to the bulb from discrete regions of the epithelium.

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Transaxonal Transport of Cadmium in the Olfactory System. L. HASTINGS and J. EVANS (Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio 45267)

Recent reports in the literature suggest that the olfactory primary sensory neurons may provide a direct route of entry for agents into the central nervous system (CNS). To investigate whether cadmium, a heavy metal which has been associated with anosmia in industrial workers, can enter the CNS via the olfactory system, rats were exposed either intranasally (unilaterally) or intraperitoneally with ^{109}Cd . The intranasal exposure was accomplished by inserting a length of PE 10 tubing into the nostril and slowly infusing the cadmium. The rats were allowed to survive 7 days, at which point they were euthanized and the kidney, liver, right and left forebrain, right and left olfactory bulb, and right and left epithelium were removed. The tissues were placed in counting tubes and the radioactivity counted in an Auto-Gamma Counting system (Packard). In rats exposed by intranasal instillation, Cd levels were significantly elevated in the kidney, liver, and ipsilateral olfactory bulb and epithelium, but not in the contralateral bulb and epithelium or forebrain areas. With the intraperitoneal exposure, cadmium levels were only elevated in the kidney and liver, the traditional target organs for cadmium exposure. These results indicate that cadmium, after intranasal intubation, is selectively sequestered in the olfactory bulbs. However the location of the cadmium within the bulbs is not known and the topic is under current investigation.

Proliferation and Cell Death Patterns During Postnatal Development in the Olfactory Bulb of Normal and Unilaterally Deprived Rats. L.L. FRAZIER (Univ. of Virginia), P.C. BRUNJES (Univ. of Virginia).

Unilateral external naris closure on postnatal Day 1 results in a dramatic decrease in the number of the olfactory bulb's postnatally born interneurons, the granule cells (*J.Comp.Neur.*, in press). The decrease may be due to changes in patterns of cell proliferation, cell death, or both. A key role for cell death is indicated by observations that granule cell number in adult rats is regulated by cell death (*J.Comp.Neur.*, 239, 177), and that unilateral deprivation has little effect on the proliferation zone of the bulb, the subependymal layer (SUB). Nevertheless, a consistent developmental lag in the number of granule to relay cells has been reported, suggesting changes in proliferation are possible. The current study assessed both proliferation and cell death patterns in normal and deprived rats. Pups were occluded or sham-operated on postnatal Day 1 and injected with ^3H -thymidine on postnatal Days 2, 5, 10, 20, or 30. Groups of subjects were sacrificed 2 hrs, 24 hrs or 30 days after injection. The first two groups were used to examine proliferation patterns within the SUB. The third group was used to identify patterns of granule cell infiltration and survivability. In each group, horizontal, 1.5 micron sections were processed via standard emulsion autoradiography. Sections through the middle of the SUB were selected and counts of labeled vs. unlabeled cells made at 25, 50, 75 and 100% of the bulb's rostral-caudal extent for the mitral and granule cell layers, and SUB. In the 2 and 24 hr survival groups, no differences in numbers or patterns of labeled cells were encountered between normal and deprived subjects indicating the procedure does not affect early cellular proliferation. However, in the 30 day survival group the percent of labeled cells was consistently less in deprived bulbs. Therefore, the dramatic decreases in the number of granule cells must be due to alterations in normal cell death processes. This hypothesis is being examined further by a qualitative study of cell death patterns using a modification of the Fink Heimer technique.

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Effects of Chloride Concentration in ACSF on Olfactory Bulb Evoked Potential. P.B. COOK (University of California Berkeley), B.K. RHOADES (University of California Berkeley), W.J. FREEMAN (University of California Berkeley).

Periglomerular cells in olfactory bulb are primarily GABA-ergic, which, through Cl^- shunt, would typically be considered inhibitory. Freeman (1974b) has shown these cells to be self excitatory while also attenuating onto the glomerular complex (Freeman, 1974a). In this study the activity of Pg cells is assessed using surface and depth electrodes measuring average evoked potentials induced through primary olfactory nerve and lateral olfactory tract volleys. Continuous application of artificial cerebral spinal fluid to the bulbar surface serves as a control for test solutions including low chloride ACSF, and GABA-ergic toxins. Results and implications towards a model of Pg action in olfactory bulb are discussed.

Freeman (1974a) Brain Research 65, 77-90.

Freeman (1974b) Brain Research 65, 91-107

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Acceleration of Reproductive Development in Female Djungarian Hamsters by Adult males. DAVID S. REASNER and ROBERT E. JOHNSTON (Department of Psychology, Cornell University, Ithaca, NY 14853).

Housing young female Djungarian hamsters with an adult male accelerates uterine and ovarian development and there is a strong relationship between uterine weight and ovarian measures (e.g., follicular size). Uterine weights of females housed with an adult male for 10 days following weaning are comparable to values from females housed alone for 25 days. Removal of endogenous androgens by castration eliminated the capacity of adult males to accelerate reproductive development in young females and treatment of castrated males with exogenous androgens maintained the production of the acceleratory chemosignal. When adult male urine and ventral gland sebum were examined as possible sources for the acceleratory chemosignal, only male urine had an acceleratory effect on reproductive development. In addition, preliminary studies indicate that central targets of the accessory olfactory system may undergo anatomical changes in conjunction with accelerated reproductive development. Thus, female Djungarian hamsters respond with accelerated reproductive development to androgen-dependent chemosignals in the urine of adult males and may offer an approach for the study of the neural basis of puberty onset.

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Olfactory Bulb Transplantation into the Olfactory Bulb of Neonatal Rats. T. ZIGOVA (Institute of Neurobiology, Slovak Academy of Science, Kosice, Czechoslovakia), P.P.C. GRAZIADEI and G.A. MONTI GRAZIADEI (Biological Sciences, Florida State University, Tallahassee).

Pattern of Reinnervation after Partial Bulbectomy. G.A. MONTI GRAZIADEI and P.P.C. GRAZIADEI (Biological Sciences, Florida State University, Tallahassee, Florida).

Severance of the olfactory axons at the intracranial level of the lamina cribrosa is followed by reinnervation of the olfactory bulb with maintenance of the topographical projections from the olfactory neuroepithelium to the olfactory bulb. In the present study we intend to find out if the olfactory axons, that have lost their target due to the partial removal of the olfactory bulb, redirect themselves to the undamaged existing glomeruli (superinnervation) or not. Neonatal and adult rats were partially bulbectomized by removing selected portions of the olfactory bulb. The animals were sacrificed after long postoperative survival times and serial histological sections prepared and stained with standard histological procedures, silver methods and processed by immunohistochemistry for OMP to visualize the regrown olfactory axons within the olfactory bulb. Consistently we have observed that the glomeruli in the undamaged portion of the olfactory bulb were reinnervated and normal in appearance. The sensory axons, deprived of the specific target (the mitral cells) randomly formed glomerular structures in every layers of the olfactory bulb and did not superinnervate the existing glomeruli. The relevance of these data will be discussed in relation to their functional implication.

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Previous experiments in rodents of partial bulbectomy, with partial or total sensory deafferentation, have shown that the olfactory bulb undergoes considerable remodeling as a consequence of its reinnervation. Given this plasticity of the olfactory bulb, we wanted to see to what extent an embryonic olfactory bulb would have integrated with the host brain after being transplanted. In the present study, after total or partial unilateral removal of the olfactory bulb in neonatal rats, the presumptive olfactory bulb from E14-E18 embryos was transplanted in place of the removed tissue. In the instances of partial bulbectomy the transplant fuses with the host olfactory bulb and no clear boundary can be observed between the two structures. The donor olfactory bulb can be recognized for the different orientation of its layers. The undamaged portion of the host bulb has maintained a normal morphology. The olfactory axons projecting to the transplant and to the damaged part of the host's bulb form randomly placed glomerular structures. When total bulbectomy is performed, the transplanted bulb acquires morphological continuity with the spared forebrain and it is innervated by the olfactory axons as described above. This study, while extending our previous observation of total and partial bulbectomy, also emphasizes the importance of concurrent events for the establishment of a normal pattern of projections. In spite of the randomly formed connections, one cannot discard a priori that a normal functional behavior can be acquired.

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#53 appears after #36

Stimulus Access to Olfactory and Vomeronasal Receptors In Utero. DAVID M. COPPOLA & ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury MA 01545)¹

Several lines of evidence have suggested that some components of the olfactory system begin to function before birth. In rats, preferential uptake of 2-deoxyglucose in the accessory olfactory system (AOS) of near-term fetuses has been used as evidence of this olfactory subsystem's functional hegemony *in utero*. Stimulus access to the vomeronasal organ (VNO), in which the receptors of the AOS are sequestered, is under the control of a sympathetically and parasympathetically innervated vascular pump. Here we describe studies to determine the functioning of the VNO pump *in utero*. Fluorescent microparticles (beads 0.6-1.15u dia) were injected into the amniotic fluid of E18 mice. On day E19, fetuses were Cesarean delivered and their heads were prepared for histology. Heads were sectioned in the coronal plane from the external nares through the entire extent of the VNO. Examination of sections under fluorescent illumination revealed beads throughout the lateral and dorsoventral extent of the nasal cavity in nearly all sections. However, beads were never found in the lumen or canal of the VNO. Importantly, connection of the VNO to the nasal cavity could not be reconstructed upon examination of stained, 10u serial sections suggesting that the VNO canal may not be patent at this age. Thus, our results fail to demonstrate stimulus access to the VNO *in utero* which may be due to the inactivity of the VNO pump or the lack of patency of the VNO canal. Detailed histological studies are currently underway to resolve this point. Nevertheless, if the rat resembles the mouse with regard to stimulus access to the VNO, the functioning of the AOS *in utero* is called into question. If not, at least the generality of AOS functioning *in utero* is in doubt.

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Effects of Intrabulbar Injections of 6-Hydroxydopamine on Ethyl Acetate Odor Detection in Castrate and Non-Castrate Male Rats. RICHARD L. DOTY, MARK FERGUSON-SEGALL, IRWIN LUCKI, MARGARET KREIDER, JUDY M. RISSER (Smell and Taste Center, Department of Psychiatry, and Department of Otorhinolaryngology and Human Communication, School of Medicine, University of Pennsylvania)*

The function of norepinephrine-containing neurons which project to the olfactory bulb is poorly understood. Although there has been suggestion that norepinephrine (NE) may modulate general olfactory sensitivity by attenuating the inhibitory feedback of granule cells upon mitral and tufted cells, behavioral indices of olfactory sensitivity have not been measured in animals with depletions of bulbar NE. The present study used computerized olfactometry and signal detection methodology to assess the odor detection performance of castrate and non-castrate male rats to a range of perithreshold concentrations of ethyl acetate following 6-hydroxydopamine depletion of bulbar NE. Such depletion had no significant influence on odor detection performance at any of the odorant concentrations examined in either castrate or non-castrate animals, as indexed by the non-parametric sensitivity measure S1. This observation implies that general olfactory sensitivity is unaltered by major depletion of intrabulbar NE, but does not preclude the possibility that NE modulates sensitivity to select odorants or odorant mixtures, or alters detection ability under atypical states of arousal.

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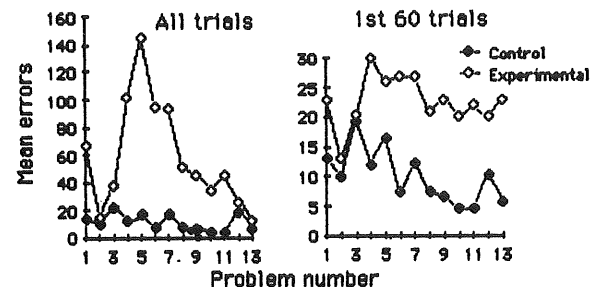
Impaired Olfactory Learning in an Animal Model of Diencephalic Amnesia. R.G. MAIR, R. KNOTH, S. RABCHENUK-PORCH (University of New Hampshire, Brockton VAMC)

In humans, diencephalic and medial temporal lobe amnesias are associated with impairments of olfactory discrimination, the neurologic basis of which is not certain. Acute pyridoxamine treatment of the rat produces a chronic animal model of diencephalic amnesia that exhibits patterns of thalamic lesions, neurochemical pathology, and behavioral impairments comparable to those associated with Korsakoff's syndrome, the most common cause of human diencephalic amnesia [cf Mair, Langlais et al *Brain Res* 360,273-284; 421,140-149 *Behav Brain Res* (In Press)]. In this experiment, we compared the performance of pyridoxamine-treated and control rats on serial reversal learning for spatial [left/right] and olfactory [α -ionone/geraniol] stimuli. Although experimental animals were able to reach criterion on both tasks (8 consecutive correct), they required significantly more trials to do so. For initial spatial learning, experimentals averaged 47.0 ± 5.8 and controls 15.8 ± 2.5 errors to criterion, and the two groups exhibited comparable positive transfer across subsequent reversals. For initial olfactory learning experimentals averaged 248.6 ± 62.8 and controls 78.8 ± 23.1 errors to criterion and experimentals were severely impaired in their performance on subsequent reversal trials. This pattern of results is comparable to observations made of humans with Korsakoff's disease [cf Mair, et al *Behav Brain Res* 9, 1-32; *Neuropsychologia* 24, 831-839]. Comparison of pathologic changes in humans with Korsakoff's disease and rats suggests several neurologic processes that might account for the consistent coincidence of olfactory and memory dysfunction.

Supported by VA Medical Research Funds.

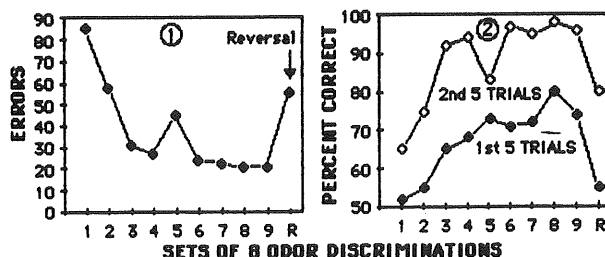
MD Lesion and Olfactory Learning. XI-CHUN LU & BURTON M. SLOTNICK (The American University)

Olfactory projections to the medial dorsal thalamic nucleus (MD) have been described in detail (e.g. Price & Slotnick, *J. Comp. Neuro.*, 1983) but their behavioral significance is unclear. To assess the effects of MD lesions on acquisition of a learning-set rats were trained on a series of 13 simple 2-odor discrimination problems and on reversal of the 13th problem. Most controls learned each problem within 60 trials and rapidly acquired a learning set. Experimental rats made significantly more errors than controls on most problems, improved more slowly over the test series, and most failed to reach criterion on one or more discriminations within 400 trials. Experimental rats also made many more errors than controls on the reversal task (265 vs 92). While these results extend those of Slotnick and Kaneko (*Science*, 1981) that rats with MD lesions perform poorly on a reversal learning-set task, the highly variable performance, including failures to solve some problems, suggest that the discrimination deficits in rats with MD lesions cannot be explained simply by a deficit in interproblem transfer.



Rats Learn to Label Lots of Odors: A Remarkable Demonstration of Learning-Set and Odor Memory in the Rat. ANGELA M. KUFERA, BURTON M. SLOTNICK, and JUDITH M. RISSER (The American University)

Six water deprived rats were tested on an operant task to discriminate among many odors generated by an olfactometer. In each of nine problems a new and unique set of 4 S+ and 4 S- odors were presented in random order. The rats were reinforced with water only for responding in the presence of S+ stimuli. In the first problem all rats initially performed at chance (50% correct responding), but in problems 6-9 all rats reached criterion of 90% correct responding (Figure 1) and most did so within 80 trials (fewer than 10 presentations of each odor). Improvement was evident even in the first 5-10 presentations of each odor (Figure 2). At the end of training, problem 6 was repeated except that the meaning of each odor was reversed. Significantly more errors were made on the reversal problem thus demonstrating retention for these odors (Figure 1). Clearly, rats are able to acquire an olfactory learning-set even when multiple stimuli are used in each task and are able to remember odors despite the ample opportunity for retrograde and anterograde interference from other odors. Preliminary results suggest that this rapid acquisition of a learning set occurs even when 16 novel odors are presented in each session. Supported in part by The National Science Foundation Grant # BNS8319872 to BMS.



Evidence for Neuromodulation of a Pheromone-Mediated Courtship Behavior in the Blue Crab, *Callinectes sapidus*. DEBRA E. WOOD*, RICHARD A. GLEESON (The Whitney Laboratory, St. Augustine, Fla.), and CHARLES D. DERBY*, *(Georgia State University, Atlanta, Ga.).

The courtship behavior of the male blue crab offers an excellent model for studying the neural control of chemosensory behaviors because it is a specific rhythmic activity elicited by a unique chemical stimulus. The courtship display behavior is produced in response to a pheromone released from pubertal females (Gleeson, 1980). This behavior is a stereotypical response which includes a wide lateral cheliped spread, a standing posture on fully extended pereopods, and most uniquely, a rostral-dorsal waving above the carapace of the most posterior pair of legs (the swimming legs) (Teytaud, 1971). Males appear to have cyclic sensitivity to the pheromone and may occasionally display spontaneously without stimulation (Gleeson and Wood, unpublished observations). The incidence of this spontaneous behavior increases following the ligation of the eyestalk (Gleeson, 1987). This suggests that the neural systems controlling display behavior may be hormonally modulated. We have bioassayed the effects of several neuroactive compounds thought to be modulators in both invertebrates and vertebrates. Each compound was tested over a range of doses in single injection and by chronic studies in which the substances were introduced using osmotic mini-pumps. Octopamine, dopamine, serotonin, and proctolin were all found to produce specific postures in a dose-dependent manner. Although some subset of the display behavior may be seen upon injection of any one of the amines, our initial results suggest that the peptide proctolin activates the most unique aspect of the behavior, leg waving.

Partially supported by the Whitehall Foundation.

Synthetic Peptide Analogs to Barnacle Settlement Pheromone. K. TEGTMEYER, D. RITTSCHOF and E. SESSIONS, DUKE MARINE LABORATORY BEAUFORT, NC 28516

Pheromones enhance the rate of settlement and metamorphosis of barnacle larvae. Analogs to the large (3000-5000 kD) heterogeneous pheromone peptides were sought. Larval settlement assays were used to assess effectiveness. Native pheromones have a lower activity threshold at approximately 10 pM. A series of dipeptides composed of acidic, neutral, and basic amino acids was tested. Neutral-basic and basic-basic dipeptides were pheromone mimics. Other likely analogs were tested. In all six di- and tripeptides with a basic carboxy-terminal amino acid and a neutral or basic amino terminal amino acid were pheromonemimetic. All other combinations of amino acids and mixtures of free amino acids comprising active peptides were ineffective. High concentrations of peptides did not enhance settlement rates. The most effective peptides were L-Ileu-Gly-Arg and L-His-Lys (threshold 0.2 nM). The molecules that mimic barnacle pheromone are similar but not identical to those that act as larval release pheromones. Peptide pheromone analogs should be useful in determining the nature and mechanisms of barnacle pheromone receptor interactions. Supported in part by NSF Grant #OCE-8603945.

A Mathematical Computer Model of the Temporal Filtering Characteristics of Chemosensory Adaptation PAUL A. MOORE and JELLE ATEMA (Boston University Marine Program)

The turbulent dispersion of odors results in a signal that is a series of fluctuating peaks and valleys. The adaptation state of chemoreceptor cells is constantly being changed by this fluctuating signal. This adaptation state of the receptor determines the "floating baseline" against which incoming signals are measured. This baseline is the response threshold of the receptor cell. Thus, chemosensory adaptation is an important part of the temporal filtering characteristics of the receptor organ. We present a mathematical computer model that calculates the adaptation state of the receptor cell based upon a weighted historical moving average. The weight is an exponential function that decays with time. The effect an incoming signal on the adaptation state of the receptor is dependent upon the amount of time since the signal was received and the absolute concentration of the signal. We predict that different rates of adaptation and disadaptation allow the receptor cell to respond best to different concentration fluctuations. Thus, slow adapting and slow disadapting chemoreceptors respond best to slow, long term changes in odor signals. While, fast adapting and fast disadapting chemoreceptors respond best to fast, high-frequency changes in signals. By having a series of receptors with varying adaptation and disadaptation rates, a chemoreceptor organ can be transformed into a frequency filter. Integration by higher neurons can then pick out and further amplify the biologically important frequencies of incoming signals.

Supported by a grant from Whitehall Foundation and NSF to JA and a Graduate Fellowship from BU to PM.

Effects of Heavy Metals and a Respiratory Poison on Chemosensory Responses in *Tetrahymena* (Cilioprotista). M. LEVANDOWSKY, DAVID AZCUE and TONY KIM (Haskins Laboratories, Pace University, N.Y., NY 10038)*

Chemosensory responses were assayed in starved cells of *Tetrahymena pyriformis* in the presence of sublethal levels of inorganic heavy metal salts and the respiratory blocker, sodium azide. Chemosensory responses were to L-methionine were assayed using a capillary method (Biol. Bull. 167, 322-30). Cells were exposed to the toxicant for 30 minutes before assays were run. Chemosensory responses were inhibited in a dose-dependent manner by salts of Hg, Pb, Ni and Cd; Inhibition was detected at concentrations at least 3 orders of magnitude below lethal levels. The lowest inhibitory levels detected were (mM): Cd .01, Ni .1, Pb .01, Hg .0001. By contrast, sublethal concentration of the respiratory poison sodium azide enhanced the chemosensory response. Cells exposed to 1 mg% (.17mM) Na azide were four to five times as responsive to L-methionine as unexposed cells. We conclude that: (a) inhibition of the chemosensory response is an extremely sensitive index of heavy metal toxicity, and (b) *Tetrahymena*, which is tolerant of reducing environments such as sewage digesters, and can tolerate low oxygen levels, has a greatly enhanced chemosensory response in the presence of sublethal levels of a respiratory blocker.

*This work was supported by a grant to ML from the Whitehall Foundation

Comparison of Pheromone Receptor Neurons on the Antennae of 3 Closely Related Noctuid Moths: Implications for the Evolution of Species Isolation Mechanisms. R. W. MANKIN and M. S. MAYER (Insect Attractants, Behavior and Basic Biology Laboratory, USDA, Gainesville, FL)

Three phylogenetically related moths in the subfamily Plusiinae, the cabbage looper, soybean looper and celery looper, have an identical major sex pheromone component, (Z)-7-dodecen-1-ol acetate (Z7-12:Ac), and their geographic distributions overlap. One hypothesis to explain how these moths are isolated reproductively is that the males of each species respond differently to minor pheromone components, some of which are shared and some of which may be unique to a given species. We have investigated receptor neurons on male antennae to determine their responses to pheromone components emitted by the conspecific and interspecific females. Cabbage looper receptor neuron types have been found that respond to low concentrations of Z7-12:Ac, (Z)-7-dodecen-1-ol (Z7-12:OH), or (Z)-7-tetradecen-1-ol acetate (Z7-14:Ac). On soybean looper antennae, Alan Grant and coworkers have found types which respond to low concentrations of Z7-12:Ac, Z7-12:OH, or (Z)-5-dodecen-1-ol acetate (Z5-12:Ac). In preliminary experiments with celery looper, we have found types that respond to low concentrations of Z7-12:Ac, Z7-12:OH, or another minor component. In each case, these neurons cross-react with other minor components at higher concentrations, and there is considerable variation in the responsiveness of each type of receptor neuron to the other minor components. Apparently, when it is reproductively advantageous, natural selection has acted on this inherent variation to increase the sensitivities and numbers of neurons responding to minor components, which results eventually in the development of new receptor neuron types.

Relative-to-ideal Ratings and their Relationship with Hedonic and Intensity Ratings. R. SHEPHERD, K. SMITH and C.A. FARLEIGH (AFRC Institute of Food Research, Norwich, UK)

Subjects are able to rate a sample relative to their own ideal of an attribute but the relationship between these ratings and those on conventional hedonic and intensity scales are not well understood. Forty five subjects tasted soup with 8 concentrations of salt (98-1278 mg Na/100g). Three rating scales were used, intensity, relative-to-ideal and hedonic, comprising 100mm lines with anchors at the extremes and in the centre. For the relative-to-ideal ratings the anchors were "Not nearly salty enough", "Just right" and "Much too salty". Each scale was used at four separate sessions. For each individual subject, regressions were calculated of the intensity ratings against log(concentration) and the slope was used in subsequent analyses. Similar regressions for the relative-to-ideal ratings yielded the slope and ideal concentration. The concentration with the highest hedonic rating was taken as the break point. The hedonic break point correlated with the ideal ($r=0.76$, $df=42$, $p<0.001$). The results were similar when the break point was calculated by fitting a quadratic equation or using a curve smoothing procedure. The slopes of the intensity and relative-to-ideal functions correlated at $r=0.31$ ($df=43$, $p<0.05$), although the overall mean responses on the two scales were highly correlated ($r=0.998$, $df=6$, $p<0.001$). Unfolding the hedonic ratings about each individual's break point gave a close approximation to the relative-to-ideal ratings but not so when the group data were used. Discrimination between concentrations was lower for the hedonic than for the relative-to-ideal and intensity scales. There was good agreement between the ideal and hedonic break point, but not between the slopes of relative-to-ideal and intensity functions. This is probably because the relative-to-ideal ratings include an element of how concerned the individual is about deviations from ideal. The simplicity of the linear relationship and lower variability in the relative-to-ideal ratings make this scale useful for obtaining estimates of maximally preferred concentration from individual subjects.

Simple Responses to Complex Mixtures: Implications for Gustatory Coding in the Colorado Potato Beetle. B. K. MITCHELL (Department of Entomology, University of Alberta, Edmonton, Alberta, Canada. T6G 2E3).

A field of gustatory sensilla on the galea (one of the mouthparts) of adult Colorado Potato Beetles, provides information on potential host-plants as the beetle bites into a leaf. These sensilla have four chemosensory cells, but when fresh saps from host plants in the genus *Solanum* are used as stimuli, only one of these cells responds vigorously, and with little variance within a single sensillar field or upon multiple stimulations of a single sensillum. This is in contrast to the multi-cellular and variable responses obtained when leaf saps from non-host plants are used as stimuli. This unusually simple response to a very complex natural stimulus provides a gustatory preparation which can be used to study the effects of mixtures at the level of a single peripheral chemosensitive cell. Since responses to saps from host and non-host plants are so different, it is possible that the essentially unicellular response to host-saps is acting as a labelled line to signal acceptability, while the multicellular and variable responses to non-host saps represent a variety of nonsense inputs which signal unacceptability. Data are presented on the nature of the simple response to host saps which document its exceptional reproducibility, its low variability within and between sensilla, its slow adaptation under continuous stimulation and the effectiveness of even five fold dilutions of plant saps. These unusual characteristics suggest that it will be possible to use an electrophysiological bioassay to monitor fractions of potato leaf sap in an attempt to identify the factor, or more likely factors, involved in adequately stimulating this cell.

Sequential Sensitivity Analysis for Sensory Difference Testing: Tasting Salt and Water Stimuli. M. O'MAHONY (Dept. Food Science and Technology, University of California, Davis, CA 95616).

Sequential Sensitivity Analysis is a system for predicting the ability of sensory difference tests to discriminate between two food stimuli. It considers the possible sequences in which stimuli can be tasted during a given test and makes predictions for the signal strengths of each of these stimuli in the sequences. These signal strengths can be measured independently by signal detection procedures, and their values applied to any sensory difference testing protocol for a given food system. For a low concentration NaCl and water system, these signal strengths depend on the effects of adaptation, salivary interference, signal learning, and differential supra- and subadapting taste sensitivity. A difference testing sequence in which the signal strengths of the stimuli are high will show more discrimination than one in which they are low. The signal strengths of all stimuli in the various possible orders of tasting for a given difference testing protocol can be examined to predict the test's efficacy. Sequential sensitivity analysis has been applied to make successful predictions for food and model systems for the discrimination performance of triangle, paired comparison, duo-trio and R-index tests. It has also been used to develop more sensitive testing protocols.

Thurs. Eve Talk 7:30 PM Abst. * 66

Estimating JNDs from Ratings. D.A. BOOTH (University of Birmingham, England)

Quantitative judgments such as ratings of intensity or preference can readily be obtained under test conditions in which the individual's objective psychophysical scaling performance is not distorted by effects from stimulus or response context, e.g. for tastants in real foods (Appetite 4:301,1983; LWT 19:486,1986). The ratings are then proportional to the stimulus difference for a signal detection $d' = 1$, i.e. the just noticeable difference (JND), taken in Weber's ratio to the stimulus level at each level in the tested range. For moderate levels of many tastants, for example, concentrations produce a virtually constant Weber ratio. In such cases, ratings plot in a straight line against log concentrations. We have also found thus far that the variances among ratings of a particular stimulus level are constant over the tested range. An equal-variance (Thurstone Case V) signal-detection model can be applied to such data, to yield an estimate of the individual's Weber ratio in that stimulus range, i.e. their JND for any of those stimulus levels (J Food Sci,1988; J Appl Psychol in press). Thus, there is no ineradicable difference between discrimination experiments and rating experiments. Also, we can bypass the laborious traditional technique of "indirect scaling", i.e. measuring JNDs in discrimination designs at different stimulus levels and then cumulating JNDs over the range of interest. Finally, if there prove to be any differences between individually bias-minimised descriptor intensity judgments and supposed scales of sensation magnitudes (whether from functional measurement or still weaker models for group experiments), contextual distortions of some individuals' ratings are likely to have occurred.

Thurs. Eve Talk 8:00 PM Abst. * 67

Categorization of Ambiguous Odors in Restricted and Unrestricted Classification Tasks with Multidimensional Scaling Analysis. HARRY T. LAWLESS (S.C. Johnson & Son, Inc.)

Two groups of subjects categorized a set of 18 odorants on the basis of perceived similarity. Six odors were of a primarily woody character, six of a citrus character, and six were of a mixed citrus-woody type, e.g. a pine/ lime odor such as dihydromyrcenol. One group of subjects was allowed to form as many categories as they wished, while the second group was required to divide the set into only two categories. The number of times odorants were placed in the same category was used as an estimate of perceived similarity, and the data subjected to nonmetric multidimensional scaling (MDS) via ALSCAL. Odors were also rated on attribute scales for woody and citrus character and as to whether they smelled like a single odor or a mixture. These ratings confirmed the a priori classification of the stimuli, including the mixed quality of the six ambiguous (citrus/woody) odorants. The group restricted to two categories produced two clusters in the resulting MDS configuration, one woody and one citrus with the ambiguous odors on the edge of each cluster. In contrast, the group allowed an unlimited number of categories produced four clusters, with lime and pine/line odorants falling between more homogeneous citrus and woody groups. These results indicate that 1) some ambiguous odors may be weak exemplars of odor classes, 2) the nature of the classification task can affect the type of model which is constructed by MDS, 3) single chemicals can behave, perceptually, like odor mixtures, and 4) measures of inter-observer agreement in classification tasks offer a potential shortcut over exhaustive paired comparisons as estimates of similarity for MDS input. These procedures are recommended for further exploration of concept formation and qualitative categorization in the chemical senses.

Thurs. Eve Talk 8:15 PM Abst. * 68

A Multidimensional Theory of Similarity. DANIEL M. ENNIS (Philip Morris Research Center, Commerce Road, Richmond, VA 23261).

A multidimensional theory of similarity is described in which the mental representations of stimulus objects (like odorants and tastants) are assumed to be drawn from multivariate normal distributions. A distance-based judgment function is defined and a measure of similarity is derived. In vision and hearing, this theory is the basis for explaining apparent differences between confusable and discriminable stimuli with regard to the form of the distance metric and the nature of the judgment function. The theory of similarity presented is used in a Thurstonian extension of Shepard's model of identification performance. A stochastic approach to multidimensional scaling based on confusion matrices is demonstrated using an artificial data set.

Differential Effect of Odorant Maskers on Odorant Identification. H.N. Wright, Ph.D. (Otolaryngology), Beth M. Cady, M.D., (Otolaryngology), Paul R. Sheehee, Sc.D. (Preventive Medicine) (Clinical Olfactory Research Center, State University of New York Health Science Center at Syracuse)*

A closed set 10 x 10 odorant confusion matrix was administered to two observers in 4 background levels (.03, .12, .48, and 1.92M) of 3 competing odorants (benzaldehyde, butanol, and octane). Comparison of the confusion matrix results among the competing odorants to the matrix without a competing masker showed decreased odorant identification as a function of increased concentration for benzaldehyde, and no pronounced change for either butanol or octane. Such results are interpreted to illustrate that an odorant may not only elevate the detection of another, but also alter its identification. Further, this effect is odorant and concentration dependent.

*Supported by PHS Program Project Grant NS19658 from the National Institute of Neurological and Communicative Disorders and Stroke.

A Method for Establishing a Five Odorant Identification Confusion Matrix in Rats. S.L. YOUNGENTOB, M.M. MOZELL and D.E. HORNUNG (Clinical Olfactory Research Center, SUNY Health Science Center at Syracuse, NY).

