The Association for Chemoreception Sciences

Book of Abstracts

AChemS

20 YEARS

Sarasota, Florida
April 22-26, 1998

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Assembly of Program and Abstract Books by Jennifer Lawrence, University of Arizona

GENERAL INFORMATION

1. Registration will be in the Longboat Room on Wednesday evening, 5:00-7:00 P.M., and in the morning during the meeting.

2. All slide sessions will be held in the Sara Desoto Room. All speakers in slide sessions should meet with the session chairperson and give the slides to the projectionist at least 20 minutes prior to the start of the session.

3. All poster sessions will be held in the Hernando Desoto Room. All morning posters should be removed by 3 P.M. and all evening posters should be removed by midnight. Posters should be placed on the board with the number that corresponds to the assigned board in the program book.

4. On Thursday evening, Dr. Maxwell Mozell will present a special lecture on the History of AChemS in honor of the Twentieth Anniversary of AChemS. A social will follow immediately after this lecture.

5. The Clinical Luncheon will take place on Saturday from 1:00-3:00 P.M. in the Florida Room. Tickets are on sale in the Longboat Room.

5. The Industrial Reception will take place on Thursday from 5:30-7:00 P.M. in the Florida Room. Tickets are on sale in the Longboat Room.

6. The Wine Tasting will be held in the Florida Room on Saturday from 5:00-7:00 P.M. Tickets are on sale in the Longboat Room. Admission also includes a bottle of your favorite wine.

7. There will be a van from the hotel to Lido Beach Thursday, Friday, and Saturday afternoons. The van will leave from the front of the hotel on the hour, beginning at 1:00 P.M. and returning on the half-hour from Lido Beach. The last van will leave the beach at 4:30 P.M.

8. There will be a van from the hotel to the softball game. The van will leave from the hotel at 2:30 P.M. and return to the hotel at the end of the game.

9. AChemS will sponsor an opening buffet reception on Wednesday from 6:30-8:00 P.M., and a limited number of breakfast pastries will be available each morning beginning at 7:00 A.M.

10. The Hyatt will provide a cash "Quick-Lunch Sandwich Cart" at the conference center daily at 12:00 P.M. The Prefunction area is reserved for eating your lunch and socializing if you do not care to go outside and wish to meet with other conferees.

Please refer to the Program Book or program summary in this volume for listings of Symposia, Special Lectures, and other Special Events.

ASSOCIATION FOR CHEMORECEPTION SCIENCES
TWENTIETH ANNUAL MEETING
PROGRAM SUMMARY

	WEDNESDAY, APRIL 22, 1998
12:00 P.M.	Executive Committee Meeting
5:00-7:00 P.M.	Registration (*Long Boat Room*)
6:00-6:30 P.M.	Minority Fellows Organizational Meeting (*Palm Room*) *Organizer: Diego Restrepo*
6:30-8:00 P.M.	Opening Buffet (*Gallery*)
8:00-8:30 P.M.	Welcome, Opening Remarks, and Awards Ceremony (*Hernando Desoto Room*) *Thomas Scott, Executive Chairperson*
LECTURE 8:30-9:30 P.M.	Givaudan-Roure Lecture (*Hernando Desoto Ballroom*) Dr. Christine Petit Unité de Génétique des Déficits Sensoriels Institut Pasteur, CNRS, Paris *Chairperson: Gail Burd* "The X chromosome-linked form of Kallmann syndrome: An early developmental defect of the olfactory system"
9:30 P.M.	Social Reception and Cash Bar (*Gallery*)
9:40 P.M.	Organizational Meeting for Students with Travel Awards (*Hernando Desoto Ballroom*) *Organizers: Joel White and Alan Nighorn*
	THURSDAY, APRIL 23, 1998
Thursday Morning	
SLIDES 8:00-9:30 A.M.	Taste Physiology: From Receptor Cell to Brain *Chairperson: Tim Gilbertson*
9:30-9:45 A.M.	Refreshment Break

SYMPOSIUM 9:45 A.M.- 12:00 P.M.	Mosquito Olfaction Symposium *Organizer: John Hildebrand* Dr. Willem Takken Laboratory for Entomology, Wageningern Agricultural University, Netherlands "Differentiation in the behavior of anthropophilic and zoophilic malaria mosquitoes in response to host odors" Dr. Martin Geier Department of Entomology, University of California at Riveside "Olfactory host finding of yellow fever mosquitoes: Exploring the attractive odor blend and the effect of odor plume structure on upwind flights" Dr. Alan Grant American Biophysics Corp. "Electrophysiological investigations of sensory neurons involved in mosquito host-seeking behavior" Dr. John Carlson Department of Biology, Yale University "*Drosophila*, a model system for the study of mosquito olfaction" Dr. Laurence Zwiebel Department of Biology, Vanderbilt University "A molecular characterization of olfaction in the malaria vector mosquito, *Anopheles gambiae s.s.*" *Sponsored by National Institutes of Health (NIDCD)*
POSTERS 8 A.M.- 12 P.M.	Olfactory Receptor Structure Structural Studies of Central Olfactory Pathways Human Taste and Oral Chemesthesis
Thursday Afternoon	
1:00-2:30 P.M.	Open Discussion on Cloning Olfactory Receptor Genes (*Sara Desoto Ballroom*) *Organizer: Peter Mombaerts*
3:30-5:30 P.M.	Educational Outreach Workshop (*Sarasota Room*) Lecture by Ken Kubo, BIOTECH Project, University of Arizona and Discussion with Sarasota Teachers *Organizer: Christine Byrd* *Sponsored by the National Institutes of Health (NIDCD)*

5:30-7:00 P.M.	Industrial Reception (*Florida Room*) *Organizer: Grant Dubois*
Thursday Evening	
SLIDES 7:00-9:00 P.M.	Human Olfaction *Chairperson: Pam Dalton*
9:00-9:15 PM	Refreshment Break
POSTERS 7:00-9:15 P.M.	Physiology of Central Olfactory Pathways
LECTURE 9:15 P.M.	AChemS: The Beginning (*Sara Desoto Ballroom*) AChemS XX Celebration Lecture Dr. Max Mozell *Chair: Thomas Scott*
10:00 P.M.	AChemS XX Social (*Gallery*) *Sponsored by AChemS and Oxford University Press*
	FRIDAY, APRIL 24, 1998
Friday Morning	
SLIDES 8:00-9:15 A.M.	Coding in the Olfactory System *Chairperson: John Kauer*
LECTURE 9:15-9:45 A.M.	Special Lecture on Olfactory Coding Dr. Gilles Laurent Department of Biology, California Institute of Technology "Spatio-temporal codes for odors in oscillating neural assemblies" *Chairperson: John Kauer* *Sponsored by the National Institutes of Health (NIDCD)*
9:45-10:00 A.M.	Refreshment Break
SLIDES 10:00-11:45 A.M.	Olfactory Receptor Cells: Functional Aspects *Chairperson: Randy Reed*
POSTERS 8 A.M.-12 P.M.	Taste in the CNS: Structure and Function Human Olfaction and Nasal Chemesthesis
Friday Afternoon	
12:00-1:30 P.M.	Minority Fellow Luncheon (*State Room*) *Organized by: Diego Restrepo*

1:30-3:00 P.M.	NIH Grant Review Workshop (*Sara Desoto Ballroom*) *Chairperson: Jack Pearl, NIDCD* Speakers: Drs. Judith Finkelstein, NIA; Craig Jordon, NIDCD; Laurence Stanford, DRG
3:00-5:00 P.M.	Annual Smell vs Taste Softball Game (location: TBA) *Organizer: John Caprio*
Friday Evening	
SLIDES 7:00-8:15 P.M.	Human Taste Perception *Chairperson: Beverly Cowart*
LECTURE 8:15-8:45 P.M.	Special Lecture on Human Taste Psychophysics Dr. Linda Bartoshuk Department of Surgery, Yale University "From sweets to hot peppers: Genetic variation in taste and oral pain" *Chairperson: Beverly Cowart*
8:45-9:00 P.M.	Refreshment Break
SLIDES 9:00-10:30 P.M.	Chemical Communication *Chairperson: Brian Smith*
POSTERS 7:00-11:00 P.M.	Life and Death in Taste Buds Development and Plasticity of Central Olfactory Pathways Vomeronasal, Nervus Terminalis and Trigeminal Systems: PNS Mechanisms
	SATURDAY, APRIL 25, 1998
Saturday Morning	
SLIDES 8:00-9:00 A.M.	Factors in PNS Development and Plasticity *Chairperson: Linda Barlow*
9:00-9:15 AM	Refreshment Break

SYMPOSIUM 9:15-11:30 A.M..	Developmental Mechanisms Symposium *Organizers: Gail Burd and Leslie Tolbert* *Chairperson: Leslie Tolbert* Dr. Connie Cepko Department of Genetics, Harvard Medical School "Extrinsic and intrinsic cues that affect the development of vertebrate photoreceptors" Dr. Scott Fraser Biological Imaging Center, California Institute of Technology "Animating developmental neuroanatomy" Dr. Piali Sengupta Department of Biology, Brandeis University "Development and function of the olfactory system in *C. elegans*" *Discussants: Monica Vetter and Christopher Nosrat* *Sponsored by the National Institutes of Health (NIDCD)*
POSTERS 8:00-11:30 A.M.	Clinical Taste, Olfaction, and Chemesthesis Taste: Peripheral Mechanisms
11:45 A.M. - 1:00 P.M.	AChemS Annual Business Meeting (*Sara Desoto Ballroom*) *Chairperson: Thomas Scott, Executive Director* Address on the status of NIDCD Dr. James Battey, Director of NIDCD
Saturday Afternoon	
1:00-3:00 P.M.	Clinical Luncheon (*Florida Room*) *Organized by: Daniel Kurtz* Speakers: Drs. Barry Green and William Cain "Clinical Aspects of Trigeminal Chemoreception"
3:30-5:00 P.M.	Panel Discussion on Careers in the Chemical Senses (*Sara Desoto Ballroom*) *Organized by: Michael Meredith*
5:00-7:00 P.M.	Wine Tasting (*Florida Room*) *Organized by: Charles Greer and Jack Kinnamon*

Saturday Evening	
SYMPOSIUM 7:00-9:00 P.M.	AChemS Awards Symposium *Chairperson: Charles Greer* Speakers will be the winners of the: Takasago Award for Research in Olfaction Moskowitz Jacobs Inc. Award Ajinomoto Award AChemS XX Award for Outstanding Achievement in the Chemical Senses
9:00-9:15 P.M.	Refreshment Break
SLIDES 9:15-10:30 P.M.	Human Chemesthesis *Chairperson: Barry Green*
POSTERS 7:00-11:00 P.M.	Olfactory System: Peripheral Mechanisms
	SUNDAY, APRIL 26, 1998
Sunday Morning	
SLIDES 8:00-9:30A.M.	Plasticity in the Olfactory Epithelium *Chairperson: Virginia Carr*
9:30-9:45 A.M.	Refreshment Break
SLIDES 9:45-10:45 A.M.	Physiology of Central Olfactory Pathways *Chairperson: Kathy Hamilton*
POSTERS 8:00-11:00 A.M.	Chemical Communication Gustatory Behavior and Psychophysics

Lectures and Symposia

1 Givaudan-Roure Lecture

The X chromosome-linked form of Kallmann syndrome: An early developmental defect of the olfactory system. CHRISTINE PETIT *Unité de Génétique des Déficits Sensoriels, Institut Pasteur, CNRS, Paris, France* FAX: 33-1-45-67-69-78

Kallmann syndrome is defined by the association of hypogonadism with anosmia or hyposmia. Hypogonadism in Kallmann syndrome is due to gonadotropin-releasing hormone (GnRH) deficiency. Anosmia has been related to the absence or hypoplasia of the olfactory bulbs and olfactory tracts. Three different modes of inheritance of the syndrome have been reported, X chromosome-linked (KAL-1 gene), autosomal dominant and autosomal recessive. Only, the KAL-1 gene has been identified. In a human 19 week old male fetus carrying a deletion of the KAL-1 gene, it has been observed that the olfactory, vomeronasal and terminalis nerve fibers had no contact with the brain, nor were the GnRH neurons present in any area of the brain ; both nerve fibers and GnRH cells were observed in the upper nasal area and within the dural layers of the meninges beneath the forebrain (Schwanzel-Fukuda M et al. (1989) Mol. Brain Res. 6: 311-326. This observation indicates that the developmental defect of the X-linked Kallmann syndrome is an early developmental defect of the olfactory system, which does not impair the initial steps of differentiation of olfactory and GnRH neurons within the olfactory placode. The study of the KAL-1 gene in a number of patients has led us to conclude that some of the additional symptoms or anomalies occasionally reported in Kallmann patients, such as renal aplasia, mirror movements, hearing loss, cerebellar ataxia, high arched palate are also underlied by defects within the KAL-1 gene. We named the protein encoded by the KAL-1 gene, anosmin-1. The predicted sequence of anosmin-1 suggests that it is an extracellular protein component with anti-protease and anti-adhesion activities.

In order to progress in the understanding of the pathophysiological processes involved in this developmental defect, the expression pattern of the KAL-1 gene has been studied in chicken, during embryogenesis as well as in adult. Anosmin-1 has been produced in mammalian cells, purified and in vitro functional studies undertaken. Finally, an analysis of the expression of anosmin-1 in primate embryos, mainly focusing between weeks 4 and 9 of development, has been performed. The results obtained shed light on the role of anosmin-1 during early stages of the development of the olfactory system.