The diagnosis of olfactory dysfunction is now hampered by the fact that when a patient presents with an olfactory complaint, it may be attributed to a number of causes. One approach to relate various distortions in olfactory perception to particular underlying mechanisms is to develop an animal model in which specific lesions or functional compromises can be selectively created and evaluated with respect to patient performance on identical tasks. Therefore, we have developed the training and testing procedures for an animal analogue to the Olfactory Confusion Matrix (OCM) administered in our clinic. Using a cross-modal association paradigm, rats were trained to associate a particular tunnel and response location with one of five different odorants (amyl acetate, propyl acetate, acetic acid, phenethyl alcohol, and anethole). Each of the five tunnels differed with respect to: 1.) the illuminated pattern on the response key; 2.) the brightness of the illuminated pattern; and 3.) the somesthetic quality of the tunnel floor. Standard operant techniques were used to shape trial initiating and sampling behavior at a central odorant presentation point. Following acquisition training (>90% on ten consecutive blocks of 20 trials), the animals were tested using a standard 5 x 5 confusion matrix design. The resulting data were recorded in a matrix such that the rows represented the odorant presented and the columns represented the same odorants as alternative response choices. The entries in the cells of the matrix were the frequency with which the presentation of a particular odorant was responded to with each response alternative. Since the mathematical properties of confusion matrices are such that they may be analyzed using multidimensional scaling techniques, these data represent the first instance in which the perceptual odor space of an animal can be determined.

Supported by NIH Grant # NS19658

Selected Results of the National Geographic Smell Survey CHARLES J. WYSOCKI AND AVERY N. GILBERT (Monell Chemical Senses Center, Philadelphia, PA)

We designed a scratch-and-sniff survey of the sense of smell that was sent to the 10.5 million members of the National Geographic Society in the September 1986 issue of the Magazine. Participants supplied demographic data as well as responses to microencapsulated samples of androstenone, amyl acetate, Galaxolide™, eugenol, mercaptans, and synthetic rose. Nearly 1.5 million surveys were returned--the resulting 160 megabytes of information represents the largest human olfactory data set yet compiled. Effects of gender, age, smoking and fragrance use on olfactory perception will be presented. Attention will be given to disparities between self-perceived olfactory ability and measures of performance. Selective anosmia to androstenone also will be addressed.

Psychophysical and Physiological Responses of Humans to Odorant Stimulation of Nose and Eyes. JAMES C. WALKER & DANIEL B. KURTZ (BGTC; R. J. Reynolds Tobacco Co.; Winston-Salem, NC 27102)

A range of concentrations of each of four compounds (acetic acid, n-amyl acetate, nicotine, propionic acid) was presented to the nose and/or eyes of sixteen 16-24 year old males who had no suspected loss of nasal or ocular chemosensory function. During each odorant presentation, changes in breathing and in eye blink rate were used as reflexive measures of nasal and ocular stimulation, respectively. Following each trial the subjects reported the Odor Strength (OS), Nasal Irritation (NI), Eye Irritation (EI), Eye Feel (EF) and Overall Acceptability (OA) of the stimulus. Odor thresholds ranged from about .5 ug/l (.07 ppm) for nicotine to about 5 ug/l (1.85 ppm) for n-amyl acetate. With all four odorants, both OS and NI increased monotonically as concentration was increased. At each concentration, the degree of OS was greater than the NI, although any concentration for which the OS was above zero also caused some NI. Across all four compounds there was a strong inverse relationship between OS and OA; however only slight increases in NI were related to drastic decreases in OA. Although very little EI was reported throughout the concentration range of each compound and little eye blinking was observed, respiratory behavior was different on "eye + nose" and "nose only" trials. Psychophysical responses throughout the concentration range of each odorant could be predicted based on the concentration presented and several parameters of respiratory behavior.

A Psychophysiological Study of Three Odorants
STEPHEN WARRENBURG (Int'l Flavors & Fragrances),
GARY SCHWARTZ (Yale University)

This was an exploratory study of three odorants: apple spice, neroli, and galbanum. Counterbalanced presentations of these odorants, and a non-odored control were made. Interspersed with these trials were resting baselines, and trials designed either to arouse (mental math) or relax (relaxation) subjects. Each trial was 1 minute in duration, and was followed by subjective ratings, including hedonics and intensity. Twenty-eight college-age subjects participated. Psychophysiological measures included electromyogram (EMG) from the corrugator and zygomatic muscle regions of the face, palmer skin resistance (SR), and finger skin temperature (ST). Hedonic ratings indicated, as expected, that: apple spice was pleasant, galbanum was unpleasant, neroli was split with half of the subjects liking and half disliking the odor, and the solvent was neutral. All 3 odorants were rated equally for intensity. EMGs were lowest during the solvent condition, with no differences between the 3 odor conditions. There were no significant condition effects for SR or ST. These results indicate that the EMG, SR and ST measures failed to discriminate among the 3 odorants, despite their distinctly different hedonics. Since subjects were divided concerning neroli hedonics, they were split into a "like-neroli" (LN) group and a "dislike-neroli" (DN) group. LN subjects had higher SR than DN subjects across all odor and non-odor trials. The LN group also had lower ST than the DN group during some trials. Within the LN group a higher hedonic rating for a particular odor was associated with lowered ST ($r = -.31$), but these variables were uncorrelated in the DN group ($r = .05$). These results suggest that the LN group was more relaxed and attentive to external stimuli during the experiment. Elevated SR indicates more relaxation and lowered ST is a sign of externally oriented attention. Further supportive of this interpretation are the self-ratings of the groups. The LN group was less "bored" during all 4 odor conditions. It appears that subjects who found neroli pleasant were predisposed to be generally more relaxed and attentive, as well as more interested in novel odors.

Airflow Characteristics in a Large Scale Model of the Human Nose. P.W. SCHERER*, M.M. MOZELL (Clinical Olfactory Research Center, SUNY Health Science Center at Syracuse, NY; Monell Chemical Senses Center, Phila., PA), and I. HAHN* (*Department of Bioengineering, Univ. of Pa., Phila., PA).

A model, scaled up 20-fold from coronal CT scans of the right nasal cavity of the human nose, was constructed from styrofoam panels and plaster. The airway in each slice of the CT scan was represented in a single styrofoam panel by a cutout of its enlarged form. The panels were then glued and clamped in order, and their luminal surfaces were smoothed with plaster. This yielded a physical model with a volume 8000 times that of the life-size human nose. Provision was made in the model for removable sections and holes were drilled through the sides to access all airway regions. A variable speed DC fan was attached with a cowl at the "pharyngeal" end to produce controlled "inspiratory" airflows. With this large model a variety of air monitoring devices can, unlike with life-size models, be inserted, permitting direct measurement of airflows in different regions under different "sniffing" strategies. From the scaling laws of fluid mechanics this large size allows much slower (1/20 of real) air velocities and much longer (400x real) "sniff" durations than occur in the human nose. Three airflow rates through the model were studied: a low flow rate typical of normal resting inspiration and two higher flow rates typical of medium and vigorous sniffs. A hot-wire anemometer (Kurz Inst.) was used to measure local air velocity (cm/s) in different regions of the airway by insertion into the various drilled reference holes. It was found that at all three flow rates, airflow was spread rather uniformly throughout the nasal cavity with similar air velocities in all three meatuses. Air velocities in the olfactory region were surprisingly high, being similar to those in the middle meatus.

Supported by NIH Grant # NS19658.

Preliminary freeze-fracture observations on rapidly-frozen taste bud microvilli of rat gustatory papillae rotary-replicated with tantalum/tungsten. BERT PH. M. MENDO, ALBERT I. FARBMAN (Department of Neurobiology and Physiology, O. T. Hogan Hall, Northwestern University, Evanston, IL 60208), and GORAN HELLEKANT (Department of Veterinary Sciences, University of Wisconsin, Madison, WI 53706)

Rat taste papillae were dissected from the tongues and their apical parts were cut away. This was done in order to increase the chance that the taste buds would be in the plane of optimal freezing, which is about 15 μ m from the surface. The remainders of the unfixed papillae were rapidly frozen with a Gentleman Jim bounce-free liquid nitrogen/copper-block impact-freezer. The specimens were then fractured at -140°C and highly reproducibly replicated with tantalum/tungsten (Ta/W) at angles of 20° in 4 to 6 seconds using a newly designed Ta/W electron beam gun. The replicas were reinforced with carbon obliquely evaporated from above. During both evaporations the sample stage rotated at 300 to 400 rpm. The Ta/W replicas were, on average, about 0.2 nm to 0.3 nm thick, which is much thinner than the 2 nm to 3 nm obtained with conventional platinum/carbon (Pt/C) electron beam gun evaporation. Hence, with Ta/W evaporation fracture plane structures are considerably more accurately replicated than with Pt/C; more substructure can be discerned in the replicated structures, especially at high electron microscope magnifications (Menco et al., 1988 and references therein). In our preliminary studies we obtained fractures through pore regions of taste buds of several vallate papillae. Two types of taste bud microvilli could be discerned within the pores. Both types have membrane-associated particles with distinct substructures. Some of these may contain transmembranous channels. However, in one type of microvillus the majority of the particles are more shallow than in the other one. To determine to which taste bud cell either type of microvillus belongs is a matter for further investigations.

Literature: Menco, B. Ph. M., Minner, E. W., and Farbman, A. I. (1988) J. Electron Microsc. Techn.: Submitted.

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The effect of cyclic AMP on neurite outgrowth in explant cultures of developing chick olfactory epithelium. ALBERT I. FARBMAN, RANDALL R. JOHNSON AND FEDERICO GONZALES, (Northwestern University, Evanston, IL 60208)

The effect of cyclic AMP (cAMP) analogs and phosphodiesterase (PDE) inhibitors on neurite outgrowth was studied in explant cultures of olfactory neurons. Nasal pits from 5 or 6 day chick embryos were minced, explanted into culture dishes, and grown in a serum-free medium. One of the cyclic AMP analogs, N⁶,2'-O-dibutyrylguanosine 3',5'-cyclic mono-phosphate (dbcAMP) or 8-bromoadenosine 3',5'-cyclic mono-phosphate (8-Br-cAMP), or one of the PDE inhibitors, theophylline or isobutylmethylxanthine (IBMX), was added to the culture medium in concentrations of 10⁻⁵ to 10⁻³M. The explants were examined for neuritic outgrowth after 2 days in vitro. Addition of dibutyryl cAMP at a concentration of 10⁻⁴ and 10⁻³M resulted in a 25-27% increase in the number of explants expressing neurites, while 8-Br-cAMP had a small but non-significant effect at the same concentrations. Treatment of the explants with similar concentrations of N⁶,2'-O-dibutyrylguanosine 3',5'-cyclic mono-phosphate (dbcGMP) resulted in no increase in neuritic outgrowth, thus demonstrating that the effect of enhancing neuritic growth is specific to cAMP and not cyclic nucleotides in general. The resulting increase in neurite outgrowth is likely due to the cyclic nucleotide component of dbcAMP, since both IBMX and theophylline, which act to elevate intracellular cAMP, also increased neurite outgrowth significantly at 10⁻³M. The requirement for cAMP in the developing neurite may be related to the phosphorylation of proteins associated with extension of neurites during development and regeneration of axons.

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L-Amino Acid Stimulated Adenylate Cyclase in Catfish Barbel Epithelium: Specificity and Characterization. D.L. KALINOSKI*, V.J. LA MORTE*, L.C. JOHNSON* and J.G. BRAND* (*Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104; and *Veterans Administration Medical Center and U. of PA, Philadelphia, PA 19104).

Biochemical assays have demonstrated that fractions from barbel (taste) epithelium have low basal levels of adenylate cyclase (AC) activity (1.7 pmol/mg protein/min) even when measured in the presence of the phosphodiesterase inhibitor IBMX (2 mM). Basal activity was stimulated 3-fold by GTP, approximately 8-fold by Gpp(NH)p and forskolin and 25-fold by GTP γ S. Sodium fluoride stimulated cyclase in a dose-dependent manner with 20 mM NaF yielding a 10-fold increase in catalytic activity. AC activity of barbel epithelium was affected by calcium ion concentration. Addition of 0.63 mM CaCl₂ to the assay medium suppressed forskolin, guanine nucleotide, and L-alanine stimulation of AC essentially to zero. Addition of EGTA to the assay medium increased basal and stimulated catalytic activity. Titration of Ca²⁺ with EGTA indicated that calcium concentrations in a physiologically relevant range could regulate AC activity. The taste stimulus L-alanine enhanced AC activity in a dose-dependent manner. Stimulation of AC by L-alanine was more pronounced in the presence of Gpp(NH)p suggesting a guanine nucleotide dependence for AC stimulation by amino acid taste stimuli. Time course studies in the absence of IBMX indicated that L-alanine stimulation of cyclase activity could be measured at time points as early as 20 secs. Maximal accumulation of cyclic AMP in the absence of IBMX was measured at 2 mins, followed by a rapid return to basal levels. When IBMX was present, cyclic AMP levels plateaued by 3 mins with only a minor further increase in stimulated cyclic AMP accumulation seen at 15 mins. The taste stimulus L-arginine which has been demonstrated to open cation channels in epithelial membranes (Teeter & Brand, Soc. Neurosci. Abstr. 13, p. 361, 1987), had no effect on AC activity even in the presence of guanine nucleotides. These data support a role for AC in the transduction pathway of the gustatory system and suggest that different classes of taste receptors act to regulate that pathway.

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Structure-Function Correlations of Isolated Mudpuppy Taste Bud Cells. JANET B. MEEHL¹ (Univ. of Colorado, Boulder), SUE C. KINNAMON², STEPHEN D. ROPER² (Colorado State Univ.), JOHN C. KINNAMON¹.

The correlation of structure with function in taste cells is a goal that has heretofore never been achieved at the cellular level. Previous studies have shown that isolated mudpuppy taste cells are electrically excitable and possess voltage-dependent Na⁺, Ca²⁺ and K⁺ conductances, but the magnitudes of the different ionic currents vary greatly in the different isolated taste cells. To determine if the variability in membrane properties can be correlated with taste cell type, we are performing studies in which isolated taste cells are characterized using the giga-seal whole-cell recording technique and then subsequently analyzed ultrastructurally. Cells were isolated from pieces of lingual epithelium using previously described procedures. Cells from which recordings were made were subsequently relocated using finder grids. Identified cells were then immediately fixed with 2% glutaraldehyde followed by secondary fixation with osmium tetroxide. After ethanolic dehydration, the cells were embedded in LX-112, sectioned serially at 1/4 μ m, stained with uranium and lead, and then examined with the high voltage electron microscope. Based upon the presence of granular packets in the apical cytoplasm and the electron density of the cytoplasm and nucleoplasm, we can distinguish between light and dark cells after isolation. The membrane of light cells appears more fragile, however, and to date we have only correlated structure and function in dark cells. Isolated dark cells possessed inward Na⁺ and Ca²⁺ currents and outward K⁺ currents. We are currently experimenting with the taste cell isolation procedure to increase the viability of light cells. In future experiments we plan to examine the differential sensitivity of taste bud cells to applied taste stimuli.

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Phosphatidylinositol-4,5-Bisphosphate (PIP2-PDE) Activity of Catfish Taste Tissue. TAUFIQUL HUQUE* and JOSEPH G. BRAND*, (*Monell Chemical Senses Center, 3500 Market Street, and *Veterans Administration Medical Center, University of Pennsylvania, Philadelphia, PA 19104).

Broken cell preparations of catfish barbel (taste) epithelium were shown to possess basal PIP2-PDE activity (E.C. 3.1.4.11). In assays using exogenous radiolabeled phosphatidylinositol-4,5-bisphosphate (PIP2) as the substrate, the principal labeled product formed was inositol triphosphate (IP3), which was therefore used as the index of enzyme activity. The activity and calcium-dependency of the enzyme was tested under various conditions at levels of free Ca²⁺ spanning the range 14 nM-12 μ M. When assayed in Ca²⁺/EGTA buffers with PIP2 alone as the substrate, the enzyme readily degraded its substrate in a Ca-dependent manner. However, when assayed under ionic conditions more nearly resembling conditions *in vivo* (2 mM MgCl₂, 100 mM KCl), IP3 production was inhibited at all levels of Ca²⁺, suggesting that the enzyme is normally inactive *in vivo*. Similar lack of activity was observed when PIP2 was presented as a component of a lipid mixture resembling the plasma membrane bilayer. Calcium-dependent activity could, however, be induced by mixing PIP2 with a ten-fold molar excess of dipalmitoyl phosphatidylethanolamine (PE), a technique which converts the substrate mix into a non-bilayer configuration. An even greater level of activity, peaking at 0.5 μ M Ca²⁺, was observed when PIP2 was mixed with excess plasmalogen-rich PE. However, no enhanced calcium-dependent activity was observed using a soybean-derived PE fraction. These observations suggest that conditions which lead to a transient change in the configuration of the lipid bilayer - e.g., when a taste stimulus interacts with its membrane receptor - will also lead to PIP2 breakdown, with the resultant generation of intracellular second messengers such as IP3. Rapid (10 sec) production of IP3 was demonstrated in response both to the taste stimulus L-alanine and also the G-protein activator NaF. The rapidity of the reaction is consistent with the hypothesis that IP3 may act as an intracellular second messenger in taste tissue.

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Ultrastructure of Rabbit Foliate Taste Buds. SUZANNE M. ROYER and JOHN C. KINNAMON. (University of Colorado, Boulder, CO 80309).

As part of an ongoing comparative study of mammalian taste buds, we have examined the foliate taste buds of the rabbit using a combination of serial section analysis and computer-assisted three-dimensional reconstruction. Among our goals were the classification of cell types within the taste buds, the identification of the gustatory receptor cells, and the characterization of synaptic contacts between the receptor cells and intraganglionic nerve fibers. Although our findings regarding the general architecture of rabbit foliate taste buds are similar to previous reports, we have made additional observations regarding the details of taste cell ultrastructure and the relationship between taste cells and nerve fibers. Our results indicate that the classification of rabbit foliate taste cells into dark (Type I), light (Type II), and Type III cells is an oversimplification, since we have found a number of subtypes of cells which may be distinguished on the basis of their cytoplasmic and nuclear characteristics. Some of these are: 1) a small number of light cells located at the periphery of the taste bud and having very electron-lucent cytoplasm and large, round nuclei; 2) cells with very densely-staining cytoplasm containing a complex system of electron-lucent elements of the endoplasmic reticulum; 3) cells having cytoplasm of intermediate electron-density containing closely packed lamellae and whorls of granular endoplasmic reticulum in their apical portions; and 4) dark cells having nuclei most often located toward the periphery of the taste bud and sending narrow processes between and around cells of other types. The latter may represent a class of supporting cells. We have also observed the presence of dividing cells within taste buds. In addition, we have contributed to the understanding of the Type III cells by serially sectioning and reconstructing a number of such cells and their regions of synaptic contact with nerve fibers. We have not yet identified afferent synapses from taste cells of any other type onto nerve fibers. Compared with our previous studies in the mouse, these results demonstrate a significant difference between the rabbit and mouse in the patterns of synaptic connections within their taste buds.

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Histological Changes of the Mouse Taste Bud Following Single Dose Radiation. BARBARA A. ESSES, M.D., BRUCE W. JAFEK, M.D., GEOFFREY S. IBBOTT, PAMELA M. ELLER, University of Colorado Health Sciences Center and Rocky Mountain Taste and Smell Center

Taste dysfunction (dysgeusia) following a routine course of radiation therapy to the head and neck is well established. The histological changes which occur within the taste buds and/or the perigemmal structures in response to radiation treatment, however, are not well documented. In this study CH57BL6/J mice from Jackson Laboratories, each approximately 20-25 grams in size, underwent single dose radiation exposure to the head and neck region. Three dose groups were used in order to establish isodose curves and to determine maximal effects upon individual taste buds and perigemmal components. Our previous work had already determined the effects at 1500 cGy of radiation (AChemS 1986). This study utilized 1200 cGy and 1800 cGy. In each single dose group mice were sacrificed at 2,4,7,9 and 12 day intervals following radiation treatment. Circumvallate papillae were harvested at the time of sacrifice. Each specimen was studied histologically and evaluated for taste bud number, taste bud size, and cells per bud. Perigemmal structures were also evaluated. We have previously reported a decrease in bud number, size and cellularity as a function of time and dosage. The isodose curves and histological results of the three dose groups will be reported.

Human Taste Pore Quantification with Videomicroscopy. INGLIS J. MILLER, JR. and FRANK REEDY, JR. (Bowman Gray Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC)

We have developed a method for counting taste pores in human fungiform papillae with topological examination of the tongue using videomicroscopy. We plan to apply the method to fungiform papillae in living human subjects so that a correlation can be made between taste perception and taste bud quantification. On human cadaver tongues, a map of papillae is made by applying a mixture of methylene blue dye and red food coloring to stain the pores. Each taste pore appears as a dot on the surface of the papilla. One sample with a .73 cm² surface area contained 29 fungiform papillae. Ten of them (34%) had no visible pores, and 19 papillae had from 1 - 14 taste pores. The average number of taste pores/gustatory papilla was 6.7 ± 3.6 (s.d.). The pores have not been confirmed yet by light microscopy as representing taste buds, but these results are consistent with observations from sectioned material of a contralateral specimen of the same tongue. Taste pores are not evenly distributed but clustered into groups. Some have a linear distribution, while others are grouped in two-dimensional arrays. The papillae with taste pores are found together with the higher densities on the tongue tip. Mapping taste pores in living human subject requires a two-stage process. The first session involves mapping fungiform papillae within a region at magnifications of about 50 times for identification. The second step uses magnifications up to 300x of individual papillae to resolve taste pores. This method would be useful for the study of normal taste perception and also for determining whether patients with lowered taste sensitivity have fewer taste buds than normal subjects.

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Functional Recovery of the Gustatory System Following Sodium Deprivation During Development: Oral vs. Postingestional Consequences. P.R. PRZEKOP, JR., D.G. MOOK & D.L. HILL (Dept. Psychology, Univ. of Virginia)

The development of taste function in the rat involves a dramatic increase in sodium responsivity. This increase results from the addition of amiloride-sensitive membrane components to taste receptors. Normal development of sodium-transduction elements can be attenuated if rats are deprived of dietary sodium during a prenatal "sensitive period": the taste system will remain immature provided sodium deprivation is maintained. However, mature responses emerge if dietary sodium is restored. The present study examines the effect on the deprived taste system of various ingested amounts of NaCl. Rats fed a 0.03% NaCl diet from 3 days gestation to adulthood ingested 6, 10, 20, or 30 ml. of isotonic NaCl solution and then returned to their deprived regimen. Thirty days after the single exposure to the NaCl solution, multifiber chorda tympani responses to concentration series (0.05-0.5M) of NaCl, NH₄Cl and KCl were recorded and compared to responses from both normal and continuously-deprived rats. Results demonstrated that 30 ml. of NaCl was sufficient to restore mature sodium responsivity, while 6 ml. had no effect upon deprived sodium responses. The responses to the ingestion of 10 and 20 ml. were intermediate between normal and deprived responses. This implies that recovery depends upon the amount of sodium ingested and that functional recovery is not "all or none". To examine whether recovery relates to contact of sodium with taste receptors or to postingestional consequences, deprived rats were injected (i.p.) with 1 mg furosemide 1/2 hour before and after the ingestion of 30 ml. of NaCl. Furosemide induces natriuresis and inhibits the compartmentalization of sodium. Recordings obtained 30 days after the NaCl exposure revealed that chorda tympani responses in furosemide-treated rats were similar to rats never exposed to a NaCl solution. Therefore, recovery appears to depend upon the incorporation of sodium into the body and not upon direct contact of receptors by NaCl.

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Early Postnatal Development of Laryngeal and Palatal Taste Buds in the Hamster. TERI L. BELECKY and DAVID V. SMITH (University of Cincinnati College of Medicine)

Taste buds are found on the epiglottis and soft palate at birth in humans (Lalond & Eglitis, 1961). Neonatal cats and sheep also have epiglottal taste buds (Stedman, Mistretta & Bradley, 1983; Bradley, Cheal & Kim, 1980). The development of taste buds in these regions has not been quantified for animals with short gestational periods, except in the rat (Settembrini, 1987), which shows postnatal development of nasopalatine taste buds. In the rat and hamster, taste buds in the vallate and foliate papillae also develop postnatally (Hosley & Oakley, 1987; Smith & Miller, 1986). The present study quantifies the early development of taste buds in the epiglottis, aryepiglottal folds, nasopharynx, soft palate and nasopalatine ducts in the hamster. Tissue was taken at postnatal ages of 1-5, 7, and 10 days, embedded in paraffin, and sectioned at 8 µm. Sections were stained with a modified Heidenhain's iron-hematoxylin and with eosin. Individual taste buds were located on successive serial sections and mapped to ensure that each bud was counted only once. The presence of a distinct taste pore was also noted. At birth, taste buds are present on the soft palate in near adult numbers (cf., Miller & Smith, 1984), only half of which have a distinct taste pore. By day 10, about 3/4 of the palatal taste buds have pores. A few immature taste buds are seen on the aryepiglottal folds at birth. There are no taste buds or pores in any of the other regions until day 2 or 3. Epiglottal taste buds show a steady growth over the first 10 days of life, with the number of taste buds reaching about 1/2 the adult number by the 10th day, although about 1/2 of these are immature (i.e., without pores).

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Fri. Morn Post. Sess. A Abst. # 85 Post. A11

A Factorial Analysis of Taste Mixtures in the Hamster Chorda Tympani. ETSURO OBATA, ROBERT A. FRANK and DAVID V. SMITH (University of Cincinnati College of Medicine)

Sodium cyclamate (50 mM) generalized only to NaCl and quinine in a taste aversion experiment in gerbils (Jakinovich, 1981), although it tastes sweet to humans (Moskowitz, 1970). Hamsters do not prefer 15.1 mM Na-cyclamate to water in a two-bottle preference test (Hellekant & Roberts, 1983). These data suggest that Na-cyclamate is not a sweet stimulus for these rodents. The present experiment examined the chorda tympani (CT) response to three concentrations each of NaCl (0.5, 2 and 10 mM) and Na-cyclamate (1, 5 and 30 mM), factorially combined in all possible pairs. This resulted in 9 binary mixtures of the two and 6 self-mixtures of each. These self-mixtures and the individual components produced concentration series (0.5 - 20 mM NaCl, 1 - 60 mM Na-cyclamate) against which to compare the binary mixtures. Integrated CT responses were quantified by comparing each response to a 10 mM NaCl control delivered before and after each mixture. By comparing the mixture responses to those evoked by the two concentration series it could be seen that these stimuli produced a homogeneous mixture, i.e., they behaved as if they were the same stimulus. Mixtures of NaCl and Na-cyclamate produced responses of the same magnitude as those produced by the self-mixtures of these stimuli. Since Na-cyclamate is sweet to humans at these concentrations, this suggests that it might not be sweet for hamsters. In a further experiment, CT responses were recorded to three concentrations (3, 10 and 30 mM) of each substance, factorially combined in a cross-adaptation design. The cross-adaptation results suggested that Na-cyclamate behaved like a slightly weaker NaCl, i.e., that the Na⁺ ion was the effective stimulus for both substances. Supported by NINCDS Grant NS23524-02 to D.V.S.

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Processing Mixtures of Taste Stimuli by Specialist and Generalist Chorda Tympani Afferents in Hamsters. MARION E. FRANK (University of Connecticut Health Center, Farmington, CT 06032).

Mixture interactions are seen in responses of the nerve that innervates taste buds of fungiform papillae in mammals (Hyman and Frank, J. Gen. Physiol. 76, 1980). They are not reactions within chemical solutions but are the consequence of membrane properties of individual receptive cells and/or interactions within/between taste buds (Miller, J. Gen. Physiol. 57, 1971). The effect of mixture interactions on information processing was studied by applying binary mixtures of stimuli with different tastes to the anterior tongue of anesthetized adult golden hamsters. Responses from single chorda tympani fibers, including ten "specialists" that were activated by sucrose or sodium chloride and six "generalists" that were activated by hydrochloric acid, ammonium chloride and sodium chloride, were recorded. Neither combining individual stimuli ("stimulus substitution") nor individual responses ("response summation") could account for the effects of mixtures. Each specialist chorda tympani neuron responded with more nerve impulses in a five-second period to its activator than to a mixture of its activator and a stimulus with a different taste. Responses were lessened by 25-65%, depending upon stimulus and afferent type. In contrast, each generalist chorda tympani neuron was most-responsive to mixtures of stimuli with different tastes, not pure stimuli. In fact, responses of generalist afferents to mixtures containing hydrochloric acid were 12.5% larger than the sum of responses to mixture components. Specialist taste afferents may be "tuned" to pure nutrient sources and generalist taste afferents may signal nutrient contamination.

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Fri. Morn Post. Sess. A Abst. # 88 Post. A13

The Effect of von Ebner's Glands Salivary Secretion on Taste Responses. SUAT GURKAN and ROBERT M. BRADLEY (University of Michigan, Ann Arbor, MI 48109)

Von Ebner's glands supply the microenvironment of the majority of oral taste buds. Recently, we have shown that electrical stimulation of the glossopharyngeal nerve results in secretory granule depletion and fluid secretion from von Ebner's gland acini. Because the rat has a single circumvallate papilla that is bilaterally innervated, we now have been able to initiate salivary secretion by electrical stimulation of one glossopharyngeal nerve while simultaneously recording circumvallate papilla taste responses from the contralateral glossopharyngeal nerve. Both glossopharyngeal nerves were exposed and one nerve was placed on platinum recording electrodes while stimulating electrodes were positioned on the contralateral nerve. The following solutions, at tongue temperature, were applied to the circumvallate papilla: 0.5 M NH₄Cl, citric acid, KCl, NaCl, Na saccharin and 0.1 M quinine HCl. Integrated responses were recorded from the whole glossopharyngeal nerve. After 30 sec of application of each stimulus, the contralateral glossopharyngeal nerve was electrically stimulated for 15 sec to elicit salivary secretion from von Ebner's glands. Ninety sec after termination of the electrical stimulation a distilled water rinse was applied. This stimulation sequence was repeated five times in each rat. The ratio of the response during the nine seconds following electrical stimulation was calculated relative to the nine seconds prior to electrical stimulation. Salivary secretion from von Ebner's glands caused more than 50% reduction in taste responses to all stimuli. Administration of atropine, which blocks salivary secretion from von Ebner's glands that is initiated by electrical stimulation of the glossopharyngeal nerve, also blocked the depression of the taste response produced by electrical stimulation of the glossopharyngeal nerve. This indicates that depression of the taste response is due to salivary secretion into the papilla cleft. These results are the first direct evidence that secretion of saliva by von Ebner's glands can influence taste responses of circumvallate papilla taste buds.

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Fri. Morn Post. Sess. A Abst. # 89 Post. A14

Pentadin, the sweet principle of Pentadiplandra brazzeana Baillon; isolation, characterization and neural taste response in rhesus monkey and rat. H. van der WEL, G. HELLEKANT, G. LARSON. (Department of Veterinary Science and Wisconsin Regional Primate Center, University of Wisconsin, 1655 Linden Dr., Madison, WI 53706 USA) An aqueous extract from the pulp of the plant Pentadiplandra brazzeana Baillon (fam: Pentadiplandraceae) yielded a strong sweet tasting material. This sweet principle was isolated by water extraction, gelfiltration and ultrafiltration. The conclusion that this substance must be of a proteinaceous nature is based on the amino acid analysis, the characteristic UV-absorption spectrum and the positive colour reaction with Coomassie Brilliant Blue. The molecular weight of the sweet subunit was estimated around 12000 Da. The sweetness intensity of the whole protein is about 500 times sucrose on weight basis. The taste response in a Rhesus monkey of a 0.1% solution was comparable to the response of a 0.02% monellin solution. No response was recorded in the rat. We propose the name "Pentadin" for this sweet-tasting protein. Supported by NIH Grant #NS17021

Isolated Xenopus olfactory receptor neurones and two major whole cell currents. D. Schild
 Physiologisches Institut der Universität Göttingen
 Humboldtallee 23, D-34 Göttingen, FR Germany

In order to investigate the olfactory transduction processes by electrophysiological means, an isolated cell preparation of Xenopus olfactory neurones has been developed. Adult animals were decapitated after brief anaesthesia with MS 333. The mucosa of the main olfactory cavity was cut out and rinsed in Ca-free amphibian Ringer. For immediate use, one of the mucosa pieces was put in a Petri dish filled with divalent-ion free solution and gently mechanically disrupted. An alternative trypsin treatment (0.025%, 30 min) did not result in a considerably higher yield. The tissue was then gently tritu-rated 3 to 5 times and applied to a Concanavalin A-coated cover slip in the experimental chamber. The experimental chamber allowed for a continuous flow of the bath solution and changing of the solutions. Characteristically, olfactory receptor neurones could be well distinguished from other cells by morphological criteria: the typical features are a small soma and one long dendritic process (at least 30 μ m, often more than 70 μ m) ending in the dendritic knob and cilia. The neurones were analysed using the whole cell patch clamp technique. Seals were between 4 and 15 G Ω . The cells' resting potentials were determined as -34mV+8mV, n=19, the membrane's capacitance was about 7pF, and the resting impedance was between 1.2 and 3.3 G Ω . For a holding potential of -90mV and voltage pulses up to -65mV no voltage activated channels were observable. At -55mV an inward current developed with maximal amplitudes of 100 to 150 pA at -35mV. The inward current was followed by a slowly and incompletely inactivating outward current. Ion exchange experiments indicate the existence of Ca- and Ca-dependent potassium channels on these cells.

Channels of Bullfrog Olfactory Cilia. S.J. KLEENE, R.C. GESTELAND (Nose City, Department of Anatomy & Cell Biology, University of Cincinnati), and R.C. MacDONALD (Department of Neurobiology & Physiology, Northwestern University).

We are attempting to characterize membrane channels in olfactory cilia from the bullfrog, *Rana catesbeiana*. The combined dorsal and ventral olfactory epithelia of a bullfrog are incubated for 30 minutes in a Ca²⁺-free medium. Then CaCl₂ (final concentration 10 mM) is added, causing the cilia to detach at their bases. A pellet of cilia is obtained by differential centrifugation.

Three cell-free systems are being used to study the ciliary channels. In the first, the cilia are frozen and thawed with an exogenous lipid, usually azolectin. A drop of the resulting vesicles spontaneously forms a lipid monolayer at its surface, and this monolayer includes ciliary channel proteins. Passing a recording electrode twice through the monolayer causes a bilayer to form at the electrode tip. Currents through single channels in the bilayer can then be monitored.

In the second cell-free system, the cilia are again frozen and thawed in the presence of exogenous lipid. Conditions are chosen such that lipid vesicles 10-30 μ m in diameter result. These vesicles are large enough that standard patch clamp techniques may be used to study single-channel currents due to the olfactory cilia.

Finally, we have found that subjecting isolated olfactory cilia to a threefold dilution of osmotic strength causes them to assume a spherical or toroid shape typically 1-2 μ m in diameter. We will be attempting to make direct patch clamp measurements on these single cilia.

Supported by NIH NS23348.

Effects of Lithium on Chemoresponse and Ion Regulation in Paramecium. MARK WRIGHT (University of Vermont) and JUDITH VAN HOUTEN (University of Vermont)

Paramecia, unicellular ciliates, recognize and accumulate in solutions of certain chemicals. The primary event in response to an attractant stimulus (e.g. folic acid) is a plasma membrane hyperpolarization. The change in membrane potential is directly responsible for the observable alteration in swimming behavior, which eventually causes accumulation of the cells. The basic biochemical and biophysical mechanisms underlying the hyperpolarization are not yet elucidated. We find now that lithium may be an important pharmacological tool in the study of these mechanisms. Using a simple behavioral assay (T-maze), which is indirectly indicative of the membrane potential, we have found that 1-4 mM LiCl treatment of cells dramatically inhibits the cells' normal behavioral response to folic acid. We have further studied the perturbation effect of lithium on basic membrane physiology in an attempt to identify the specific pathways involved in *Paramecium* stimulus response coupling. Here we report that LiCl exerts several effects on normal membrane function: 1) Li inhibits the rate of ⁴⁵Ca²⁺ efflux; 2) Li competes for K⁺ uptake and causes a depletion of internal K⁺; 3) Li may inhibit normal adenylate cyclase activity; 4) Li does not appear to perturb phosphoinositide metabolism. Continued study of LiCl as a tool to perturb membrane functions should help elucidate which (or all) of these factors are essential in the normal signal transduction mechanism of this chemoreceptor cell.

Supported by NSF and the VRCC.

Ionic composition of toad olfactory mucus measured with ion selective microelectrodes. DAVID CHIU, TADASHI NAKAMAUARA*, and GEOFFREY H. GOLD* (Dept. of Cell. and Molec. Physiol., Yale U. School of Medicine). *Present address: Monell Chemical Senses Center, Philadelphia, PA.

To understand the role of olfactory mucus in the physiology of the olfactory epithelium, we have used ion-selective microelectrodes (ISM's) to measure the ionic composition of toad olfactory mucus. ISM's offer several advantages over existing techniques, e. g., they are non-perturbing, have excellent time resolution, and measure free rather than total ion concentrations. Here we report steady state measurements of Na, K, Ca, and Cl in the absence of odor stimulation.

The measurements were carried out on excised epithelia, which were mounted in a humid chamber at room temperature. The ISM's were made from silanized borosilicate glass micropipettes which were tip-filled with commercially available liquid ion-exchange cocktails. The tip of the ISM was mounted adjacent to the tip of a Ringer's-filled reference microelectrode, and the pair was advanced into the mucus layer under visual control. The ISM's were calibrated in standard salt solutions before and after each mucus measurement. Due to the imperfect selectivity of the Na ligand for Na with respect to K, the K measurements were used to correct the Na measurements for K interference. To confirm the validity of these methods, we measured the composition of plasma and the results agree well with published data.

	Na	K	Ca	Cl
Mucus	85 \pm 16	11 \pm 5	0.32 \pm 0.16	93 \pm 9
Plasma	104 \pm 11	2.7 \pm 0.4	1.1 \pm 0.2	88 \pm 2

The data indicate that olfactory mucus is typical of respiratory tract mucus, in that K is elevated and Na is reduced with respect to plasma levels. Na absorption and K secretion by the olfactory epithelium may play a role in regulating mucus water content, as occurs in respiratory epithelia. Free Ca concentration is considerably smaller than total Ca (10 mM, Joshi et al., 1987), suggesting that most mucus Ca is bound to mucus glycoproteins. Our results are consistent with evidence that odor-induced currents originate in the cilia and dendritic knob, i. e., the observed concentrations of Na and K would enable a depolarizing current to be carried by the cation-selective channels which are present in the ciliary and dendritic membranes (Nakamura and Gold, 1987).

Ion Transport Across the Frog Olfactory Mucosa: Basal and Odorant-Stimulated States K.C. PERSAUD, G.L. HECK, J.A. DESIMONE (Department of Physiology, Virginia Commonwealth University, Richmond, VA 23298) M.L. GETCHELL and T.V. GETCHELL (Department of Anatomy and Cell Biology, School of Medicine, Wayne State University, Detroit, MI 48201)

We have developed an isolated preparation of bullfrog olfactory mucosa in which both the basal short-circuit current (Isc), and the odorant-evoked current (Δ Isc) can be measured. When bathed in Ringer's, Isc = 53.0 ± 14.5 μ A/sq cm (directed from ciliated side (c) to submucosa (s)). The current reflects an active ion transport process. Replacement of the Cl ion with gluconate reduced Isc to zero while replacement of Na ion with N-methylglucammonium reduced Isc by 60%. Furosemide added to side c reduced Isc by an average of 70%. The results indicate that the major part of the basal current is linked to a Na/Cl cotransporter, probably associated with sustentacular cell function. This current was blocked by ouabain added to side s, but not by amiloride on either side. The basal I-V curve was linear; the open-circuit potential was -3.55 ± 0.43 mV, and the resistance 67 ± 26.5 ohm sq cm. The odorant-evoked current was inwardly directed, superimposed on the basal current, and odorant concentration dependent. At transmucosal voltages clamped to 15 mV or higher (referenced to s), Δ Isc reversed direction becoming outward. Furosemide had no effect on Δ Isc, but amiloride reversibly reduced Δ Isc by as much as 75%. Symmetrical Na replacement had a similar effect. The results indicate that odorants activate a Na current source, probably a channel in receptor cell apical membranes.

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Evidence for Indirect Activation of Rat Olfactory Bulb Neurons During Odor Stimulation. DAVID P. WELLIS and JOHN W. SCOTT. (Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.)

Vertebrate olfactory bulb neurons show temporally patterned odor responses, presumably reflecting the complex circuitry within the olfactory bulb. To begin delineating the circuitry involved in the generation of odor induced responses, we recorded intracellularly from rat olfactory bulb output neurons and interneurons with HRP (in either KAC or KCl solutions) filled electrodes. Nineteen cells were held long enough for the presentation of at least six concentrations of an odorant. Stimulating electrodes placed at antidromic and orthodromic sites helped electrophysiologically identify neurons not recovered.

These studies suggest that output neurons may have more than one input path for odor activation. First, many mitral cells could not be activated by single shocks to the olfactory nerve, even though they respond strongly to odorants. The recordings also suggest that output cells are not equipotential; the changes in the membrane potential do not always correlate well with the spike activity. Suppression of spike activity was not always correlated with hyperpolarization of the membrane at the recording site, implying either presynaptic inhibition or disfacilitation of the cell. Recordings from the apical dendrites of two mitral cells included fast prepotentials (FPPs). The ability of a FPP to initiate full soma spikes in these cells varied with the intensity and quality of odor presented. If the FPP represents the summed activity from the glomerular layer, another input must modulate spike activity at the soma. Finally, we observed interneurons that turned off with odor stimulation, possibly removing a tonic inhibition of output cell basal dendrites.

Together, these results indicate that some odor induced responses in output neurons may be mediated through an indirect interneuronal input path (i.e. through basal dendrites) in addition to the direct olfactory nerve input through the apical dendrite.

Supported by NIH NS-12400.

Effects of Stimulus Concentration and Duration on the Dynamic and Temporal Properties of the Response of Pheromone-Sensitive Olfactory Receptor Neurons. PAOLA F. BORRONI and ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA).

Recent physical descriptions of the non-uniform pheromone distribution in natural odor plumes, as well as behavioral and physiological results have prompted a new interest in the adaptation properties of olfactory receptor neurons in insects, especially in reference to their ability to follow rapid stimulus pulses. However, little is known about the fundamental temporal properties of adaptation of these (or any other) chemosensory receptor neurons. As a first step towards determining these properties, we measured the relationship between stimulus concentration and duration and the time course of chemosensory responses. Extracellular recordings from single olfactory neurons were obtained by inserting a tungsten microelectrode through the cuticle at the base of sensilla trichodea of male cabbage looper moths, *Trichoplusia ni*. Stimulus delivery through computer controlled valves insured a relatively rectangular stimulus profile. Stimulus dose-response and duration-response functions were obtained from single receptor neurons, using several identified pheromone components. For the dose-response experiments, stimulus concentrations ranged in log steps from 0.00001 to 0.1 μ g/ μ l (stimulus duration = 1 s); for the duration-response experiments, stimulus durations were: 0.1, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 3.0, 5.0, 10.0, 20.0, and 40.0 s (stimulus concentrations = 0.0001 and 0.01 μ g/ μ l). The dynamic and temporal properties of the chemosensory response were analyzed as follows: response intensity was measured both as the total number of spikes in a given response period, and the number of spikes per second; the duration of a given response period was defined as the time comprised between the first spike following stimulus onset, and the return of the spiking activity to the experimentally determined pre-stimulatory level. The results suggest that response intensity and duration are exponential functions of stimulus concentration, but linear functions of stimulus duration.

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Inhibition in the periphery: occurrence in olfactory and gustatory receptor cells of aquatic crustaceans, correlation with mixture suppression, and effect on quality coding. CHARLES DERBY (Georgia State University, Atlanta, GA), MARIE-NADIA GIRARDOT (Georgia State University), SHEENAN HARPZ (Monell Chemical Senses Center, Philadelphia, PA)

For chemoreceptor cells of vertebrates and invertebrates alike, chemically-induced excitation (increase in the spiking frequency from spontaneous level) is much more frequently observed than is inhibition (decrease in spiking frequency from spontaneous level). One reason for this observation is that chemoreceptor cells in general have a very low rate of spontaneous activity, usually < 1 Hz, making detection of inhibitory responses by means of extracellular techniques difficult. We report that primary chemoreceptor cells of aquatic crustaceans are capable of being inhibited by chemicals. This is true for olfactory (antennular) cells of spiny lobsters (*Panulirus argus*) and gustatory (leg) cells of freshwater prawn (*Macrobrachium rosenbergii*). Some single receptor cells respond with excitation to one chemical but inhibition to another chemical. There is a correlation in the occurrence of inhibition and mixture suppression: chemicals that inhibit the spontaneous activity of a cell usually suppress that same cell's response to excitatory chemicals. This correlation raises the possibility that the mechanism responsible for inhibition may also be responsible for some types of peripherally generated mixture suppression. The occurrence of inhibitory responses to select chemicals could also improve the ability of the nervous system to resolve differences in the quality of chemicals: as a result of single receptor cells having both inhibitory and excitatory responses, more complex and unique across-neuron response profiles for a population of receptor cells can be created, resulting in better discrimination of differences in chemical quality than is possible with excitation alone. A model is presented that tests this hypothesis.

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Analog (More-or-less) Electronic Modules for Electrophysiology.
ROBERT C. GESTELAND (Oshreshiopolis, Dept. Anat. & Cell Biol., Univ. of Cincinnati)

Most electrophysiology laboratories need general purpose electronic instruments for signal generation and for signal processing. Instruments which are small, simple, and inexpensive often prove more useful than larger special purpose equipment. Signals can be generated by a microcomputer with digital-to-analog and digital input-output boards but programming is time consuming and costs are relatively high. Stimulators and amplifiers designed for the electrophysiologist are generally complex and are difficult to trouble shoot. They are difficult to modify when performance does not meet demands and often are not supplied with a circuit schematic. It is convenient to work out protocols for a new experiment by patching together appropriate modules without writing programs and buying specialized electronic instruments.

The laboratory staff has designed several useful new instruments. Signal generation equipment includes a clock module which generates gated trigger pulses over a wide interval range, a pulse generator with pulse end triggers for time delays, and a staircase generator with several unusual applications. An inexpensive commercially available ramp generator is mounted in a compatible module. Inputs to all of these devices are accepted from panel controls and from digital logic pulses from data acquisition equipment. Signal processing modules include an amplitude discriminator of unusual versatility, an analog charge-coupled bucket brigade signal delay unit with high fidelity and wide delay range, a signal processing amplifier with jumper selected gain ranges and multiple d-c offset modes, and a high-speed quad amplifier controlled by external binary commands. Available commercial boards which are useful for signal processing are a ratiometer and a gated integrator.

The 4.5" x 6.5" double-sided etched boards plug into standard 44-pin backplane connectors and are driven by a common power supply. The instruments have compatible signal levels and pinouts so that any board plugs into any slot. Typical modules cost about \$35 for the etched, drilled and plated circuit board and \$50 to \$100 for electrical components. Some useful laboratory applications will be described.

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Intensity Discrimination Measurements in Lobster Olfactory Cells. C.L. MERRILL, R. VOIGT, J. ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA) and B.R. JOHNSON (Cornell University, Ithaca, NY).

The lateral filament of the antennule of the american lobster, *Homarus americanus*, contains several populations of chemoreceptor cells narrowly tuned to specific amino acids. Cells sensitive to hydroxy-L-proline (Hyp) were chosen to study dynamic tuning in olfaction. The intensity of a given chemical signal to which receptor cells are sensitive is measured against the background concentration to which cells have adapted. Hyp cells tend to have a shallow response function and individual cells, when adapted to low backgrounds (e.g. artificial seawater (ASW)) appear to function over three or more log units of concentration. While various authors have analyzed the intensity discrimination capability of chemoreceptor cells statistically based on population dose-response functions, this capability has never been measured directly. Here, we investigated the reliability with which single cells code intensity information. Dynamic change in the concentration of a stimulus may be encoded in the number of spikes arriving from specific, narrowly tuned receptor cells or be encoded by across-fiber patterns associated with a population of cells. Responses of individual Hyp sensitive cells were recorded extracellularly. Cells were stimulated by repetitions of highly reproducible concentration pulses alternating between 10^{-4} and 10^{-5} or 3×10^{-5} and 3×10^{-6} M Hyp in ASW. The number of spikes recorded from individual cells often did not provide sufficient information to distinguish even 10-fold differences in concentration because of large variation in response to identical pulses. However, mean response of relatively small populations ($N = 16$ and $N = 8$) of Hyp cells varied less than 20 % to identical pulses while mean responses to the two different concentrations differed by approximately 50 %. Response functions over a concentration range from 10^{-6} to 5×10^{-4} M at 0.5 log-steps were also determined for individual cells and for populations of Hyp cells. As in the repetition study, single cell response functions often deviate from a monotonic increase in response with increasing stimulus intensity; whereas response functions for small populations are a more reliable indicator of stimulus intensity.

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Responses of olfactory and vomeronasal epithelia to airborne odors in garter snakes. JUN INOUCI and MIMI HALPERN (SUNY Health Science Center at Brooklyn)

Most terrestrial vertebrates possess both main olfactory and vomeronasal systems with many features in common. There have been few studies using electrophysiological methods to characterize and compare the properties of these two systems, especially in the same animal. To examine the physiological properties of both systems in garter snakes (*Thamnophis sirtalis parietalis*), we first focused on recording electroolfactograms (EOGs) from the olfactory and vomeronasal epithelia using an air delivery system to stimulate the epithelia with airborne compounds (amyl acetate ($10^{-2.25}$ - 10^0), butanol ($10^{-2.25}$ - 10^0) and saturated earthworm wash vapor. For each of the three odors, response magnitude recorded from the olfactory epithelium increased exponentially with logarithmic increase in stimulus concentration. We also successfully recorded from the vomeronasal epithelium, obtaining reliable EOG responses to amyl acetate and butanol. No response to saturated earthworm wash vapor was observed. The response amplitude from vomeronasal epithelium increased exponentially with logarithmic increase in stimulus concentration. Response amplitudes recorded from the vomeronasal epithelium were consistently lower (10 times) than EOGs recorded from the olfactory epithelium and thresholds for eliciting a response from the vomeronasal epithelium were consistently (10 times) higher than thresholds for response from the olfactory epithelium.

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Effects of stimulus profile on temporal response profile and disadaptation time course. RAINER VOIGT and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, MA 02543, USA)

The taurine-sensitive cells on the lateral flagellum of the American lobster form a spectrally narrowly tuned cell population which provides an excellent model to examine adaptation and disadaptation properties of chemoreceptor cells. When stimulated with low taurine concentrations at low ambient background concentration single cells are able to resolve relatively high stimulus pulse rates. This feature requires fast interpulse disadaptation rates. When stimulated with short 1 s pulses taurine cells give short, mostly phasic-tonic responses which last less than 3 s. The temporal response profile resembles the stimulus profile. To examine to what degree the stimulus profile determines the response profile and to what degree intrinsic cellular processes determine the time course of a chemoreceptor response we tested taurine sensitive cells with stimulus pulses where we varied and duration. Preliminary results suggest that taurine sensitive receptor cells adapt rapidly during single pulses. The adaptation time courses of individual cells vary with their stimulus response functions.

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Role of cations in olfactory reception in the spiny lobster. I. SCHMIEDEL-JAKOB, P. A. V. ANDERSON, and B. W. ACHE (Whitney Laboratory and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086)*

Olfactory receptor cells of the spiny lobster respond to odorants (amino acids, extracts of fish food or crab) with spikes superimposed on slow depolarizing receptor potentials. To identify the ionic basis of the receptor potential, the ionic composition of the saline superfusing the outer dendritic segment of the receptor cells was changed and the resulting effect on the receptor potential measured. With most receptor cells it was not possible to test more than one modified saline. All effects were reversible within 3-12 min: (i) When the Na concentration was reduced from 480 mM to 48 mM and substituted either by choline-Cl or TMA, the receptor potential was abolished or markedly reduced in 50% of the cells tested. (ii) When the K concentration was reduced from 14 to 1-2 mM and substituted by equimolar Na, the receptor potential was abolished or reduced in 63% of the cells tested. (iii) When the Ca concentration was reduced from 13 to 2-4 mM and substituted by equimolar Mg, the receptor potential was reduced in 60% of the cells tested. (iiii) In six cells, it was possible to superfuse the sensilla sequentially with all 3 modified salines. In 3 cells elimination K or Ca, but not Na, reduced the receptor potential; in the other 3 cells, the receptor potential remained unchanged upon elimination of either Na, K, or Ca. These results are consistent with the hypothesis that more than one ionic mechanism underlies the receptor potential in lobster olfaction.

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How the Anterior Tongue and Taste Responses Are Mapped onto the NST in Fetal and Perinatal Sheep. CHARLOTTE M. MISTRETTA (University of Michigan, Ann Arbor, MI 48109)

The afferent innervation of taste buds and fungiform papillae on the anterior two thirds of the tongue projects to second order neurons in the nucleus of the solitary tract (NST). To learn whether there is a functional somatotopy and/or chemotopy in NST for tongue and taste projections, multiunit responses to salt and tactile stimuli were sampled systematically throughout the nucleus in sheep. Five fetuses at 130 days of gestation (term = 147 days) and four perinatal animals (5 days before or after birth) have been studied. Multiunit responses were recorded with a low impedance microelectrode and neural data were passed through an ac to dc converter to measure response magnitude. With an initial coordinate in the anterior tongue taste projection, responses were sampled at 0.2 mm steps in rostral or caudal, and medial or lateral, directions through the nucleus. Within each electrode track, responses were recorded at 0.1 mm steps. Stimuli were 0.25 and 0.50M NH_4Cl , NaCl , and KCl , and light touch with a glass probe. Throughout the nucleus, responses to salts were recorded most dorsally in electrode tracks, then responses to salts and touch, and most ventrally, responses to touch only. For tactile responses, the posterior tongue was represented most dorsally and the anterior tongue most ventrally. For salt taste responses, NH_4Cl elicited larger responses than NaCl , and NaCl larger than KCl , throughout the dorsal to ventral representation. Even throughout a 2.2 mm caudal-rostral extent of NST, areas of very high responsiveness to one particular salt were not encountered. However, there was a trend for NaCl to elicit larger response magnitudes, relative to NH_4Cl , at more rostral locations. Preliminary results indicate a tongue taste projection that is dorsal to tongue tactile, a dorsal to ventral somatotopy for posterior to anterior tongue tactile projections, and a trend for some chemotopy with larger NaCl responses represented most rostrally. However, a well defined chemotopy for salts has not been observed. General conclusions apply to both age groups studied, but it remains to be seen whether there are differences in older animals. (Supported by NSF Grant BNS 8311497.)

Rapid activation and desensitization of single channels on chemosensory neurons of the crayfish walking leg. HANNS HATT (Physiologisches Institut der Technischen Universität München Biedersteiner Straße 29, 8000 München 40, FRG) Single channel currents were recorded from the soma membrane of chemoreceptive neurons by means of G Ω seal patch clamp technique (cell attached and outside-out configuration). Until now two types of ion channels could be identified in different neurons activated by either nicotinic (and analogous substances) or purine nucleotides.

Open and close times, reversal potential and the single channel conductance of the two types were determined. A new method for the controlled application and wash out of activator at excised membrane patches within less than 0.5 ms was used (details see Franke, Hatt, Dudel; Neurosci Lett. 1987, 73, 137). The purine nucleotide channel is activated within few ms and after an initial peak of openings desensitizes with a time constant of about 200 ms to a low steady state level. Using different concentrations of the applied nucleotides detailed information about the kinetic of the molecular reaction can be obtained.

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Single-unit Recording and Anatomical Characterization of Cells in the Solitary Nucleus of the Hamster. MARTHA MCPHEETERS, SARAH C. NUDING, THOMAS P. HETTINGER & MARION E. FRANK (BioStructure and Function Department, University of Connecticut Health Center).

Many sensory systems have a clear spatial organization based upon stimulus characteristics. Usually an organization is apparent in receptor populations and continues in one form or another throughout ascending neural levels. In the taste system, the existence of any such organization is difficult to ascertain. One possible scheme is based on the stimulus characteristic of taste quality. According to this hypothesis, cells responding to the same chemical stimuli are anatomically grouped together in taste buds or at higher levels. Our study involved locating medullary neurons responding to rapid stimulation of the anterior tongue and determining if a spatial organization based on taste quality was present in the solitary nucleus. We recorded from 44 single taste-responsive units in the medulla of hamsters (*Mesocricetus auratus*) and subsequently described their anatomical locations. Only three taste stimulants [NaCl (0.03M), sucrose (0.1M), and KCl (0.1M)] were used because they distinguish distinct populations of afferent fibers to the solitary nucleus (Frank et al. 1988). Each unit was classified by its relative responses to the stimulants. After physiological characterization, horseradish peroxidase (HRP) was iontophoresed at the recording site. Histological processing revealed spheres of reaction product (50 μm in diameter) which were defined in three-dimensional space. Their positions were transposed to a computer model of a typical solitary nucleus where they were compared to one another on the basis of location and taste quality.

Supported by NIH grant NS16993.

Fri. Morn Talk 8:30 AM Abst. # 105

Response Characteristics of Lamb Pontine Neurons to Chemical, Mechanical and Thermal Stimulation of the Oral Cavity and Epiglottis. ROBERT D. SWEAZEY and ROBERT M. BRADLEY. Univ. Michigan, Ann Arbor, MI.

Using neuroanatomical techniques, we have shown that the cranial nerves which relay sensory information from lamb oral cavity and epiglottis terminate in three different nuclei of the brainstem: the nucleus tractus solitarius (NTS), the caudal half of the spinal trigeminal nucleus (SPVn) and the caudal pons (J. Comp. Neurol., 245:471, 1986). We already have reported response characteristics of lamb second-order neurons in two of these nuclei: the NTS (Brain Res., in press) and SPVn (Neurosci Abstr., 13:779, 1987). We now report response characteristics of single neurons in the third nucleus located in the lamb pons. Neurons were characterized by their responses to stimulation of the oral cavity and epiglottis with chemical, mechanical and thermal stimuli, and the location and size of receptive fields. The anatomical locations of neurons isolated in the pons were determined by examining stained sections for small lesions made at the recording sites. Responsive neurons were located 11.0-11.5 mm rostral to obex and 5.2-5.8 mm lateral to the midline. Neurons which responded to chemical stimuli were located in a compact group of cells just medial to the dorsomedial tip of the trigeminal nucleus. Cells with anterior tongue receptive fields were medial to neurons with receptive fields on the caudal tongue. Neurons which responded exclusively to mechanical stimuli were intermixed or ventral to neurons responsive to chemical stimuli. Neurons responsive to thermal stimuli were located lateral in the trigeminal nucleus. Like neurons in the lamb NTS, pontine neurons responded best to salts and acids, but showed little response to sugars or quinine hydrochloride. Furthermore, as was previously observed for cells in the NTS, most pontine neurons responsive to chemical stimuli responded to one of the other stimulus modalities as well. Unlike the NTS and SPVn, neurons in the pons rarely had more than one receptive field. This is the first report showing that neurons in the lamb pons respond to chemical stimuli. In contrast to results reported in studies using other species, the responsive area in the pons is not located in the parabrachial area, but more ventral near the trigeminal nucleus. Our studies suggest that this region comprises a second-order relay for afferent information from the oral cavity and upper airway.

Supported by NIH Grant DE05728.

Fri. Morn Talk 9:00 AM Abst. # 107

Preliminary Findings on Hamster Gustatory Cortex Studied with Voltage Sensitive Dyes. J. A. LONDON (University of Connecticut Health Center, Farmington, CT 06032)

Preliminary work is described in which optical recording methods were used to record hamster cortical activity in response to electrical stimulation (ES) of the anterior and posterior tongue. In this technique, externally applied dyes change their fluorescence as a result of changes in the membrane potential of neurons and glia. The fluorescence changes are recorded by a 122 element array of light detecting diodes (Orbach, Cohen and Grinvald, 1985). A 3-5 mm craniotomy was performed 5-8 mm lateral to bregma. The gustatory cortex was identified as the area of the brain activated when ES was applied to the tongue. ES of the anterior 2/3 of the tongue resulted in activation of a small, discrete area of the cortex. Stimulation of the posterior tongue did not activate this area but did activate a more caudal part of the cortex. Surrounding areas of the brain (e.g. the somatosensory cortex) were not activated by either the anterior or posterior tongue ES. Other more distant cortical areas were not examined. Bilateral destruction of the chorda tympani resulted in cessation of rostral gustatory cortex activity in response to anterior tongue ES but not the caudal gustatory cortex response to posterior tongue ES. In conclusion, it appears that a discrete area of the hamster cortex which responds to ES of the tongue has been identified via voltage sensitive dyes. This area can functionally be divided into two parts: an area activated by ES of the anterior tongue and an area activated by ES of the posterior tongue. It seems possible that information to the rostral gustatory cortex, identified via voltage sensitive dyes, is carried by the chorda tympani.

Supported by NSF grant NS16993.

Fri. Morn Talk 8:45 AM Abst. # 106

Peripheral versus Neocortical Processing of Oral Thermal and Chemical Signals. GARY J. SCHWARTZ and EVA KOSAR (Monell Chemical Senses Center, 3500 Market St., Phila., PA 19104)

Previous electrophysiological studies have shown that a variety of chemical stimuli elicit oral afferent neural activity similar to that elicited by thermal stimulation. "Thermomimetic" chemical stimuli, such as menthol (M) and capsaicin, have been shown to elicit thermal sensations in man. These experiments were designed to compare the neural responses to both thermal and chemical stimulation at the level of the peripheral nerve to elicited neural activity in rat neocortex. Electrophysiological responses to oral thermal and thermomimetic stimulation were recorded from chorda tympani (CT), lingual nerve single fibers, and single neocortical units. At least two distinct patterns of lingual afferent activity emerged, and these patterns were paralleled in cortical single unit activity. Both CT and lingual nerve fibers responded similarly to thermal stimulation, yet they differed in their response to M stimulation. CT and lingual nerve fiber activity increased in response to cold water (CW) stimulation, while warm water (WW) stimulation suppressed activity below spontaneous baseline levels. In contrast, M elicited vigorous activity in lingual nerve fibers, while M failed to stimulate activity in thermally-sensitive CT fibers. At least two different groups of cortical neurons were identified that have patterns of activity similar to either CT or lingual nerve fibers. One group showed an increase in activity to CW stimulation and a decrease to WW stimulation with no activation by M. The second group showed both thermal responsiveness and increased activity in response to M stimulation. In addition, cortical neurons demonstrate more diverse patterns of activity to these stimuli, suggesting a more complex processing of oral thermal and chemical information.

This research was supported by NSF grant BNS85-19629 to EK, NIH training grant 5T32 NS07176-08 to GJS, and the Monell Chemical Senses Center.

Fri. Morn Talk 9:15 AM Abst. # 108

Golgi Studies of the Gustatory Zone of the Nucleus of the Solitary Tract in the Hamster. BARRY J. DAVIS and TAICHANG JANG (University of Alabama at Birmingham)

The Golgi method was used to study the typologies of neurons within the dorsal, intermediate and ventral levels of the gustatory zone of the nucleus of the solitary tract (NST). Two classes of neurons are observed across all levels. Class I neurons are characterized by long, relatively unbranched dendrites that often extend outside the gustatory zone. These neurons tend to be large and fusiform in shape. Class II neurons are multipolar and possess significantly more and shorter secondary and tertiary dendrites than Class I neurons. Both classes are generally spine-poor. Both Class I and II neurons exhibit a preferred orientation in the horizontal plane. Class I neurons and their dendritic arborizations appear planar when viewed coronally or sagittally after 3-dimensional rotation analyses. Class II neurons are also oriented mostly in the horizontal plane but show some radiation of their dendritic processes when viewed coronally or sagittally. Together, these classes produce horizontal tiers of dendritic processes that frequently extend outside the traditional cytoarchitectonic subdivisions of the NST and outside the target zones of primary gustatory afferent fibers. Peripheral gustatory fibers also enter the solitary tract, form collaterals and then terminate in the gustatory zone as individual fibers that are best visualized in the horizontal plane. Consequently, these fibers are in the requisite orientation to maximize interactions with the dendrites of second order gustatory neurons. Neurons situated in the projection zone of the glossopharyngeal nerve possess dendrites that could extend into the projection zone of the chorda tympani and vice versa. Such anatomical findings are consistent with electrophysiological studies that show single NST neurons are responsive to taste stimuli applied to widely spaced population of taste buds. Dendrites of neurons located in the adjacent reticular formation are seen to extend into the gustatory NST. The dendritic arborizations of these neurons are very different from those of gustatory NST neurons but such arborizations are also oriented with respect to incoming primary gustatory fibers. These findings may provide a basis for the interactions between gustation and ingestional reflexes mediate via the reticular formation.

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Morphological Types of Neurons in Taste-Responsive Sites in the Hamster's Solitary Nucleus. M.C. WHITEHEAD, S.A. AHMED (Ohio State U.), L.D. SAVOY and M. MCPHEETERS (UCConn).

We combined HRP histochemistry and microelectrode mapping to study the sizes, shapes, and orientations of neuronal cell bodies and dendrites located at sites of taste-elicited single unit activity in the solitary nucleus. Cells responding to sapid stimuli applied to the anterior tongue were recorded using micropipettes containing 0.5M KCl and 4% HRP. Iontophoretic injections (n=15) of HRP into the recording sites resulted in small (50-125 μ m diam.) opaque zones bordered by a small number (2-15) of neurons with well-labelled cell bodies and dendrites. The cell bodies were near (50-250 μ m) the injection sites into which they sent labelled dendrites. The cells were drawn, measured and their locations and orientations plotted in relation to cytoarchitectonic subdivisions of the solitary nucleus. Three types of neurons were commonly HRP-filled. Large elongate cells (n=46) had oval-spindle-shaped cell bodies (12x22 μ m) oriented mediolaterally. Two primary dendrites extended 100-300 μ m from the cell body, one medial and one lateral, and branched within a cylindrical field oriented mediolaterally. A minority of large elongate cells had unusually long (400 μ m) or caudally extending dendrites. Large stellate cells (n=34) had round-oval cell bodies (14x18 μ m) and 3-5 primary dendrites, coursing 100-300 μ m in all directions and branching as spheroidal fields. A minority of large stellate cells had 3 unbranched dendrites resembling tentacles. Small stellate cells (n=23) had small cell bodies (10x15 μ m) and short dendrites. The labelled cells and their entire dendritic fields were remarkably confined to the rostral solitary nucleus. An exception to this was 11 large cells (15x24 μ m) of the reticular formation that sent labelled dendrites to 4 injection sites located on the edges of the solitary nucleus. Within the solitary nucleus labelled cell types were differentially distributed. All but 4 labelled cells were located in either the rostral lateral subdivision, where elongate cells predominated, or in the rostral central subdivision, where stellate cells predominated. Thus, neurons of the gustatory solitary nucleus can be classified into morphological types based on cell body size and shape (compare Davis&Jang, 1986), dendritic structure and location. It remains to be determined whether these types can also be distinguished by their physiological properties.