8 Mosquito Olfaction Symposium

Differentiation in the behavior of anthropophilic and zoophilic malaria mosquitoes in response to host odors, WILLEM TAKKEN and TEUN DEKKER, *Laboratory for Entomology, Wageningern Agricultural University, PO Box 8031, 6700 E. Wageningen, the Netherlands.* willem.takken@medew.ento.wau.nl

Female mosquitoes locate their hosts guided chiefly by olfactory cues. The latter exist of emanations produced by vertebrates from which the insects take their blood meal. While animals produce a large number of volatile compounds, originating from expired air and skin emanations, it is assumed that mosquitoes respond to a limited number of host chemicals only. Carbon dioxide, a compound present in the emanations of all vertebrates, is the kairomone that most mosquitoes have in common, but next to it other compounds are known to play a behavioral role as well. The reported differences in host preference are likely to be based on differences in response to host odors, and this must be reflected in the nature of the chemosensory perception of the mosquito. Recently we have conducted experiments to reveal the differences in anthropophily and zoophily within members of the Afrotropical malaria mosquito *Anopheles gambiae s.l.*, a group that exists of six morphologically identical mosquitoes but with large differences in host preference. It was found in the laboratory and field that the highly anthropophilic *An. gambiae sensu stricto* responds poorly to carbon dioxide, but strongly to human odors. In contrast, the zoophilic *An. quadriannulatus* was not attracted to human odors but responded strongly to carbon dioxide and cattle odor. The opportunistic *An. arabiensis* varied in its responses to human and animal emanations, responding more strongly to carbon dioxide than *An. gambiae* but less than *An. quadriannulatus*. The implications of these behaviors will be discussed in relation to adaptation to specialistic and generalistic host-seeking behavior.

9 Mosquito Olfaction Symposium

Olfactory host finding of yellow fever mosquitoes: Exploring the attractive odor blend and the effect of odor plume structure on upwind flights. MARTIN GEIER[1,2], JÜRGEN BOECKH[2], [1] *Department of Entomology, University of California at Riveside, CA 92521,* [2]*Institut für Zoologie, Universität Regensberg, Germany.* martin.geier@biologie.uni-regensburg.de

Female mosquitoes use olfactory cues for finding and identifying their specific hosts to suck blood from them. We are interested in (1) which component the mosquitoes use for identification of their hosts, (2) how the receptor cells code this olfactory information, and (3) which behavioral mechanisms and strategies they use to locate the odor source. As a model system we use the yellow fever mosquito *Aedes aegypti* (L.), which is active at day and prefers humans for blood sucking. We measure the attractiveness of odors in a Y-tube olfactometer. We found that the very attractive effect of human skin odor is based on a synergism between lactic acid, which is a major component on human skin, and several other components. Lactic acid plays a key role in attracting mosquitoes, because the skin odors lose its attractiveness without this component. A blend of lactic acid and three synthetic compounds, which are emitted from human skin, is nearly as effective as an extract of the skin residues, which attracts 80-90% of the mosquitoes within 30 sec. Electrophysiological studies show that each of these compounds excites a different cell type in the A3 sensilla on the antenna of the mosquito.

Besides the quality of the host odor also the structure of the attractive odor plume seems to play an important role in host finding. We tested two different attractive odors: (1) carbon dioxide, which is an activating and attractive component in the breath of the host, and (2) the complex odor given off from human skin. The percentage of upwind flights increased in carbon dioxide plumes with increasing intermittence of the signal. If the carbon dioxide concentration was completely homogeneous inside the wind tunnel, the upwind progress was greatest reduced. The opposite effect was found with skin odor: A high percentage of mosquitoes (90%) flew sustained upwind in the homogeneous odor, whereas in intermittent plumes the percentage was at most 40 %.

Electrophysiological investigations of sensory neurons involved in mosquito host-seeking behavior. ALAN J. GRANT[1,2,3],BRUCE E. WIGTON[2], and ROBERT J. O'CONNELL[1,3]. [1] *Worcester Foundation from Biomedical Research, Shrewsbury, MA 01545,* [2]*American Biophysics Corp., East Greewich, RI 02818,* [3]*Univesity of Massachuttes, Medical School, Worcester, MA 01655.* ajgrant@edgenet.net

Mosquitoes rely on a range of cues, including olfactory signals, to locate appropriate hosts for blood feeding. To investigate one of the sensory channels involved in host odor detection, we conducted electrophysiological studies of the olfactory receptor neurons found in the *sensilla basiconica* on the maxillary palps of mosquitoes. Our results describe a class of receptor neurons whose properties could provide the afferent input required for some aspects of odor-mediated host-seeking behavior. One neuron within the maxillary palp sensilla is sensitive to stimulation with carbon dioxide (CO_2). This neuron has an threshold (150-300 ppm) which is near ambient atmospheric concentrations of CO_2 (300-350 ppm). The concentration-response function, for this neuron, to CO_2 stimulation is steep. Small (50 ppm) fluctuations in concentration elicit reliable changes in activity. The neuron behaves like an absolute CO_2 detector in so far as the back ground concentration of CO_2 does not modulate the respond to step increases in CO_2 concentration. However, it can also detect rapid fluctuation in concentration by means of an interesting reciprocal phasic-tonic temporal pattern of discharge to step increases and decreases in concentration. In addition to this neuron, the maxillary palp sensillum contains two other receptor neurons, neither of which responds to CO_2. One of these neurons responds to stimulation with very low doses of another behaviorally relevant compound, 1-octen-3-ol (octenol). Octenol, in combination with CO_2, modulates the behavioral response of the mosquito as measured in field experiments. However, the electrophysiological response function to a mixture of octenol and CO_2 is not different from the response to the individual compounds. This suggests that octenol's behavioral effects are the consequence of central integration and not a modulation of the sensory channel involving CO_2 detection.

Drosophila, a model system for the study of mosquito olfaction. JOHN R. CARLSON, MARIEN DE BRUYNE, PETER J. CLYNE, *Dept. of Biology, Yale Univ., PO Box 6666, New Haven, CT 06520-8103,* john.carlson@yale.edu

The genetics and molecular biology of *Drosophila* make it a useful model system in which to investigate basic principles of olfaction which may have applications in the control of mosquitos and other insect vectors of human disease. The olfactory system of *Drosophila* shows similarities to those of other insects both in overall design and in molecular detail *e.g.* the presence of OBPs.

We have isolated a number of genes required for olfactory function and CO_2 response in *Drosophila* and have characterized several of them at the molecular level. We have explored the functional organization of the system with single-unit electrophysiology and have learned that one olfactory organ consists of six classes of neurons, paired in three types of sensilla according to a strict pairing rule. With this foundation, we have begun to analyze selected mutants and have learned that neuronal identity relies on the *acj6* gene. In *acj6* mutants, a subset of neurons acquires a different odorant response profile. Molecular analysis of *acj6* shows that it encodes a POU domain transcription factor. Other members of this class of transcription factor have previously been shown to act in the development of mammalian visual and auditory neurons.

Our data suggest a model in which the odor response spectrum of an olfactory neuron, and perhaps the choice of receptor genes, is determined through a process requiring the action of POU domain transcription factors.

Supported by NIH grant DC02174 and the HFSP.

A molecular characterization of olfaction in the malaria vector mosquito, *Anopheles gambiae s.s.* LAURENCE J. ZWIEBEL[1,2], and FOTIS C. KAFATOS[2], [1]*Department of Biology, Vanderbilt University, Nashville, TN 37235,* [2]*European Molecular Biology Laboratory, Heidelberg, Germany.* l.zwiebe@vanderbilt.edu

The ability to sense and discriminate a large collection of both chemical and visual cues is critical for several critical behaviors in arthropods. In particular, olfaction plays a significant role in host seeking behaviors of blood feeding mosquitoes and makes an important contribution to the vectorial capacity of these and other insect disease vectors. Therefore, an understanding of the chemosensory mechanisms that are employed by these arthropods may aid in the design and development of novel biological control strategies such as odor baited traps which may prove effective in reducing the transmission of pathogens responsible for a number of human and veterinary diseases. A molecular characterization of olfaction within the *Anopheles gambiae* species complex has been undertaken initially focused on *Anopheles gambiae s.s.* This study has been carried out using novel PCR based methods to generate chemosensory specific cDNA pools which have been used as substrates for the isolation of olfactory-specific cDNAs by subtractive hybridization. This approach has resulted in the isolation of several classes of olfactory tissue specific cDNA clones. One class of cDNA clones corresponds to members of the Odorant Binding Protein (OBP) family of proteins will be discussed. Northern blot analysis suggests that one putative OBP cDNA is specifically expressed in the sensory appendages of female *Anopheles gambiae s.s.* and may therefore play a role in host seeking behaviours in this vector mosquito. Additional antennal cDNAs have been isolated during this screen including several cDNAs corresponding to members of the Arrestin gene (super)family which have been shown to play essential roles in the regulation of specific G-protein coupled receptors (GPCRs) in several vertebrate signal transduction processes. The isolation of Arrestin cDNAs that are shown by RT-PCR analysis to be expressed in specialized olfactory tissues further supports the hypothesis that insect olfactory chemoreception is mediated by GPCRs. These approaches will be extended to isolate olfactory cDNAs that are specific to the anthropophilic vector *Anopheles gambiae s.s.* by subtractive hybridisation against a non-vector sibling species, *Anopheles quadriannulatus* which displays a strong zoophilic preference.

AChemS: The Beginning. M.M.MOZELL* & THE MEMBERSHIP OF ACHEMS, **SUNY Health Science Center at Syracuse, Syracuse,N.Y.,13210*

This paper unfolds the events, the people and the times that led up to the founding of AChemS and fashioned its character during its early formative years. A wealth of papers and records is used to document and describe the path over which AChemS came, going from the original assertions and denials for the need of such an organization to its later inception and nascent development. This narration highlights such topics as: the debate over the need for AChemS, the role of N.S.F. in the founding of AChemS, the derivation of the Association's name, the choice of Sarasota and the Hyatt House as the meeting site, the generation of the programs for the early annual meetings, the adoption of the bylaws, the process of incorporation and tax deferment, and the birth of the Givaudan Lectureship. Most emphatically highlighted, however, is the enthusiasm, commitment and hard work that the members of the chemosensory research community dislayed in bringing AChemS to fruition.

87 Special Lecture on Olfactory Coding

Spatio-temporal codes for odors in oscillating neural assemblies. GILLES LAURENT, *Division of Biology, California Institute of Technology, Pasadena, CA 91125*, laurentg@cco.caltech.edu

Stimulus evoked oscillatory synchronization of neural assemblies has been most clearly documented in the olfactory and visual systems of several vertebrates and invertebrates. Recent results from the olfactory system (antennal lobes and mushroom bodies) of locusts (*Schistocerca americana*) show that information about odour identity is contained in the identity of the recruited neurons as well as the timing of their action potentials in an oscillatory population response. This suggests that brain oscillations may reflect a common reference for messages encoded in time, allowing combinatorial representations of odors in time as well as in space.

Although stimulus-evoked oscillatory phenomena are reliable, their roles in sensation, perception, memory formation and pattern recognition remained to be demonstratedÑ a task requiring a behavioral paradigm. Using honeybees (*Apis mellifera*), we have recently demonstrated that odor encoding involves, as in locusts, the oscillatory synchronization of assemblies of projection neurons, and that this synchronization is, here also, selectively abolished by the GABA receptor-associated chloride channel blocker, picrotoxin. In collaboration with the group of Brian Smith (OSU), we then showed, using an associative odor conditioning paradigm, that picrotoxin-induced desynchronization impairs the discrimination of molecularly similar odorants, but not that of dissimilar odors.

It appears, therefore, that oscillatory synchronization of neuronal assemblies is functionally relevant and essential for fine sensory discrimination, but not necessarily for odor learning. This suggests that oscillatory synchronization and the kind of temporal encoding it affords provide an additional dimension by which the brain could segment spatially overlapping stimulus representations.

Supported by the National Science Foundation (USA) and the Sloan Center for Theoretical Neuroscience at Caltech.

133 Special Lecture on Human Taste Psychophysics

From Sweets to hot peppers: Genetic variation in taste and oral pain. LINDA M. BARTOSHUK, *Surgery (*Otolaryngology*), Yale School of Medicine, New Haven, CT 06520-8041.* Linda.Bartoshuk@yale.edu

Thresholds for PROP (6-*n*-propylthiouracil) are bimodally distributed: high thresholds - nontasters; low thresholds - tasters. Family studies showed that nontasting is mediated by two recessive alleles.

In the 1970s, our studies of PROP using suprathreshold scaling revealed three groups. Even saturated PROP was essentially tasteless to nontasters while tasters found it to be either moderately bitter (medium tasters) or intensely bitter (supertasters). In the United States, the proportions are roughly 25% nontasters, 50% medium tasters, and 25% supertasters. Females have the highest incidence of supertasters.

Supertasters perceive the most intense taste sensations from a variety of substances (e.g., sucrose, quinine, NaCl) but the degree to which supertasters perceive greater intensities varies across tastants. For example, quinine shows a larger difference across non to supertasters than sucrose which in turn shows a larger difference than aspartame. Miller's videomicroscopy revealed that supertasters have the greatest density of fungiform papillae(structures that contain taste buds).

Supertasters also perceive the greatest oral burn from irritants like capsaicin (chili peppers) and ethanol, and the greatest tactile sensations from fats (e.g., dairy fat, canola oil) and thickeners (guar gum). This occurs because fungiform papillae are innervated by the trigeminal nerve (pain,touch) as well as the chorda tympani (taste). Supertasters, with the most fungiform papillae, presumably also have the most abundant trigeminal innervation.

The conclusions above depend on comparisons of sensory experience across individuals. Since we cannot share one another's experience, there is no way to make absolutely correct comparisons. Thus we must make assumptions (e.g., "strong" reflects the same intensity to all subjects) that are difficult to validate. The association between taste intensity and number of fungiform papillae provides a new tool for validating psychophysical methodology for PROP studies. That is, the psychophysical differences obtained with various scaling methods can be compared to see which associates most closely with the anatomy.

Supported by NIH grants DC 00283 and DC 03003.

180 Developmental Mechanisms Symposium

Extrinsic and intrinsic cues that affect the development of vertebrate photoreceptors. CONNIE CEPKO, TAKAHISA FURUKAWA, ERIC MORROW, MICHAEL BELLLIVEAU, and DIALA EZZEDDINE, *Department of Genetics, Harvard Medical School, Boston, MA 02115,* FAX: 617-432-7595

Photoreceptors are a very specialized cell type that can be exquisitely sensitive to light. Those found in vertebrate eyes comprise the rods and the cones, those that are active in dim and bright light, respectively. We have been studying the development of these cells using several approaches. To begin to precisely characterize the development of these cells, the normal in vivo kinetics of opsin expression among rat retinal cells born at different times was examined. For rods born after E19, a strong correlation was found between the time a cell became postmitotic and the time it expressed opsin. The kinetics were different for those rods born before E19, and the kinetics were found to be intrinsically controlled as they were unaltered when the cells were exposed to the later environment.