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Continuous and Pulsatile (5 hz train) Taste Stimulation Differing Effects On Judged Maximum Taste Intensity, But Similar Adaptation. STEVEN T. KELLING, DONNA L. MANEY, BRUCE P. HALPERN (Cornell University, Physiol./Psych./NBB, Ithaca NY 14853-7601).

Both continuous (Pantzer, K & H. *ISOT IX*) and pulsatile (K & H. *ISOT IX*) taste stimulation produce increased judged intensity as liquid flow duration is lengthened. High resolution (10/sec A/D) time-intensity (TI) tracking of 2 mM Na saccharin (NaSac) also shows more sustained responses for longer stimulation (K & H. *Chem. Sen.* 12(4), 1987). To reveal differences between TI tracking of continuous and 5 hz 2 mM NaSac, 5 practiced subjects received randomized solution and control (H₂O) trials under both conditions for 10 sessions. Total stimulation durations were 0.1, 0.3, 0.5, 1, 2 sec for continuous; 0.2, 0.6, 1, 2, 4 sec for 5 hz. [total duration = cumulative solution duration for continuous; = twice cumulative solution duration for 5 hz]. A 10 sec prestimulation H₂O rinse was used. A closed stimulus delivery system was used (K & H. *Chem. Sen.* 11, 89-104, 1986). **RESULTS:** No intensity increases were indicated on control trials. On solution trials, median response latencies were 800-900 msec (= simple reaction time, K & H. *Chem. Sen.* 12(4), 1987). For equal total durations, continuous stimulation produced larger median maximum magnitudes at all durations; for equal cumulative solution durations, at ≥ 1 sec. However, equivalent total durations produced similar overall extent of responses (time >0). **CONCLUSIONS:** Intensity information starts at simple RT. Processing is cumulative during flow, but asymptotes sooner for 5 hz trains than for continuous solution.

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Medullary convergence of taste and tactile inputs onto visceral motor neurons in the channel catfish, *Ictalurus punctatus*. J.S.KANWAL and T.E.FINGER, (Dept. Cell. & Struct. Biol., Univ. of Colorado Sch. of Med., Denver, CO 80262).

Various oromotor reflexes and physiological responses to taste and tactile stimuli suggest the presence of relatively direct sensorimotor interconnections between orofacial sensory inputs and the visceral motor output of the medulla. To verify the existence of such connections, electrophysiological responses of vagal motoneurons to taste and tactile stimulation of the gustatory epithelium were recorded. Recordings were obtained either centrally, with glass microelectrodes or peripherally, by means of a Ag wire hook-electrode on which the central out end of a branchial or coelomic branch of the vagus nerve was placed. For tracing the neural connections, HRP (10% solution in 3M NaCl) was injected iontophoretically through the glass electrode after recording the relevant neural activity. The visceral motor column of ictalurid catfish is divisible into a lateral motor column (LMC, =n. ambiguus) which innervates orobranchial musculature, and a medial motor column (MMC, =dorsal motor nucleus X) which innervates coelomic viscera. Previous studies have also shown that primary orofacial sensory afferents do not directly contact either the LMC or the MMC. Our results indicate that neurons in the LMC exhibit rhythmic patterns of activity. This activity can be modified by electrical stimulation of the facial lobe as well as by oral or extraoral application of liver extract and high concentrations ($>10^{-2}$ M) of an amino acid mixture (alanine, arginine, & proline) and oral application of quinine ($>10^{-3}$ M). Neurons in the MMC do not show a rhythmic pattern of discharge but respond to taste and tactile stimulation of extraoral or oral surfaces. Response patterns similar to those of both populations of vagal motoneurons are obtained also from cells in the medial reticular formation (RF). The neuroanatomical and electrophysiological results obtained so far suggest that at least some of the gustatory responses of vagal motoneurons occur due to the medullary convergence of orofacial sensory inputs onto neurons of the visceral motor column via two premotor nuclear groups, the nucleus intermedius of the vagal lobe and the RF.

Cross Adaptation of Saltiness Among NaCl, KCl, and NH₄Cl.

LINDA M. BARTOSHUK (Pierce Foundation and Yale University) and GREG DEMBER (Yale University)

Cross adaptation studies on salty-tasting salts are of special interest because they are relevant to mechanism(s) of salt transduction. If the saltiness of two salts completely cross adapts, then we conclude that the salty taste of these salts is mediated by a common receptor mechanism. If they fail to cross adapt, then we conclude that at least two mechanisms are required to mediate saltiness. Smith and McBurney (1969) showed that adaptation to NaCl cross adapted the salty taste of a variety of salts including KCl. Bierer (cited in Bartoshuk, 1980) showed not only that NaCl cross adapted the salty taste of KCl but that KCl also cross adapted the salty taste of NaCl. Since this study was done on a localized area on the tongue tip (to replicate the conditions used by Hahn in his classic cross adaptation studies), we repeated the experiment using the McBurney Gustometer which stimulates the entire extended tongue. In addition, we added a third salt: NH₄Cl. Adaptation concentrations were .1 molar for all three salts.

The results showed cross adaptation in both directions for all three salts. However, the magnitude of cross adaptation was not equivalent for all three. The order of effectiveness was: NH₄Cl, NaCl, and KCl. That is, the salts behaved as if the effective concentration of salty stimulus was not the same for all three salts.

These results suggest that the saltinesses of NaCl, KCl, and NH₄Cl are mediated by a common mechanism. This contradicts the conclusion based on the effects of amiloride on human taste (Schiffman, Lockhead, and Maes, 1983) that the saltinesses of NaCl and KCl are mediated by different mechanisms.

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An Assessment of Binary Sweetener Interactions Using Factorial Mixture Designs and Self-Mixtures. R.A. FRANK, S. HUBAY, R. CARTER & S. J. MIZE (Univ. of Cincinnati)

Factorial mixture designs were used to investigate binary mixtures of eight sweeteners including sucrose, fructose, glucose, aspartame, cyclamate, xylitol, acesulfame K and stevioside. Three concentrations of each sweetener were chosen to approximately match the sweetness of 0.1, 0.3 and 0.5 M sucrose. Each of the nine possible binary combinations of each sweetener pair was judged for its sweetness on a 21 point category scale. In addition, the subjects judged the sweetness of the unmixed component stimuli and distilled water. At least 18 subjects participated in each of the mixture experiments. Prior to testing, the subjects received distilled water, the highest concentration of each mixture component and the mixture of the two high components. This was done to provide a context for rating the range of sweetener values. (Mixtures were obtained by adding solutes, not by mixing solutions.) Thirty second intertrial intervals and a between sample distilled water rinse were used. It was found that the mixtures tended to exhibit superadditivity (i.e., the mixtures were sweeter than the sum of the components) at lower concentrations, but showed subadditivity (i.e., the mixtures were less sweet than the sum of the components) at higher concentrations. By using "mixtures" of the component substances with themselves, i.e., by examining "self mixtures," the non-linearity of the component psychophysical functions was removed from the super- and subadditivity of the mixtures. It was found that the sweetness of some mixtures (e.g., acesulfame K-cyclamate) consistently exceeded the sweetness function of the component substances, i.e., showed synergism. In other cases, the sweetness of the mixtures was identical to the sweetness of the components (e.g., glucose-fructose). The results of the present study indicate that synergistic interactions exist for some sweeteners. Although the mechanism for this effect is unknown, synergism may result from activation of multiple sweetness transduction mechanisms.

This research was supported by an Ohio Board of Regents Research Challenge grant to R. Frank.

The Effects of Nutritional Status and Age on Threshold for Detection of an Amino Acid Mixture. CLAIRE MURPHY AND MAGDALENA M. JENSEN (San Diego State University)*

In earlier work, elderly persons and those of lower nutritional status preferred higher concentrations of casein hydrolysate in an amino acid deficient soup base than young persons and those of higher nutritional status (Murphy and Withee, *J. Gerontol.*, 1987). The present experiment was designed to assess detection threshold for casein hydrolysate in order to determine whether or not subjects with compromised nutritional status are differentially sensitive to the amino acid mixture. Eighteen young (mean age = 19.3, $SD = 2.1$) and 18 elderly subjects (mean age = 71.7, $SD = 4.6$) two thirds of whom were female and one third male, were asked to fast 12 hours prior to blood assays for nutritional status. An independent laboratory provided assays of serum total protein, albumin and Blood Urea Nitrogen (BUN). On the same day that his or her blood was drawn, a subject's detection threshold was determined for casein hydrolysate, in the amino-acid deficient soup base, using a two-alternative, forced choice, staircase method. The procedure was continued to a criterion of six reversals. The last five were used to determine thresholds. Because pilot work as well as previous research suggested that subjects' measured threshold sensitivity will depend upon practice, all subjects first performed a practice threshold test using the identical procedure with sucrose in deionized water before beginning the experiment. Subjects' serum total protein values were divided into low and high groups using the median value and a t-test was performed on the casein hydrolysate thresholds for the two groups. Subjects who were low in protein status were more sensitive to the casein hydrolysate than those who were high in protein status, $p < .05$. The present results suggest that increased preference for an amino acid mixture in those of lower nutritional status may be driven, at least in part, by chemosensory cues.

*Supported by NIH grant NO. AGO4085 to C.M. We gratefully acknowledge the assistance of the San Diego State University Health Services in providing phlebotomy services, and Jill M. Sniffen, Kathleen McCann, and Scott Hofer in testing subjects.

Chemosensory Dysfunction: Analysis of 750 Patients from the University of Pennsylvania Smell and Taste Center. DANIEL A. DEEMS, RICHARD L. DOTY, R. GREGG SETTLE, AND JAMES B. SNOW, JR. (Smell and Taste Center; Department of Otorhinolaryngology and Human Communication, School of Medicine, University of Pennsylvania)*

Information concerning the nature, etiology, and severity of the chemosensory dysfunction of an initial group of 750 patients evaluated at the University of Pennsylvania Smell and Taste Center will be presented. Although "loss of taste and smell" was the most common complaint of this group (66.7%), true taste loss was rare (< 5%), with olfactory dysfunction alone being the most prevalent sensory disorder (72.5%). Mild to severe depression, as measured by the Beck Depression Inventory, was present in 23.8% of the patients, with the greatest prevalence occurring in patients reporting dysosmias or dysgeusias (34.5%). Patients with viral-related or idiopathic etiologies had symptom onsets primarily after the age of 50, whereas patients with trauma-related etiologies had symptoms which typically occurred before the age of 35. Of the 7 major etiologic classifications, patients with head trauma and polyposis demonstrated the greatest impairment of olfactory function. Patients presenting with smell loss accompanied by dysosmia had significantly better olfactory function than patients presenting with smell loss alone. Transient and non-transient cases of dysosmia were reported by some patients to be exacerbated by inhalation of warm vapors. Systemic corticosteroid treatment has proven effective in treatment of many transport-related olfactory disorders (e.g. polyposis, allergic rhinitis), suggesting that a significant proportion of cases presenting with olfactory loss may result from edema or inflammatory processes above the superior turbinate. Endocrine relationships, prognosis and other findings concerning chemosensory dysfunction and treatment will be discussed.

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Clinical Categorization of Olfactory Loss. D.A. LEOPOLD, H.N. WRIGHT, M.M. MOZELL, S.L. YOUNGENTOB, D.E. HORNUNG, R.A. RICHMAN, P.R. SHEEHE (Clinical Olfactory Research Center, SUNY Health Science Center at Syracuse, New York)

Efforts have been made to categorize dysosmic individuals based on their histories and clinical testing. More than 200 such dysosmic patients seen and followed at the Clinical Olfactory Research Center in Syracuse, N.Y. form the basis of this study. Elements of the history which seem to be important include the duration of onset of loss (sudden vs. prolonged), degree of loss, character of loss (trigeminal vs. olfactory), association with viral upper respiratory infections, and fluctuation in olfactory ability. Efforts have been made to use the Odorant Confusion Matrix (OCM) to categorize dysosmic patients and these efforts have so far proven successful in separating trigeminal from olfactory dysfunction. During rapid losses of olfactory ability or during recovery of olfactory ability, there is often an associated phantosmia or parosmia. Most commonly, olfactory losses have been found to be associated with nasal airway obstruction and head trauma, or following a viral upper respiratory infection.

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Surgical Correction of Olfactory Disorders. GERALD LEONARD (Division of Otolaryngology, Dept. of Surgery, and Connecticut Chemosensory Clinical Research Center [CCCRC], University of Connecticut Health Center, Farmington, CT 06032), WILLIAM S. CAIN (John B. Pierce Foundation Laboratory, New Haven, CT 06519 and CCCRC), and GAIL CLAVET (CCCRC).

Paranasal sinus disease figures prominently as a cause for olfactory dysfunction among patients evaluated at the CCCRC. Approximately one-third show indications of such disease on clinical evaluation, on x-ray, or by history. When the patients display serious loss and when the objective signs of disease appear commensurate with the loss, the patients may be offered sinus surgery. We report here the pre- and postoperative olfactory function of 25 patients who underwent unilateral or bilateral transantral ethmoidectomy for their chemosensory problem. Questions of particular interest were: a) Did the procedure correct the olfactory problem? b) Was recovery complete? and c) Did surgery on one side have any influence on olfactory functioning on the other side? Olfactory functioning was measured on a 7-point scale with the CCCRC test. Altogether, 41 nostrils were involved. Average score equalled 0.27 before surgery and 3.34 after surgery. Nine of the 25 patients achieved normal status (score of 6 to 7) in one or both nostrils postsurgically. Seven, however, showed no improvement. Six of these patients had bilateral surgery and failed to improve on either side, which suggests that something other than sinus disease caused their olfactory problem. Of the remaining nine patients, four exhibited only mild hyposmia (i.e., almost normal functioning) and five moderate or severe hyposmia. Such incomplete cures may represent instances where surgical intervention should be supplemented with medication. Finally, surgery on one side does improve functioning in the contralateral nostril significantly. Whether the improvement derives from bilateral release of obstruction or from some more obscure mechanism (e.g., bilateral neural connections) remains to be established.

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Topical Corticosteroids Can Alleviate Olfactory Dysfunction. APRIL SCOTT (Dept. Medicine, & Conn. Chemo. Clin. Res. Ctr. [CCCRC], UCONN Health Ctr., Farmington, CT 06032), WILLIAM S. CAIN (John B. Pierce Found. Lab. New Haven, CT 06519 and CCCRC), & GAIL CLAVET (CCCRC).

Corticosteroids are effective in the treatment of seasonal rhinitis and in selected patients with perennial rhinitis and nasal polyposis. Insofar as such diseases may cause anosmia/hyposmia, it is reasonable to expect that corticosteroids may improve olfactory functioning also. Unfortunately, the use of oral corticosteroids is restricted owing to deleterious systemic effects, most notably suppression of the H-P-A axis. Topical steroids lack significant local or systemic effects, even with chronic use. We sought to determine whether their anti-inflammatory action was powerful enough to bring back smell. In order to assess long-term changes in smell functioning, we devised a home test. It required identification of twenty odors, ten per nostril, generated by PolyIFF beads. The particular odorants in the test varied from one administration to another. Normals achieve somewhat less than perfect scores on the test and anosmics usually score zero. Patients participated in a protocol designed to optimize both the efficacy and distribution of the topical steroid. First, a two-week course of antibiotics was administered. Flunisolide and nasal decongestant sprays were introduced day 15. The latter was continued for one week only, and, in conjunction with participant's use of Moffat's position, sought to enhance dispersal. Daily subjective ratings of functioning revealed generally about a two week latency for any effect of the flunisolide. This conformed to the outcome of the home odor test, which revealed that, after considerable latency, functioning increased gradually to an asymptote in the mid-hyposmic range. Topical steroids may not offer the magnitude of relief obtainable with systemic steroids (a matter under investigation), but the benefits may often outweigh the risks of alternate medical or surgical treatment.

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Friday Evening

Time-Intensity Quantification of Oral, Nasal and Retronasal Perception of Citral and of Vanillin. Yu-Ling Kuo and Rose Marie Pangborn (Food Science and Tech., Univ. of California, Davis, CA 95616)

Perceived intensity of 0.01% citral and of 0.14% vanillin was quantified over time via computerized entry by 19 subjects, when presented nasally (by sniffing), retronasally (by tasting) and orally (by tasting with nose pinched). For oral sensations, maximum intensity was recorded a few seconds after the 7-sec expectoration, with extinction between 20-35 sec. As expected, little aroma was perceived orally except for ascribing low vanillin intensity to solution with 10% sucrose (SUC+VAN) and of moderate citral intensity to solutions with 0.1% citric acid (CA+SUC, CA+CIT, CA+SUC+CIT). T-I curves for oral sensation by individual subject showed two distinct populations: nine subjects ascribed little or no citral intensity to all samples, whereas the other ten judges gave high citral intensities to all acidified solutions, even CA+SUC without citral. However, no sub-grouping was observed for vanillin. For retronasal perception, the duration was much longer (100-120 sec), and maximum intensity was observed between 16-19 sec after stimulation. For citral, the same ten judges again gave much higher intensities to acidified samples. The enhancement order of citral intensity by added tastants was: CA > CA+SUC > SUC > 0.8% NaCl. Retronasally, vanillin intensity was enhanced by SUC, but depressed by CA and NaCl. For both odorants, the onset and decay of nasal perception was very rapid, with maximum between 6-8 sec and extinction between 12-15 sec. The two groups of subjects scored similarly for nasal perception of citral. The variations were ascribed to tastant interactions or associations rather than subjects' use of intensity of scale.

Development of the Portable Olfactory Sensitivity Meter (POSM). MICHAEL D. RABIN (State University of New York at Purchase), WILLIAM S. CAIN, FRANC T. SCHIET (John B. Pierce Foundation Laboratory and Yale University), and GAIL J. CLAVET (University of Connecticut Health Center).

The goal of the experiments was to produce a portable, home version of the Connecticut Chemosensory Clinical Research Center (CCCRC) olfactory threshold test for patients to self-administer for follow-up self evaluations. Objectives for a portable test included the ability to mail it, ease of self-administration by patients, and agreement with CCCRC threshold procedure results. Interflo pellets were used as the source of odorant to avoid spillage problems in the mail. These pellets are made of compressed filaments of polypropylene and act as excellent adsorbents capable of retaining up to approximately 0.24 ml of liquid. Pellets were found to be uniform in shape, weight, and fluid retention capacity. Headspace analysis from the pellets via GC revealed a linear relation between vapor-phase and liquid-phase concentration. A small scale version of the CCCRC threshold test was designed using small squeeze bottles containing pellets soaked with butanol diluted in mineral oil. Threshold evaluations from 35 subjects (including normals and patients) resulted in an overall correlation of 0.74 between the two tests. In a further attempt at miniaturization, a small, hand held Portable Olfactory Sensitivity Meter (POSM) has been designed. It consists of two turrets, each containing 12 Interflo pellets (9 dilutions of butanol and 3 blanks) that can be dialed to present any combination of concentration vs blank. Preliminary testing shows promise. Further testing of the device is underway to evaluate its utility.

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Automated Human Odor Testing. JAMES C. WALKER, DANIEL B. KURTZ, F. MITCH SHORE (BGTC; R. J. Reynolds Tobacco Co.; Winston-Salem, NC 27102) & VALARIE CLAYBORN (UNC Medical School, Chapel Hill, NC 27514)

A completely automated system for the measurement of the psychophysical and physiological responses of humans to odorant stimulation of the nose and eyes was developed. All aspects of the generation and production of odor stimuli, the monitoring of the output of the olfactometers, the recording of the psychophysical and physiological responses of the subjects and the storage of data were handled by an Apple IIe microcomputer. The nasal and ocular olfactometers were based on Teflon-lined solenoid valves and electronic mass flow controllers; these devices were used to control the ratios of volume flow rates of odorant-saturated and clean air. The output of each olfactometer was measured by photo-ionization detector, the signals from which were led to the microcomputer. Odor stimuli were delivered to custom-fitted odor masks, that allowed separate stimulation of the nose and eyes, through pneumatically operated Teflon flow valves. A video camera was used to record the responses of the eyes. A pneumotachograph, in combination with a pressure transducer, was used to record changes in respiratory behavior. An electronic "mouse" was used to enter the subject's psychophysical responses directly into the microcomputer.

EEG Activity During Administration of Undetected Odors. TYLER S. LORIG, GARY E. SCHWARTZ (Yale University)

To test the hypothesis that undetected odors may alter brain activity, two odors of varying concentrations were administered to sixteen subjects as EEG data were collected. The odors used in this experiment were spiced apple and lavender oil and were presented in three concentrations: threshold, medium and high intensity based on the self-reports of pilot subjects. Odors were administered via a remote olfactometer which provided odorant material mixed with room air. Following each odor administration, subjects completed a questionnaire indicating detection of the odor, its intensity and other characteristics as well as their mood. For purposes of the present hypothesis, comparisons of only the threshold and no odor conditions were appropriate. In order to assure that results were unconfounded by subjects which might have smelled the lowest odorant concentrations, only those subjects which indicated no detection in the threshold odor condition and had limited false alarms to the no odor condition were used in the analyses ($n=7$). Results of the analysis of variance of these data indicated that EEG beta activity differed significantly between the two odorant conditions even though subjects were unaware that odors were being presented ($F(1,6)=8.29$, $p=.0281$). The lavender odorant produced significantly more beta activity across posterior brain regions than did the spiced apple odorant or the no odor control condition. These results support the hypothesis that odors existing in undetectable concentrations affect brain activity and may thus influence mood and behavior.

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Qualitative and Quantitative Responses of Osmic and Anosmic Subjects to Various Odors. ROBERT J. O'CONNELL*, DAVID A. STEVENS (Clark University, Worcester MA 01610), DAVID M. COPPOLA*, ALAN J. GRANT* AND R. PATRICK AKERS* (*Worcester Foundation for Experimental Biology, Shrewsbury MA 01545)¹

Individuals with specific anosmias are generally defined as those who have a good sense of smell but lack the ability to perceive the aroma of a particular odorant. In anosmics this deficiency is usually expressed by an elevated threshold for the substance in question and often by a pronounced shift in the perceived odor quality. The diastereoisomeric ketone, *cis*-4-(4'-t-Butylcyclohexyl)-4-methyl-2-pentanone (pemenone), shares with 5 α -androst-16-en-3-one (androstene) a pronounced urine-sweaty type odor. A specific anosmia for this latter compound has been described. In this study, we determined the prevalence of the anosmia for these two compounds and for other materials which may be described as urinous. We also estimated the generality of these defects by evaluating other compounds for which specific anosmias have been described. Further, we determined the odor descriptors used by osmic and anosmic subjects for each of the compounds for which quantitative data had suggested that a particular anosmia existed. Several hundred subjects were screened with a moderate concentration of pemenone (375 μ M). Of those screened, 38% reported some odor quality with 22% choosing the urine-sweaty descriptor. A total of 39 subjects were examined in detail, 60% of these were classified as anosmic for the intense urine-sweaty odor of pemenone. The subject's magnitude estimates and quality reports for pemenone were significantly correlated with their estimates and reports for androstene. The odor descriptors for both materials, in those judged to be anosmic for the urinous note, were clustered into several distinct categories. These data suggest that different processes are activated during the detection of these odors and that a specific anosmia results when one of these processes is reduced or absent.

¹ We thank Dr. G. Ohloff, Director of Laboratory Research, FIRMENICH SA, Geneva, Switzerland for providing the sample of pemenone. Supported by NINCDS grant NS 14453.

Olfactory Adaptation and Aging. JOSEPH C. STEVENS, WILLIAM S. CAIN, and MICHAEL W. OATLEY (John B. Pierce Foundation Laboratory, New Haven, CT 06519)

Four studies in our laboratory suggest that olfactory adaptation is faster and recovery from it slower in elderly subjects (over 65 yrs) than in young subjects (under 36 yrs). In one study, two groups of five persons each, matched for threshold sensitivity, were adapted to butanol (8-10 sniffs of 155 ppm in air), followed by repeated forced choices between air and the threshold concentration at intervals from 15 to 180 sec. This procedure was repeated 14 times. On the average at 60 sec all five young had recovered to 90% accuracy, whereas at 180 sec no old subject showed any discrimination at all. A second study compared 10 young and 10 old under an identical procedure, except that the subjects were not matched exactly for threshold and adaptation level was 27 times the individual threshold. The average difference between groups was a little smaller here but still pronounced. A similar third study, still underway, compares larger groups of subjects under longer periods of recovery and with certain methodological improvements. So far nearly every old subject has shown much slower but in many cases eventual recovery. A fourth study screened 77 old and 63 young for thresholds to pyridine, and, of these, 31 from each group were selected for equal sensitivity. These then served in three 54-min sessions in which pyridine was gradually infused into a test chamber at either 2.5 L/min, 1.25 L/min, or 0 L/min (sham). The task was line-rating of odor intensity. Both groups showed high false-positive responding, especially the old. At 2.5 L/min infusion both groups were able to track the growth of odor intensity and its decline when the infusion ceased and concentration level began to diminish. The young did the same at 1.5 L/min. After correcting for false-positives (sham condition) it became clear that the elderly failed to detect the lower rate even though the concentration level eventually well exceeded threshold, presumably because of adaptation. This finding bears on the question of detection of slow gas leaks.

This study was supported by NIH Grant AG04287

Dietary Assessment of Patients with Taste and/or Smell Disorders. RICHARD MATTES, BEVERLY COWART, CATHY ARNOLD, PEGGY SCHTAVO, BETSY GARRISON, MORLEY KARE (Monell Chemical Senses Center and Jefferson Medical College)

Diminutions in chemosensory function generally have not been associated with changes in dietary behavior. Discrepant observations have been reported for dysgeusic patients. To further explore these issues, patients evaluated at the Monell/Jefferson CCRC, undergo a dietary assessment which is based upon food recalls, a food frequency questionnaire and a questionnaire eliciting general information on dietary behavior. To date, 74 patients have been evaluated. The incidence of taste and/or smell decrements was 40% and 60% reported dysgeusia and/or dysosmia. Levels of ingestion of 42 nutrients as well as items in 9 food groups (dairy, meat, fat, fruit, vegetable, grain, alcohol, salty snacks and sweet snacks) in the total patient population and in sub-groups with reductions or with distortions of chemosensory function were comparable to levels consumed by a comparative database of 78 healthy individuals. The median body mass index of patients was similar to that of the comparative group and well within normal ranges. However, among all patients, approximately 27% reported a loss of appetite, 82% enjoyed food less, 59% stated their chemosensory problem altered the way they eat, 38% have altered their use of spices, 36% have developed food aversions and 16% have food cravings. Compared to patients with partial or complete taste or smell losses, dysgeusic and dysosmic patients were twice as likely to report problems with appetite, food aversions and cravings. These observations indicate that while patients with chemosensory disorders may experience a decrement in quality of life, they are not necessarily at nutritional risk.

Supported by NIH 5 P50 NS19616.

Diminished Trigeminal Sensitivity in Nasal Disease. B.J. COWART^{1,2}, L.M. YOUNG², B. GARRISON¹, M.R. KARE¹, & L.D. LOWRY² (¹Monell Chemical Senses Center and ²Jefferson Medical College)*

Olfactory evaluation at the Monell-Jefferson Chemosensory Clinical Research Center includes measures of threshold for phenyl ethyl alcohol (PEA) and pyridine (PYR) as well as an odor identification task (ID). We have observed significant age-related declines in performance on each measure in a control sample of healthy individuals (n=117), but in contrast to the distribution of scores for PEA and ID, PYR shows a compression with aging: no control subject has obtained a threshold above the 13th binary dilution of a neat solution. Since others have found absolute nasal trigeminal sensitivity to be unaffected by aging (Stevens & Cain, *Physiol. & Beh.*, 1986, 37, 323-328), we speculated the typical trigeminal threshold for PYR must occur at or below our 13th dilution. Among hyposmic/anosmic clinical patients, however, over a third have obtained PYR thresholds above dilution 13. Assuming this represents a diminution in trigeminal sensitivity, we hypothesized the diminution might be associated with mucosal changes accompanying nasal disease. A comparison of patients whose PYR was above dilution 13 (n=28) with patients whose PYR fell at or below this level but who also evidenced diminished olfaction as measured by PEA and/or ID (n=46) revealed those with elevated PYR were significantly more likely to show clinical signs of nasal disease (chi-square=6.11, p<.02). Moreover, all patients with PYR at or above dilution 11 (n=12) have shown signs of nasal disease. These findings suggest nasal disease may lead to generalized nasal insensitivity to chemical stimuli, not simply diminutions in olfactory sensitivity, and that markedly diminished trigeminal sensitivity may provide a highly specific marker for nasal disease.

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Olfactory Thresholds in Alzheimer's Disease are Correlated with Neuropsychological Assessment of Dementia. MAGDALENA M. JENSEN AND CLAIRE MURPHY (San Diego State University)*.

Deficits in odor recognition (Serby et al., 1985; Doty et al., 1986; Koss et al., 1987) and in odor memory (Murphy et al., 1987) have recently been shown to occur early in the degenerative process of Alzheimer's Disease. Since conflicting results have been reported regarding olfactory thresholds in these patients, we wished to examine further this relationship by increasing the sample size of our population from a previous study (Murphy et al., 1987). We also wished to examine the relationship between degree of dementia and olfactory threshold. The subjects were 22 persons (mean age = 72.8) diagnosed as Probable or Possible Alzheimer's patients by two independent neurologists at UCSD Medical Center's Alzheimer's Disease Research Center. Neuropsychological assessment included the Blessed Dementia Rating Scale (Blessed), the Mini-Mental State Exam (MMS), and the Dementia Rating Scale (DRS). On each of these tests, as the number of errors increases, so does the dementia score. Typical questions are "What year is this?" and "What is your birthdate?" Generally, normal elderly controls do not make errors in answering these questions. The Alzheimer's patients in this study missed a mean of 13.6 of 33 questions (SD = 6.4) on the Blessed, and answered correctly 19.95 of 30 questions (SD = 4.4) on the MMS, and 108.3 of 144 questions on the DRS (SD = 12.6). The olfactory thresholds of the Alzheimer's patients were compared to those of 31 normal elderly (M = 72.5 yrs) and 29 young (M = 20.2 yrs) controls previously tested. The odorant n-butyl alcohol, presented in a two-alternative, forced-choice, ascending series, was used to assess threshold. ANOVA showed significant differences between olfactory thresholds for the three groups, p < .05. A Newman-Keuls Test, p < .05, confirmed that Alzheimer's patients had significantly higher thresholds than the elderly or young controls, and elderly controls had higher thresholds than young controls. Correlations were used in order to determine the relationship between olfactory threshold and dementia for the Alzheimer's patients. The correlations of -.57 (Blessed), .53 (MMS), and .51 (DRS), all p < .05, suggest that as patients make more errors on the dementia scales, they also show decreased sensitivity to odor.

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Endoscopic Intranasal Surgery as an Approach to Restoring Olfactory Function. ALLEN M. SEIDEN and DAVID V. SMITH (Taste and Smell Center, University of Cincinnati Medical Center).

Nasal and sinus diseases have long been implicated in olfactory loss and comprise most of the few etiologies amenable to therapy. Treatment of these conditions, however, has traditionally been correlated more with the alleviation of nasal obstruction and discharge than with any effect on olfaction. Diffuse polypoid disease presumably interferes with smell because of the obstruction of nasal airflow. However, we have seen a number of patients whose allergic rhinitis has been well-controlled with standard immunotherapy and medication, but who remained severely hyposmic or anosmic. Appropriate examination revealed significant ostiomeatal disease in many of these cases. A relatively new surgical technique, functional endoscopic sinus surgery, has been utilized as a treatment for these patients' olfactory impairment. Less invasive than traditional surgical techniques and theoretically providing more long-lasting results, this approach takes advantage of modern endoscopic technology to specifically address the ostiomeatal or middle meatal complex. The olfactory ability of 5 patients was evaluated preoperatively and postoperatively with the University of Pennsylvania Smell Identification Test (UPSIT). Preoperatively, these patients ranged from anosmic to moderately hyposmic (X UPSIT score = 16.5). Four to eight weeks following endoscopic intranasal ethmoidectomy and antrostomy, all 5 of these patients showed significant improvement in their olfactory ability, both objectively (X UPSIT score = 33.5) and subjectively. None have required the use of systemic steroids. Although continued followup is required, we feel that intranasal functional endoscopic sinus surgery is a promising procedure for patients with olfactory loss secondary to nasal and sinus disease.

Fri. Eve Post. Sess. A Abst. # 130 Post. A12

Thirst in Chronic Renal Failure Patients C.A. FARLEIGH, R. SHEPHERD (Institute of Food Research, Colney Lane, Norwich, Norfolk, NR4 7UA, U.K.), J.S. PRYOR (Norfolk and Norwich Hospital, Brunswick Road, Norwich, NR1 3SR, U.K.).

Thirst is reported to be a symptom in patients with chronic renal failure, and in a previous study (Farleigh, Shepherd and Pryor, Clinical Science 1986, 71, Suppl 15, 77p) we have demonstrated a greater overall thirst in these patients when compared to control subjects. The greater thirst is due, in part, to restrictions in fluid intake and removal of body fluid during dialysis. This study was designed to quantify and assess changes in thirst in patients with chronic renal failure. A questionnaire comprising 17 questions relating to thirst, prospective drinking, and previous drinking, was developed and validated. Twenty-five patients undergoing regular haemodialysis were recruited on to the study. Observations were made on three separate visits to the outpatients dialysis unit and on three corresponding control days (24 hours following dialysis). On each occasion, at the beginning and end of dialysis, blood samples were taken and analysed for electrolytes along with renin, aldosterone, arginine vasopressin, and atrial natriuretic peptide. Body weight was measured to determine fluid loss during dialysis. Patients were required to complete the thirst questionnaire at the start, middle and end of dialysis and at the equivalent times on the following day. The ratings on the questionnaires were measured and data analysed by analysis of variance. Preliminary analysis of the results indicate that in general the thirst ratings were correlated with the time since the last drink. Thirst was greater on the day following dialysis than on dialysis day, whilst there was no difference in drinking patterns on these days. The relationship between the thirst ratings and changes in hormone status, electrolyte balance and fluid balance are currently being examined.