We examined the effects of environmental factors on the development of postnatally generated rods, identifying several factors that reduce or increase the number of rods and cones in vitro. Following treatment with CNTF, LIF, or OSM, (members of a growth factor family that act through common receptor components) cells that ordinarily differentiated as rods instead differentiated into bipolar interneurons. The effect of the environment on the production of cones has also been examined. The number of cells differentiating as cones was found to increase when embryonic rat retinal cells were exposed to a rod-producing environment. The production of extra cones occured via recruitment of cells from the pool that normally differentiate as amacrine interneurons. These data show that there is an intrinsic bias towards cone vs. rod production among embryonic retinal progenitors, and that the number of cones produced can be regulated by environmental factors.

Transcription factors that might influence the development of photoreceptors have also been studied. A novel homeobox gene, Crx, was isolated and has been characterized. It is expressed only in photoreceptors and can bind to an oligonucleotide encoding a sequence found upstream of many photorereceptor-genes, such as opsin genes. In addition, in transfected cells, it can transactivate promoters with this sequence appended. We isolated a Crx gene from humans and found that it mapped to the cone rod dystrophy 2 locus. Subsequent work showed that Crx can have mutations in individuals with this disease in which photoreceptors degenerate.

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Animating developmental neuroanatomy. SCOTT E. FRASER, *Biological Imaging Center, Beckman Institute, California Institute of Technology, Pasadena, CA 91125.* FAX: (626) 449-5163

A major challenge in developmental neurobiology is to understand the assembly and refinement of neuronal connectivity. Although great advances have been made by examining the anatomy of fixed specimens, such approaches are largely blind to the dynamic processes that are now known to play major roles in sculpting the nervous system. Thus, our goal has become to follow cell interactions, migrations and lineages within the living embryo. This provides some special challenges, as the cells of interest can change shape, size and depth in the embryo over the span of minutes.

Cell microinjection can be used to introduce indicator dyes or cell lineage dyes into single precursor cells, thereby rendering them and their descendants unique and identifiable in the living embryo. Because the dyes are carried within the cells and parsed to the descendants at mitosis, the injected dye permits them to be followed as development proceeds. The challenge therefore becomes the imaging of these labeled cells, which often lie at inconvenient depths for conventional microscopies.

Selected results will be used to demonstrate the relative merits of laser scanning confocal, two-photon and magnetic resonance imaging (MRI) microscopies to follow not only cell lineages but also cell movements and axonal patterning in species ranging from the frog to the mouse. Each technique offers a different compromise between price, performance and ease of use. Some of the most striking intravital images have been obtained with our two-photon microscope, constructed from a Molecular Dynamics Sarastro laser scanning unit and a Coherent Ti-sapphire ultrafast laser. The modifications to the laser scanning unit are simple, permitting rapid changes between conventional confocal and two-photon microscopy, using the same optics and same specimen. The dramatically reduced photobleaching and the ability of the infra-red wavelengths to penetrate the specimen with less scattering has permitted us to record three-dimensional time lapse movies, even in tissues where phototoxic effects had previously been a difficulty. Cells labeled by dye injection or GFP expression can be followed as they execute neuronal development and patterning.

Development and function of the olfactory system in *C. elegans*. PIALI SENGUPTA, *Department of Biology and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254.* piali@volen.ccs.brandeis.edu.

We are interested in understanding how the sensory functions of chemosensory neurons are defined. The nematode *C. elegans* responds to multiple chemicals using a few chemosensory neurons. Genetic and behavioral screens have resulted in the identification of signalling molecules that are required for the function of these neurons. These experiments have shown that each neuron expresses a distinct subset of olfactory receptors and signalling genes.

We are investigating the developmental mechanisms that lead to the expression of cell-type specific olfactory genes. We are using a combination of behavioral and visual screens to identify mutants where the expression of olfactory genes is altered, resulting in altered sensory response profiles. Using these screens, we have identified mutants where the expression of olfactory receptor genes in specific neuron types is lost. We have also isolated mutants where olfactory neuron-specific genes are ectopically expressed in the lineage. These screens have also yielded mutants in the processes of sensory neuron migration, axon outgrowth and dendritic differentiation. In addition, we are using a reverse genetic approach to identify the roles of genes predicted by the genome sequencing project in the development of these neurons. We expect that further characterization and eventual cloning of the genes affected by these mutations will allow us to define key molecules in the cascade of events that results in the distinct identity and function of each chemosensory neuron type.

Poster and Slide Sessions

Slide 2

Rapid kinetics of receptor cell firing and second messenger formation in response to sucrose. KARA D. FOSTER[1], ANDREW I. SPIELMAN[2], and LINDA M. KENNEDY[1], *Neuroscience Program, Dept. of Biology, Clark University, Worcester, MA 01610 and [2]New York University College of Dentistry, Basic Science Division, New York, NY 10010,* kfoster@clarku.edu

Incubation of sucrose with rat gustatory tissue for several minutes leads to increases in cAMP levels (Striem et al., 1986, 1989), and these increases are generally thought to mediate sweet taste transduction. Since rat behavioral responses to sweet compounds occur within 250msec (Halpern and Tapper, 1971), it is not yet clear whether these increases in cAMP occur soon enough to be involved in transduction. Fly (*Phormia regina*) behavioral responses to sucrose are observed within 100msec (Dethier, 1955), and receptor cell responses are observed within 10msec of contact of the taste sensillum with 50mM sucrose. By 50msec, the response rate declines, indicating the onset of adaptation. In our earlier work, inhibition of the first 100msec firing rates of *P. regina* receptor cells to 50mM sucrose by the nonspecific phosphodiesterase inhibitor, IBMX, suggested a decrease, rather than increase in cAMP as a more likely transduction mechanism (Foster and Kennedy, 1994). Here, we have used rapid kinetics and the quench flow system (QFM5) to measure real time changes in second messengers in response to 50mM sucrose. cAMP and IP3 concentrations were monitored in homogenates of *P. regina* sensilla in the presence of 50mM sucrose for 10, 25, 50, 75 or 100msec. After 25, 50 and 75msec, sucrose induced clear decreases in cAMP (to 50%, 50%, 33% of basal levels), however limitations of the detectability of the assay precluded measurement of further decreases in cAMP. There was a marked increase in cAMP (to 540% of basal levels) after 100msec of stimulation. There was no change in IP3 from basal levels in sucrose stimulated tissue at any of the time points measured. These data suggest a decrease in cAMP as a possible mechanism for sweet transduction and that increases in cAMP may mediate adaptation after 75msec.

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Slide 3

A novel mechanism for bitter taste is mediated through cGMP. SOPHIA ROSENZWEIG, MAXIMILLIAN DASSO, WENTAO YAN, and ANDREW I. SPIELMAN, *Basic Science Division, New York University College of Dentistry, New York, NY 10010.* andrew.spielman@nyu.edu.

Several bitter taste mechanisms have been proposed: direct inhibition of potassium channels, activation of the inositol phosphate pathway, direct activation of G-proteins, or through gustducin-mediated reduction of cyclic nucleotides.

Here we report a novel mechanism that increases the second messenger guanosine 3',5'- cyclic monophosphate (cGMP). Monitoring cGMP production in real time with a quench flow system (QFM5) demonstrated a rapid and transient increase in the second messenger. The bitter stimulants theophylline (10 mM) and caffeine (25 mM), but not strychnine (2 mM) or denatonium (1 mM), increased cGMP levels at 50 mseconds ($p<0.001$). cGMP production declined throughout the following 400 mseconds and remained at baseline levels during the subsequent 5 seconds. The caffeine-induced cGMP response demonstrated concentration dependence between 5 and 50mM. Application of the soluble guanylyl cyclase inhibitors ODQ (10 mM) and methylene blue (30 mM) reduced the caffeine-induced response by 54% and 44%, respectively. This reduction was significant ($p<0.05$ and $p<0.01$).

We propose that the cGMP mediated signal transduction is achieved by direct inhibition of phosphodiesterase(s) by caffeine and theophylline, possibly leading to opening of the recently identified cyclic nucleotide-dependent cation channel.

Supported by NIH grant 10754.

Slide 4

On the relationship between taste qualities and taste fibers in higher primates. GÖRAN HELLEKANT, VICKTORIA DANILOVA AND THOMAS ROBERTS. *Department of Animal Health and Biomedical Sciences and Wisconsin Regional Primate Center, University of Wisconsin, Madison WI 537 06.* gh@ahabs.wisc.edu

A longstanding enigma is the relationship between the human taste qualities and the distribution of taste fiber found in animal models. The taste fibers could be categorized, but the categories derived conformed poorly with the human taste qualities. However, we and others found in non-human primates a considerably closer relationship.

Another way to study this relationship is to use a taste modifier, such as miraculin. Miraculin is a tasteless glycoprotein which, after it is bound to the tongue, adds sweetness to every sour compound. However, miraculin has no effect on most laboratory species. Also we really don't know what constitutes a taste quality in an animal. Once again the solution was to identify species which were affected by miraculin and could be expected to perceive a similar taste quality as humans do, if not the same. As a consequence we have used miraculin in simian primates. If sweet taste is the result of activity in one particular group taste fibers, taste fibers normally responding to sweet stimuli should be the only ones that change their impulse activity to sour compounds after miraculin. This opens up an opportunity to test coding theories in taste.

To date we have single taste fiber recordings in the chimpanzee, rhesus and marmoset which suggest that sweet taste is the result of activity in one particular group of taste fibers. Fibers which responded to sweeteners, after miraculin increased their taste responsiveness to sour compounds. No other fibers were affected. Further, we have corroborative whole taste nerve recordings from all simian primates tested: chimpanzee, gibbon, rhesus, baboon, cynomolgus, grivet and marmoset. Finally, our behavioral data show increased preference for sour solutions after miraculin in chimpanzee, rhesus and marmoset.

Thus in higher primates the data support the idea that the sweet taste quality is related to activity in a particular group of taste fibers. This presentation will present a summary of our data.

Slide 5

Effect of miraculin on behavioral and single taste fibers responses in common marmoset, *Callithrix jacchus jacchus*. VICKTORIA DANILOVA, GÖRAN HELLEKANT, ZHEYUAN JIN, *Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, WI 53706,* danilova@ahabs.wisc.edu.

The purpose of this study was to investigate the relationships between the activity in different types of taste fibers in both chorda tympani (CT) and glossopharyngeal (NG) nerves and the gustatory behavior in marmosets. As a tool we used the glycoprotein miraculin, which in humans adds a sweet taste quality to sour stimuli.

Methods. In electrophysiological experiments we recorded the responses of single taste fibers in both CT and NG to 3 salts, 4 acids, 6 bitter compounds and 30 sweeteners before and after tongue application of miraculin (1 mg/ml for 3 min). In two-bottle preference (TBP) experiments we measured preference ratios before and after licking 1-2 ml miraculin (3 mg/ml).

Results. Both in CT and NG hierarchical cluster analysis of the fibers' response profiles revealed 3 major clusters of fibers characterized by predominant sensitivity to sweeteners (S cluster), bitter compounds (Q cluster), or acids (H cluster). We recorded the responses of 7 S-cluster fibers (4 CT and 3 NG), 2 Q cluster fibers (1 CT and 1 NG) and 2 H cluster CT fibers before and after miraculin. Application of miraculin did not affect the responses to any of the compounds in Q and H cluster fibers. In contrast the response profiles of all the S cluster fibers were changed. Before miraculin they responded exclusively to sweeteners; after miraculin all acids elicited responses in every S fiber.

In short-term TBP tests, after licking 1-2 ml of miraculin, the hedonic reactions changed from rejection of all acids to preference.

Conclusions. In a previous study we demonstrated that marmosets preferred only compounds which stimulated S cluster fibers. These results further support the idea that the activity in a particular cluster of taste fibers is associated with the type of the behavioral response. These results, in connection with our earlier corroborative observations in rhesus monkey and chimpanzee, suggest that the responses to acids in S cluster fibers after application of miraculin underlie their changed hedonic reacitons to acids.

Slide 6

The role of amiloride-sensitive and -insensitive mechanisms in NaCl- and KCl-evoked responses in the hamster solitary nucleus. STEVEN J. ST. JOHN, JOHN D. BOUGHTER, Jr., and DAVID V. SMITH, *Dept. Anatomy & Neurobiology and Program in Neuroscience, Univ. Maryland School of Medicine, Baltimore, MD 21201.* sstjo001@umaryland.edu.

Lingual application of amiloride inhibits acid responses in NaCl (N)-best, but not HCl (H)-best cells in the hamster solitary nucleus (NST). There is some suggestion that amiloride-blockable channels play a role in transducing other ionic stimuli such as KCl, but the extent to which this contributes to central processing of nonsodium salts is unclear. Of interest in the present study was whether amiloride sensitivity or the concentration functions for NaCl or KCl varied with a neuron's best stimulus classification. The best stimulus for each cell (n=46) was determined by its response to 0.1 M sucrose, 0.03 M NaCl, 0.003 M HCl, 0.001 M quinine, and 0.1 M KCl applied to the anterior tongue. Amiloride (10 μM) significantly suppressed activity of *both* 0.1 M NaCl and 0.5 M KCl, but *only* in N-best cells (n=16). Concentration series also differed as a function of neuron type. NaCl and KCl were roughly equally effective in stimulating both H- and sucrose-best neurons at isomolar concentrations (0.001–1 M, 1-log steps). In contrast, isomolar NaCl was at least 4X more effective than KCl in stimulating N-best cells at all concentrations except 1.0 M. This resulted in part from a consistent non-monotonic NaCl concentration-response function, which was further explored by extending the concentration range of NaCl to 0.001–3 M (½-log steps) in some cells. Across cell types and stimuli, only N-best cells responding to NaCl displayed non-monotonic concentration-response functions, which typically had a peak response around 0.1 M. The mechanism behind this biphasic response demands additional study. These results show that the amiloride sensitive pathway plays some role in centrally evoked KCl activity. Although NaCl and KCl are equally effective in sucrose- and H-best neurons, KCl is far less potent for N-best cells at concentrations below 1.0 M. Thus, the amiloride-sensitive pathway may not contribute greatly to the neural code for KCl, at least at midrange and low stimulus concentrations.