This research was carried out with the aid of a grant supplied by the East Anglian Regional Health Authority.

Fri. Eve Post. Sess. B Abst. # 131 Post. B1

Two Transduction Pathways Identified in Distinct Subpopulations of Rat Taste Cells. MYLES AKABAS, JANE DODD & QAIS AL-AWQATI (Depts. of Medicine & Physiology, Columbia Univ.)

The final step in the taste cells' response to rapid stimuli is the secretion of neurotransmitter. Because in almost all other neurosecretory systems an increase in the cytoplasmic calcium concentration ($[Ca_i]$) is one step in the process of neurotransmitter secretion, we measured the $[Ca_i]$ in individual rat taste cells, loaded with the fluorescent calcium sensitive dye fura-2, as an assay for response to stimulation. Stimulation with denatonium, a membrane impermeant bitter substance, causes an increase in the $[Ca_i]$ from a mean level of 65 (± 12) nM in the resting state to 127 (± 32) nM ($N=8$ cells) in a small subset of the taste cells. A similar rise is seen when the cells are stimulated in the absence of extracellular calcium implying that denatonium induces release of calcium from internal stores. Following exposure to denatonium, depolarization of the cells by raising bath potassium to 70 mM, does not cause a further rise in the $[Ca_i]$ in the cells that responded to denatonium. However, the $[Ca_i]$ does increase in a separate subpopulation of taste cells.

This implies that the transduction of bitter stimuli occurs in a distinct subpopulation of taste cells. It involves binding of the bitter ligand to a cell surface receptor. This results in the generation of an intracellular second messenger and release of calcium from internal stores. There appears to be no voltage-dependent calcium influx pathway in these cells. There is a second separate subset of taste cells which do possess a voltage-dependent calcium influx pathway and these cells may be involved in the transduction of other taste modalities.

Fri. Eve Post. Sess. B Abst. # 132 Post. B2

Transduction Mechanisms of Iontophoretic Stimuli: The Receptor Potential. M. SCOTT HERNESS. (The Rockefeller University, New York, NY 10021)

The receptor potential to iontophoretic stimuli, a method of presenting ionic tastants with a weak electric current, was studied using intracellular recording techniques on frog taste cells. Receptor potentials to NaCl presented rapidly and iontophoretically differed in at least two obvious manners, the rise/fall slope and the conductance change. Iontophoretic stimuli produced receptor potentials with very fast slopes from resting to stimulated state compared to rapid receptor potentials. This is thought to reflect the rate of stimulus delivery/removal. Receptor potentials to rapid NaCl showed a decrease in membrane resistance (i.e., a conductance increase) in the stimulated state while those to iontophoretic presentations showed no change in resistance (i.e., no conductance change). Reversal potential measurements had similar implications to the conductance change. Rapidly presented NaCl demonstrated a clear reversal potential at positive membrane potentials though iontophoretic presentations showed no reversal potential even at positive membrane potentials. Receptor potentials to iontophoretically delivered Mg^{++} were somewhat larger than to Na^+ at the same current values indicating that the phenomenon of ion specificity, observed with iontophoretic stimuli at the neural level, may also be present within the receptor cells. The lack of conductance change and of a reversal potential argue for different transduction mechanisms for rapid and iontophoretic stimuli. Iontophoretic receptor potentials may be produced without the activation of ion channels.

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Effects of calcium channel blockers on chorda tympani nerve taste responses in the rat. S.D. FELTON and C.M. MISTRETTA (Schools of Nursing and Dentistry, University of Michigan, Ann Arbor, MI 48109)

Investigators have demonstrated that amiloride and 4-aminopyridine selectively depress responses from the rat chorda tympani nerve to NaCl and KCl, respectively. To learn whether there are effects of calcium channel blockers on salt taste responses, we examined electrophysiological responses in the presence of verapamil and diltiazem. Taste responses were recorded from the whole chorda tympani nerve in 30 adult rats. Stimuli included CaCl_2 (0.01M to 0.75M) and NaCl, KCl, and NH_4Cl (0.05 to 0.25M), dissolved in water or a solution of the channel blocker. Ten ml of stimulus dissolved in water were applied to the tongue for 30 seconds, followed immediately with a second application of 10 ml of stimulus dissolved in the blocker. Summated response magnitudes were measured at 10 seconds after each stimulus application. A ratio of the response to the stimulus in the presence of the blocker was calculated relative to the response in water alone. This ratio was compared to a control ratio obtained from 2 consecutive applications of the stimulus in water. Concentrations of 0.5mM to 25mM verapamil and 1mM to 20mM diltiazem were studied, and 5mM was subsequently used as a standard concentration because it suppressed taste responses and did not itself elicit a large response. Chorda tympani responses to CaCl_2 at concentrations above 0.10M were reduced in the presence of the blockers. A maximal effect was observed at 0.25M CaCl_2 , with verapamil producing a 16% reduction and diltiazem producing a 12% reduction in taste response. Responses to NaCl and NH_4Cl were not affected by the blockers. The data suggest that membrane components sensitive to calcium channel blockers may play a role in taste responses to CaCl_2 . However, the magnitude of the reduction in taste response with these blockers is much less than that observed with chemicals that block sodium and potassium transport.

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Video Monitoring of Taste Cell Activity in Tongue Slices Using Voltage-sensitive Dyes. M.M. DASTON, H.J. DUNCAN, J.M. LUNA, J.N. BROUWER, R.C. GESTELAND, M.T. SHIPLEY (Dept. of Anatomy and Cell Biology, University of Cincinnati, College of Medicine).

Taste receptors are small, densely packed cells with no morphological subclasses at the light microscope level. Intracellular microelectrode recordings have yielded conflicting results with respect to the existence of differential responses to different stimulus substances. The use of voltage-sensitive dyes (VSDs) may provide a means to overcome the sampling problem that is inherent in single-unit studies. VSDs are molecules that bind to excitable membranes and indicate changes in membrane potential by changes in fluorescence, absorbance, or birefringence. They allow activity of many cells to be recorded simultaneously with adequate temporal and spatial resolution. Diode detector arrays resolve single action potentials with limited spatial discrimination. Video techniques have very high spatial resolution but are limited in time resolution to about 30 milliseconds. They are adequate for observing slower receptor events and average nerve activity changes.

In this study VSDs (RH414 and RH160, obtained from Molecular Probes, Inc.) were used with cross-sectional slices (250-300 μ) of frog tongue. The slice preparation was chosen over the whole tongue or whole animal because it provides optimal visualization of the organization of cells in the taste papilla, and it eliminates mechanical and electrical interference due to heartbeat and respiration. The slice was laid flat in a shallow chamber and immobilized with a coverslip. Tastants (NaCl, sucrose, quinine hydrochloride, and citric acid) were delivered and removed via cotton wicks which were placed in the chamber on opposite sides of the slice. Changes in the fluorescence intensity of the dyes were recorded, enhanced, and analyzed using a video-based image analysis system.

Stimulus-specific changes were observed in discrete subregions of the papilla. In addition, changes similar to those reported in whole nerve electrophysiological recordings were observed in papillary nerve stumps. Correlative electrophysiological studies are in progress.

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Cross-Adaptation of the Response to Sodium and Chloride Salts by the Hamster Chorda Tympani Nerve. BRADLEY G. REHNBERG, MARION E. FRANK, AND THOMAS P. HETTINGER. (University of Connecticut Health Center, Farmington, CT 06032, USA)

The transient response of a peripheral taste nerve will decline (adapt) during the continuous presentation of chemical stimuli to its receptive field. Cross-adaptation occurs if a reduced response to a stimulus is observed when the test stimulus is presented immediately after some other different stimulus (Smith & Frank, *Physiol. & Behav.* 8: 213). The degree of cross-adaptation between two stimuli is thought to reflect the overlap of their "transduction processes" (Caprio & Byrd, *J. Gen. Physiol.* 84: 403). We measured self- and cross-adaptation of the whole-nerve response by the hamster chorda tympani during reciprocal presentations of stimuli that gave equal responses when applied to the tongue singly: 0.1 M NaCl, 0.1 M NaNO_3 , 0.1 M NaBr, 0.4 M NaAcetate, 0.09 M LiCl, and 0.4 M NH_4Cl . Nearly complete reciprocal cross-adaptation was seen for NaCl, NaNO_3 , and NaBr. Results for those sodium salts paired with LiCl also showed strong but somewhat asymmetrical cross-adaptation. The response to NaCl, NaNO_3 , or NaBr after adaptation to LiCl was slightly stronger than the reciprocal cross suggesting similar but nonidentical transduction pathways. Strongly asymmetrical cross-adaptation was found between NaAcetate and the other sodium salts. The response to NaCl, NaNO_3 , or NaBr after adaptation to NaAcetate was minimal. In contrast, the response to NaAcetate during the reciprocal cross was strong. This complex role for the acetate anion is presently being examined using single fiber data. NH_4Cl showed the least tendency to cross-adapt with other stimuli suggesting the involvement of a relatively separate transduction pathway.

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Calcium Chloride depresses specific tastes in rat. GILL, J. M. II, ERICKSON, R. P., and SCHIFFMAN, S. S., (Departments of Psychiatry and Psychology, Duke University, Durham, NC, 27706)

The divalent cation calcium has become a popular food additive and may also play a significant role in taste transduction. Multi-unit responses were recorded in the rat NST to 100mM NaCl, 500mM sucrose, 10mM HCl, 10mM QHCl, 50mM NH_4Cl , and 30, 100, and 300mM KCl before and after a 5 minute application of 5mM CaCl_2 (comparable to some commercial calcium supplements) to the anterior tongue. Responses were measured 10 seconds after the initial change from baseline activity. Applications of 5mM CaCl_2 for 5 minutes resulted in significant changes in the NST response to all concentrations of potassium chloride, as well as QHCl, and NH_4Cl . 30mM KCl was suppressed 76% while higher potassium concentrations were depressed to a lesser degree (100mM and 300mM were suppressed 24% and 19% respectively). The response to NH_4Cl was decreased 57%, and QHCl was suppressed by 84%. 100mM NaCl, 10mM HCl, and 500mM sucrose not were significantly affected. All stimuli recovered to pre-adaptation levels after a deionized water rinse. These results are similar to those observed by Schiffman, Simon, Gill, and Erickson (AChES X abstract) after a 5 minute application of BaCl_2 . While it is unclear whether the results reported here are due to calcium's interaction with potassium transduction mechanisms or are the result of an adaptation phenomenon it is certain that the addition of CaCl_2 in concentrations similar to those used commercially can alter the gustatory systems response to specific tastants.

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Receptor Site Types for Amino Acids in the Facial Taste System of the Channel Catfish. S. WEGERT and J. CAPRIO (Department of Zoology & Physiology, Louisiana State University, Baton Rouge, LA. 70803)

Although the gustatory system of the channel catfish, *Ictalurus punctatus*, responds to L-amino acids, the number of different receptor site types for these compounds has not been thoroughly investigated. Previous electrophysiological experiments from this laboratory (see Caprio 1987 in Sensory Biology of Aquatic Animals) have indicated independent gustatory receptor sites for L-alanine and L-arginine, respectively, which were subsequently confirmed biochemically by receptor binding experiments (Cagan, 1986, Comp. Biochem. Physiol., **85A**:355-358). In addition, a third receptor site type was proposed for L-proline in the oral (IX-X) taste system of this species (Kanwal & Caprio, 1983, J. Comp. Physiol., **150**:345-357). The present study identifies a relatively independent L-proline receptor site type in the facial (VII) taste system of the channel catfish and confirms the existence of D-alanine receptor sites (Brand et al., 1987, Brain Res., **416**:119-128). Preliminary results further indicate a D-arginine site distinct from that which binds its L-isomer. The relative independence of the gustatory binding sites for amino acids was indicated from *in vivo* electrophysiological cross-adaptation (i.e. competition) experiments. The single "adapting" and eight "test" amino acids were adjusted in concentration to provide approximately equal peak integrated responses in the unadapted state. The extent of cross-adaptation was determined by comparing the facial taste responses to a series of "test" amino acids under the control regime (carrier flow of water superfusing the maxillary barbel) to those during each of four "adapting" amino acid regimes: L-alanine, L-arginine, a mixture of L-alanine and L-arginine, and L-proline, respectively. A quantitative analysis will be presented to support the existence of these additional relatively independent gustatory receptor sites.

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Actions of Modulcin: Dynamics of Suppression of Receptor Cell Responses to Sucrose and Surface Activity. L.M.Kennedy & D.E.Kolodny (Clark Univ., Worcester MA 01610).

Modulcin (from *Hovenia dulcis* leaves) selectively suppresses sweetness perception in humans(1) and behavioral and receptor cell responses to sucrose in flies(2). Modulcin appears to be a triterpene saponin glycoside(1,3), probably a surfactant. To characterize modulcin actions, we used the paradigm in (2) to measure the magnitudes and durations of suppression for a concentration series (0.01-0.1% w/v) of purified modulcin (HDE)(1,3) on *Phormia regina* receptor cell action potential responses to sucrose (50mM in NaCl 50mM). To test a role for surface active properties in those actions, we determined the critical micelle concentration (CMC) of HDE by measuring the fluorescence at 525nm (excitation at 436nm) of acridine orange (AO) (4.75 μ M) in various HDE concentrations (0.01-1.0%). For the receptor cell responses, firing was initially suppressed and then gradually increased over the 10 min post-HDE. HDE concentration affected the magnitude of suppression ($p < 0.000$, Kruskal Wallis ANOVA): The lowest effective concentration was 0.025% ($p < 0.02$). Suppression increased with HDE concentration (slope -0.97) to a maximum at 0.075% ($p < 0.001$) and then decreased (slope 1.92) at 0.01% HDE ($p < 0.000$) (Mann Whitney Tests). As the magnitude of suppression increased, the duration also increased. For AO fluorescence, there was no change from 0.01-0.015% HDE, a sharp change from 0.015-0.067% (slope -1.38), and a slow change (slope -0.13) to 0.1% HDE. The fluorometry indicates a CMC of 0.041% HDE. The narrow range of effective HDE concentrations for suppression of receptor cell responses, together with correspondence of the concentration-effect curves for the physiology and chemistry suggest that physical-chemical factors (eg. hydrophobic and dielectric effects) that contribute to the energy relationships which drive micelle formation are necessary factors for the taste actions of modulcin.

- 1) Kennedy, L.M. et al., Chem. Senses, 1988, in press.
- 2) Kolodny, D.E. & L.M.Kennedy, Chem. Senses, 1988, in press.
- 3) Sefacka, R. & L.M.Kennedy, Ann.N.Y.Acad.Sci. **510**, 1987, 602.

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Differential Actions of Propanol or Propanol/Salt Mixtures on Receptor Cell Responses to Sucrose or NaCl. D.M.BOURASSA & L.M.KENNEDY (Clark Univ., Worcester MA 01610).

Normal alcohols suppress fly behavioral and taste receptor cell action potential responses to sucrose. The receptor cell effects are biphasic over time-- firing is first suppressed and then increased and irregular, as are effects of the sweetness-suppressing taste modifiers, gymnemic acids, ziziphins and modulcin(1,2). For a NaCl stimulus, a propanol (prop)/NaCl mixture produces similar biphasic effects, but the effects of prop without NaCl are different(3). Electrolytes are known to alter physical-chemical activities. Since physical-chemical surface active properties may play a role in the actions of taste modifiers(4), we studied the effects of mixtures of prop 1.2M with NaCl 50 or 500mM on *Phormia regina* receptor cell action potential responses to sucrose 50mM (in NaCl 50mM) or NaCl 500mM, using the paradigm in (1-3). The solvent for all treatment solutions was Tris(15mM, pH7) (1-3). Treatments were with prop, prop/NaCl 500mM, prop/NaCl 50mM, prop/sucrose 50mM, NaCl 50mM or Tris (9 flies/ condition). Prop significantly suppressed initial posttreatment responses to both sucrose and NaCl; then sucrose responses increased, but NaCl responses did not. For NaCl responses, both prop/NaCl mixtures produced significant initial suppressions of longer duration and then increased responses, but prop/NaCl 50mM had no significant effects on sucrose responses. Neither NaCl 50mM nor Tris significantly affected responses ($p < 0.01$, Kramer Tests). Thus prop affects responses to sucrose and also to NaCl, but affects them differently. An electrolyte changes the effects of prop and changes them differently for sucrose or NaCl responses. These data support the proposal that physical-chemistry plays a role in the taste actions of propanol, and perhaps also of taste modifiers, and indicate that those actions affect transduction mechanisms for sucrose or NaCl differently.

- 1) Kennedy, L.M. Ann. N.Y. Acad. Sci. **510**, 1987, 409.
- 2) Kolodny, D.E. & L.M. Kennedy, Chem. Senses, 1987, 672.
- 3) Bourassa, D.M. & L.M. Kennedy, Soc. Neurosci. Abstr., 1987, 139.
- 4) Kennedy, L.M. & B.P. Halpern, Chem. Senses, 1980, 123.

Supported by NIH NS 24159 to L.M.K.

Distribution of Na, K, Ca, and Ca-mediated Channels in Taste Cells of the Mudpuppy. DON W. MCBRIDE, JR., STEPHEN D. ROPER, (Colorado State Univ.)

Ion channels play a fundamental role in taste transduction. We have investigated the localization of ion channels in taste cells. The lingual epithelium from *Necturus maculosus* was dissected from the animal and placed in a modified Ussing chamber. This permitted us to isolate the mucosal and serosal surfaces of the epithelium: solutions and agents applied to one surface do not diffuse to the opposite side because of the junctional complexes found near the apical ends of the epithelial and taste cells. Taste buds were visualized under a water immersion objective at 400X and individual cells impaled with microelectrodes. During an impalement, the mucosal and serosal chambers were perfused independently and with several changes. In the presence of symmetrical solutions of amphibian physiological saline action potentials (a.p.) can be elicited by intracellular current injection through the recording electrode. When 100nM TTX was added to the serosal chamber the a.p. disappeared. Addition of TTX to the mucosal chamber had little or no effect on the a.p. This indicates that Na channels are present on the basolateral membrane and suggests that the number of Na channels on the apical membrane is much less than on the basolateral. The addition of 8mM TEA to the mucosal chamber changed the duration of the a.p. from 10-20 msec to 1-5 sec. Addition of TEA to the serosal chamber did not significantly change the shape of the a.p. This indicates the K-delayed rectifier channels are located preferentially, if not exclusively, on the apical membrane. A series of substitution (Mg^{++} for Ca^{++}) and Ca-channel blocker (0.1mM Cd^{++}) experiments showed that Ca-channels are present in both the apical and basolateral membranes. Another series of substitution experiments (Ba^{++} and Mg^{++} for Ca^{++} in the presence of TEA) revealed the existence of a Ca-mediated, TEA-insensitive current on the basolateral and not on the apical membrane. The possible significance and role in taste transduction of these asymmetrical distributions will be discussed.

This work was supported by NIH grants NS24107, NS20486, and AG06557.

Fri. Eve Talk 7:00 PM Abst. # 141

An Ultrastructural Comparison Between the Synapses of Fungiform, Foliate and Circumvallate Taste Buds of the Mouse. JOHN C. KINAMON, DAVID M. HENZLER and SUZANNE M. ROYER (University of Colorado, Boulder, CO 80309).

One of our goals is to understand how taste receptor cells are connected to sensory nerve fibers. Our initial approach to solving this problem has been to study the structure of taste buds from the circumvallate (CVP), foliate (FOP), and the fungiform (FUP) papillae of the mouse. Serial sections of taste buds are sliced and then examined with the high voltage electron microscope. Next we prepare 3-D reconstructions of taste cells, nerve fiber arborizations, and synapses. Features common to synapses from all three papillae are: 1) Synaptic clefts ranging from 16-30 nm, 2) Clear vesicles 40-70 nm in diameter, and 3) Asymmetric synapses in which the presynaptic thickening is more developed than the postsynaptic thickening. We find that CVP and FOP synapses are generally similar in structure and distribution, but that FUP synapses are significantly different, both qualitatively and quantitatively. For example, approximately 20-30% of the taste cells in a CVP form synapses onto sensory nerve fibers. FUP synapses are much less common, being associated with only about 2-5% of the FUP taste cells. Of greater significance is our observation that, although FUP synapses are much less common, they have many more synaptic vesicles associated with their synapses than CVP or FOP synapses. We speculate that fewer taste cells with synapses in a FUP taste bud suggest that these taste cells might be more active, especially because they contain so many more synaptic vesicles. The question remains, however, as to why FUP synapses are so different from FOP and CVP synapses. Are FUP taste cells qualitatively different or does the difference in synaptic structure result from the different innervation of the FUP cells? It is possible that innervation by different sensory nerves may cause alterations in both synaptic structure and the number of synapses within a taste bud. We propose to test this hypothesis by carrying out cross-reinnervation experiments to determine if glossopharyngeal innervation of FUP taste buds (or chorda tympani innervation of CVP taste buds) results in altered synaptic ultrastructure.

This work was supported in part by NIH grants NS21688 and RR-00592 and a grant from the Procter & Gamble Co.

Fri. Eve Talk 7:30 PM Abst. # 143

The Putative cAMP Chemoreceptor of Paramecium. JENNY BAEZ, JIN ZHANG, BRIAN COTE, and JUDITH VAN HOUTEN (University of Vermont)

Cyclic AMP is an external chemical stimulus for *Paramecium tetraurelia*. Cells accumulate in cAMP and there is a saturable, specific, low affinity binding of ^3H -cAMP that correlates with chemoresponse (Smith et al., *Biochim. Biophys. Acta* 928: 171-8, 1987). In order to identify the membrane protein that is the cAMP receptor, we have used 2 approaches. 1) Affinity chromatography has been used to identify one major cAMP binding protein from the cell body membrane. This protein is 48,000 in molecular mass and shows specificity for binding that parallels the specificity of the chemoresponse. 2) 8- N_3 -cAMP is a photoaffinity probe that will covalently bind to surface proteins when cells are exposed to uv light in its presence. 8- N_3 -cAMP specifically inhibits chemoresponse to cAMP and not other responses to folate and acetate. ^{32}P -8- N_3 -cAMP labels all surface-exposed cAMP binding proteins when photolyzed with whole cells. These labeled proteins include a protein of 48 kd. Our current approaches to the study of this putative receptor are to a) clone the gene for this protein and b) quantify this protein in a mutant that is conditionally not responsive to cAMP. The probes being prepared for cloning from a genomic *gt11 Paramecium tetraurelia* library are oligonucleotides based on microsequencing of CNBr cleavage fragments and polyclonal antibodies produced against this 48 kd protein.

Supported by NSF.

Fri. Eve Talk 7:15 PM Abst. # 142

High Voltage Electronmicroscopic Analyses of Olfactory Bulb Granule Cell Spine Geometry. CHARLES A. GREER (Sections of Neurosurgery & Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510)

The spiny dendrites of olfactory bulb granule cells are of unique interest due to their mixed afferent and efferent properties. It is through the spiny appendages of these dendrites that the reciprocal dendrodendritic synaptic interactions with mitral and tufted cells occur. As part of an ongoing interest in characterizing dendrodendritic microcircuits, Golgi procedures were developed for studying spine geometry with high voltage electronmicroscopy (HVEM). Granule cells were impregnated with gold chloride following a modification of a technique first described by Fairen et al. (*J. Neurocytol.* 1977, 6:311). Sections 80um thick, containing well impregnated neurons, were embedded in EPON and serially sectioned at 1-3um. Lead staining was not employed so that optimal contrast between impregnated and non-impregnated structures would be attained. Impregnated neurons were studied with HVEM at 1,000kV; both flat and stereo pair micrographs were obtained. Analysis of the data revealed a complexity in the morphological geometry of granule cell spines that was not previously recognized. Spine necks tended to follow long complex courses, often in excess of 4um. This exceeds prior estimates based on light microscopy by approximately 2um (Greer, *Soc. Neurosci. Abst.* 1987, 13:1412). Of greatest significance, spinous appendages were frequently observed to bifurcate resulting in multiple spine heads continuous with the parent dendrite through a single common neck. These observations force us to revise our concepts of the functional properties of granule cell spines and consider the possibility that the functional role of bifurcated spiny arrays may differ from single unbranched spines. It may be that bifurcated arrays function in a more isolated fashion with less effectual transfer of graded potentials than unbranched spines.

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Fri. Eve Talk 7:45 PM Abst. # 144

Immunocytochemical localization of Neuronal Growth-Related Membrane Proteins in Rodent Taste Buds. THOMAS E. FINGER (Univ. Colorado Medical School), LARRY BENOWITZ (Harvard Medical School), & KARL PFENNINGER (Univ. Colorado Medical School).

The membrane proteins GAP 43 (= F1, = B-50) (Benowitz et al, *TINS* 1987) and 5B4 antigen (Wallis, et al., *J Cell Biol.* 1985) are two chemically distinct proteins that are developmentally regulated in neurons. The receptor cells in taste buds are neuron-like cells that undergo a continuing cycle of generation, differentiation and maturation which necessitates remodeling of synaptic connections at the base of the taste buds. Thus taste buds appeared to be a likely site in which neuronal growth-related proteins might be expressed, either in the taste receptors or in the continuously changing nerve fibers that innervate them. Accordingly, antisera directed against GAP 43 and 5B4 were utilized for immunocytochemical investigations of lingual taste buds in the laboratory rat. Tissue was fixed in 4% paraformaldehyde in phosphate-buffered saline, and prepared for routine immunocytochemistry. Virtually all taste buds were found to contain cells immunoreactive for each antiserum. For both antisera, the immunolabeling appears to be membrane associated; i.e. the entire taste cell from apex to basal lamina is outlined by reaction product. The 5B4 immunolabeling is relatively homogeneous along the length of each reactive cell. GAP 43-immunolabeling is more heterogeneous; a single cell will exhibit regions of intense punctate label scattered along a more lightly labeled membrane. Double label experiments indicate that the 5B4- and GAP 43-immunoreactive cell populations within each taste bud are mutually exclusive. Virtually no cells are immunoreactive for both antisera. Thus 5B4 and GAP 43 antibodies may label different developmental phases of the taste receptor cells. In addition, the presence of two presumed neural membrane proteins in taste cell membranes may be indicative of an embryonic neuronal lineage for these cells. That is, taste cell precursors may arise from neural plate or neural placodes rather than from indifferent ectoderm or endoderm.

Fri. Eve Talk 8:00 PM Abst. # 145

Localization of Adrenergic Nerve Terminals in the Olfactory Mucosa. B. ZIELINSKI, M.L. GETCHELL and T.V. GETCHELL (Dept. of Anatomy and Cell Biology, Wayne State University School of Medicine Detroit, MI 48201)

Previous studies demonstrated adrenergic and cholinergic regulation of secretory granule content in Bowman's glands of the salamander (Getchell and Getchell, J Comp Physiol A 155:435-443, 1984; Getchell et al., J Comp Physiol A 160: 155-168 1987). In the present study we describe the electronmicroscopic appearance of axonal varicosities with small dense core vesicles that are characteristic of "en passant" release of adrenergic neurotransmitters. The electron density of small dense core vesicles was enhanced by pre-treatment with 5-hydroxydopamine and by tissue fixation with a modified chromaffin method. Adrenergic terminals present in the lamina propria were adjacent to blood vessels, Bowman's glands and melanocytes and were located within 0.05 um to 1.0 um of presumed target cells. The mean diameters of small dense-core vesicles of terminals associated with blood vessels, Bowman's glands and melanocytes were 54 ± 7 nm, 50 ± 9 nm and 56 ± 8 nm respectively. Nerve endings between acinar cells of Bowman's glands contained agranular vesicles with a mean diameter of 65 ± 8 nm that are characteristic of cholinergic terminals. Adrenergic terminals in the olfactory epithelium were adjacent to blood vessels located alongside ducts of Bowman's glands. The mean diameter of the small dense-core vesicles was 56 ± 6 nm. Nerve terminals with agranular vesicles were not observed in the olfactory epithelium. The present study identifies adrenergic terminals, localizes them adjacent to their presumed target cells and characterizes the adrenergic vesicles by electron density and size. Supported by NSF Grant BNS-07949 (MLG) and NIH Grant NS-16340 (TVG).

Fri. Eve Talk 8:15 PM Abst. # 146

Immunohistochemical Study on the Rabbit Olfactory Epithelium in Adulthood, During Development, and Following Unilateral Bulbectomy, Using Monoclonal Antibodies. NORIHIKO ONODA (Dept. Physiol., Sch. Med. Gunma Univ.)

Immunohistochemical staining patterns for monoclonal antibodies produced against the rabbit olfactory bulb were studied in the rabbit olfactory epithelium in adulthood, during development, and after olfactory bulbectomy. One monoclonal antibody, 112D5 (MAB 2D5), stained all of olfactory receptor cells in the adult rabbit, whereas the other, 114G12 (MAB 4G12), stained the upper 2/3 to 3/4 of the receptor cell layer. Both MABs did not stain the supporting cells, basal cells, and Bowman's glands. MAB 2D5 stained all of receptor neurons in the olfactory epithelium and vomeronasal organ during development. 4G12-positive cells scatteringly appeared in the epithelium of the embryonic day 17 fetus. At embryonic day 25/26, 4G12-positive cells were situated in the superficial receptor cell layer. The arrangement in the 4G12-positive and -negative receptor neurons was 'superficial-positive' and 'deep-negative'. Thereafter, a gradual increase in the 'superficial-positive' cells was accompanied by a decrease in the 'deep-negative' cells. These changes continued until postnatal day 30. A slight decrease in olfactory receptor neurons occurred at 24 hours after the bulbectomy. MAB 2D5 stained all receptor neurons including degenerating neurons, but MAB 4G12 showed a rapid decrease in immuno-staining so that 4G12-positive cells disappeared within 7 days after lesion. 4G12-positive cells reappeared at 4 weeks following lesion. By three months, 4G12-positive cells were arranged in a line at the apical region of the receptor cell layer, suggesting a recapitulation of developmental pattern of receptor neurons. Thereafter, the 4G12-positive cells increased progressively and staining pattern of the olfactory epithelium recovered by 6 months. MAB 4G12 is thus the first marker, that is not specific to the olfactory neurons, and that can be used to characterize certain embryonic traits during the degeneration and regeneration of the olfactory epithelium in the adult mammal.

Fri. Eve Talk 9:00 PM Abst. # 147

2DG Activity and Monoclonal Antibody Staining in the Olfactory System of the Fetal Rat. P.E. PEDERSEN, B. FRIEDMAN, G.M. SHEPHERD and S. HOCKFIELD. (Yale University School of Medicine).

The accessory olfactory bulb (AOB) is the site of intense 2-deoxyglucose (2DG) activity in E(embryonic day)22 fetal rats *in utero*. Together with behavioral and anatomical evidence, this finding suggested that fetuses may use this pathway to sample and detect their chemical milieu. We report the use of monoclonal antibody Rat 204 generated in this laboratory in combination with 2DG autoradiography to compare the biochemical and functional differentiation of components of the peripheral olfactory pathways. In the adult rat, Rat 204 recognizes a subset of olfactory neurons situated in the dorsal recess of the main olfactory epithelium and in the vomeronasal organ and their respective axonal projections. In the fetal rat, at E13, the immunoreactivity is similarly spatially restricted to dorsal recess and to the vomeronasal axons. Fetuses that receive 2DG *in utero* according to previously reported methods exhibit differentially dense areas of metabolic activity throughout the neuraxis. At the earliest age examined, E14, areas of dense 2DG uptake are evident within the olfactory mucosa and presumptive olfactory bulb. Within the olfactory mucosa, these areas appear in the dorsal recess. There is also dense uptake in the vomeronasal organ. Of interest is the finding that the areas of high metabolic activity in the mucosa overlap with the areas that are recognized by Rat 204. The early expression of antigens in subcomponents of the olfactory system, correlated with metabolic activity, suggests that the neurons in the dorsal recess and in the vomeronasal organ may undergo early functional differentiation. This may be significant for the development of odor detection.