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Slide 7

Water applied to the human tongue elicits robust cortical activation. DAVID H. ZALD AND JOSÉ V. PARDO, *Cognitive Neuroimaging Unit, VA Medical Center, Minneapolis, MN 55417 and Division of Neuroscience Research, Dept. of Psychiatry, Univ. of Minnesota, Minneapolis, MN 55455.* zald@james.psych.umn.edu

Studies of gustatory processing frequently utilize water as a control stimulus. However, the neural representations of "tasting" water have rarely been studied in humans. To examine which brain regions activate during stimulation of the tongue with water, we measured regional cerebral blood flow (rCBF) with a high resolution H_2O^{15} PET technique while eight healthy subjects attempted to taste room temperature, deionized, distilled water. Relative to a resting control condition, water elicited dramatic bilateral rCBF increases in perisylvian cortex (extreme ventral pre- and post- central gyrus, the underlying operculum, and insula), as well as activating Rolandic and cerebellar cortices bilaterally. Increased rCBF also localized the cerebellar vermis, brainstem, right fusiform gyrus and the right amygdala. Supplemental studies involving horizontal movement and mechanical stimulation of the tongue reveal that Rolandic activity may largely reflect motor or somatosensory processing. In contrast, most of the perisylvian activity (especially in the right hemisphere) could not be attributed to mechanical stimulation or movement of the tongue. These data indicate that stimulation of the tongue with water produces powerful rCBF increases in perisylvain cortex including the insula and operculum, and this activity may obscure rCBF changes induced by gustatory stimuli in these regions. The data also implicate the area at the base of the central sulcus in intraoral perception, and suggest that the tongue representation in this area is functionally dissociable from the representation of the tongue occurring in more superior Rolandic cortex.

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Poster 13

Morphological evidence (using lanthanum) for continuity between the hemolymph and sensillar lymph of the olfactory sensilla (aesthetascs) of the blue crab, *Callinectes sapidus*. RICHARD A. GLEESON[1], LORRAINE M. MCDOWELL[2], and HENRY C. ALDRICH[2], [1]*The Whitney Lab., University of Florida, St. Augustine, FL 32086,* [2]*Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611.* FAX:(904) 461-4008.

Several lines of evidence reported previously suggest that, in low salinity conditions, the structural and functional integrity of the dendrites of the blue crab's olfactory receptor neurons are sustained by an ionic/osmotic microenvironment that is dynamically maintained within the aesthetascs [Cell Tissue Res. 284:279-288 (1996); J. Exp. Biol. 200:445-456 (1997)]. Continuous diffusion of ions from the hemolymph, driven by an actively maintained concentration gradient between the hemolymph and the external environment, is proposed to generate and sustain this microenvironment. In this study we used the electron-dense, extracellular tracer, lanthanum, as a probe to examine the relationship between the extracellular fluid bathing the soma of olfactory receptor neurons (i.e., the hemolymph) and that bathing their outer dendritic segments within the aesthetascs (i.e., the sensillar lymph). In a first set of experiments, isolated antennules were internally perfused for 30 min with crab saline containing 1% lanthanum nitrate. In a second set, the antennules of restrained crabs were externally incubated for 30 min in seawater (seawater-acclimated animals) or freshwater (freshwater-acclimated animals) containing lanthanum. In both experimental conditions, examination of the aesthetascs using transmission electron microscopy revealed the presence of lanthanum in the extracellular fluids bathing the olfactory receptor neuron soma and their dendrites. These results suggest there is extracellular continuity and, therefore, a pathway for direct ion diffusion between the hemolymph and the sensillar lymph.

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Poster 14

Structural analysis of the peripheral olfactory organ of *Monodelphis domestica*. JULIA M. COUPER LEO and PETER C. BRUNJES, *Program in Neuroscience, University of Virginia, Charlottesville, Virginia, 22903.* jmc6g@virginia.edu.

Monodelphis domestica, the gray, short-tailed opossum, is well suited for studying the early development of the olfactory mucosa since offspring are born after a short, 14-day gestation in an very immature state. To date a detailed developmental analysis of the opossum olfactory mucosa providing needed structural and normative data has not been reported. In the present study, animals were sacrificed at postnatal (P) days 1, 10, 20, or 40, and paraffin sections used for morphometric analysis. Epithelial area and thickness, and nasal cavity perimeter were measured using MCID Image Analysis software. In order to examine age-related patterns of cell proliferation, a second group of animals was injected with bromodeoxyuridine (BrdU) and allowed to survive an additional 2, 24 or 48 hrs before sacrifice. Paraffin sections were prepared and processed via immunocytochemistry. Nasal cavity perimeter is largest in the caudal third of the nose in all ages examined. In older animals, the caudal nasal cavity is three times larger than the rostral region. Epithelial thickness of the septal and lateral walls is higher in the anterior third of the olfactory mucosa than in posterior portions. Dorsal mucosal thickness is more variable and is greatest towards the caudal portion of the nasal cavity. Mucosal area increases in the caudal third of the nasal cavity, increasing by about 200% in older animals over more rostral regions. Results from the BrdU study_reveal a robust decrease in cellular proliferation with age; the number of proliferating cells decreased by more than 50% from P10 to P40. Results from these studies show that there is dramatic structural and cellular postnatal growth in the opossum peripheral olfactory organ.

This research was supported by grant DC 02400 from the NIDCD.

Poster 15

OX-42 immunostaining in peripheral and central olfactory structures of goldfish. JEANINE S. STEWART, C. RAMEY HARRIS, and KIMBERLY A. FREEMAN, *Dept. of Psychology, Washington and Lee University, Lexington, VA 24450.* jsstewart@wlu.edu

Our laboratory is investigating the potential for macrophages and microglia to influence olfactory receptor neuron (ORN) turnover in goldfish (*Carassius auratus*). ORNs, with somata in the nasal cavity and axon terminals in the olfactory bulb, link the external environment with the central nervous system (CNS). Perhaps because ORNs offer an entry point for pathogenic invasion of the CNS (Lima & Vital, *Mycopathologia 126*, 1994), macrophages and microglia reside in olfactory bulbs of both rats and fish (Dowding, et al., *Glia 4*, 1991; Caggiano & Brunjes, *Neuroscience 52*, 1993). Immune system cells secrete locally-acting substances, including growth factors which may exert effects on nearby neurons (Nathan, *J. Clin. Invest. 79*, 1986; Sunderkotter, et al., *J. Leukocyte Biol. 55*, 1994). Thus, immune system cells may modulate a variety of cellular processes, possibly including olfactory neurogenesis.

Staining of cryosections with alpha naphthyl butyrate esterase (Sigma) and immunostaining for OX-42 (Chemicon), both of which identify macrophages in tissue sections, reveals labeled cells in olfactory nerve bundles within rosettes, and in the first nerve and olfactory nerve layer of olfactory bulbs. Less dense label is also seen in the epithelium and granule cell regions of the bulb. This apparent concentration of macrophages among olfactory nerve fibers suggests an important role for these cells, and perhaps for their secretory products, in the removal and regrowth of ORN axons. We are currently mapping the normative regional distribution of phagocytic cells in these structures. We plan to compare this distribution with patterns of cell genesis (with PCNA immunostaining) and apoptotic activity in rosettes and bulbs of goldfish.

Poster 16

Distribution of amino acid immunoreactivity in the developing and adult olfactory organ of zebrafish. WILLIAM C. MICHEL, *Dept of Physiology, University of Utah School of Medicine, Salt Lake City, UT 84108.* mike.michel@m.cc.utah.edu.

From fertilization until hatching occurs approximately 72 h later, the development of the olfactory system of zebrafish is a time of dynamic morphological change (Hansen and Zeiske, J. Comp. Neurol. 333:289, 1993). The olfactory placode is evident 12-14 hours after fertilization (AF). The olfactory pit, first noted 30-36 hours AF, widens to an oval-shaped structure by hatching. During post-embryonic life, secondary morphological features, such as the mid-line raphe and lamellae, begin to develop and the olfactory pit gradually assumes the morphology of the adult olfactory rosette. Conventional immunohistochemical techniques were used to compare the distribution of amino acid immunoreactivity in the neuroepithelium of time-staged embryos and adults. Olfactory organs were glutaraldehyde-fixed, embedded in plastic and sectioned at 0.5 μm. After incubation in anti-amino acid primary antibodies (the rabbit polyclonal IgG antibodies generated against amino acid/BSA conjugates were generously provided by Dr. Robert Marc), the distribution of amino acid signal was visualized using a nanogold secondary antibody and silver intensification. Patterns of taurine, glutathione (GSH), glutamate, glutamine, aspartate and GABA immunoreactivity are useful in distinguishing sensory and non-sensory epithelium in both adult and larval fish. Taurine-immunoreactivity is pronounced in the olfactory placode 24 h AF (the earliest time point thus far examined). In adults, GSH immunoreactivity is highest in the transitional region between the sensory and non-sensory epithelium. Similar variability in the concentration of other amino acids within the sensory epithelium suggests that classification of cell types is possible.

This work was supported by NIH NIDCD DC-01418-07 and NINDS NS-07938-25.

Poster 17

Olfactory mucosal metabolism and toxicity of the anti-hyperthyroid drug carbimazole in the Long-Evans rat. MARY BETH GENTER, *Department of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH 45267-0576* gentermb@popmail.uc.edu.

Carbimazole (2-carbethoxythio-1-methylimidazole) has been associated with deficits in or loss of the senses of smell, taste and hearing in humans who are prescribed this drug for treatment of hyperthyroidism. This study examined the potential for carbimazole to cause olfactory mucosal damage via two routes of exposure. Carbimazole was administered orally (by gavage) or by i.p. injection to groups of male Long-Evans (L-E) rats at doses ranging from 0 (vehicle) to 200 mg/kg. Carbimazole caused olfactory mucosal degeneration by both routes of exposure. Comparison of the oral No-Observed-Effect Level for carbimazole's olfactory toxic effects (100 mg/kg) with the daily oral doses administered to humans (ca. 0.1 to 0.7 mg/kg) reveals an approximately 200-fold margin of safety for this adverse effect of carbimazole. In order to study the mechanism of olfactory toxicity of carbimazole, *in vitro* metabolism studies were performed using S9 fractions prepared from male L-E rat olfactory mucosa. Spectrophotometric analysis of reaction mixtures following incubation of carbimazole with olfactory mucosal S9 preparations in the presence of exogenous NADPH revealed that the olfactory mucosa is capable of metabolizing carbimazole to methimazole. Thus it appears that the olfactory toxicity of carbimazole is attributable, at least in part, to target tissue-specific production of methimazole, a previously-characterized olfactory mucosal toxicant.

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Poster 18

Adverse influence of dexamethasone on anterograde labeling of primary afferents in the olfactory bulb of 3-methylindole-injected rats. IGOR L. KRATSKIN[1], YASUYUKI KIMURA[2], RICHARD L. DOTY[1], [1]*Smell and Taste Center, University of Pennsylvania, Philadelphia, PA 19104 USA,* [2]*Department of Otolaryngology, Kanazawa University, School of Medicine, Ishikawa 920, Japan.* FAX: (215) 349-5266.

Dexamethasone (DM), an anti-inflammatory corticosteroid, is believed to enhance olfaction in patients with nasosinus diseases. We examined the influence of systemically administered DM on the recovery of projections from the olfactory epithelium to the olfactory bulb in rats intraperitoneally injected with 150 mg/kg of the olfactotoxicant 3-methylindole (3-MI). For this purpose, applications of 1% wheatgerm agglutinin-horseradish peroxidase (WGA-HRP) were made onto nasal epithelium, and the mean density of staining in anterogradely labeled olfactory axons was measured in transverse sections of the olfactory bulb. The basal staining in the bulb, where only the olfactory nerve layer and glomerular layer exhibited a peroxidase reaction, was established in six intact rats. Twelve rats received DM (1.5 mg/kg, i.p.) every other day for 8 days before 3-MI administration; a DM injection regimen was continued, depending on the experimental group, from one to four weeks until the application of WGA-HRP. Twelve additional rats received saline in place of DM on the same time scale. One week after 3-MI administration the mean density of staining was reduced to 12% and 21% ($P<0.01$) of the basal level in DM- and saline-injected rats, respectively. Anterograde labeling recovered gradually, and in four weeks the mean density of staining in DM- and saline-injected rats reached 87% and 83% ($P>0.05$) of the basal level. The results suggest that chronic systemic administration of DM likely exacerbates 3-MI-induced damage to the olfactory epithelium in rats. Whether similar effects of DM occur in humans requires additional investigation.

Supported by NIH research grant 5 PO1 DC 00161 from the National Institute on Deafness and Other Communication Disorders.

Poster 19

Altered neuronal morphology of the olfactory system in neuronal storage disease. E.E. MORRISON, J.C. DENNIS, S. SINNARAJAH, K. WOLFE, and V. VODAYNOY, *Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL 36849-5518.*

Neuronal storage disorders (e.g. Tay Sach) are genetic defects characterized by the accumulation of storage material within CNS neurons, resulting in progressive neuronal dysfunction. These diseases are often associated with lysosomal enzyme defects and are characterized by the presence of lamellar bodies within the neuronal cytoplasm. Neurons of the cerebral cortex show significant morphological changes particularly in the initial axon segment where new dendrite growth is stimulated. The present study examined olfactory receptor neurons (ORN) and olfactory bulb neurons in cats having neuronal storage (Gm_1) disease. Olfactory tissue from adult age matched cat (generously supplied by H. Baker, Director Scott-Ritchey Research Center, Auburn University) olfactory tissue was examined with LM, TEM and Golgi procedures. ORN's had storage vesicle accumulations in the cell body cytoplasm which also extended into the dendrite and knob. ORN's in the apical epithelium showed more storage vesicles than neurons located more basally. Both normal and mutant ORN's were OMP positive. Olfactory bulb mitral and tufted cells showed the greatest content of storage vesicles. The nerve cell body region and dendritic processes had dense accumulations of lamellar bodies. Although storage vesicles were present in the dendrites the glomerular synaptic contacts appear normal. The basal rate of cAMP was determined from cilia preparations and was similar (120 nM/mg min) between normal and mutant cats. Odorant induced accumulation of cAMP following the addition of 10 mg GTP showed mutant OE cilia at reduced levels(120 nM/mg min) when compared to normal (160 nM/mg min). These results indicate neuronal storage diseases alters neuronal morphology in primary and second order olfactory neurons and also alters the second messenger system.

Poster 20

Synapse distribution on morphologically characterized olfactory interneurons in the terrestrial snail. S. RATTÉ, R. CHASE, *Department of Biology, McGill University, Montréal, Québec, Canada, H3A 1B1.* stefanie@bio1.lan.mcgill.ca

The procerebrum is the second processing centre for olfactory information in *Helix aspersa*. It consists of a large number of small somata located laterally to a fine neuropil, where a large proportion of olfactory nerve fibres branch and terminate. The population of procerebral neurons is not morphologically homogeneous: some cells have neurites entirely intrinsic to the procerebrum while others have both intrinsic and extrinsic arborizations, and still others have only extrinsic arborizations. We are using serial sectioning and electron microscopy to investigate the distribution of synapses on morphologically different cells, in hopes of building a model of neural organization in the procerebrum.

On neurons with arborizations intrinsic to the procerebrum, we find that output synapses occur mainly, and in high density, at varicosities. This arrangement results in considerable divergence, and possibly in synchronization of multiple postsynaptic cells. The fibres of intrinsically arborizing neurons are mostly of small diameter and with few inputs. Sites of input are usually between varicosities, but are sometimes colocalized with output synapses on varicosities, giving rise to reciprocal synapses.

We also studied some intrinsically arborizing cells that have neurites confined to the internal mass region of the neuropil. The neurites of these cells typically display many fewer varicosities than the other intrinsically arborizing neurons, but they meander extensively, and serial sectioning reveals that synapses are scattered along their lengths. On the proximal part of the neurite, in the soma region of the procerebrum, 80% of the synapses are inputs and only 20% are outputs. In the internal mass region, the density of synapses on the labelled procerebral cells is four times higher than in the soma region and furthermore, 80% of those synapses are output synapses. Thus, the proportions of inputs and outputs are inverted in the somatic and internal mass regions. These results will eventually allow morphological comparisons to other model systems.

Poster 21

Ultrastructure of the glomerular neuropil and periglomerular zone of the adult salamander olfactory bulb. DIANNE M. ALLEN and KATHRYN A. HAMILTON, *Department of Cellular Biology and Anatomy, Louisiana State University Medical School, Shreveport, LA 71130-3932.*

Ultrastructural studies to determine the synaptic arrangements within the glomerular neuropil and periglomerular zone in rodents have established criteria for identifying profiles of pre- and postsynaptic cells. (A.J.Pinching and T.P.S.Powell, *J.Cell Sci.* 9:305-345, 347-377, 379-409, 1971a-c; E.L. White, *Brain Res.* 37:69-80, 1972; E.L.White, *Brain Res.* 60:299-313, 1973) To determine if similar synaptic arrangements occur in the adult salamander olfactory bulb, we have examined the glomerular neuropil and periglomerular zone at the ultrastructural level. Initial results indicate that the synaptic arrangements of the salamander layers resemble those seen in rodents.

Semi-thin sections of adult *Ambystoma maculatum* olfactory bulbs were stained with toluidine blue and used to locate the glomerular and olfactory nerve layers. Thin (silver and silver-gray) sections were then obtained. The sections were stained with uranyl acetate and lead citrate and they were examined with a Philips CM-10 transmission electron microscope.

The olfactory nerve axons were readily identified by their electron dense cytoplasm containing tightly-packed microtubules and mitochondria. Fascicles of olfactory nerve axons defined the periglomerular zone surrounding the glomeruli. Within the glomerular neuropil, the nerve terminals were densely packed with spherical or pleomorphic translucent vesicles, and they were interspersed with neuronal profiles with translucent cytoplasm. Dendritic and axonal profiles of olfactory bulb neurons, as well as a variety of synaptic contacts, were identified. Profiles of mitral/tufted cell dendrites were distinquished by their large diameter, numerous (not packed) microtubules and mitochondria, and spherical or large flattened vesicles. Profiles of periglomerular cell dendrites were distinquished by their irregular shapes and large flattened vesicles. Profiles of periglomerular axons were distinguished by their small, flattened vesicles and absence of microtubule aggregates. Dense-core vesicles were commonly observed in the cytoplasm of both dendrites and axons. Within the glomerular neuropil, the types of synapses thus far observed include dendrodendritic asymmetrical (mitral-to-mitral and mitral-to-periglomerular) and axodendritic symmetrical (periglomerular axon-to-mitral cell dendrite). In the periglomerular zone, a reciprocal dendrodendritic synapse (mitral and periglomerular) was also observed. Additional studies are being conducted to identify the cell types forming the synapses.

Supported by the Whitehall Foundation and departmental funds.

Poster 22

Distinct glomerular projection patterns of primary olfactory axons expressing different G-protein α subunits in the mouse olfactory system. KENNEDY S. WEKESA, and ROBERT R. H. ANHOLT, *Department of Zoology, North Carolina State University, Raleigh, NC 27695-7617.* FAX: (919) 515-5327.

Olfactory receptor neurons expressing the same odorant receptor are confined within restricted expression zones in the olfactory neuroepithelium and converge on the same glomeruli in the olfactory bulb. The mechanisms that govern the formation of this zonal organization and convergent glomerular projections are largely unknown. In the vomeronasal organ two expression zones have been identified. Neurons in the apical zone express VN1 type putative pheromone receptors together with G_{i2} and project to the anterior accessory olfactory bulb. Neurons in the basal zone express VN2 type receptors together with G_o and project to the posterior region of the accessory olfactory bulb.

Immunohistochemical studies using antibodies monospecific for different G-protein α subunits show in cross sections of the vomeronasal nerve that G_o expressing axons segregate to the ventral region of the nerve prior to arrival at their target sites. Furthermore, segregation of axons expressing different G-protein α subunits is observed also in the glomerular layer of the main olfactory bulb. Axons projecting to individual glomeruli express either G_{i2} or G_o α subunits, but not both, forming distinct, complementary patterns of G_{i2} or G_o expressing glomeruli. Glomeruli immunoreactive with antiserum against the α subunit of G_{i2} are found primarily on the dorsal region of the olfactory bulb, whereas glomeruli immunoreactive with antiserum against the α subunit of G_o occur more ventrally. These results indicate that axons of olfactory neurons expressing the same odorant receptor will contain the same G-protein, either G_{i2} or G_o. In addition, the presence of distinct complementary patterns of expression of different G-protein α subunits in the primary olfactory projection, suggests that, at least, two distinct, mutually exclusive classes of neurotropic signals are delivered to olfactory axons as they project to the olfactory bulb and converge on their target glomeruli.

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Poster 23

The olfactory bulb-olfactory cortex slice. A.C. PUCHE, V. ARONIADOU-ANDERJASKA, AND M.T. SHIPLEY, *Dept. Anatomy & Neurobiology, Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201.* FAX: 410-706-2512

Olfactory receptor neurons expressing a particular odorant receptor gene converge onto one or a few glomeruli where they synapse onto juxtaglomerular interneurons, and mitral/tufted cell output neurons. The organization of projections from mitral/tufted cells arborising in single glomeruli, thus, have important implications for the patterns of bulb and olfactory cortical activity during activation of the olfactory bulb (OB). It is known that mitral/tufted cells project to a variety of brain regions collectively referred to as primary olfactory cortex (POC), and the POC sends heavy centrifugal afferents back into the bulbs. However, little is known about information processing between the OB and the POC. Large anatomical tracer injections *in vivo* suggest that mitral cells have a diffuse projection to the POC, but the organization of projections from mitral/tufted cells associated with a single glomerulus has not been investigated. Axons from these mitral cells could project to the entire POC; emit collateral branches at discrete intervals; or even terminate in a single locus. These and other potential patterns of organization would predict different patterns of generated activity. To investigate these kinds of issues we are developing acute and cultured slices which include both the OB and POC. DiI/DiO tract tracing within these slices indicate that the axons and terminal branches of mitral/tufted cells to the POC are well preserved along almost the entire length of the lateral olfactory tract. In the same slices, there is extensive retrograde labeling of the perikarya and dendritic arbors of neurons in the superficial layers of the POC. Deep POC axons coursing to the bulb are also labeled. These preliminary anatomical preparations suggest that functional circuits from the bulb to the POC and back to the OB are intact. The use of acute as well as organotypic cultured slices of OB-POC should provide the opportunity to analyze the functional topography of mitral/tufted cell projections, activity patterns in the POC after stimulation of specific glomeruli, and the precise roles of centrifugal afferents in OB function.

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Poster 24

Combining information across input afferent streams gives rise to hyperacuity in Biological and Artificial Olfactory Systems. TIMOTHY C. PEARCE[a], TODD A. DICKENSON[b], DAVID R. WALT[b], JOHN S. KAUER[a]. [a]*Department of Neuroscience, Tufts University Medical, Boston, MA 02111.* [b]*Department of Chemistry, Tufts University, Medford, MA 02155.* tpearce@opal.tufts.edu.

Hyperacuity is the property of a sensory system in which the overall sensitivity to some aspect of the stimulus is greater than that of the underlying detectors. It is a result of selectively combining information from a number of sensory sources in order to increase the certainty of a particular measurement. If required, this increase in certainty may then be traded for sensitivity gained at other stages of processing. While examples of hyperacuity have been studied in other sensory modalities, such as colour perception and stereooptic depth perception in vision, it is a less well quantified phenomenon in olfaction. Our intent is to highlight key biological information processing mechanisms that may lead to olfactory hyperacuity, by investigating the combination of sensory signals in an artificial olfactory system.

First, using data gathered from an artificial olfactory system consisting of a large number of ostensibly identical chemically sensitive polymer beads, we show how such signals can be linearly summed in order to improve the overall system sensitivity. This is demonstrated by successfully solving an odour discrimination task that requires greater sensitivity than can be obtained from any individual sensor. Second, we show how the statistics of these signals can be used to estimate the number of sensors required in order to reach a particular level of system sensitivity for a given odorant. Finally, a data processing scheme is proposed to further improve the system sensitivity that selectively weights the contribution from each sensor. A solution to the set of weights that optimally encode the mutual information between the original sensor signals and the weighted summed signal is derived. Comparisons are made between the convergence of receptor signals early in the biological olfactory pathway and our processing model as a method for selecting among receptor signals to improve overall system sensitivity.

Supported by DARPA and ONR grants.

Poster 25

Efferent projections of the anterior and posterior divisions of the accessory olfactory bulb in the short-tailed opossum, *Monodelphis domestica*. ALINO MARTÍNEZ-MARCOS, and MIMI HALPERN, *Department of Anatomy and Cell Biology, Health Science Center at Brooklyn, State University of New York, New York, NY 11203*. FAX: (718) 270-3378.

The nerve and glomerular layers (N/GL) of the accessory olfactory bulb (AOB) of Brazilian opossums can be divided into an anterior and a posterior portion on the basis of their different chemoarchitectures. The rostral portion receives its main input from receptor neurons located in the middle layer of the vomeronasal epithelium whereas the input to the caudal portion of the AOB arises from cells located in the basal layer of this epithelium. Further, anterior and posterior populations of mitral/tufted cells appear to exist since their primary dendrites are restricted to the corresponding subregion of the N/GL (Jia and Halpern, 1997).

In order to investigate if this segregation is preserved further caudally in the amygdala, injections of different dextran conjugates were aimed at the anterior and posterior portions of the AOB. Our results indicate that the efferent projections from both subdivisions overlap throughout the recipient structures in the amygdala.

Supported by NIH grant DC02745 (to MH).

Jia, C. and Halpern, M. (1997) Neuroreport, **8**, 1887-1890.

Poster 26

Functional magnetic resonance imaging (fMRI) of the human brain during olfactory stimulation GERD KOBAL[3], BIRGIT KETTENMANN[2,3], MICHAEL ERB[1], WOLFGANG DITTERICH[2], AXEL KLUSMANN[4], UWE KLOSE[1], MARKUS PFISTER[2], WOLFGANG GRODD[1], *[1]Sect. Exp. NMR of the CNS, Dept. of Neuroradiol. and [2]Dept. of Otolaryngology, Univ. Tübingen, [3]Dept. of Exp. and Clin. Pharmacol. and Toxicol., Univ. Erlangen-Nürnberg, [4]Dept. of Neuroradiol., RWTH Aachen, Germany,* FAX+49-9131-856898.

The investigation of humans neocortical processing is the aim of this study. Here we report on bilateral activation of the human neocortex to olfactory stimulation identified by fMRI.

Six healthy subjects participated in the experiments. fMRI data were acquired with a 1.5 T tomograph (Siemens-Vision) using a multisclice EPI sequence (TE 46 ms, 64*64 matrix, 27 slices 4mm, scan time 3 sec). Imaging was performed for 70 measurements with a rate of 6 sec. Olfactory stimuli (vanillin and hydrogen sulfide) were delivered within a humidified and temperature controlled constant airflow to the nasal cavity without altering the thermal conditions at the mucosa. The stimulus sequence consisted of 500 ms pulses once every 30 sec, with baseline scans at the beginning, in between and at the end of each stimulation period. Each stimulus period consisted of 2x4 stimuli. fMRI data were evaluated with the z-score method and transformed in Talairach space using the AFNI program. Additional postprocessing was performed with the SPM package.

Significant activation was recorded in the orbital gyrus, in the rectal gyrus, in the medial and lateral frontal region, and in the temporal lobe. In the temporal and the lateral frontal lobe lateralisation of the right hemisphere in terms of stronger activation was observed, independent from the side of nostril stimulated.