Fri. Eve Talk 9:15 PM Abst. # 148

Growth Associated Protein (B50/GAP43) Expression in the Developing Olfactory Neuroepithelium. F. L. MARGOLIS AND J. VERHAAGEN (Roche Institute of Molecular Biology, Nutley, New Jersey) and A. B. OESTREICHER AND W. H. GISPEN (Institute of Molecular Biology, University of Utrecht, Holland)

The unique ability of the adult olfactory neuroepithelium to generate new neurons from a population of precursor cells present in the basal cell layer of this tissue is well known. This phenomenon has been studied as a model of neural development and plasticity. However, the molecular mechanisms underlying this neurogenetic phenomenon remain unknown. Membrane associated, the neuron specific, phosphoprotein B-50/GAP-43 is one of the best characterized molecular markers associated with developing and regenerating neurons. This prompted us to study the regulation of its expression in the olfactory neuroepithelium during development and in response to lesions of the olfactory nerve. We show that: 1) in neonatal rats B-50 can be visualized throughout the population of olfactory receptor neurons including the cell bodies, dendritic knobs and axons of all receptor cells, 2) by contrast, in mature receptor neurons (as defined by immunocytochemical staining for OMP) B-50 immunoreactivity is virtually absent. Thus, as development proceeds the expression of the B-50 protein becomes progressively more and more confined to those cells adjacent to the basal lamina. Finally, 3) in rats 3 1/2 and 6 months of age only a small number of cells in the basal cell layer express B-50 immunoreactivity. A significant fraction of these B-50 positive cells also bear stained dendritic and neuritic processes. The presence of this neuron specific growth-associated protein in cells adjacent to the basal cell layer of the olfactory neuroepithelium provides molecular evidence consistent with the ability of this tissue to manifest continuous neuronal turnover. The response of this protein during recovery from olfactory nerve lesions is under investigation. Expression of the B-50 phenotype will permit the identification and separation of immature olfactory receptor cells in suspensions. Finally, it should facilitate the characterization of putative olfactory cell lines *in vitro*, a problem which remains unsolved.

On the Lifespan of Olfactory Receptor Neurones

Kittel, P.W. (Department of Physiology, University of Adelaide, S.A.) Mackay-Sim, A. (School of Science, Griffith University, Nathan, Qld.), Leigh, C. (Department of Anatomy, University of Adelaide, S.A.) and Hodgson, A.J. (Department of Immunology, Flinders University Medical School, S.A., Australia).

It is often stated that olfactory receptor neurones undergo continuous turnover with a concomitant turnover of their synapses in the olfactory bulb. Indeed various studies have indicated that the average lifespan of these cells in mice is about 30 days. This latter conclusion was challenged recently with evidence that some receptor cells may live for at least 12 months (Hinds, et al. *Anat. Rec.*, 1984, 210: 375-383). These authors suggested that in adulthood most receptor cells may remain alive and connected with the olfactory bulb, and that it is mainly the newly formed cells which turnover, degenerating without forming synapses. The present study reexamines receptor cell longevity in adult mice in two experiments: first, ³H-thymidine autoradiography to test survival of nuclei labelled during cell division in the basal cell layer, and second, survival of receptor cells retrogradely labelled after injection of colloidal gold into the olfactory bulb. After periods of 7-90 days there were similar numbers of nuclei labelled with ³H-thymidine. Thus cells arising from basal cell division survived for at least 90 days, and most had migrated into the receptor cell layer of the epithelium. After injection of colloidal gold into the olfactory bulb there were significant amounts of gold remaining in the epithelium 7-90 days later. Thus receptor cells with an axon in the olfactory bulb at the time of injection survived for at least 90 days. Taken together these results indicate that receptor cells in adult olfactory epithelium live for at least 3 months - three times the commonly accepted lifespan.

(Supported in part by grants from the Australian Brain Foundation and the N.H. and M.R.C.)

Olfactory Marker Protein (OMP) Expression in Hamster CNS: Biochemical and Immunocytochemical Characterization. HARRIET BAKER (Cornell Univ. Med. Coll.), MARY GRILLO and FRANK L. MARGOLIS (Roche Inst. of Mol. Biol.)

OMP is a 19kd molecule previously thought to be expressed only in olfactory receptor neurons and their processes. However, in recent experiments OMP-like immunoreactivity was found in hypothalamus of mouse, rat and hamster. This finding raised a number of questions including: 1) Where else is it localized in CNS?; 2) Is the antigen recognized OMP?; 3) How much is present? These questions were addressed in hamster where OMP-like staining was most robust and widely distributed. Neurons containing OMP were found in the following nuclei listed from rostral to caudal: pontine central grey, pontine reticular tegmental n., prepositus hypoglossal n., medial vestibular n., lateral cerebellar n., and lateral medullary reticular n. Neurons also were found scattered throughout the medullary reticular formation. In any nucleus, only a subpopulation of neurons contained OMP-immunoreactivity. Terminal arbors were found in the red nucleus as well as in cerebellum where they formed mossy fiber bands and typical mossy fiber rosettes. Using a solid phase immunoisolation procedure, followed by Western Blot analysis, the antigen recognized in hamster brain and cerebellum was found to have the same molecular weight as OMP isolated from olfactory bulb. The amount of OMP in cerebral cortex, olfactory bulb and cerebellum was quantified by radioimmunoassay. OMP was not detected in cortex. The immunoreactivity in bulb and cerebellum had parallel displacement curves but the concentration in cerebellum was 1/1000 that in the bulb. These data demonstrate that authentic OMP is found within neurons and terminals of discrete nuclei in the hamster CNS. (Supported in part by NS23103).

#152 appears after #75

Incorporation of the Olfactory Adenylate Cyclase in Liposomes. ROBERT R. H. ANHOLT (Department of Physiology, Box 3709, Duke University Medical Center., Durham, NC 27710)*

Olfactory cilia are enriched in an adenylate cyclase that can be stimulated in a GTP-dependent manner by some, primarily hydrophobic, odorants. (Pace et al. (1985) *Nature* 316, 255-258; Sklar et al. (1986) *J. Biol. Chem.* 261, 15538-15543). Functional reconstitution of the olfactory membrane may clarify whether odorants activate this enzyme via specific odorant receptor proteins or whether activation of the cyclase via its regulatory GTP-binding protein occurs as a result of partitioning of odorants into the lipid membrane. Isolated olfactory cilia were solubilized with Lubrol PX in the presence of supplementary lipid, sodium fluoride and forskolin. Subsequent removal of the detergent by adsorption onto Biobeads SM2 results in the formation of proteoliposomes that display forskolin- and GTP γ S-sensitive adenylate cyclase activity. Sucrose gradient centrifugation of liposomes formed in the presence of a fluorescent lipid marker show that the olfactory adenylate cyclase associates with the exogenously added lipid. Forskolin stimulates the enzyme in reconstituted membranes with the same potency as in native membranes (EC_{50} = 1-2 μ M). GTP γ S is 350-fold more potent in native membranes (EC_{50} = 4.0 \pm 0.5 nM) than in reconstituted membranes (EC_{50} = 1.4 \pm 0.3 μ M). Sensitivity to odorants was in these initial studies not recovered. This may be due either to denaturation of odorant receptor proteins or to alterations in the protein-lipid ratio and lipid composition of the reconstituted membranes. These studies represent a first step toward the functional reconstitution and molecular dissection of the olfactory membrane.

* Supported by NIH grant NS-24521 and Army Research Office grant DAAL03-86K-0130

Behavioural and Electrophysiological Evidence for a P2- Type Receptor in the Stable Fly. ANN ASCOLI (Queen's University at Kingston), J.F. SUTCLIFFE (Queen's University at Kingston) P.J. ALBERT (Concordia University of Montreal)

Vertebrate purinoceptor classification was applied to a chemoreceptor involved in engorgement behaviour in the stable fly, *Stomoxys calcitrans*. An artificial feeding assay was used to determine the phagostimulatory activity of ATP analogues in a buffered saline solution. The action of methyl xanthines on the feeding response was also tested. The response mediated by ATP was antagonized by the use of the photoaffinity label ANAPP3. Single-unit tip-recordings were obtained from the labellar sensilla. A salt responsive cell was characterized by its dose response to NaCl. Its activity was decreased by ATP. The action of other adenine nucleotides on this cell was also tested. The action of ATP on this cell was antagonized by ANAPP3. Based on both the behavioural and electrophysiological data, this receptor involved in ATP detection in the stable fly was classified as a P2x- type.

Sat. Morn Post. Sess. A Abst. # 153 Post. A1

Organization of GABA and GABA-T Containing Neurons Within The Gustatory Zone of the Nucleus Solitary Tract. P.S. LASITER and D.L. KACHELE, (Dept. Psychol., Florida Atlantic University, Boca Raton, FL).

The nucleus of the solitary tract (NST) contains neurons that show glutamic acid decarboxylase-like immunoreactivity and GABA-like immunoreactivity. NST neurons also stain positively for 4-aminobutyrate: 2-oxoglutarate aminotransferase ("GABA-transaminase": GABA-T; EC 2.6.1.19), the principle degradative enzyme for neuronal GABA. Although GABAergic neurons have been well-characterized in caudal "visceroceptive" regions of the NST, little attention has been given to the organization of putative GABAergic neurons in the gustatory zone of the NST. In the present study, GABAergic neurons are characterized in the gustatory zone of the NST. Transganglionic transport of HRP was used in combination with immunohistochemistry for GABA and histochemistry for GABA-T. GABA-LI neurons are located in regions of the NST that receive projections from the facial and glossopharyngeal nerves. GABA-LI somata and terminal-like puncta are located in both the medial and lateral NST throughout the extent of the gustatory zone, although a greater number of GABA-LI somata are located in the medial NST. Within the lateral NST, the density of somata in terminal fields is quite low (ca. 4% of neurons) relative to neurons located immediately adjacent to terminal fields. Thus, terminal fields in the gustatory NST can be readily characterized on the basis of neurochemical architecture. Preliminary observations indicate that approximately 82% of neurons that show somatic GABA-LI also show positive somatic staining for GABA-T. The majority of GABA-LI puncta are similarly reactive for GABA-T. These results confirm that GABAergic neurons and terminal-like structures are located within the first-order central gustatory relay. Thus, GABAergic neurons may modulate ascending gustatory afference.

Sat. Morn Post. Sess. A Abst. # 155 Post. A3

Sensory/Motor Mapping of the Solitary Nucleus and Adjacent Structures. JOSEPH B. TRAVERS, ROBERT WALTZER & SUSAN TRAVERS (Department of Oral Biology, College of Dentistry, The Ohio State University, Columbus, Ohio)

Ingestion and rejection responses to sapid stimulation are organized in the caudal brainstem (Grill & Norgren, 1978) and involve synergies among the trigeminal, hypoglossal and ambiguous motor nuclei. It is unclear, however, how each of several premotor neuron groups (e.g. solitary nucleus (SN), parabrachial nuclei, reticular formation) influence the oro-motor nuclei. The aim of the present study was to map the input/output characteristics of these premotor areas by correlating their oro-sensory properties with responses induced in oral and pharyngeal musculature by microstimulation through the recording electrode. Under Urethane anesthesia, fine wire electrodes were inserted into the anterior digastric (AD), styloglossus (STY), geniohyoid (GEN) and thyropharyngeus (PHA) muscles of adult rats. A tungsten microelectrode (exposed tip = 15 X 5u, 1.5 megohms) was used for recording multi-unit activity and stimulating in central structures. Sites were tested for responsiveness to gustatory, tactile and proprioceptive stimuli, followed by microstimulation with 30ms trains of pulses (250 pps, duration = 0.1ms, -5 to -80uA). Sites in the SN responsive to mechanical stimulation of the soft palate or posterior tongue were low-threshold sites for microstimulation-elicited swallowing, characterized as responses in both STY and GEN (mean latency = 45ms), followed by a PHA contraction. Microstimulation of more anterior SN sites, responsive to sapid stimulation of the anterior tongue, did not elicit low-threshold swallows but did produce shorter-latency (mean = 21ms) responses in STY (tongue retractor). Microstimulation of the anterior SN could also be differentiated from microstimulation of the jaw-stretch responsive RF ventral, or the intraoral responsive spinal V nucleus lateral to the SN. Microstimulation of either of these areas was more likely to produce AD (jaw opener) responses (mean latency = 18 ms).

Supported by NIH NS24889 and NS24884.

Sat. Morn Post. Sess. A Abst. # 154 Post. A2

An in vitro brain slice preparation to study gustatory-salivatory nucleus interactions. T. A. HARRISON and R. M. BRADLEY (University of Michigan, Ann Arbor, MI, 48109).

Accumulating evidence indicates that secretomotor control of the von Ebner's lingual salivary glands in the rat resides in a small nucleus of parasympathetic neurons lying near the ventromedial border of the gustatory zone of the nucleus of the solitary tract (NST). The von Ebner's glands secrete directly into the clefts of the circumvallate and foliate papillae, which contain most tongue taste buds. Thus, interactions between afferent gustatory information and efferent salivary outflow may be extensive, both peripherally and centrally. To investigate the central components, we have developed an in vitro brainstem slice preparation which contains both nuclei. This preparation offers greatly improved stability for extracellular recording over in vivo conditions, as well as the opportunity to independently stimulate gustatory afferents and secretomotor efferents within the brain, before these fibers join and travel together in the IXth nerve. Previously established methods for preparing viable brainstem slices were used. Briefly, rats are anesthetized and decapitated. The brain is rapidly removed, blocked, mounted in a vibratome and cut into 200-400 um slices. After 1 hour recovery, slices are placed in a Hass-type chamber, perfused continually with oxygenated artificial CSF solution at 35°C. The NST and solitary tract are clearly visible through a dissecting microscope when illuminated from below. Glass micropipettes filled with 4M NaCl (2-10 Mohms) are used for extracellular recording. In coronal slices, we have successfully recorded spontaneously active respiratory neurons in the ventrolateral part of caudal NST for up to 5 hours. Some of these showed rhythmic discharges, as reported by others. So far no neurons in coronal slices from gustatory NST have been spontaneously active. Successful stimulation of the solitary tract to drive NST neurons has been accomplished using a bipolar concentric stimulating electrode visually placed on the tract. We are continuing to characterize responses of gustatory neurons to electrical stimulation of the solitary tract, and to identify secretomotor neurons by antidromic activation and characterize their responses to solitary tract stimulation.

Supported by NIH Grant NS21764

Sat. Morn Post. Sess. A Abst. # 156 Post. A4

Taste responses in the parabrachial pons of male, female and pregnant rats. PATRICIA M. DI LORENZO, SCOTT MONROE (SUNY at Binghamton)

Sex-related and pregnancy-related variations in taste preferences have long been known to exist in humans as well as animals. However, the neurophysiological underpinnings of these variations have not yet been described. In an effort to discover whether differences in hormonal state are reflected in the neural processing within the gustatory system, electrophysiological responses to representatives of the four basic taste qualities were recorded in the parabrachial nucleus of the pons (PbN) of male, diestrous female and pregnant rats. Responses to NaCl (.1M), HCl (.01M), quinineHCl (.01M), sucrose (.5M) and NaSaccharin (.004M) were recorded using standard electrophysiological techniques. PbN units were recorded: 47 units from males, 46 units from diestrous females and 42 units from pregnant females. Results from preliminary analyses can be summarized as follows: 1) Spontaneous rates of firing were not different across groups. 2) Comparison of mean 5 sec response rates across groups showed that response to NaSaccharin and sucrose were greater in female and pregnant rats compared with males. Overall response magnitudes for the other taste stimuli were not different across groups. 3) Examination of individual unit responses to each taste quality showed that the responses to NaSaccharin and sucrose were greater across the entire sample of PbN units in female and pregnant rats compared with males. Further differences between taste responses in female and pregnant rats were evident in those units that showed the largest responses to NaSaccharin and sucrose. Other differences in responses in pregnant rats compared with the other groups were also apparent. 4) Classification of units according to their best stimulus revealed that a greater proportion of units responded best to sweet stimuli in female and pregnant rats compared with males. 5) Examination of the Neural Mass Differences in the 3 groups of units showed comparable values for nearly all stimulus pairs. Further analyses are in progress to study differences in the organization of response profiles across units as a function of hormonal state.

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The Taste Area in the Caudolateral Orbitofrontal Cortex of the Primate.
 LESLIE L. WIGGINS and EDMUND T. ROLLS
 (University of Oxford, Department of Experimental Psychology, Oxford OX1 3UD, England.)

A cortical taste region has recently been identified in the caudolateral orbitofrontal cortex of the primate (Rolls et al., 1985), and it has been shown anatomically that this is a secondary cortical taste area (Wiggins, Rolls and Baylis, 1987). The properties of single neurons are being analyzed further, with the following results. First, the neurons appear to be unimodal, in that they are not activated by visual, olfactory or somatosensory stimuli. Visual neurons are found just laterally, in the inferior frontal convexity. Second, for the prototypical stimuli, neurons which responded best to glucose or NaCl were more common than neurons which responded best to HCl or quinine HCl. The responses of these neurons when tested with a wide range of tastants including glucose, NaCl, HCl, quinine HCl and foods were found in a number of cases to respond better to a real food (e.g. apple juice, tomato juice, milk and banana) than to one of the prototypical tastants. Third, it was found with binary taste mixtures that glucose often produced some suppression of the responses of neurons which responded best to other prototypical stimuli. Further, for 8 neurons it was found that the neuronal response to a mixture was greater than would be predicted by linear addition, showing that in this region some neurons respond particularly well to combinations of tastants.

Rolls, E.T., Yaxley, S., Sienkiewicz, Z.J. and Scott, T.R. (1985) *Chemical Senses* 10: 443.
 Wiggins, L.L., Rolls, E.T. and Baylis, G.C. (1987) Afferent connections of the caudolateral orbitofrontal cortex taste area of the primate. *Chemical Senses* 12: 206.

Field observations of preferences of bananaquits for mixtures of ethanol, sucrose, jams, and water. CEDRIC M. SMITH (State University of New York at Buffalo, New York 14214)

The ubiquitous yellow bird of the Caribbean tropics, the bananaquit, is notorious for its consumption of alcoholic drinks, sugar, and jams. The detection, preference and consumption of alcohol, sucrose, and marmalade mixtures were investigated in Antigua with a flock consisting of 10 to 20 bananaquits and 2 to 4 Lesser Antillean bullfinches. Solutions were made up fresh each day from 80 or 100 proof vodka. Preference tests were carried out in the morning on a patio; the solutions were presented in one to three saucers with a random assignment of their relative positions. Solutions containing up to 10% (v/v) ethanol were readily and consistently consumed; the tasting of solutions of 8-10% was accompanied by a vigorous head-shake. Using a two choice procedure, mixtures containing 6% ethanol in water, in water/sucrose, or in water/marmalade were consistently preferred over the respective non-alcoholic solutions; up to twice as much of the 6% alcohol/marmalade/water mixture was consumed as the respective control mixture. The approximate order of preference was 6% ethanol in a mixture of marmalade and water, followed by 6% ethanol/sucrose/water, marmalade/water, sucrose/water; water alone led to bathing more often than actual consumption. Although some birds consumed large quantities of ethanol (estimated at >10 g/kg consumed in an hour or less) no overt intoxication was detected. By contrast, mixtures containing more than 8% ethanol were less avidly consumed than the nonalcoholic comparison mixtures. These relative preferences for ~5-8% alcoholic solutions with sugar and fruit flavorings are, in general, similar to the drinking and feeding behaviors reported for some members of a number of species including flies, rats and humans.

Taste Detection: Severe Deficits Produced by Lesions of the Thalamic Taste Nucleus. KOLLEN M. MARTIN and BURTON M. SLOTNICK (The American University)

Lesions of the thalamic taste nucleus (VPMpc) alter preference for tastants (e.g., Ables & Benjamin, J. Neurophysiol., 1960). Do these changes stem from alterations in the perceived hedonic value of tastants or from deficits in sensory capacity? Rats with VPMpc lesions were tested on a simple taste detection task using operant conditioning (Brosvic and Slotnick, Physiol. & Behav., 1987). Preoperatively, animals were given extensive training to discriminate between delivery of NaCl (0.8% - 0.5% NaCl) and water from a stimulus sampling tube. Postoperatively, 4 rats with complete lesions of the thalamic taste nucleus performed at or near chance in 1400 test trials on the highest NaCl concentration. Three rats with more posterior lesions (which presumably interrupted brain stem afferents to VPMpc) performed somewhat better but did not achieve criterion of 85% correct responding. The performance of these 7 rats was significantly worse than those with unilateral lesions of VPMpc, lesions of other thalamic nuclei, and sham operates, most of which had perfect retention. These preliminary data indicate that the thalamic taste nucleus may play a critical role in the detection of even strong tastants. Changes in taste preference reported in earlier studies probably stem from lesion-induced alterations of gustatory capacity. Supported in part by the National Science Foundation Grant #BNS8319872 to BMS.

Taste Reactivity in the Hamster. SHERYL K. BRINING, TERI L. BELECKY and DAVID V. SMITH (University of Cincinnati College of Medicine)

Taste reactivity to gustatory stimuli in the rat consists of appetitive and aversive response components, the latter seen mostly to bitter stimuli (Grill & Norgren, 1978; Schwartz & Grill, 1984). Earlier work with hamsters, using stimuli similar to those in the rat, showed little or no aversive responsiveness (Petty & Smith, 1985). However, recent evidence from hamster IXth nerve fibers suggests that this animal is sensitive to quinine hydrochloride (QHCl) only at relatively high intensities (Hanamori, Miller & Smith, 1986). The present experiment was designed to characterize the hamster's taste reactivity to stronger stimuli. Sucrose (1 M), NaCl (1 M), HCl (0.03 M) and QHCl (0.1 M) were presented via intraoral cannulae, with their order counterbalanced across animals (n = 8). Infusions of 0.6 ml were delivered over a 1-min period from a syringe pump. Responses were recorded on videotape for later analysis and were also coded online into a computer. Appetitive responses included forward and lateral tongue protrusions and aversive responses included increased locomotion, forelimb flailing, chin rubbing, fluid rejection, gaping and aversive posturing. Each stimulus produced a characteristic pattern across these behaviors, with sucrose eliciting only appetitive behaviors and QHCl evoking aversive responses in almost all (90 - 100%) of the animals. Both NaCl and HCl produced appetitive responses, as seen previously in the rat, although these stimuli also elicited aversive behavior in the hamster. Aversive behaviors were seen in 60 - 70% of the hamsters following HCl infusion and in 30 - 40% after stimulation with NaCl. The patterns of appetitive and aversive behaviors followed the hedonic ordering of these stimuli from sucrose to NaCl, HCl and QHCl. Supported by NINCDS Grant NS23524-02 to D.V.S.

Sat. Morn Post. Sess. A Abst. # 162 Post. A9
Participation of the X and Y Chromosomes in the Individual
Chemosensory Identity of Mice According to Genotype. KUNIO
YAMAZAKI, GARY K. BEAUCHAMP, DONNA KUPNIEWSKI
(Monell Chemical Senses Center), JUDITH BARD, LEWIS
THOMAS and EDWARD A. BOYSE (Memorial Sloan-Kettering
Cancer Center).

The Major Histocompatibility Complex (MHC) of the mouse imparts to each mouse an odor that reflects its genetic constitution at this region of chromosome 17. Since sensory recognition of these differential odors influences mating behavior and evokes neuroendocrine responses critical to the maintenance of pregnancy, we wished to determine whether other parts of the mouse genome contribute to individual of odor and so may similarly exert a selective force on loci other than the MHC. To accomplish this, the sex chromosomes offer an experimental advantage over autosomes since it is possible to test an entire chromosome (X or Y) en bloc. Using the inbred mouse strains AKR and B6 (C57BL/6) together with B6.Y^{AKR} males, which differ from B6 males in having exchanged their Y chromosome for that of AKR, hybrid mice can be bred that differ genetically only in their X and/or Y chromosomes. Thus we were able to test olfactory distinction of mice with X and/or Y difference in the Y maze, previously employed to investigate MHC related scent distinctions. The data demonstrated that the X and Y chromosomes each confer individuality of scent related to genotype. However, the MHC appears to be considerably more salient since the order of odor intensity, judged by comparative ease of Y-maze training, was the MHC (most intense), the X chromosome and the Y chromosome (least intense). This conclusion was supported by subsequent studies on pregnancy block where it was shown that while Y chromosome difference were sufficient to induce significant blockage, the proportion of blocked pregnancy were greater where MHC differences were involved.

Supported by NIH grant GMCA-30296.

Sat. Morn Post. Sess. A Abst. # 163 Post. A11

Joint Determinants of Sweet Tastant Response Phenotypes
in Mice: The Interactive Contributions of Genetic
Predilection and Dietary Experience. JOHN C. MAGGIO and
GLAYDE WHITNEY (Dept. of Psychology, Florida State Univ.).

Genetic and environmental contributions to the ingestion of sweet tastants (sucrose, glucose+saccharin) were examined among the high preferring SWR/J mouse strain, and the lesser preferring A/J and DBA/2J strains. Exp. 1 revealed a sucrose concentration-specific enhancement of fluid ingestion among SWR/J, but not DBA/2J mice. At the maximally preferred concentration (0.1M), additive genetic effects on sucrose solution intakes were evidenced in reciprocally derived F₁ hybrids displaying consumption values intermediate between those of the parental strains. Maternal effects were also noted. A classical Mendelian cross of A/J and SWR/J mice (Exp. 2) subsequently showed sweet fluid ingestive behaviors to be strongly influenced by parental gene dose. Based on parental contribution (i.e., proportion of genes), a hierarchical relationship among parental, F₁, F₂, and backcross generations, in both preference for and ingestion of 0.1M sucrose, was obtained. A similar gene dose relationship in ingestive response phenotypes, obtained when mice were later tested with a 3% glucose + 0.125% saccharin mixture, indicated gene dose effects to be consistent across sweet tastants. Exp. 3 (conducted as part of a larger, diallel analysis) showed that depending on genetic constitution, sucrose experience may exert either profound or relatively minor influence on sweet fluid ingestive responses. When compared to like-genotype water-raised controls, SWR/J mice that had a life history of 0.1M sucrose solution as the sole source of fluid ingested substantially more 0.1M sucrose in a two-bottle 48-hr preference test than did similarly treated mice of either the A/J or DBA/2J strains. Reciprocal crosses of SWR/J mice to each of the latter two strains yielded F₁ progeny displaying experience-enhanced elevations in sweet fluid intakes generally intermediate between those of parental strains. Taken collectively, the present results emphasize the interactive contributions of genetic predilection and dietary experience as joint determinants of sweet tastant response phenotypes.

Supported, in part, by NINCDS Grant NS15560.

Sat. Morn Post. Sess. A Abst. # 202 Post. A10

Creation and Utilization of Congenic Taster-Nontaster
Inbred Mice. GLAYDE WHITNEY, DAVID B. HARDER, KIMBERLEY S.
GANNON, and JOHN C. MAGGIO (Dept. of Psychology, Florida
State Univ.).

SWR inbred mice avoid bitter sucrose octaacetate (SOA) solutions at weaker concentrations than do other strains. SWR/J (taster) and C57BL/6J (nontaster) inbred strains, their F₁ progeny, and 12 derived segregating generations (nearly 4,000 mice) were tested for SOA avoidance in the course of developing a set of congenic inbred strains. Phenotypic ratios of tasters to nontasters, observed across all segregating generations, were consistent with predictions for a single genetic locus with two alleles, the taster allele being dominant to the nontaster. The congenic strains have essentially the C57BL/6J genome, but avoid SOA due to a short chromosome segment transferred from the SWR/J strain. These congenic C57-taster lines can now be compared to the standard C57BL/6J (nontaster) inbred strain. Initially the taster congenics are being tested with other tastants on which the parental strains differ. The congenics resemble the SWR/J strain for some tastants (suggesting the influence of the Soa gene or closely linked genes on the short chromosome segment). For other tastants, the congenics resemble the C57BL/6J strain (suggesting the effects of genes elsewhere in the genome). In general these congenic taster-nontaster mice should be useful in investigating patterns of co-variation and mechanisms of taste.

Supported, in part, by NINCDS Grant NS15560.

Modification of the Gerbil's Taste Behavior by p-Nitrophenyl- α -D-glucopyranoside¹. CYNTHIA E. MYERS (Lehman College and Temple University School of Medicine), ANDREA NEITA (Lehman College), and WILLIAM JAKINOVICH (Lehman College).

The gerbil's chorda tympani nerve response to sucrose is inhibited by p-nitrophenyl- α -D-glucopyranoside (PNP-Glu). To help us understand the nature of this inhibition we have conducted a number of behavior experiments to see if the gerbil's behavior can be modified by adding PNP-Glu to taste solutions, focusing on the conditioned taste aversion (CTA) behavior because it appears to be the simplest as well as the most definitive method.

We have found that the gerbil's CTA of a sucrose solution can be overcome by the addition of high concentrations of PNP-Glu. Moreover, since PNP-Glu is bitter, we also tested mixtures of sucrose and quinine and found that the sucrose aversion is not affected by quinine. We have also examined the effect of PNP-Glu on animals' trained to avoid sodium chloride and observed no effect.

Therefore, these experiments indicate that PNP-Glu is both an inhibitor of the gerbil's chorda tympani sucrose response and sucrose CTA. Both of these observations indicate that this inhibitor is in a specific inhibitor of the animal's taste response.

¹This work was supported in part by grants from City University of New York PSC-BHE Research Award Program.

DOCA-Induced Salt Appetite is Partially Blocked by Hydrochlorothiazide. K. J. MOONEY and R. A. BERNARD (Dept. of Physiology, Michigan State University, East Lansing, MI 48824).

Administration of deoxycorticosterone acetate (DOCA) to rats produces a large increase in salt intake. This increased salt appetite is widely viewed as the behavioral counterpart of the salt and water retaining effects of the mineralocorticoid hormones. However, rats and other animals escape from the retention effects of DOCA within a few days and return to normal sodium and water balance in spite of continued hormone administration. It is our hypothesis that the increased salt intake is not a direct effect of DOCA, but rather the result of the activation of an endogenous diuretic system by the volume expansion produced by DOCA. To test this hypothesis we administered a diuretic along with DOCA to counteract the volume expansion. Three groups of rats (n=7) were used. One group received DOCA alone (5mg/kg in sesame oil, s.c., twice daily) while a second group received the same amount of DOCA plus hydrochlorothiazide (HCZ) in the drinking water (DOCA-HCZ group). A third group served as controls. Salt intake was measured by the 24-hr 2-bottle choice method in which .5M NaCl and distilled water were used. A 4-day control period was followed by 11 days of treatment. NaCl intake increased 7-fold in the DOCA-treated group, rising gradually from an average pretreatment intake of 5.3 ml to a plateau value of 41.1 ml on the 7th day. In the DOCA-HCZ group, NaCl intake rose rapidly from 5.8 ml to a peak of 35 ml on day 2, after which salt intake dropped to an average of 25.6 ml, an intake almost 40% lower than that of the DOCA group. The lower salt intake of the DOCA-HCZ group is consistent with the hypothesis that volume expansion is the key factor in the stimulation of salt appetite by DOCA. Since HCZ by itself also stimulates salt appetite, this would account for the early and transient elevation of salt intake in the DOCA-HCZ group. Suppression rather than additivity of effects by two agents which individually stimulate salt appetite strongly suggests that they operate by very different mechanisms. The hormonal mechanisms triggered by volume expansion are now under investigation for their possible role in the DOCA model of salt appetite.

Supported by NIH Grant NS23223.

No Significant Influence of Adrenalectomy on Taste or Odor Detection Performance Measures of Male Rats. JUDITH M. RISSER, RICHARD L. DOTY, GARY M. BROSVIC, (Smell and Taste Center, University of Pennsylvania, Philadelphia, and Department of Psychology, Glassboro State College, Glassboro, NJ)*

Although Henkin has presented clinical data which suggest that adrenal insufficiency results in heightened taste and smell sensitivity, well-controlled animal studies on this point are lacking. In this study we determined the odor and taste sensitivity of adult male rats before and after adrenalectomy or sham operations to a wide range of perithreshold stimulus concentrations using computer-controlled operant testing procedures and a non-parametric go/no-go signal detection paradigm. A total of 36 rats (half shams) were tested: 12 using half-log steps of the odorant ethyl acetate (concentrations extending from log -6.0 to -3.5 relative to saturation); 12 using the same concentrations of the odorant pyridine; and 12 using sucrose and sodium chloride concentrations ranging from .0001M to 0.2 M. No significant influences of adrenalectomy were observed for either the odor or taste detection values at any of the concentrations presented. These findings suggest that the notion of enhanced olfactory and/or gustatory sensitivity following adrenalectomy needs to be reconsidered.

*Supported by NINCDS Grant NS 16365.

Sodium Depletion Can Produce a Conditioned Taste Aversion. SANDRA P. FRANKMANN (Monell Chemical Senses Center).

In the studies reported here, the possibility that sodium depletion is a sufficient condition for the formation of a conditioned taste aversion (CTA) was evaluated. Male, Sprague-Dawley rats were made hypovolemic by s.c. injection of polyethylene glycol (PEG, 20,000 MW, 16.7 ml/kg). The PEG causes an effective loss of isotonic fluids at an edema which forms at the injection site, but the PEG is excreted and the edema is dissolved within 24-48 h after injection. Purina chow (0.5% Na⁺) was available ad lib except for 24 h immediately following injection with PEG or the isotonic saline vehicle (VEH). Thus, the effective sodium depletion was alleviated by dissolution of the edema and by consumption of sodium in the diet. For 6 PEG and 6 VEH rats, tap water was replaced with 0.1% saccharin (Na⁺ free) water and 24 h intakes were recorded for 5 days following injection. For the next 2 days, both fluids were available and preference scores were calculated. Over the 5 days following injection the PEG group consumed significantly less of the saccharin water (22 ml/day) than did the VEH group (34 ml/day). When given a 2 bottle choice, saccharin water intake was 74% for the VEH group but only 3% for the PEG group. Thus, sodium depletion, as produced with PEG and under the circumstances given above, is a sufficient condition for the formation of a CTA.

A second experiment extended this observation to sodium depletion produced by combination of injection with the natriuretic/diuretic drug, furosemide (10 mg/rat) and sodium deficient diet overnight. Sodium depleted and control rats were given either tap water or 0.1% saccharin water over sodium depletion period. Following recovery from the sodium depletion, all rats were given two, 24-h preference tests with water and 0.1% saccharin water. Intakes of the two solutions were recorded, and the preference scores calculated. The 0.1% saccharin water accounted for only 8.9% of the intake of the furosemide-saccharin paired group, whereas it accounted for 52.1-54.1% of the intakes for the furosemide-tap water and control groups.