The results extend previous data indicating functional asymmetry in the human brain in the processing of chemosensory stimulation.

Poster 27

Separate cerebellar components subserve sniffing and smelling in the *Human*. N. SOBEL*[1], V. PRABHAKARAN[1], J.E. DESMOND[2], G.H. GLOVER[3], E.V. SULLIVAN[4,1], J.D.E. GABRIELI[2,1]. *Prog. in Neuroscience[1], Depts. of Psychology[2], Radiology[3], and Psychiatry & Behavioral Sciences[4], Stanford University, Stanford, CA 94305.*

Sniffing is an integral component of mammalian olfaction. We used fMRI in order to localize the human cerebellar substrates involved in sniffing. In addition, we examined cerebellar activation induced by the presence of an odorant (smelling), regardless of sniffing. The sniffing experiments consisted of 8 alternating 40s blocks of sniffing/no-sniffing. The smelling experiments consisted of 8 alternating 40s blocks of odorant/no-odorant, with sniff-rate held constant at once every 8s over the odorant and no-odorant conditions. Sniff duration in the odorant and no-odorant conditions was held constant by following a visual sniff command that remained projected for 1s at the onset of every sniff. Odorants were delivered using an olfactometer that offered odor rise time under 500 msec. The alterations between odorant/no-odorant conditions were not accompanied by any tactile, thermal, auditory or visual cues. Functional data were acquired from six 5mm slices, 2mm skip, taken at a plane parallel to the brainstem at 1.5 T using a T2* sensitive gradient echo spiral sequence (TR=540, TE=40, flip angle=60°, 4 interleaves). Data were motion corrected, and analyzed using the cross-correlation method of Friston et al (1994). Four right handed non-smoking subjects were scanned Sniffing induced activation primarily in anterior cerebellar regions, specifically the biventral lobule and tonsil. In contrast, smelling (odorant presence) induced activation primarily in posterior cerebellar regions, specifically in the left superior semilunar lobule. Parametrically varying either sniff-rate or odor concentration, selectively increased activation in the anterior and posterior cerebellum, respectively.

Poster 28

Cytoplasmic shrinkage of Purkinje cells in cerebella of patients with schizophrenia. GREGORY S. SMUTZER[1,2], KHOA D. TRAN[1], and RICHARD L. DOTY[1], *[1]Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, and [2]Institute for Human Gene Therapy, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.* FAX: (215) 349-5266.

Recent research has demonstrated that olfactory function is decreased in individuals with schizophrenia, and that the cerebellum might be involved in some forms of olfactory processing. To address the general question as to whether the cerebellum is affected in this disease, we determined the cytoplasmic area of Purkinje cells in the cerebellar vermis of 5 patients diagnosed with schizophrenia relative to 6 matched normal controls. The cerebellar vermal samples were obtained at autopsy and quickly frozen. Sections were prepared and fixed in paraformaldehyde as previously described[1]. *In situ* hybridization with a transmembrane cDNA probe of type I IP_3 receptor (IP_3R) was carried out using ^{35}S-labeled antisense RNA to detect overall IP_3R expression. After hybridization, tissue sections were exposed for autoradiographic detection of gene expression. Since mRNA expression occurs in the cytoplasm and since IP_3 receptors are highly expressed in Purkinje cells, the cross-sectional cytoplasmic areas of individual cerebellar Purkinje cells were measured by silver grain production using computer-assisted image analysis. We found an overall 16.5% reduction in cross-sectional cytoplasmic area of Purkinje cells in the cerebellar vermis of patients with schizophrenia relative to the controls ($p=0.03$). There were no correlations between the cytoplasmic areas with either age or post-mortem interval. In addition, the average number of labeled Purkinje cells was approximately the same in both control and schizophrenia groups. Our findings suggest cerebellar involvement in schizophrenia, and is in accordance with other reports of neuronal abnormalities in post-mortem tissue from patients diagnosed with schizophrenia. To what degree such abnormalities relate to central olfactory processes mediated by the cerebellum is yet to be determined.

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1. Smutzer G. et al., *Biochimica et Biophysica Acta* 1358 (1997) 221-8

Poster 29

Possible influence of monosodium glutamate (msg) on gustatory reaction time to saltiness of NaCl, KCl and their mixtures in model systems, M. C. ZAMORA, M. MARTINEZ and M. E. OTERO-LOSADA, *Laboratorio de Investigaciones Sensoriales, CONICET, Fac. de Medicina, UBA, M.T. de Alvear 2202 4° P, CP 1122, Buenos Aires, Argentina,* cz@lis.edu.ar
Zamora, M.C.

The sensory interactions of MSG, NaCl (Na^+) and KCl (K^+) in model systems were examined by Gustatory Reaction Time (GRT) method at 25°C. Twelve panelists evaluated five concentrations of MSG (4.3, 10.8, 27.0, 67.5, 168.7 mM), Na^+ (12.5, 25.0, 50.0, 100.0, 200.0 mM), K^+ (25, 50, 100, 200, 400 mM) and the following mixtures: Na^+/K^+(12.5+25; 25+50; 50+100; 100+200; 200+400), Na^+/MSG (27mM), K^+/MSG (27mM) and Na^+/K^+/MSG (27mM). Each concentration was replicated seven times.

One way ANOVA and LSD test on seven replications indicated that 6th and 7th replications were significantly different ($p<0.05$), their GRTs were longer attributable to receptors' saturation. Hence replications 6th and 7th were not included so as to eliminate that source of variation.

Experimental design was a 4-factor randomized block model with three-way interactions (factor / numbers of levels): salts (7), concentrations (5), panelists (12), replications (5).

ANOVA results of GRT evaluations showed that salts ($F_{6,1056}=26.54$), concentrations ($F_{4,1056}=185.90$) and panelists ($F_{11,1056}=12.95$) were a significant source of variation ($p<0.001$). Salt * concentration interaction ($F_{24,1056}=1.80$; $p<0.05$) revealed that the salts behaved in different ways across concentration ranges. This result was likely due to different salts' ranges. Salt * panelist interaction ($F_{66,1056}=1.43$; $p<0.05$) indicated that the same salts were differently rated due to interindividual differences in sensitivity.

Cluster analysis was performed in order to identify which salts mixtures containing the lowest Na^+ and K^+ concentrations had same GRT values. According to this same GRTs were found for the following salts and salts' mixtures (mM): a).$Na^+(200) = K^+(200) = Na^+(100) + K^+(200)$; b).$Na^+(100) = Na^+(50)$+MSG; c). $Na^+(50) = Na^+(25)$+MSG; d). $Na^+(25) = K^+(50) =$ MSG(27); e). $Na^+(12.5) = K^+(25) =$ MSG(10.8). These results show GRT shortening by 27mM MSG, the usual concentration in foods. Practical consequence of this would be saltiness signal amplification resulting in earlier perception of saltiness.

Poster 30

Chirality of sweetness and sweetness inhibition. RACHEL W. SIERTSEMA, GORDON G. BIRCH[1] and LUCIO MERLINI[2] [1]*Department of Food Science and Technology, P.O. Box 226, University of Reading, Whiteknights, Reading, Berkshire, RG6 6AP UK, and* [2]*Dipartimento di Scienze Molecolari di Agroalimentari, University of Milano, Via Celoria 2, 20133, Milano, Italy*

The identical sweet taste intensities and identical solution properties of **D**- and **L**- arabinose have been further explored with the sweet taste inhibitor, sodium propoxy methoxy propanoate (NaPMP). The inhibitor is itself chiral, only the **S**- enantiomer inhibiting sweetness. Therefore its action (if any) on enantiomers is mechanistically illuminating. All commercial preparations of NaPMP, are, however, racemic, and a human taste panel has now revealed that commercial NaPMP at 150 ppm inhibited the enantiomers of arabinose equally. When the S enantiomer of NaPMP, was tested on the arabinose enantiomers, it once again inhibited their sweetness equally. However the percentage inhibition was now about twice as great as with the commercial racemic inhibitor. These findings eliminate the possibility that the inhibitor acts on individual conformers of arabinose which are present at mutarotational equilibrium of the sugar. A reasonable explanation of the action of NaPMP is competitive inhibition and these findings support the concept of one single sweet receptor for the two enantiomers of arabinose.

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Poster 31

Isentropic compressibility as a sensitive indicator of taste. SNEHA A. PARKE and GORDON G. BIRCH *Department of Food Science and Technology, University of Reading, Whiteknights, P.O. Box 226, Reading, RG6 6AP, U.K.* FAX: +44(0) 118 931 0080

The isentropic compressibility of sapid molecules in water has been the focus of a recent study. A knowledge of the hydration behaviour of solute molecules in water is of prime importance to understand the underlying mechanism of taste chemoreception. Solute molecules in solution interact with the solvent in a way which depends on the stereochemistry and nature of both. Isentropic apparent specific compressibilities ($K_{2(s)}$) are a measure of the extent to which the hydration layer around the solute can be compressed. The measurement accounts for the compression of the first two layers of water surrounding the solute which are under the direct influence of attractive forces exerted by the solute. $K_{2(s)}$ values are calculated from density and velocity of sound data and, assume that the solute itself is incompressible. Compressibility data reveal valuable information about the type and extent of solute-solvent interaction of sapid molecules and may well be related to the taste properties of the molecules. Large negative $K_{2(s)}$ values reflect a highly incompressible hydration layer, as in the case of ionic structures, but increased solute-solvent interaction. Solutes exhibiting similar taste modalities seem to be confined to specific compressibility ranges, for instance -2.20×10^{-5} to -8.06×10^{-5} $cm^3g^{-1}bar^{-1}$ for salty molecules, -6.131×10^{-6} to -2.99×10^{-5} $cm^3g^{-1}bar^{-1}$ for sour molecules, and -3.38×10^{-7} to -2.34×10^{-5} $cm^3g^{-1}bar^{-1}$ for sweet molecules. $K_{2(s)}$ values may also yield information about the ease of accession of the molecule to the appropriate receptor site. $K_{2(s)}$ values may be used to discriminate between the various modes and extents of solute-solvent interaction within a specific taste quality group, for example, bulk and intense sweeteners (sucrose: -6.329×10^{-6} $cm^3g^{-1}bar^{-1}$; sodium cyclamate: -42.25×10^{-6} $cm^3g^{-1}bar^{-1}$; acesulfame K: -17.68×10^{-6} $cm^3g^{-1}bar^{-1}$).

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Poster 32

Human taste mechanisms for pyranose and furanose sugars. MELANIE A. ARMSTRONG, NUSRAT FEROZ, MARCY L. HENDERSON, YELENA KATSMAN, HARJEET S. PARMAR, LAURA M. SCARSELLA and LINDA M. KENNEDY, *Neuroscience Laboratory, Biology Department, Clark University, Worcester, MA 01610,* lkennedy@clarku.edu.

Response functions from human psychophysical studies with furanose (D-fructose) and pyranose (D-glucose, L-sorbose and D-galactose) monosaccharides, and two disaccharides: maltose (pyranose-pyranose) and sucrose (pyranose-furanose), suggest different receptor cell mechanisms for the furanose and pyranose monosaccharides (Eylam et al., 1995; Armstrong and Kennedy, 1997). The data also suggest that the pyranose-furanose disaccharide sucrose and the furanose mono-saccharide (D-fructose) stimulate the same mechanism, while the pyranose-pyranose disaccharide (maltose) may stimulate a mechanism similar to that of the pyranose monosaccharides. Response functions from *Drosophila adiastola* behavioral and electrophysiological studies with D-fructose and D-glucose also suggest different receptor cell mechanisms for pyranose and furanose monosaccharides (Kennedy et al., 1997). These hypotheses were further tested by human psychophysical studies using different pyranose (L-arabinose) and furanose (D-ribose) monosaccharides. Pyranose and furanose designations were based on the percentage of each form in solution for each sugar, as in Shallenberger (1993). However, L-arabinose, which is primarily pyranose in solution, is structurally, a C-2 epimer of D-ribose. Subjects were asked to taste various concentrations of the sugars, each paired with water, and to identify the sweeter in each pair. Functions for recognition indices (proportions of subjects identifying the sugar solution as sweeter) were constructed for the data, as in previous work (Eylam and Kennedy, 1995; Kennedy et al., 1997). Based on the percentage of furanose forms in solution, the ribose function was expected to be similar to those of fructose and sucrose, the furanose sugars, while the arabinose function was predicted to resemble the pyranose functions. Contrary to this prediction, the ribose function was different from those for the furanose sugars and resembled the pyranose functions. The arabinose function was similar to the other pyranose functions. Three groups were distinguished by the slopes of regression lines for rising phases of the response functions: sucrose and fructose; glucose, arabinose and ribose; and maltose, sorbose and galactose. These data suggest three mechanisms for tasting these sugars.

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Poster 33

The effects of mono- and di- valent salts on taste profiles of twelve sweeteners. SUSAN S. SCHIFFMAN[1], ELIZABETH A. SATTELY-MILLER[1], BREVICK G. GRAHAM[1], and BARBARA J. BOOTH[2], [1]*Dept. of Psychiatry, Duke University Medical Center, Durham, NC 27710,* [2]*NutraSweet Kelco Co., Mt. Prospect, IL 60056,* FAX: 919-684-8449.

The purpose of this study was to determine whether monovalent and divalent cations (Na^+, K^+ and Ca^{2+}) alter responses to sweeteners when applied extracellularly. It was hypothesized that Na^+ and Ca^{2+}, which are found in high concentrations in extracellular fluid, would have different effects than K^+, which is found in high concentrations within the cell. A trained panel of 18 subjects evaluated 12 food grade sweeteners: 3 sugars (fructose, glucose, sucrose), 2 *N*-sulfonylamides (acesulfame-K, sodium saccharin), 2 polyhydric alcohols (mannitol, sorbitol), 1 chlorodeoxysugar (sucralose), 1 dipeptide derivative (aspartame), 1 protein (thaumatin), 1 sulfamate (sodium cyclamate) and 1 terpenoid glycoside (rebaudioside-A). Five levels of each sweetener were tested that reflected the range of sweetness achieved for each sweetener. Expected sweetness intensities reached for a given concentration of sweetener were determined according to formulae developed by DuBois et al. (Sweeteners. Discovery, molecular design, and chemoreception; 1991;261-276). The sweeteners were mixed in solutions with three salts, each at 5mM: NaCl, KCl and $CaCl_2$. Responses for sweet, bittter and salty ratings were the most affected by the addition of salts to the sweet solutions. However, there were very few significant changes in the overall taste profile of the sweeteners. Thus, at the concentration used in this study, these mono- and di- valent cations had little effect on sweetness intensity ratings of a trained panel.

Poster 34

The effect of number of categories on the estimated bitterness, saltiness, sourness and sweetness of taste mixtures. ILSE A. POLET, JAN H.A. KROEZE, *Psychological Laboratory, Utrecht University, Utrecht, the Netherlands.* FAX: +31-30-2534511.

Several researchers have reported the so called "dumping effect": when subjects have to rate only one quality of a chemosensory mixture they tend to judge this quality higher than when they have to rate several qualities of the same mixture simultaneously (e.g. Clark and Lawless, 1994; *Chem. Senses,* **19**: 583-594). In this study this effect is examined for quaternary taste mixtures using cross modal magnitude matching.

In a "Profile" condition subjects rated bitterness, sweetness, sourness, and saltiness of each taste stimulus simultaneously and loudness of tones. In a "One quality" condition subjects estimated only one quality of the taste solutions and loudness of the tones. Subjects in the "profile" group participated in one session, while subjects in the "one quality" group participated in four, one session for each of the four qualities.

Taste stimuli were three quaternary mixtures of NaCl (N), sucrose (S), citric acid (C) and QHCl (Q) in demineralized water (Stimulus 1: 0.10 M N, 0.165 M S, 3.2 mM C and 0.1 mM Q; Stimulus 2: 0.20 M N, 0.33 M S, 6.4 mM C and 0.2 mM Q; Stimulus 3: 0.40 M N, 0.66 M S, 12.8 mM C and 0.4 mM Q). Four 500 Hz tones of 60, 73, 80 and 90 dB served as auditory stimuli. Taste stimuli were presented in medicine cups and auditory stimuli were presented using the speakers of a personal computer. Auditory and taste stimuli alternated with a 45 sec ITI.

Cross-modal magnitude matching was used. Each group had to judge the taste stimuli and auditory stimuli in proportion to the loudness of a standard tone of 73 dB to which a magnitude estimate of 10 was assigned.

The loudness function is the same for both groups. The estimates for the taste stimuli were significantly higher for the "one quality" group than for the "profile" group. This difference was present for bitterness, saltiness and sourness ratings, but not for sweetness ratings. Furthermore, the difference is stimulus dependent: the psychophysical function for the "one quality" group was steeper than for the "profile" group. For stimulus 1 the groups did not differ.

Poster 35

Quantitative measures of performance from a taste confusion matrix. THOMAS P. HETTINGER[1], JANNEANE F. GENT[1], LAWRENCE E. MARKS[2,3], MARION E. FRANK[1], [1]*UConn Health Center, Farmington, CT 06030,* [2]*J.B. Pierce Lab., New Haven, CT 06519,* [3]*Yale University, New Haven, CT 06520.* thetting@neuron.uchc.edu

Performance of normal human subjects on a taste identification task, in which 10 stimuli were each presented 10 times, was evaluated by means of a 10x10 taste confusion matrix (Frank *et al.*, 1994). Two measures of performance obtained from the set of identifications were *percent correct* and *transmitted information, T.* Percent correct quantifies the proportion of times that people identify stimuli by the label defined as correct. Transmitted information quantifies the degree of consistency with which people give correct responses to different stimuli. Untrained subjects (N=42) made 17 to 92% correct identifications (mean 57%) and T_{10} ranged from 1.54 to 2.97 bits (mean 2.25) out of a maximum of 3.32 bits. There was a strong correlation between T_{10} and % correct (r=0.89), yet some subjects made consistent though incorrect identifications. The discriminability of each possible pair of taste stimuli was evaluated by means of the 45 2x10 confusion matrices. In this case, a maximal value of T_2, 1 bit, is obtained as long as the two stimuli have no overlap in their responses. In these pairwise comparisons, sweet-nonsweet pairs were most readily distinguished, producing T values close to 1 bit. Salt pairs were harder to distinguish, with T_2 close to 0.5 bit, while the two sweeteners: sucrose and aspartame, were most confused (T_2 = 0.2 bit). In another experiment, trained subjects (N=40) performed somewhat better (T_{10} = 2.58 bits) than subjects with no training (N=20) (T_{10} = 2.37 bits). Performance reached asymptotic values after 2 presentations of the 10 stimuli with feedback. Nonetheless, some subjects achieved near perfect performance without training, although the sense of taste is thought to be cognitively simpler than the sense of smell.

Reference. Frank ME, Hettinger TP, Gent JF, Marks LE. (1994) Chem Senses 19: 471.

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Poster 36

Experience with a flavor in mother's milk modifies the infant's acceptance of similarly flavored cereal. JULIE A. MENNELLA and GARY K. BEAUCHAMP, *Monell Chemical Senses Center, Philadelphia, PA 19104.*

The present series of studies aimed to investigate whether experience with a flavor in mother's milk modifies the infant's acceptance of similarly flavored foods at weaning. First, we established, using methods developed in our laboratory, that the ingestion of carrot juice by lactating women (n=6) produced a sensory change in their milk approximately 2 to 3 hours after the ingestion of the beverage. Second, we randomly formed two groups of breast-fed infants (n=38) who had been fed cereal for a few weeks but had only experienced cereal prepared with water. Their mothers were asked to consumed one of two types of beverages (i.e., carrot juice, water) during the 7-8 day exposure period. Each mother was observed feeding her infant cereal during 4 test sessions. The first two sessions occurred during the two days before the exposure period; in counter-balanced order, infants were fed cereal prepared with water on one testing day and cereal prepared with carrot juice on the other. The infants' behaviors, including the rate at which they were fed, were monitored by videotape and the amount of cereal consumed was assessed by weighing the infants immediately before and after each feed. These two test sessions were then repeated following the exposure period. The results demonstrated that the infants who had exposure to carrot in their mothers' milk during the exposure period consumed the carrot-flavored cereal faster when compared to the control infants whose mothers consumed the water, although the total amount consumed did not differ between groups. These data are consistent with the hypothesis that flavor exposure during nursing impacts of the acceptance of that flavor in the context of weaning foods.

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Poster 37

The relationship between salivary glutamate and sodium levels and taste perception of sodium chloride and monosodium glutamate. MARIA G. BUSCARELLO[1] AND MIRIAM R. LINSCHOTEN[2]. [1]*Dept. of Food Science and Human Nutrition, Colorado State Univ., Fort Collins, CO 80523 and* [2]*Rocky Mountain Taste and Smell Center, Univ. of Colorado Health Sciences Center, Denver, CO 80262.* FAX: (303) 315-8787.

Monosodium glutamate (MSG) has been used to flavor foods for centuries; however, its specific mode of action is not well understood. Recently, a taste receptor for glutamate was identified as mGluR4 (Chaudhari et al., 1996). Previous research reports that subjects with low levels of salivary sodium have lower NaCl thresholds than those with higher salivary sodium levels (Bartoshuk, 1980). Based on dietary intake data, Asians consume more MSG but do not generally report adverse reactions to ingestion of MSG.

Thus, the objectives of this study were: 1) to measure levels of glutamate and sodium in human saliva; and 2) to determine if a relationship exists between salivary levels and psychophysical responses to NaCl and MSG.

Sixty subjects, evenly divided between males and females, and Asians and non-Asians, expectorated 2 ml of whole mouth saliva. Next, thresholds to NaCl and MSG were determined using the Adaptive Maximum-Likelihood Staircase Procedure (Linschoten et al., 1997). Last, subjects gave magnitude estimates of five concentrations of NaCl (0.01, 0.032, 0.1, 0.32 and 1.0 M) and MSG (0.63, 1.25, 2.5, 5.0, and 10 mM).

No significant main effects for race or gender were found for the salivary constituents. However, the interaction between these factors was significant ($p = .0062$) for salivary sodium: female Asians had a higher salivary sodium level than all other groups ($p = .003$). Asians had significantly lower thresholds for NaCl than non-Asians ($p = .004$). No other differences in sensitivity were found. Finally, the three lowest concentrations of NaCl were rated as significantly more intense by non-Asians compared to Asians ($p = .024$). These data suggest that the relationship between salivary sodium and NaCl sensitivity is not as straightforward as previously thought. Furthermore, perceptual measures of MSG appear to be independent of salivary glutamate levels.

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Poster 38

Prop (6-*n*-propylthiouracil) genetics and trigeminal innervation of fungiform papillae. JORDAN M. PRUTKIN, KATHARINE FAST, LAURIE A. LUCCHINA, LINDA M. BARTOSHUK, *Otolaryngology, Yale University School of Medicine, New Haven, CT 06520-8041.* jordan.prutkin@yale.edu.

Two cranial nerves innervate the fungiform papillae on the anterior tongue. Taste is carried by the chorda tympani branch of the facial nerve (VII) and pain, touch, and thermal sensations are carried by the trigeminal nerve (V). Trigeminal neurons enter the fungiform papillae, surround the taste buds, and terminate at the apex of the papillae (Whitehead, et al., 1985).

Individuals can be classified into three groups based on their ability to taste saturated PROP. Those to whom it is nearly tasteless (nontasters) have two recessive alleles and comprise about 25% of the American population, those to whom it is intensely bitter probably have two dominant alleles and comprise 25%, and those to whom it is moderately bitter probably are heterozygotes and make up the remaining 50%. PROP tasting ability has been shown to correlate with the number of fungiform papillae on the anterior tongue.

We used the genetic variation in perception of PROP to test which sensations are mediated by the trigeminal neurons that enter fungiform papillae. Since supertasters have the most fungiform papillae, they have the most trigeminal neurons. If only certain types of trigeminal neurons enter fungiform papillae, then supertasters should perceive the sensations mediated by those types as more intense.

Oral irritation was assessed by swabbing ethanol (30-70%) and capsaicin (1-100 ppm) onto the anterior tongue. Oral touch was assessed by asking subjects to sip and spit viscous solutions of guar gum (.1-1 g/ml) and canola oil (0-100%). Warmed or cooled metal probes were placed on the left anterior tongue, and subjects rated the intensity of the warmth or coolness, respectively. Subjects also rated NaCl and PROP; supertasters have the highest PROP/NaCl ratios. Intensity ratings were obtained using the Labeled Magnitude Scale (Green et al., 1993).

Perception of irritation and touch correlated significantly with PROP ratio (i.e., supertasters perceived the most intense sensations); however, neither thermal sensation showed a significant correlation.

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Poster 39

Alteration in lingual somatosensation as a result of transection of the chorda tympani nerve (VII). SETH R. SCHWARTZ, TANVEER JANJUA, JOHN KVETON, BARRY G. GREEN, LINDA M. BARTOSHUK. *Otolaryngology, Yale University School of Medicine, New Haven, CT 06520-8041.* schwarsr@Biomed.med.Yale.edu.

Fungiform papillae (the structures that contain taste buds on the anterior tongue) are innervated by two cranial nerves. The chorda tympani (VII; taste) and the trigeminal (V; somatosensation, i.e.,pain, touch, temperature). The chorda tympani fibers innervate receptor cells in the taste buds while the trigeminal fibers surround the taste buds and terminate in the apical epithelium of the fungiform papillae (Whitehead et al., 1985).

Animal studies investigating the effects of chorda tympani transection on taste bud and fungiform papillae anatomy generally report some degeneration of the the taste structures (although there is species variation). Since the chorda tympani and trigeminal nerves show close anatomical association in the fungiform papillae, we examined the effects of chorda tympani transection on somatosensation in humans. We studied a group of eight patients with unilateral surgical transection of the chorda tympani nerve peripheral to the geniculate ganglion during mastoidectomy.

Patients rated the perceived intensities of irritation/pain, touch, and thermal stimuli applied to the operated and nonoperated (i.e., control) sides of the anterior tongue using the Labeled Magnitude Scale. Irritation/pain stimulation was produced by capsaicin (1-100 ppm), ethanol (50%), and citric acid (.1 M). Thermal stimulation was produced by metal probes heated or cooled and placed on the anterior tongue. Touch stimulation was applied via a small brush.

All somatosensory sensations were signficantly reduced on the operated side. Thus the reduction in somatosensations is a secondary effect of transection of VII. The magnitude of the reduction was not equal for all somatosensations. The largest effect was for the irritants citric acid and capsaicin (10 ppm); the smallest effect was for temperature and touch.

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Poster 40

Acceptance of salty, sweet and bitter foods across pregnancy. VALERIE B. DUFFY[1], LINDA M. BARTOSHUK[2], RUTH STRIEGEL-MOORE[3], and JUDITH RODIN4, [1]*Univ. of Connecticut, Storrs, CT 06269,* [2]*Yale Univ. School of Medicine, New Haven, CT 06520,* [3]*Wesleyan Univ. Middletown, CT 06457,* [4]*Univ. of Pennsylvania, Philadelphia, PA, 19104,* vduffy@uconnvm.uconn.edu.

We have reported that taste changes across pregnancy (Duffy et al, ISOT, 1997) with data from the Yale Pregnancy Study (J. Rodin, PI). Presently, we examined the effect of pregnancy on food acceptance in the same cohort. All of the 46 women completed a 142-item food acceptance questionnaire when they were non-pregnant and at each trimester. Four women with severe dietary restrictions were removed from analyses. Sixty-five of the questionnaire foods fit a conceptual grouping of salty, sweet, or sour/bitter. With principal component analysis and varimax rotation, 3 groups of salty, 5 sweet, and 3 sour/bitter foods were formed. The groups had sufficient internal reliability and explained a majority of the variance. The Friedman ANOVA statistic with planned comparisons between non-pregnant and 1st trimester and between 1st and 3rd trimesters were used to test each food group. Of the salty groups, acceptance of Salty Meats rose significantly from 1st to 3rd trimesters, but no change was observed for Salty Snacks or Cheeses. Acceptance for 3 sweet groups (Fruit, Candy, and Dessert) rose significantly from 1st to 3rd trimesters; 2 groups (Sweets or Baked Desserts) did not show significant change. For bitter groups, women reported significant reduction in acceptance of Bitter Liquids and 1 of 2 Vegetable groups from non-pregnant to 1st trimester.