Thus, sodium depletion as produced by at least two methods can result in the formation of a CTA. At this time it is not clear whether it was the sodium depletion per se or an aggravation of the sodium depletion state by the consumption of water (which may have further diluted the sodium concentration of the extracellular fluids, plasma and interstitial) that produced the aversions. Future studies will evaluate these two possibilities.

Sat. Morn Post. Sess. A Abst. # 168 Post. A16

Electrolyte Excretion and Sodium Appetite of Adult Sprague-Dawley Rats Modified by Maternal NaCl Intake. ROBERT J. CONTRERAS & KELLY W. RYAN (Univ. of Alabama, Dept. of Psychology, Birmingham, AL)

The time preceding and following birth up to the time of weaning is an important ontogenetic period during which there are vast changes in the structure and function of the water and electrolyte systems, including their neural and hormonal regulatory mechanisms (Zicha, et al., Hypertension, 12: 1096, 1986); environmental stimuli early in development can lead to permanent changes in these systems. We have shown previously that differences in maternal dietary NaCl lead to both short-term (Dev. Psychobiol., 20:111, 1987) and long-term (J. Nutr., 113:1051, 1983) changes in need-free NaCl preferences. In the present report, we extend these initial observations to studies of electrolyte excretion and salt intake under sodium deficient conditions. Adult female rats were fed a normal diet that varied only in NaCl with either 0.12, 1, or 3% NaCl. After mating, the dams and litters were kept on their diets until 30 days postpartum, when they were all fed the same pelleted stock diet. At 90 days of age, the adult offspring were placed in metabolism cages for 7 days and fed 1% NaCl chow for days 1-2, and 0% NaCl chow for days 3-7. On days 6-7, the rats were free to consume water or 0.3 M NaCl solution. When dietary NaCl was available, male and female rats raised on the high salt diet excreted significantly more Na⁺ on days 1-2 and 6-7 than did the adult rats raised on the mid or low salt diets. There were no differences in electrolyte excretion during sodium deprivation on days 3-5. The animals raised on the high salt diet also consumed significantly more .3 M NaCl solution on days 6-7 than did mid or low salt rats. There were no differences in water intake. In another group of adult rats raised on either the low or high NaCl diets, we measured their 24-h intakes of water and 0.3 M NaCl solution in response to treatments consisting of 48-h of dietary Na⁺ deprivation and an injection (5 mg, sc) of the diuretic furosemide. The animals received 4 such treatments at weekly intervals. The rats raised on 3% NaCl consumed significantly more NaCl solution over the 4-week period than did rats raised on 0.12% NaCl. There were no differences in water intake. We do not yet know whether the present findings are due to primary changes in excretion or appetite mechanisms. We suspect that the changes in both Na⁺ intake and excretion are related to recently discovered changes in brain angiotensin II receptors due to differences in maternal NaCl intake (Moe, Soc. Neurosci. Abstr., 12:1169, 1987).

This research was supported by NIH grant HL 38630.

Sat. Morn Post. Sess. A Abst. # 169 Post. A17

A Detailed Analysis of Sodium Chloride Drinking in the Rat. JAMES C. SMITH (The Florida State University) and ROBERT J. CONTRERAS (University of Alabama at Birmingham).

In the present report a detailed description is presented of the 24-hr. home cage drinking and eating patterns of rats presented with sodium chloride solutions, water and powdered laboratory chow. The concentration of the NaCl was systematically varied from .02 M to 0.4M across days with a single concentration presented for four consecutive days. In addition to the daily consumptions, the number and duration of ingestion bouts were measured. Also the inter-bout-intervals and eating efficiency were recorded. The juxtaposition of salt, water and food bouts was noted. The dependent variables were analyzed separately for day and night time ingestions. In one group of rats the salt concentration was systematically increased over days and in a second group the salt concentration was decreased. The rats which were given the ascending series of salt concentrations show an increase in salt intake up to .15M and a marked decrease at higher concentrations. The correlation between mean salt solution intake and mean daily number of bouts is .85. The correlation between mean daily intake and bout length is .95. Therefore, it can be concluded that an increase in salt intake is accomplished by an increase in both the number of bouts and the bout length. Rats given a descending series of salt concentrations show a profound decrease in salt intake at most concentrations. The significance of these findings are discussed.

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Sat. Morn Post. Sess. B Abst. # 170 Post. B1

Immunological and Genetic Approach to the Folate Chemoreceptor of Paramecium. J. MICHAEL SASNER, JACQUELINE ISAKSEN, and JUDITH VAN HOUTEN (University of Vermont)

The cell body membrane of paramecia binds the chemical stimulus folate specifically and saturably. This binding has been correlated with the change in membrane potential that leads to attraction by a chemokinetic mechanism (Schulz et al., J. Comp. Physiol. 155:113-119, 1984). To circumvent potential problems that low affinity binding poses for conventional receptor biochemistry, we have developed a method to covalently crosslink folate to its binding sites and have made an antibody against this ligand as a means to locate the binding proteins. Western blot techniques were not particularly successful in detecting folate-crosslinked proteins among the total membrane proteins. However, we are now using two methods to increase the sensitivity of immunodetection of folate binding proteins: 1) Affinity-purified antibody to folate is attached to Protein A-Sepharose columns. Solubilized proteins from crosslinked membrane preparations are eluted from this column with a low pH wash. A consistent profile of proteins includes proteins of 52 and 32 kD. 2) Iodinated folate is being used in crosslinking to quantitate the efficiency of crosslinking, to increase the specific signal after the proteins eluted from the antibody affinity column have been separated by SDS-PAGE, and to demonstrate that the antifolate antibodies are recognizing proteins physically bound with folate. The folate receptor will be identified by correlative evidence such as the loss of a folate binding protein from the cell body membrane coincident with the loss of chemoreponse in a mutant. Toward this end, we have mutagenized cells with x-rays and isolated mutants that do not bind fluorescein-folate. Among these cell lines we have identified mutants that are not attracted to folate. The membrane proteins of these binding mutants will be compared to those of wild-type cells in order to help identify the chemoreceptor for folate.

This work has been supported by NSF and the Whitehall Foundation.

Sat. Morn Post. Sess. B Abst. # 171 Post. B2

Serotonin-like immunoreactivity in primary olfactory neurons of the spiny lobster. ULRIKE GRÜNERT, BARBARA-ANNE BATTELLE, and BARRY W. ACHE (The Whitney Laboratory, and Depts. of Zoology and Neuroscience, University of Florida, St. Augustine, FL 32086)*

The primary sensory transmitter(s) in olfaction has not been clearly identified. In arthropods, acetylcholine (ACh) is generally regarded to be the sensory transmitter. Here we report that serotonin as well as ACh are present in the olfactory organ of the spiny lobster. The olfactory organ of the spiny lobster consists of a tuft of hair-like sensilla, called aesthetascs, located on the distal end of the lateral filament of the antennule. Each aesthetasc is innervated by a large number of bipolar sensory neurons. About 70% of the somata of these cells show immunoreactivity to an anti-serotonin antibody. The remaining 30% are either lightly stained or not stained at all, suggesting chemical heterogeneity of the olfactory neurons. Significant levels of endogenous serotonin were detected in extracts of antennular tissue using HPLC and electrochemical detection, giving evidence for the specificity of the immunohistochemical findings. The ability of the antennular tissue to synthesize acetylcholine from radioactive choline indicates that acetylcholine also is present in the spiny lobster antennule. Although the exact localization of acetylcholine is not known, our findings suggest that spiny lobster olfactory neurons, like some insect sensory neurons (Lutz et al. 1985, Brain Res 325:353) and vertebrate photoreceptor cells (Nishimura et al. 1986, Nature 320:753), contain more than one neuroactive substance. Whether acetylcholine and serotonin coexist in the same cells and whether or not they function as neurotransmitters and/or neuromodulators needs further investigation.

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Sat. Morn Post. Sess. B Abst. # 172 Post. B3

Binding of chemoattractant from electric shock-induced earthworm secretions to garter snake vomeronasal epithelium. XIAN-CHENG JIANG, DALTON WANG, MIMI HALPERN (SUNY Health Science Center at Brooklyn)

Earthworms (*Lumbricus terrestris*), when shocked with electric current, secrete substances that act as chemoattractants to garter snakes (*Thamnophis sirtalis*) (Halpern, J. et al., *Chemical Senses* 11:607, 1986). Response to the attractant is mediated by the snake's vomeronasal system (Schulman et al., *Chemical Senses* 11:658, 1986). Previously, a 20k glycoprotein was isolated from the secretion and some of its characteristics were described (Jiang et al., *Chemical Senses* 12:667, 1987). We labeled the protein with I-125 and used dot blot techniques (Maggi & Catapano, *J. Lipid Res.* 28:108, 1987) to study its ability to bind to vomeronasal tissue. The 20K I-125-protein bound to vomeronasal sensory epithelial membrane extracts in a dose dependent fashion, but not to membrane fractions of vomeronasal mushroom body. The former tissue is believed to contain binding sites for chemicals that stimulate the vomeronasal system. In addition, we observed that membrane extracts from the snake's main olfactory bulb, olfactory epithelium, brain and tongue root, but not tongue tips, also bound the 20K I-125-protein. Pretreatment of vomeronasal sensory epithelial membrane extract with non-labeled 20K protein blocked further binding of labeled 20K protein. Other I-125 labeled glycoproteins, such as 2-microglobulin did not bind to vomeronasal epithelial or brain membrane extracts but did bind to tongue root, olfactory epithelium and main olfactory bulb membrane extracts.

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Sat. Morn Post. Sess. B Abst. # 173 Post. B4

Nasal Trigeminal Chemoreception: Response to Optical Isomers. WAYNE L. SILVER and DIANNE B. WALKER. (Department of Biology, Wake Forest University, Winston-Salem, NC 27109)

Trigeminal receptors in the nasal cavity respond to a variety of chemical stimuli. Although some of these stimuli appear to be non-irritating, trigeminal chemoreception is often considered a part of the common chemical sense whose primary function is to elicit protective reflexes when stimulated with irritating compounds. Previously we have suggested that trigeminal chemoreceptors in the nasal cavity are unable to discriminate between odorants matched for equal intensity (Silver et al., *AchemS*, 1986). In the present experiment we tested this hypothesis further by examining trigeminal nerve responses to three pairs of optical isomers (d- and l-limonene, d- and l-menthol, and d- and l-carvone). Since optical isomers have the same physico-chemical properties, differences in response characteristics would suggest that trigeminal receptors can discriminate odorants based on their structures. Multiunit responses to stimuli (menthol and carvone obtained from Fluka; limonene from Serva) delivered via a microprocessor controlled, air-dilution olfactometer were recorded from the ethmoid branch of the rat trigeminal nerve. Concentration-response curves and thresholds (defined as the concentration which first elicited a response discernible from baseline) were obtained for each of the six compounds. Neither thresholds nor concentration-response curves differed for the optical isomers of menthol and limonene. Three of 7 rats responded to d-menthol at ≈ 27 ppm while 4 of these rats also responded to l-menthol at this concentration. One of 6 rats responded to both d- and l-limonene at ≈ 500 ppm. There was no significant effect of chirality (detectable by a 2-factor ANOVA) on the concentration-response curves for either d- and l-menthol ($F = 0.8$, $p > 0.25$) or d- and l-limonene ($F = 0.2$, $p > 0.25$). However, d- and l-carvone appeared to produce different results. Four of 9 rats responded to l-carvone at ≈ 23 ppm while only 1 responded to d-carvone at this concentration. In addition there was a significant effect of the chirality of carvone on the concentration-response curve ($F = 7.7$, $p \leq 0.01$). These results suggest that trigeminal receptors in the nasal cavity may be able to discriminate odorants based on their structure.

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Sat. Morn Post. Sess. B Abst. # 174 Post. B5

Effects of Unilateral Nare Occlusion on the Development of Olfactory Epithelium in Rabbits. H. DISTEL, B. STAHL and R. HUDSON (Inst. Med. Psychol., Univ. München, D-8000 München, FRG)

Although it has long been known that closing one nare in newborn rabbits leads to a reduction in the size of the corresponding olfactory bulb (Gudden, 1870), the extent to which sensory deprivation effects the development of the olfactory epithelium remains unclear. To investigate this, unilateral nare occlusion was performed surgically on day 1 and the development of the epithelium on the deprived side was compared to that of the intact side on day 30 using morphometric methods. For this, both rostral and caudal pieces of the septal epithelium were epon embedded, and semithin sections stained using Richardson's method. While no significant difference between sides in the number of basal and supporting cell nuclei was found, the number of olfactory neurons was on average 11% less on the deprived than on the intact side. However, the clearest effect was on the number of olfactory knobs which was reduced by an average of 25%. In addition, the amount of lymphatic tissue lining the bottom of the nasal cavity was significantly less on the side protected from the external environment. Interestingly, reopening nares on days 5 or 10 resulted in normal cell and knob densities in animals examined on day 30, and even reopening nares for just 24 hrs (day 5) was sufficient to stimulate the development of the occluded side. This paradigm could thus provide a ready means of investigating the role of exogenous factors in the regulation of receptor cell development.

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Sat. Morn Post. Sess. B Abst. # 175 Post. B6

Postnatal Development of Nasal Olfactory Structures in the Rat and Rabbit as Seen in Reconstructed 3-D Models. ESMAIL MEISAMI, THOMAS KEATING, MARK PATERNOSTRO & LIN-HA TRAN (Physiol Dept, Univ of Illinois, Urbana, IL 61801)

After birth in the rat and rabbit olfactory mucosa (OM) widens by several fold, expansion occurring more or less uniformly in all parts (1). Since this growth and the increase in receptor neuron number has major functional implications for peripheral olfactory processing, we have begun to study OM growth in relation to maturational changes in nasal conchal morphology and associated cavities by developing 3-D reconstructions of the OM and the conchae using cardboard models and computer graphic 3-D image analysis. Serial H&E stained sections of the olfactory region were obtained from newborn and weanling of rats and rabbits. The outline of OM and respiratory mucosa were traced in serial sections about 0.2 to 0.4 mm apart, depending on the animal and age. The tracings were used to build a 3-D cardboard model and a computer generated 3-D image. For the cardboard model, the tracings were copied onto cardboard sheets, the cavities were cut out and the OM and adjoining respiratory epithelium were colored in. The cardboard blocks were mounted such that the conchae, cavities and air pathway through them could be visualized. The conchae of these models were then examined, named and numbered. The results indicate that in both rat and rabbit the basic adult conchal pattern of the weanling is established at birth. Thus all the primary conchae and many secondary ones are present in the newborn, although in this regard the rabbit newborn is definitely more advanced than the rat's. In fact in newborn rats several conchae are still partially fused with the adjoining ones while in the rabbit this was rare. This process explains the patchy discontinuity of the OM sheet in the adult. Growth in conchal and OM surfaces occurs in all conchae by increase in size of the existing conchae and formation of secondary ones. We are currently digitizing the tracings for 3-D image analyzer and graphic reconstruction.

1. Meisami, *Neurosci Abst* 12:953, '86; Meisami et al *Chem Sen* 11:636, '86. Supported by Univ. Illinois Research Board Grant

Thyroid Hormones and Postnatal Growth of the Olfactory Mucosa in the Rat. MARK PATERNOSTRO AND ESMAIL MEISAMI (Physiol & Biophys Dept, Univ Illinois, Urbana, IL, 61801)

We have recently shown that the rat's olfactory mucosa (OM) shows several fold increase in surface area and in receptor cell number after birth and that this may underlie the observed postnatal improvement in olfactory sensitivity (1). Thyroid hormones (TH) which increase markedly after birth are essential for optimal body and brain development. (2). Little is known about the role of TH on development of the olfactory system. Here we report on OM growth in rats made hypothyroid from birth by adding propylthiouracil (PTU) to the litter's water (0.1% w/v). By day 25 (weaning) the pups' body weight was reduced by about 50% and brain weight by about 20%. Serial frontal sections of the entire OM was prepared from paraffin embedded tissue from normal and hypothyroid 25-day-old male rats and stained with H&E. Quantitative morphometric studies revealed a marked reduction of about 40% in both surface area and total receptor number in the hypothyroid animals, compared to control values (about 130 sq.mm, 7 million receptor cells per side). Interestingly, TH deficiency had no detrimental effects on the OM's zonal histoarchitecture, nor on its thickness (about 60 u). The receptor cell density, basal cells/neurons ratio (1:5) and the supporting cells/neurons ratio (1:4) were also unaltered. Although conchae and nasal cavities of hypothyroid animals appeared similar in shape, they were smaller in size and the underlying cartilage & bone appeared thin and immature. Since in the normal postnatal animal, new receptor cells are added to the OM causing increase in its thickness (1), the results of the present study suggest that the effects of TH on proliferation of olfactory receptor neurons may not be exerted directly on the neural OM tissue, but indirectly, by influencing the growth of submucosal connective tissue which is heavily dependent on TH for growth.

1. Meisami, E. *Neurosci. Abst.* 12:953, '86.
 2. Dussault, J.H. *Ann. Rev. Physiol.* 49:321-334, '87
- Supported by a grant from Univ. of Illinois Research Board.

Olfactory Placode Transplantation in *Xenopus* Embryos. H. KIM, G.A. MONTI GRAZIADEI and P.P.C. GRAZIADEI (Biological Sciences, Florida State University, Tallahassee, Florida).

Previous experiments in our laboratory have shown that, if the olfactory placode from 22/23 stages larvae is transplanted in the eye position of same stage hosts when the eye cup has been removed, the transplanted placode gives origin to a nerve that reaches the diencephalon (at the level of the eye peduncle) where it forms glomeruli. In the present study, we transplanted the olfactory placode of *Xenopus laevis* in place of the eye cup of *Xenopus borealis* to exploit the differential staining with quinacrine of the two strains of *Xenopus* (Thiebaud, 1983). The chimerae were then able to show precisely where the host cells were located as opposed to the cells of the donor. Observations were conducted at stage 48-50. Usually the transplanted placode fused with the host's homolateral olfactory placode producing a large and irregularly shaped olfactory organ from which two olfactory nerves originate. The nature of the olfactory neurons present in this olfactory organ is the surprising finding of this study. While we were expecting to observed a differential staining of the neurons present in the two fused placodes, we could only observed the presence of neurons showing the characteristic fluorescence of the *Xenopus borealis* (host). At this time we do not know if the olfactory neurons of the transplanted olfactory organ come from the olfactory organ of the host or, since the Q bands are a phenotypic character of the chromosomes, if they assume the host's trait under the influence of the new environment. The investigation of several different post-transplantation survival times will clarify some aspects of the mechanisms involved in this phenomena. (Supported by NSF grant BNS 86 17022).

Localization of Rat Odorant-Binding Protein to the Lateral Nasal Gland Suggests an Odorant Transport Function. PAUL M. HWANG, JONATHAN PEVSNER, PAMELA B. SKLAR, J. CLARK VENABLE and SOLOMON H. SNYDER (Depts. Neuroscience and Pharmacology & Molecular Sciences, Johns Hopkins Sch. Med., Baltimore MD 21205).

Odorant-binding protein (OBP) is an abundant, soluble protein that binds several odorants with micromolar affinities. Based on its cDNA sequence it is homologous to a family of ligand-carrying proteins. We previously localized bovine OBP to mucus-secreting glands in nasal epithelium. We now report the localization of rat OBP and its mRNA to the lateral nasal gland (LNG) by in situ hybridization, immunohistochemistry and odorant autoradiography. For in situ hybridization, run-off transcripts were generated from a cDNA clone for rat OBP. [³⁵S]RNA transcripts that were antisense (complementary to mRNA) but not sense (identical to mRNA) intensely labelled the LNG. The same gland was specifically labelled using antibodies against rat OBP and staining by an avidin/biotin/peroxidase technique. The LNG was also labelled by odorant autoradiography. Coronal sections of rat nasal epithelium were incubated in 100 nM [³H]dimethyloctanol, [³H]2-isobutyl-3-methoxypyrazine or [³H]amyl acetate. The binding was saturable and specific. [³H]Isovaleric acid, which fails to bind OBP, also fails to label the LNG by autoradiography. We propose that OBP is synthesized in the LNG and released through its long duct into mucus where it transports hydrophobic odorants toward the olfactory neuroepithelium.

Supported by a grant from International Flavors and Fragrances, Inc.

Neural Plasticity in the Aging Brain. Richard M. Costanzo and Edward E. Morrison (Medical College of Virginia).

During the normal aging process the mammalian central nervous system undergoes morphological changes. This is particularly true in the olfactory nervous system. In old age the number of olfactory receptors is reduced, the size of the olfactory bulb decreases and olfactory function is said to decline. In young animals the olfactory system shows a remarkable capacity for neurogenesis and plasticity. Receptor cells undergo continuous neurogenesis and, following lesion and neural degeneration, replacement neurons are capable of growing new axons that reestablish connections with the brain.

In the present study we examined the olfactory system of old hamsters (12-24 months) to determine to what extent plasticity exists in the aging brain. Unilateral olfactory nerve lesions were made to induce degeneration of olfactory receptor cells. Following recovery periods 4-126 days, horse-radish peroxidase (HRP-VI and WGA-HRP) was applied to the nasal cavity and was used to determine if replacement axons were capable of forming terminal structures (glomeruli) within the old brain. The results indicated that newly formed axons reached the old brain. HRP reaction product was observed within the glomerular layer and in ectopic regions within the granular cell layer. Electron microscopic examination of glomeruli confirmed the formation of synapses between olfactory receptor axons and dendrites of second order cortical cells. WGA-HRP reaction product observed in second order cells provided evidence of transynaptic labelling.

These results indicate that neural plasticity persists within the aging mammalian brain.

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SEM Observations of the Human Olfactory Epithelium. Edward E. Morrison, Nitya R. Ghatak and Richard M. Costanzo (Medical College of Virginia).

The morphology of the human olfactory epithelium has been carefully studied with both light and transmission microscopy. However, only a few studies using scanning electron microscopy (SEM) have been reported. In these studies observations have primarily focused on the examination of surface morphology. In the present study we used SEM to examine the full extent of the olfactory epithelium from the epithelial surface to the basal lamina region.

Human olfactory tissue was obtained 4-8 hours post mortem, fixed in 0.6% paraformaldehyde - 2% glutaraldehyde and processed for SEM examination. Fracture planes in the epithelium that occurred during tissue preparation made it possible to examine the morphology and relationships among cellular structures below the epithelial surface. Human olfactory neurons appear to be similar to those of other mammalian species. A dendritic process emerges from an elongated cell body and reaches the epithelial surface where it terminates in a knob like structure giving rise to olfactory cilia. Axon processes were observed originating from receptor cell bodies, passing through the basal lamina and forming axon bundles within the lamina propria. The apical surface of the columnar shaped supporting cells was covered with microvilli. Supporting cells were observed to extend from the epithelial surface to the basal lamina. Irregularly shaped cells resembling basal cells were located in the lower regions of the epithelium.

These preliminary results indicate that scanning electron microscopy provides a valuable technique for studying the morphology of the human olfactory epithelium.

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Cultured Cells from Olfactory Tissue could be Either Bowman's Gland Cells or Neuronal Progenitor Cells. S.K. PIXLEY (Dept. of Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45208).

Studies of the multiple cell types in the olfactory epithelium would be greatly facilitated by the development of *in vitro* systems in which each cell type can be identified, isolated and maintained. A culture system was developed in which a unique cell type can be isolated and maintained for over two months.

Adult rat olfactory tissue was dissociated and maintained in monolayer cell culture. After one week in culture the majority of cells were fibroblast-like with patches of flat, epithelioid cells. Some of the latter were arranged radially around central noncellular areas, while other cells contained large, lipid or mucoid droplet-like inclusions, with the cytoplasm and nucleus stretched into a thin circumferential rim. Since the epithelioid cells were more resistant to trypsin treatment, it was possible to remove the more rapidly dividing fibroblast-like cells, preventing overgrowth and providing fairly pure cultures. Long-term growth was supported by Ham's F-10 medium containing 15% horse serum and 2.5% fetal calf serum, plus 50% conditioned medium from the fibroblast-like cells and 20% conditioned medium from growth hormone and prolactin-secreting anterior pituitary cell cultures.

The epithelioid cells were positive for the monoclonal 2B8 antibody (weak) and anti-keratin antiserum (strong). They were negative for olfactory marker protein, 6B7 and glial fibrillary acidic protein. In tissue sections, anti-keratin antiserum stained olfactory basal cells and Bowman's glands. The 2B8 antibody bound strongly to a subset of olfactory neurons, weakly to olfactory basal cells and not to Bowman's glands. Thus, these cells may be Bowman's gland cells or olfactory basal cells, the precursors to olfactory primary neurons and sustentacular cells. These cells should prove useful to develop additional antibody markers and to investigate the nature of similarities between olfactory basal cells and Bowman's gland cells.

Cell Cultures that Smell. H. G. COON, F. CURCIO, M. L. BRANDI*, K. SAKAGUCHI*, R. SWERDLOW. (Lab. of Genetics, NCI, and Metabolic Diseases Branch*, NIADDK, NIH, Bethesda, MD)

We have cloned 16 cell strains from the olfactory epithelium of newborn rats. Some of these cell lines accumulate cyclic AMP (cAMP) and/or release intracellular Ca^{++} in response to submicromolar quantities of odorants in the culture medium. We believe that at least some of these lines are derived from the progenitors of the sensory neurons. Because our cultures do not form fully differentiated neurons, we have reasoned that the best test of olfactory function would be the sensitive and selective response to chemical odorants as measured by second messenger production. Other markers for the sensory cells, olfactory marker protein and carnosine, have not been found. We have studied cAMP accumulation employing standard RIA assays where dose-response data were obtained with commonly used odorants at 10^{-4} to 10^{-11} M for 10 mins and time-course data from 1 - 30 mins at 10^{-7} M. In addition to those cell strains that do not respond, our negative control is the mouse neuroblastoma (C-1300) N-18 which has been proposed as a model of the olfactory system since it will depolarize in medium with 0.1-1.0 mM odorant (Kashiwayanagi and Kurihara, Brain Res. 293:251-258, 1983). N-18 shows no cAMP accumulation after exposure to citralva from 10^{-4} to 10^{-9} M, however, one of our cell lines, SNIF-12, responded on different trials with a 2-5 fold increase (with a peak about 10^{-7} M). The same experimental run showed no response from SNIF-12 to β -ionone. Both SNIF-12 and another clone, SNIF-11 gave 2-fold increases in cAMP after exposure to 10^{-7} M coniferan. SNIF-11 and SNIF-8 did not respond to citralva. Using fura-2 we have obtained evidence that a rapid release of intracellular Ca^{++} occurs when 10^{-7} M pyridine or citralva is added to the medium with SNIF-12 or 2. SNIF-8 has shown a Ca^{++} response to citralva but not coniferan or pyridine. SNIF-11 gave a Ca^{++} response to coniferan only. N-18 have not shown any Ca^{++} response. Both kinds of response, although unequivocal when they occur, have proven to vary erratically with different passages of the same cell lines. These cultures are complex mixtures of dividing and differentiating cells; we do not yet know the passage conditions that will give consistently responsive populations.

Cation Channels in Reconstituted Catfish Taste Epithelial Membrane Preparations Activated By Arginine in an Enantiomerically Specific Manner. JOHN H. TEETER*, JOSEPH G. BRAND*, D.L. KALINOSKI* and BRUCE P. BRYANT* (*Monell Chemical Senses Center, 3500 Market Street; and *VA Med. Ctr., U. of PA; †Dept. of Physiology, U. of PA, Philadelphia, PA 19104).

L-Arginine (L-ARG) is a potent taste stimulus for the channel catfish (*Ictalurus punctatus*). Receptor binding studies have demonstrated specific high affinity binding of L-ARG to a partial membrane preparation (P2) from catfish taste epithelium with K_D of $\sim 10^{-7}$ M. The enantiomer, D-ARG, is a poor electrophysiological stimulus, but a moderate to strong cross-adaptor of L-ARG responses. In biochemical receptor binding assays, D-ARG inhibits L-ARG binding, suggesting that it may act as a partial antagonist to L-ARG receptor sites. A plasma membrane-enriched fraction (concentrated in nucleotidase activity) was collected from a 30% (w/w) sucrose/buffer interface after centrifugation of P2 for 30 mins at 40,000 x g. Incorporating membrane fragments from this fraction into artificial phospholipid bilayers (soybean phospholipids), formed over the tips of patch pipettes, allowed measurement of single-channel currents. Spontaneous and voltage-gated channel activity with slope conductances ranging from 8 pS to 230 pS was observed in many bilayers. In about 50% of the bilayers showing no channel activity between -80 and +80 mV, concentrations of L-ARG greater than 0.5-1 μ M elicited single-channel fluctuations. The amplitude of the open channel current varied approximately linearly with voltage between -80 and +80 mV, corresponding to a slope conductance of about 40 pS. Increasing L-ARG concentration from 1 to 100 μ M resulted in increased bilayer conductance. The L-ARG-activated currents reversed at about 0 mV with Ringer in the bath and a pseudo-intracellular solution in the pipette (mM: 85 KCl, 12.5 NaCl, 1.6 MgCl₂, 0.25 CaCl₂, 0.5 EGTA, 5 HEPES, pH 7.4), indicating that the channel was cation selective with similar permeability to Na⁺ and K⁺. In contrast to the activity induced by L-ARG, neither D-ARG nor the potent taste stimulus L-alanine elicited channel activation up to concentrations of 100 μ M. However, in agreement with its ability to act as a partial antagonist of L-ARG receptor binding, D-ARG at levels below 100 μ M effectively reduced activity due to L-ARG in a manner consistent with competitive kinetics.

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Sugar-activated Ion Transport in Canine Lingual Epithelium: A Role In Sugar Taste Transduction. SHEELLA MIERSON, GERARD L. HECK, SHIRLEY K. DESIMONE, AND JOHN A. DESIMONE (Medical College of Virginia, Virginia Commonwealth University, Dept. of Physiology, Richmond, VA 23298, U.S.A.)

A significant body of evidence indicates that ion transport pathways in the apical regions of lingual epithelial cells serve as salt taste receptors in mammalian taste buds. We have now obtained evidence that, in the case of the dog, there exists a sugar-activated ion transport pathway that is linked to sugar taste transduction. The conclusion is based on two parallel lines of experiments (1) ion transport studies on the isolated canine lingual epithelium, and (2) recordings from the canine chorda tympani. Mono- or disaccharides added to 30 mM NaCl or to Krebs-Henseleit buffer (K-H) in the mucosal bath stimulate a dose-dependent increase in the short-circuit current (I_{SC}) *in vitro* over the concentration range coincident with mammalian sugar taste responses. This sugar-evoked increase in I_{SC} is partially amiloride-blockable. Sodium supports a larger sugar-evoked I_{SC} than does potassium. A mixture of 0.5 M NaCl + 0.5 M sucrose produces no increase in I_{SC} over that of 0.5 M NaCl alone, analogous to an experiment in which 0.5 M NaCl + 0.5 M sucrose stimulated no additional chorda tympani activity after adaptation of the canine tongue to 0.5 M NaCl (Andersen, Funakoshi and Zotterman, 1963, *Olfaction and Taste*, 1:177-192). Flux measurements with ²²Na, ³⁶Cl, and tritiated 3-O-methylglucose during stimulation with 0.5 M 3-O-methylglucose in K-H *in vitro* show that (1) the sugar-evoked I_{SC} is due to an increase in the sodium influx, and (2) ouabain or amiloride reduces the sugar-evoked sodium influx but not sugar transport. Amiloride inhibits the canine chorda tympani response to 0.5 M NaCl by 70-80% and the response to 0.5 M KCl by about 40%. This agrees with the percent inhibition *in vitro* by amiloride of I_{SC} due to 0.5 M NaCl or KCl. Amiloride also partially blocks the chorda tympani responses to sucrose and to fructose. The results indicate that in the dog: (1) the ion transporter subserving sodium taste also subserves part of the response to potassium, and (2) a sugar-activated, sodium-preferring ion transport system is one mechanism mediating sugar taste transduction. Psychophysical experiments indicate a similar sweet taste mechanism for humans (Schiffman, Lockhead and Maes, 1983, *PNAS*, 80:6136-6140).

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Ionic Mechanisms of Ammonium Chloride Responses in the Hamster Chorda Tympani Nerve. HARRY WMS. HARPER (Eastern Research Center, Stauffer Chemical Co., Dobbs Ferry, N.Y. 10522)

Whole-nerve concentration-response curves for NH₄Cl differ in two notable respects from those for other salts. First, the maximum response obtainable is very large, about as great as the sum of the maximum responses to NaCl and KCl. Second, there is a marked inflection in the response curve. This author has proposed a Diffusion Potential Model for salt taste transduction which involves two types of receptor cells, one having receptor membranes permeable to Na-like ions (Na-cells), and the other being permeable for K-like ions (K-cells). In this model the ion channels in the receptor membranes of Na-cells are closed until the concentration of Na-like ions achieves some minimum level, while the K-cell channels are always open. If NH₄⁺ is permeant for both Na-cells and K-cells, then at lower concentrations it will activate only K-cells, but at higher concentrations both K-cells and Na-cells will be stimulated. This would account for both the response curve inflection (as the Na-cells "turn on") and the large maximum response. To test this, both 0.2 M and 1.0 M NH₄Cl were tested against the Na-channel blocker amiloride and the K-channel blocker 4-AMP. Amiloride at .01 mM had no effect on responses to 0.2 M NH₄Cl, but substantially inhibited responses to 1.0 M NH₄Cl. In contrast, 4-AMP at 5 mM did inhibit responses to 0.2 M NH₄Cl. (4-AMP did not inhibit responses to 1.0 M NH₄Cl, but it is not effective against KCl at high concentrations, either.) These results are consistent with the response mechanisms suggested by the Diffusion Potential Model of salt taste transduction.

4-Aminopyridine and BaCl₂ block taste responses to KCl in rat. SCHIFFMAN, S. S., SIMON, S. A., GILL II, J. M. and ERICKSON, R. P. (Departments of Psychiatry, Physiology and Psychology, Duke University, Durham NC, 27706)

The finding that a sodium transport pathway mediates the taste of NaCl (DeSimone et. al., 1981 and Schiffman et. al., 1983) suggests the possibility that an analogous mechanism may mediate the taste of KCl. Multi-unit recordings to a range of tastants were obtained from the rat NST before and after two known potassium conductance blockers 4-aminopyridine (4-AP) and BaCl₂ were applied topically to the anterior tongue of rat. When the tastants were applied simultaneously with 5mM 4-AP for 20 seconds, the magnitude of the response measured 10 seconds after the initial change from baseline to 30mM KCl and 100mM was reduced by 43% and 62% respectively. No suppression was found for 300mM KCl. A slight reduction (10%) was found for 100mM NaCl. These data for KCl are similar to observations first presented by Kim and Mistretta (1986). When the tongue was preadapted for 5 minutes to 4-AP, the responses to salts, sweeteners, acids, and bitter compounds were significantly and irreversibly suppressed. When tastants were applied simultaneously with 5mM BaCl₂, there was a significant increase (35%) to 30mM KCl. None of the other stimuli tested was significantly affected. However, when the tongue was preadapted for 5 minutes with 5mM BaCl₂, the NST responses to all concentrations of KCl were significantly and reversibly suppressed (30mM, 100mM, and 300mM were suppressed 57%, 32% and 20% respectively). 100mM NaCl was not significantly affected. Studies in the isolated rat epithelia confirm these findings in that 4-AP reduced the short circuit current to low concentrations of KCl. These data suggest the possibility that transduction mechanisms for potassium taste may involve conductance pathways pharmacologically similar to those found in other tissues.

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Effects of thaumatin on monkey taste nerve response to sweeteners and non-sweeteners
G. HELLEKANT, A. I. FARBMAN and T. W. ROBERTS
(University of Wisconsin, Madison, WI and Northwestern University, Evanston, IL)

In previous work we showed that thaumatin, an intensely sweet protein, binds to several elements in taste pores of rhesus monkey. These include the microvilli, vesicle-like structures shed from the microvilli, and the amorphous, dense secretion released by Type I cells (Farbman et al., 1987, Scan. Mic., 1:351-357). We suggested the vesicle-like structures are composed of fragments of membrane containing the stimulus-binding site complex shed from the taste cells. To test this hypothesis we recorded the nerve response to repeated stimulation of taste buds with thaumatin and taste stimuli which cause no shedding, small molecular stimuli. Repeated stimulation with thaumatin results in declining neural response of thaumatin and other sweeteners but not that of non-sweeteners. If the taste buds are stimulated with sequences of thaumatin interspersed with longer intervals with no thaumatin stimulation, partial recovery of the ability of the taste cells to respond to sweeteners occurs. Sucrose or any other small molecular stimuli don't exert these effects. The results support our earlier hypothesis that thaumatin binding sites on taste microvillar membranes are shed as a stimulus-binding site complex into the pore. This imposes on the cell the need to replace receptors. The reduced neural response to rapid stimulation with thaumatin indicates that the cells have not had time enough to replace the receptors.

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Survey of the chemosensory response properties of lobster mouthparts: spectral populations and tuning breadth.
FRANK COROTTO and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole MA).

This is the first investigation of the physiological response properties of primary chemoreceptor cells from the third maxilliped of the lobster, *Homarus americanus*. Of interest were the presence of different spectral populations, and their tuning breadth, particularly in comparison with known populations in three other chemoreceptor organs in the same animal. Chemoreceptor cells were located by injecting a one second search stimulus pulse (equimolar mixture of L-aspartate, L-glutamate, ammonium chloride, L-proline, sucrose, L-lysine, L-glutamine, betaine, L-arginine, hydroxy-L-proline, ethanol, L-alanine, glycine, taurine, and leucine), each at applied peak concentrations of 10 uM (mixture concentration of 150 uM) into a carrier flow of artificial seawater which bathed the excised appendage. Subsequently all cells were tested with all fifteen compounds injected separately as one second pulses with applied peak concentrations of 10 uM. Action potentials were recorded extracellularly from afferent axons. Many cells were narrowly tuned (responses to other compounds were minimal), of these, some responded best to betaine, others to L-glutamate, ammonium, and taurine. Less narrowly tuned cells were also found: some responded best to hydroxy-L-proline, others responded best to glycine, L-arginine, and L-glutamate. If receptor physiology reflects behavioral function, we can speculate that taurine cells, common in antennules and rare in maxillipeds, take advantage of the naturally high signal to noise ratio of taurine - due to its low background in seawater - to detect distant odor plumes. Maxillipeds guard food intake (Derby and Atema 1982, J. exp. Biol. 98: 317-327) which sets different constraints on the receptor cells of that taste organ.

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Cross Adaptation of Bitter Compounds: Single Receptor Site. PAMELA E. SCOTT and JOSEPH FARLEY
Department of Psychology, Princeton University, Princeton, N. J. 08544.

Human psychophysical and animal behavioral studies have suggested the presence of multiple sub-classes of bitter perception which may reflect the presence of multiple sub-classes of peripheral bitter receptors. We attempted to obtain direct evidence for multiple receptor sites by recording from the glossopharyngeal (IXth) nerve and through the use of a cross adaptation paradigm. The IXth nerve of male Sprague Dawley rats was dissected using the surgical and recording procedures described by Frank (1965). Initial experiments determined the responsiveness of the IXth nerve to different classes of bitter compounds. The control stimulus was distilled water; test stimuli were quinine hydrochloride (QHCl: 0.0001M - 0.10M), urea (0.03M - 4.00M), phenylthiocarbamide (PTC: 0.0001M - 0.01M), potassium chloride (KCl: 0.001M - 1.0M), and sucrose octa-acetate (SOA: 0.0001M - 0.01M). QHCl, urea, and KCl all evoked robust and concentration-dependent responses. Neither PTC nor SOA evoked measurable neural responses at the concentrations used. In subsequent cross-adaptation experiments, the neural response to QHCl was attenuated when urea was used as the adapting solution. When QHCl was used as the adapting solution, the neural responses to both urea and KCl were dramatically attenuated. When KCl was used as the adapting solution, the neural responses to QHCl was attenuated. In conclusion, complete and reciprocal cross adaptation was observed for urea and quinine and for KCl and quinine. We thus failed to obtain evidence for multiple classes of bitter receptors subserving the IXth nerve response. These results suggest that central mechanisms account for subclasses of bitter perception.

Responses of Single Facial Taste Units to Amino Acids in the Sea Catfish, *Arius felis*. W. MICHEL and J. CAPRIO. Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

Although amino acids are known to be highly effective gustatory stimuli in fishes, systematic examinations of responses of single, peripheral taste fibers to these compounds are few. The present report identifies and defines the taste specificity of single facial nerve fibers to amino acids in the sea catfish, *Arius felis*. This study is a portion of a continuing effort to determine common principles in the processing of amino acid taste information in catfishes and to identify species specific differences. Single and few unit preparations were obtained *in vivo* from a branch of the ramus mandibularis that innervates the maxillary barbel. Identified single units from few fiber preparations were isolated by a time-amplitude window discriminator, converted to standard pulses and analyzed off-line by computer. At least three types of amino acid taste units were identified: L-alanine/glycine-best, D-alanine-best and L-histidine-best. Units that responded best to L-alanine and glycine were most numerous. D-alanine also stimulated this unit type, but was only approximately 60% as effective as L-alanine at equal stimulus concentration. Conversely, other taste units were identified that responded best to D-alanine. In these units, the responses to L-alanine and glycine were 50-70% and 0-15% of the response to D-alanine, respectively. Basic amino acids were relatively nonstimulatory to either of the alanine unit types. A third fiber type was stimulated only by basic amino acids with an order of effectiveness of: L-histidine > L-arginine > L-ornithine > L-lysine. Characteristics of unit types for amino acids in the facial taste system of the sea catfish will be compared with the L-alanine-best and L-arginine-best fiber types found in the freshwater channel catfish, *Ictalurus punctatus*.

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Voltage Clamp Analysis of Generator Currents in Vertebrate Olfactory Receptor Neurons. STUART FIRESTEIN and FRANK WERBLIN, University of California at Berkeley.

A description of the olfactory transduction event which includes direct observation and measurement of the generator current is lacking. Without these measurements issues such as receptor cell sensitivity, selectivity, and adaptation remain impossible to resolve. Utilizing a new preparation, the olfactory slice, we are now able to record odorant responses under whole cell patch clamp. In the slice preparation, which will be described in detail, receptor cell soma are accessible to the patch recording electrode and an odorant filled pipette is positioned near easily observed cilia. The stimulus, a cocktail of six odorants chosen to represent a wide range of odor qualities, was delivered by pressure ejection. An inward current with amplitude up to 200 pA was elicited by stimulus puffs of varying concentrations. In response to short (<1 sec) puffs the current rose with an S-shaped trajectory and decayed exponentially. Time to peak decreased and decay time increased with increasing concentration. Even in response to maintained stimulation (>5 sec) the generator current was transient with a decay half-time of c. 3 sec. The I-V relation for the generator current was linear in the physiological range with a reversal potential at +5 mV. The shortest latency recorded was 180 msec, at least half of which must be due to cellular processes. Odorant concentrations of 10^{-4} M to 10^{-3} M were required in the pipette, however concentrations at the cilia were measured to be as much as 2 orders of magnitude lower. A dose response curve for the receptors suggests a requirement for cooperativity. The results show a relatively slowly developing inward generator current whose amplitude and kinetics are a function of odorant concentration.

Single channel properties of the ciliary cyclic nucleotide-gated conductance. TADASHI NAKAMURA* and GEOFFREY H. GOLD* (Dept. of Cell. and Molec. Physiol., Yale U. School of Medicine). *Present address: Monell Chemical Senses Center, Philadelphia, PA.

Cyclic AMP and cyclic GMP reversibly increase the conductance of the olfactory ciliary membrane (Nakamura and Gold, 1987). In the normal ionic environment, i. e., with divalent cations present on both sides of the membrane, the magnitude of the cyclic nucleotide-gated conductance in different patches varied between 50 and 300 pS (mean, 190 pS). Removal of divalent cations from both sides of the membrane increased the magnitude of the conductance ~20-fold, varying between 0.7 and 12 nS (mean, 4 nS) in different patches. In the absence of divalent cations, low concentrations of cyclic AMP or cyclic GMP induced single channel currents which increased in frequency with cyclic nucleotide concentration. At +60 mV, the open channel conductance was 25-30 pS, and the mean open time was ~50 msec. The channel properties were the same whether elicited by cyclic AMP or cyclic GMP, consistent with our earlier evidence that both cyclic nucleotides act on the same ion channels. Dividing the total conductance of a patch by the single channel conductance yields the number of channels in a patch, which varied between 30 and 440. We do not presently know whether this large variability results from variations in channel density or variations in patch area. However, if we assume that patch area is determined by the size of the patch pipette tip opening (0.2 μ m, as measured by scanning EM), the channel density would vary between 450 and 7000 μ m⁻².

We are presently determining the permeability ratios of the cyclic nucleotide-gated conductance for various monovalent cations. Thus far, it appears that Na and K are almost equally permeable. This may explain why the reversal potential for the cyclic nucleotide-gated conductance is near 0 mV.

EOG amplitude is correlated with odor-stimulated adenylate cyclase activity in the bullfrog olfactory epithelium. GRAEME LOWE*, TADASHI NAKAMURA* and GEOFFREY H. GOLD* (Dept. of Cell. and Molec. Physiol., Yale U. Sch. Med). *Present address: Monell Chemical Senses Center, Philadelphia, PA.

Recent work suggests that odor-stimulated adenylate cyclase activity mediates olfactory transduction in vertebrates (Pace et al., 1985; Nakamura and Gold, 1987). However, stimulation of the cyclase by certain odorants is weak or undetectable, leading to the suggestion that the cyclase may not mediate transduction for all odorants (Sklar et al., 1986). An alternative explanation for these data is that differences in the ability of odorants to stimulate the cyclase may simply reflect differences in the number of receptor proteins or receptor cells which are sensitive to those odorants. If the latter hypothesis is correct, we would expect the amplitude of the EOG to be correlated with the magnitude of adenylate cyclase stimulation by various odorants.

The EOG was recorded from the excised bullfrog olfactory epithelium (dorsal surface), mounted in a perfusion chamber (active area, 12 mm²). Odors were applied to the apical side, dissolved in Ringer's solution, to approximate the conditions used for Sklar et al.'s cyclase assay. All odors were applied at an estimated concentration of 100 μ M. Stimulus duration was not calibrated, but the elicited EOG responses lasted 5-10 sec (duration at half-maximal amplitude). Of 29 odorants tested, thus far, 25 odorants elicited a monophasic negative EOG, and 4 odorants elicited a multiphasic negative EOG. The multiphasic responses may contain contributions from currents unrelated to transduction. Therefore, the odors which produced the multiphasic responses were not included in the analysis below. The peak amplitude of the EOG for each odorant was measured and compared to the percentage of maximal cyclase stimulation reported by Sklar et al. The correlation coefficient relating the EOG to the cyclase data was 0.89.

Our data show that the ability of odorants to stimulate adenylate cyclase is correlated with their ability to generate an EOG response. This result is consistent with the adenylate cyclase model of olfactory transduction.

VOLTAGE-DEPENDENT IONIC CURRENTS IN LOBSTER OLFACTORY RECEPTOR NEURONS. T. S. McClintock and B. W. Ache. The Whitney Laboratory, University of Florida.

Four voltage-dependent currents: I_{Na} , I_{Ca} , I_{KCa} and I_K , were found in the isolated somata of spiny lobster olfactory receptor cells. Somata were removed by suction from enzyme-treated (papain and trypsin) hemisections of the olfactory organs (antennules). Currents were recorded under voltage clamp using the whole-cell patch configuration. 1) I_{Na} : This fast transient current was isolated by blocking the other currents with Cs, TEA and Co. It begins to activate at about -25 mV and peaks at 0 mV. Time to peak is about 0.25 ms at room temperature. Inactivation is half maximal at -48 \pm 4 mV (n=6). It is blocked by 10^{-7} M TTX. 2) I_{Ca} : A second inward current, which peaks in 3 - 4 ms and then decays slowly, is visible when outward currents are blocked by Cs and TEA. This Ca current is carried by Ca, Ba, or Sr and is blocked by millimolar concentrations of Co or Cd. It begins to activate at about -35 mV and "washes out" within minutes after membrane breakthrough. Inactivation is half maximal at -32 mV (n=1). 3) I_{KCa} : At voltage steps to potentials above -30 mV, a large outward current is obvious. After a 1 - 2 ms delay, it activates rapidly to peak at 6 - 10 ms and then decays slowly. It is blocked by Co and Cd and washes out within minutes of membrane breakthrough. It is absent when Cs or Na replaces K in the patch pipette solution and is resistant to 20 mM internal or external TEA. 4) I_K : This current can be resolved in isolation when TTX and Ca channel blockers are present. It activates during voltage steps to potentials above -25 mV, peaks after 15 - 20 ms and then inactivates very slowly. It is blocked by 5 mM external TEA and is absent when Cs or Na replaces K in the patch pipette solution. Inactivation caused by 250 ms prepulses is half maximal at -41 \pm 5 mV (n=4).

These data show that receptor potentials below -30 mV pass through these somata with little or no contribution or attenuation from voltage-dependent currents. The large outward currents are probably the cause of rectification in the current-voltage relationships at potentials above -30 mV (Schmeidel-Jakob, Anderson, and Ache, submitted).

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The Recording of Odorant-Induced Mucosal Activity Patterns with a Voltage-Sensitive Dye. P. F. KENT, M.M. MOZELL (SUNY Health Science Center at Syracuse, NY)

One possible mechanism for discriminating odorants is their spatio-temporal response representations upon the mucosa. Unlike with the usual electrophysiological techniques, there is the potential with voltage-sensitive dyes to sample odorant induced activity simultaneously from a large number of fine-grain points covering the entire mucosal surface. In the present experiment fluorescence changes in the dye (WW781) were obtained from 100 contiguous sites on the bullfrog olfactory mucosa (10 x 10 pixels, each composed of 12 bits) every 10 ms in response to an odorous stimulus. The odorants were d-limonene, butanol, and amyl acetate each presented at two concentrations. Each odorant was humidified and puffed uniformly upon the entire mucosa for a duration of 0.7s at a rate of 300 cc/min. The fluorescent signals elicited by these stimuli were nearly identical in shape and time course to their EOGs which were recorded in separate experiments. Like the EOGs, the fluorescent signals exhibited adaptation and were abolished or reduced by either Triton X-100 or ether. There were no fluorescent signals when non-odorized, humidified air was presented as the stimulus or when the tissue was unstained. The data indicates that the spatio-temporal response pattern across the mucosa was more uniform for amyl acetate than for butanol and d-limonene. These latter two showed ordered shifts in response amplitude and response time as the mucosa is crossed.

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Perireceptor Events in Purinergic Olfaction: Properties of Ectonucleotidases Associated with Chemosensory Organs of the Spiny Lobster. H. G. TRAPIDO-ROSENTHAL, R. A. GLEESON, J. T. LITTLETON, and W. E. S. CARR (The Whitney Laboratory, University of Florida)

The olfactory organ of the Florida spiny lobster, *Panulirus argus*, consists of rows of aesthetasc sensilla present on the antennule. Each sensillum consists of a porous sheath of chitin enclosing the dendrites of chemosensory neurons. Physiological studies have revealed that many of these neurons are differentially excited by nucleotides (AMP, ADP, or ATP) present externally in seawater (Derby et al., J. Comp. Physiol. 155:341, 1984; Carr et al., J. Comp. Physiol. 158:331, 1986). Biochemical studies have shown that aesthetascs contain ectonucleotidases that dephosphorylate excitatory nucleotides (Trapido-Rosenthal et al., J. Neurochem. 49:1174, 1987); these perireceptor events can exert pronounced effects on the physiological responses to nucleotides. Kinetic measurements of the ecto-AMPase (5'-nucleotidase) activity indicate that for AMP concentrations of up to 10 uM, the half-life of an AMP molecule in the receptor environment is less than 500 msec. This value coincides well with the notion that ectoenzymes function to limit the lifetime of signal molecules in the vicinity of receptors, thus increasing the ability of the organism to detect changes in concentrations of environmentally important chemicals. Kinetic measurements of the sensillar ecto-ATPase are currently in progress. Preliminary studies indicate that the ATPase requires divalent cations for activity, with Ca^{++} being preferred to Mg^{++} . This enzyme thus appears to be similar to ecto-ATPases found on mammalian neurons and protozoan cilia. Like the antennules, the walking legs of the spiny lobster contain a large number of chemosensory sensilla, which also exhibit potent ectonucleotidase activities.

Supported by NSF grant BNS-8607513.

Implications of Perireceptor Ectonucleotidase Activity in the Physiological Responses of Purinergic Chemosensory Neurons. R. A. GLEESON, H. G. TRAPIDO-ROSENTHAL, and W. E. S. CARR (The Whitney Laboratory, University of Florida)

Previous physiological studies demonstrated the existence of P_1 -like (AMP-best) and P_2 -like (ATP-best) purinergic chemoreceptor cells within the olfactory organ (antennule) of the spiny lobster, *Panulirus argus* (Derby et al. J. Comp. Physiol. A 155:341-349, 1984; Carr et al., J. Comp. Physiol. A 158:331-338, 1986). Recent biochemical studies have revealed the presence of ectonucleotidases associated with the antennule that rapidly dephosphorylate both ATP and AMP to adenosine; adenosine is subsequently removed from the receptor environment by an uptake system (Trapido-Rosenthal et al., J. Neurochem 49:1174-1182, 1987; Trapido-Rosenthal et al. this meeting). To assess the potential effect of AMP dephosphorylation on the physiological response of P_1 -like chemoreceptor cells, AMP was focally delivered to the receptor cells in a series of pulses (2 sec apart) with no rinsing between pulses. Some cells rapidly adapt in this stimulus paradigm and do not respond after the first presentation of AMP; other cells, however, recover from adaptation and respond to each pulse. These latter findings suggest that AMP is cleared from the receptor environment, presumably by ectonucleotidase activity. In experiments with P_2 -like chemoreceptor cells, the products of ATP dephosphorylation, ADP and AMP, were found to strongly antagonize these cells when presented in binary mixture with ATP. The suppression by ADP, the most potent antagonist, gives rise to an apparent parallel right shift in the dose-response curve for ATP. Modifications in the structure of ADP at the ribose or phosphate moieties, or substitution of a pyrimidine ring for adenine, results in a dramatic loss of its suppressing potency. The antagonism exhibited by ADP and AMP suggests that the response profile of the P_2 -like receptor cell population may be tempered by the generation of these products via ectonucleotidase activity.

Supported by grant BNS-8607513 from the NSF.

A Filter Model for Chemoreceptor Cells Jelle Atema (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA)

Receptor cells interface with the stimulus environment. Their filter properties serve both to enhance contrast between biologically important signals and environmental noise and to provide diversity in neural response patterns coding for a great variety of signals. The CNS appears capable, based on previous experience, of "choosing patterns" generated across its receptor cells. Certain patterns are no doubt genetically fixed or preferred due to natural and sexual selection. In an attempt to describe the different filters through which an animal views its chemical environment, we study four different chemoreceptor organs in one aquatic species, the lobster *Homarus americanus*. These organs have different behavioral functions and operate in different micro-environments; they are also differently "tuned" in terms of receptor cells. Spectral tuning is required for stimulus identification; temporal tuning facilitates orientation to a stimulus source. Molecular mechanisms turn receptor cells into spectral and temporal filters. Generally, stimulus patterns that reach the receptor membrane have been prefiltered by more peripheral physical structures. Recent results suggest that spectral filtering may be based on (variable) distribution of differently tuned receptor sites across receptor cells. Mixture effects and cross-adaptation may be based on competitive binding and differential coupling of membrane receptors with cellular transduction mechanisms. Together, site distribution and cross-adaptation result in unique spectral responses for each cell. Temporal filtering results from adaptation and disadaptation rates of receptor cells. These rates were found to vary greatly among cells, adding to each cell's uniqueness as it responds to natural mixtures in turbulent odor plumes that determine lobster's feeding and social behavior.

Supported by grants from the Whittell Foundation and NSF (BNS 8512585)

Sun. Morn Talk 8:00 AM Abst. # 199

A Lack of Synergism in Protistan Chemosensory Responses May Reflect Basic Differences in Protistan and Metazoan Sensory Transduction. M. LEVANDOWSKY and ANDREA NEITA (Haskins Laboratories, Pace U., N.Y., NY 10038)

The cilioprotist, *Tetrahymena pyriformis*, responds to several amino acids when these are presented singly. In recent studies with small metazoans occupying a niche similar to that of the ciliate, it has been observed that amino acids that were inert when tested singly elicited chemosensory responses when tested in combination. We looked for similar synergistic effects with *Tetrahymena* using a capillary assay method (Biol. Bull. 167, 322-30). Combinations of chemicals that were not active individually did not elicit responses when tested in combinations. On ecological grounds, the same selective pressures that would lead to the evolution of metazoan responses to combinations rather than to single chemicals should operate also in the protist. It is suggested that, in the metazoan, separate specialized cells detect the individual chemicals, and a decision to respond to a combination of signals is then made in the central nervous system. Such a mechanism may not be possible within a single cell.

This work was supported in part by a grant to ML from the Whitehall Foundation.

#202 appears after #163

Sun. Morn Talk 8:30 AM Abst. # 201

Genotype - Tastant Interactions in Bitter Avoidance by Mice. DAVID B. HARDER and GLAYDE WHITNEY (Dept. of Psychology, Florida State Univ.).

Genetically based differences among inbred mouse strains have been reported for avoidance of sucrose octaacetate, phenylthiourea, quinine sulfate, and other bitter compounds. The relative strain sensitivities have varied across the several compounds, however. This inconsistency, and the available segregation data, suggest that a number of loci may contribute to differences in bitter taste sensitivity. A further implication is that, for mice, the human bitter taste quality may encompass a number of chemical classes, potentially discernable through the differential effects of variation at these loci. To begin a genetically defined categorization, concentration response functions for strains known to differ in avoidance of the three above named compounds, were compared across bitter substances varying widely in chemical structure. Several distinct patterns of relative sensitivity were found, and the substances tested were grouped according to similarity of pattern. Substances representative of each group were then tested on panels consisting of the hybrid offspring of all possible reciprocal pairings of the inbred strains (a diallel cross) to examine the genetic architecture underlying the strain by tastant interactions. The diversity of patterns observed, and the differences found in some aspects of their genetic bases, support suggestions (which have also been made regarding sweet and salty) that bitter is not a uniform quality, possibly mediated by multiple transductive mechanisms. Marked dissimilarities in responses to particular compounds, between humans and the mice in the present study, suggest potentially useful species differences in gustatory functioning.

Supported, in part, by NINCDS Grant NS15560.

Sun. Morn Talk 8:15 AM Abst. # 200

Aggregation in infant corn snakes (*Elaphe guttata*) and garter snakes (*Thamnophis radix*). GARY TEN EYCK and MIMI HALPERN (SUNY Health Science Center at Brooklyn)

Adult and new born garter snakes use chemical cues during aggregation and shelter selection and depend on their vomeronasal systems to detect these conspecific odors (Burghardt, 1980; Heller & Halpern, 1982). In the present study we compared aggregation and shelter selection in infant corn snakes and infant garter snakes. One group of corn snakes (N=8) and one group of garter snakes (N=8) were each tested three times a day in aquaria for aggregation and shelter preference. Each aquarium contained four cardboard shelters, four water dishes and two rocks. A trial began by placing the eight snakes in a container and releasing them from the center of the aquarium. After 3, 9 or 12 hours, the location of each snake was noted and a new trial was begun. The test aquaria and contents were left undisturbed between trials for the first 37 trials. Corn snakes and garter snakes were found under shelters more frequently than in the open field (97%, corn snakes, and 87.5%, garter snakes). Both groups developed strong shelter preferences with aggregates of 3 or more snakes found in some shelters more often than would be expected by chance ($p < .001$). Corn snake aggregation scores were significantly higher than garter snake aggregation scores ($t = 2.03$, $p < .05$). In another set of experiments snakes were tested in freshly prepared aquaria as above for 15 trials and then tested individually in the same, uncleaned aquaria. Individually tested corn snakes predictably returned to shelters preferred during group testing ($r = +.8847$, $p < .05$) but garter snakes did not ($r = -.713$, $p > .05$).

Supported by NIH Grant NS11713.

Sun. Morn Talk 9:30 AM Abst. # 203

Chemospecific Deficits in Taste Detection Following Bilateral Chorda Tympani Section in Rats. A. C. SPECTOR (Univ. of Penn.), G. SCHWARTZ (Monell Chem. Senses Ctr.), H. J. GRILL (Univ. of Penn.).

Results from electrophysiological and behavioral studies employing taste stimuli have suggested a certain degree of regional chemospecificity in the oral cavity of the rat. This study was designed to examine the effects of bilateral chorda tympani (CT) nerve section on the behaviorally expressed sensitivity to "near-threshold" concentrations of sucrose and NaCl. Three rats were trained, in a specially designed gustometer, to maintain spout contact to receive intermittent water reinforcement (70 μ l). Next, they were trained to cease spout contact when suprathreshold concentrations of either sucrose or NaCl were delivered (70 μ l). Failure to cease spout contact to taste stimuli resulted in a brief footshock; failure to maintain spout contact during water delivery resulted in a 15 sec time-out which further delayed the next water reinforcement. Once rats were responding at asymptotic performance levels the concentration ranges of the taste stimuli were lowered (sucrose: 0.001 M - 0.3 M; NaCl: 0.0001 M to 0.1 M). Avoidance was quantified and the data from taste trials were standardized to the performance on water control trials and statistically fitted to a sigmoidal function which permitted the evaluation of the concentration representing the 1/2 maximum response. The rats exhibited a log unit upward shift in the 1/2-max concentration for NaCl following CT section. In contrast, sucrose sensitivity was marginally affected by CT nerve section. We then tested these same rats following bilateral section of the glossopharyngeal nerve (IXn). However, this proved to be injudicious since 7 weeks had elapsed since CT section and postmortem histology indicated that the CT nerve had regenerated. This explained the surprising recovery of NaCl sensitivity following section of the IXn in rats that seemingly did not have an intact CT. At the writing of this abstract we are trying to replicate the observed effects of CT nerve section and plan to sacrifice the rats for histology before the nerve has had an opportunity to regenerate. These results and their implications will be discussed.

A SINGLE-BOTTLE GUSTATORY PREFERENCE TEST FOR USE WITH MONKEYS. T.C. Pritchard, R.B. Hamilton, and R. Norgren. Dept. of Behavioral Science, College of Medicine, The Pennsylvania State University, Hershey, PA 17033.

Gustatory preference tests with non-human primates are reported rarely, presumably due to the tediousness and expense of maintaining a suitable number of subjects for an extended period. This report describes a single-bottle test that yields reliable preference and aversion data when used with a small number of monkeys. Six cynomolgus monkeys, maintained on 18 hrs of water deprivation, were given daily access for 30 min to a single bottle of a sapid stimulus or distilled water (dH₂O). The monkeys were provided with 2 hrs of tap water ad libitum 3 1/2 hrs after the test period. Sapid stimuli were tested on alternate days because pilot data indicated that more than 24 hrs are needed to recover normal drinking behavior following a 30 min exposure to concentrated sapid stimuli. On intervening days, the monkeys were given dH₂O during the 30 min test session. Average intake was determined by presenting dH₂O periodically during regular test sessions. On 34 such days, the mean water consumption was 80 ml/30 min. The volume of sapid stimuli consumed was corrected for the average water intake of each monkey. To date, the monkeys have been tested with ascending series of the following stimuli: sucrose, NaCl, HCl, quinine HCl, citric acid, glycine, proline, glucose, histidine, and monosodium glutamate (MSG), as well as the 5'nucleotides GMP and IMP. Preference was defined as intake that exceeded water intake by at least one std. dev.; aversion, as intake at least one std. dev. below the average water consumption. Of the 81 solutions tested (12 chemicals and a single mixture pair), the monkeys preferred 10 and found 5 aversive. Sucrose, glucose, and glycine were preferred stimuli with peak intake for 0.3 M sucrose and 1.0 M glucose almost 4 times that of water (2.5 X for 1.0 M Gly). Quinine HCl was rejected at 0.003 M. In contrast to some other species, the monkeys were indifferent to all NaCl and MSG concentrations below 1.0 M, which was aversive. Neither HCl nor citric acid was rejected at any concentration tested (up to 0.03 M). Proline, which humans describe as sweet-tasting, and histidine, a flat to bitter gustatory stimulus, were both neutral stimuli at midrange concentrations. These preliminary data suggest that the taste preferences of cynomolgus monkeys differ from that of both rodents and humans. Supported by the Int. Glutamate Tech. Com., NS 20518, and MH 00653.

No Presentation. Abst. # 205

Perception of Sweetness and Bitterness in different Vehicles. CALVIÑO, A. M., COMETTO-MUNIZ, J. E. and GARCÍA-MEDINA, M.R. (Laboratorio de Investigaciones Sensoriales, CONICET - Esc. Sal. Públ., Fac. Medicina, UBA, C.C. 53, 1453 Buenos Aires, Argentina).

Supported in part by a grant to project # 03-908203 from the CONICET, República Argentina.

The aim of this work was to study taste-taste, taste-vehicle, and taste-vehicle-taste perceptual interactions. Stimuli comprised three sucrose concentrations (292, 585 and 1170 mM), three caffeine concentrations (13, 26 and 52 mM) and three binary mixtures between low (292mM-13mM), middle (585mM-26mM) and high (1170mM-52mM) levels of both components. These nine taste stimuli were dispersed in water (W), carboxymethylcellulose 1% W/V (CMC) and gelatine 6% W/V (G). The sweetness and bitterness responses of 20 subjects were analyzed and the following effects were noted: a) Six of the nine sucrose-vehicle-caffeine combinations showed that bitter intensity appears significantly weaker in mixture than in equimolar unmixed solution (taste-taste interaction). Although caffeine presents a general significant effect on sweet intensity the nine individual comparisons between alone and mixed conditions showed absence of a significant decrement of sweetness in mixture. b) A significant suppression of sweetness and bitterness was observed when 292 mM of sucrose or 26 mM of caffeine were added to gelatine instead of water (taste-vehicle interaction). c) The increase in vehicle consistency and the simultaneous addition of another taste reduced the perceived intensity of a taste presented alone and dissolved in water (taste-vehicle-taste interaction). This holds for both, sweetness and bitterness. The observed magnitude reductions seen in c) are approximately equivalent to the sum of taste suppression (see in a) plus vehicle suppression (see in b).

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