Sensory changes may contribute to changes in acceptance of salty and bitter foods. In taste testing, concentrated salt solutions are rated less aversive across pregnancy. This may contribute, in part, to an increase in acceptance of some salty foods across pregnancy. From non-pregnant to 1st trimester, reduction in bitter food acceptance parallels an increase in perceived intensity of .00032M QHCl in solution. The increase in sweet food acceptance across pregnancy appears a genuine hedonic shift. A sensory explanation is less likely as we were previously unable to detect a change in intensity or preference of sweet solutions across pregnancy.

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Poster 41

6-N-propylthiouracil (PROP) tasters assign higher sweetness ratings to sucrose and high-intensity sweeteners. LAURIE A. LUCCHINA[1]*, OTIS F. CURTIS, V.[2], PETER PUTNAM[2], and LINDA M. BARTOSHUK[1], [1]*Department of Surgery, Yale University School of Medicine, New Haven, CT 06520,* [2]*Technical Service, Cultor Food Science, Ardsley, NY 10502.* laurie.lucchina@unilever.com.

Perceived bitter taste intensity of 6-n-propylthiouracil (PROP) is a marker for genetic taste status. PROP tastes moderately bitter to "medium tasters" (MT), intensely bitter to "supertasters" (ST), and tasteless to "nontasters" (NT). In prior studies PROP perception was correlated with the perceived intensity of other compounds including those tasting sweet. In this larger study the perception of PROP and sweeteners was measured in seven separate experiments in an industry setting. All studies included groups of 234 (mean N=98) reportedly healthy volunteers (84 males, 150 females) aged 19-62 years recruited at Cultor Food Science in Groton, CT. In each panel, bitterness intensity of PROP-saturated paper or solutions (0.001M, 0.0032M), and perceived sweetness of sucrose (1.0M), and one or a series of sweeteners: saccharin, aspartame, neohesperidin dihydrochalcone (NHDC), alitame, and polyols (xylitol, sorbitol, maltitol, lactitol), were assessed. All ratings were indicated on a labeled magnitude scale [labeled line with descriptors (no taste---strongest imaginable)] and normalized to auditory stimuli (85 and 98dB tones). In all experiments, water rinses were included between each tastant and PROP was the final stimulus. Statistical analyses included descriptive statistics and simple regression. Based on the mean PROP status distribution obtained, 8.1, 44.6, and 47.4% of subjects are NT, MT, and ST, respectively. Those with higher PROP perception assigned higher sweetness ratings to sucrose and all high-intensity sweeteners. However, PROP status was not correlated with perceived sweetness of polyols. As in prior studies, a higher frequency of females were ST than males. Also, the PROP effect on sweet perception was most evident in female ST. Since genetic taste status appears to affect sweet perception, food and flavor panelists should be screened for PROP status prior to sensory evaluation of new sweetening agents or sweet food formulations.

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Poster 42

Irritant properties of nicotine and piperine: psychophysical evidence for asymmetrical cross-desensitization effects. J.-M. DESSIRIER[1,2], M. O'MAHONY[2] AND E. CARSTENS[1], [1]*Section of Neurobiology, Physiology & Behavior,* [2]*Department of Food Science & Technology, University of California, Davis, Davis, CA 95616,* jadessirier@ucdavis.edu.

When delivered to the oral mucosa, a variety of naturally occurring chemicals such as capsaicin from red chili peppers, piperine from black pepper, and nicotine from tobacco, cause a diffuse burning sensation often referred to as irritation. Irritation evoked by capsaicin increases when delivered repeatedly to the tongue at 1-min intervals (sensitization) and then decreases markedly following a 10-15 min rest period (self-desensitization). Also, following desensitization by capsaicin, irritant sensations evoked by other chemicals, including piperine and nicotine, are reduced (cross-desensitization). Piperine is structurally similar to capsaicin and is believed to stimulate a subset of the capsaicin (vanilloid) receptor. Like capsaicin, piperine exhibits sensitization when applied repeatedly and self-desensitization following a rest period. Piperine also cross-desensitizes to capsaicin. Finally, nicotine self-desensitization has not effect on capsaicin evoked irritation. To further test for piperine and capsaicin similarities, we investigated the interactions between piperine and nicotine irritation. Thus, using a new'relative' bipolar category scale, subjects rated the perceived irritation intensity evoked by repeated unilateral applications (10 times at 1 min interval) of piperine 75 ppm and nicotine 0.12% solutions. Confirming earlier results, and thus providing validation of our new scale, repeated piperine stimulation resulted in a significant increase in ratings while nicotine repeated stimulation led to a significant decrease in irritant sensation. After unilateral application of piperine or nicotine, a rest period ensued followed by bilateral application of piperine or nicotine. Subjects then indicated which side of the tongue yielded a stronger irritation sensation (2-alternative forced choice), and also provided a rating of the magnitude of irritant sensation perceived on each side of the tongue. Following piperine pretreatment, a significant majority of subjects indicated that piperine evoked a stronger sensation on the previously untreated side, and the mean rating for the untreated side was significantly higher. This confirmed piperine self-desensitization. Nicotine self-desensitization was similarly confirmed. Furthermore, like with capsaicin, nicotine irritation was reduced by prior piperine treatment (cross-desensitization) while nicotine treatment had no effect on piperine perceived irritation. These results demonstrate additional similarities between capsaicin and piperine.

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Poster 43

Reduced duration of thermal hyperalgesia from capsaicin in the orofacial region. BARRY G. GREEN[1,2] AND ALBERTO CRUZ[1], *John B. Pierce Laboratory[1] and Section of Otolaryngology, Yale School of Medicine[2], 290 Congress Avenue, New Haven, CT 06519.* FAX: (203) 624-4950.

Consistent with its ability to stimulate heat-sensitive nociceptors, topical exposure to capsaicin causes a hypersensitivity to heat. If applied in sufficiently high concentrations, the threshold for induction of heat pain can fall from its typical value near 45°C to as low as resting skin or oral temperature (32°- 37°C). Indeed, perception of painful burning during consumption of capsaicin reflects this shift in sensitivity, as heat-sensitive pain fibers become active at normal oral temperature. Common experience testifies that in the mouth this thermal hyperalgesia is relatively short-lived, lasting only minutes after capsaicin consumption has stopped. In contrast, we had previously observed that on other body sites hyperalgesia from capsaicin can persist for 24 hrs or longer. We therefore designed an experiment to measure the threshold for heat pain on the tongue tip, face (cheek) and forearm before capsaicin exposure and at 0 min, 30 min, 1 hr, 4 hr and 24 hr after. Capsaicin concentrations of 330 μM and 33 mM were used to produce comparable initial levels of hyperalgesia on the three sites, and heat pain was measured via the ascending method of limits using a computer-controlled 0.64 cm^2 Peltier thermoelectric module. Whereas on the forearm the heat pain threshold remained lower even 24 hr after exposure, on the tongue tip threshold returned to baseline levels within 30 min. Surprisingly, the time-course of hyperalgesia on the face was more similar to that on the tongue than on the forearm. Thus, independent of epithelial structure, capsaicin-induced hyperalgesia was much shorter-lived in the trigeminal field. This result, which may have implications for injury-related primary thermal hyperalgesia caused by endogenous chemical mediators, is discussed in terms of the possible effects of regional differences in dermal blood flow and/or receptor affinities.

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Poster 44

The immediate alerting effects of hot beverage ingestion: mediated by caffeine or sensory factors? JENNIFER M. ASPEN and PAUL T. QUINLAN, *Cell Biology and Physiology Unit, Unilever Research, Colworth House, Sharnbrook, Bedford, United Kingdom, MK44 1LQ,* Jennifer.Aspen-Brouwer@unilever.com.

Previous work in our laboratory has established immediate changes in autonomic nervous system parameters upon ingestion of hot, caffeinated beverages (Quinlan *et al.*, 1997). The onset of these'alerting' effects occurred more quickly than would be expected if mediated by caffeine alone. The present study sought to determine whether oral sensory stimulation with a hot beverage was sufficient to produce changes on both physiological and psychological parameters. Sixteen habitual caffeine users were asked to either consume or rinse the mouth and then expel one of several beverage treatments (i.e., tea, coffee, caffeinated water, water). All of the treatments produced changes in heart rate, blood pressure, skin conductance and skin temperature within the first three minutes of administration relative to the'no drink' condition, but sustained effects were only seen in the'swallowed' treatment conditions. There was an initial decrease in skin temperature at the time of beverage administration in all of the treatment conditions, which suggests that the expectation of a warm beverage may be sufficient to influence physiological parameters. Subjects who were allowed to consume the beverage showed an increase in skin temperature at three-minutes post-ingestion, while subjects who were asked to expel the beverage before swallowing showed a further decrease in skin temperature. Self-ratings of mood showed a profile of effects specific to tea during the first ten minutes after the beverage treatment (e.g., greater alertness, refreshment), while effects after thirty minutes were linked to caffeine and always necessitated that the beverage was actually swallowed. Taken together, these results suggest that oral sensory factors, presumably taste and temperature in the mouth, are sufficient to produce immediate physiological and psychological stimulation, while effects of longer duration are dependent on the presence of caffeine in the beverage.

Poster 45

Time course of capsaicin burn to repeated application. CAREY D. BALABAN[1], DONALD H. McBURNEY[2], and MINDY STOULIS[3], [1]*Departments of Otolaryngology and Neurobiology,* [2,3]*Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15213.* cbalaban@vms.cis.pitt.edu

Sensory processes are dynamic events incompletely characterized by a single static measurement. A previous study examined the time course of the burn produced by the steady application of capsaicin to the human tongue. We modeled the response as the sum of three processes: a phasic (or "change" detection) mechanism, a tonic (or "level") detection) mechanism, and a rising function that may be characteristic of painful stimulation. Each process is characterized by a gain and time constant. Changes in response over days (adaptation) could be explained as a decrease in the gain of the tonic mechanism.

The present study addressed intermittent stimulation, which may result in the initial response to the second period of stimulation being either greater or less than the initial response to the first period. These have been called *sensitization*, and *desensitization*, respectively.

Human subjects rated burn for 40 minutes. 100 ppm capsaicin was presented for minutes 1-10 and 21-30 of the session. Blank stimuli were presented the rest of the time. Repeated-measures ANOVA tested for adaptation between cycles. Although there was only a marginal ($p = 0.08$) main effect of cycle, there was a significant time X cycle interaction. Qualitatively the rising phase of the second cycle was reduced more than the falling phase.

Computer simulation using time constants from our previous study shows that the model provides a satisfactory fit to the data ($r^2 = 0.92$). Simulation further shows that changing the cycle length and duty cycle predicts either sensitization or desensitization. These findings indicate that our parallel phasic-tonic model is sufficient to explain phenomena previously characterized as *sensitization* and *desensitization*. Thus, these terms describe consequences of the dynamic nature of sensory processes.

Poster 46

The effect of a reference sample on sweetness ratings in two intensity scaling methods. JEANMARIE DIAMOND, and HARRY T. LAWLESS, *Department of Food Science, Cornell University, Ithaca, NY 14853.* jd44@cornell.edu.

The influence of context in intensity judgement has been a persistent concern in sensory testing. Experimentally, the magnitude of context bias appears to decrease when Magnitude Estimation with a reference is used. We wondered if this outcome was due to inherent advantages of the scale or whether due simply to the presence of a reference. Therefore the present experiment investigates whether there was a systematic reduction in context effects (simple contrast) due to the presence of a reference sample. Thirty-one individuals rated a 5% sucrose Kool Aid solution in both high and low context conditions. All panelists used both Magnitude Estimation (ME) and Labeled Magnitude procedures. Fifteen of the panelists made all judgements relative to a reference sample while the remainder did not have access to a reference. Overall, LMS showed smaller context effects than ME. Additionally, the presence of a reference sample helped to stabilize ratings using LMS. However, context effects were smaller in ME when the solutions were rated in the absence of a reference. This shift in the magnitude of context effects indicates that judges use these two scales differently.

Poster 47

Functionality of taste localization in humans. JEANNINE F. DELWICHE and PAUL A.S. BRESLIN, *Monell Chemical Senses Center, Philadelphia, PA, 19104.* delwiche@monell.org

We have previously demonstrated that people can localize a punctate gustatory stimulus on the lingual epithelium in the absence of discriminative tactile cues (*Chem. Sens.*, 22: 650-651, 1997). The purpose of this study was to determine whether humans can use gustatory spatial information to remove a target taste stimulus from the mouth when simultaneously presented with somatosensorily identical distractors. Using small 1 cm gelatin cubes, we investigated whether people could localize the position on the tongue stimulated by a quinine flavored gelatin cube and spit out this target while retaining the nonflavored gelatin cube(s) in the mouth. On any given trial, subjects blindly placed under the tongue two to four gelatin cubes, only one of which was flavored with quinine hydrochloride. At the experimenter's signal, the subject moved all the cubes out from this nongustatory area under the tongue, pushed them forward and gently held them stationary against the inside of the teeth with the tongue tip. As soon as the subject located the bitter target (~ 3 secs), they spat the suspected target into a cup. Identification of the cube as either the correct target (colored) or a distractor (uncolored) was then made by the experimenter and subject. Twelve subjects participated in ninety experimental trials each, with 30 trials conducted with one, two and three distractors. All subjects performed significantly above chance when one or two distractors were presented, and eleven of the subjects performed significantly above chance when three distractors were presented. As the number of distractors increased, all subjects took significantly longer to respond ($p<0.001$) and made significantly more errors ($p<0.001$). These findings indicate that humans are capable of localizing and removing a gustatory target from a field of distractors via taste sensations alone. We conclude that spatial localization information derived from taste sensations in the oral cavity can be used for the selective removal (or retention) of portions of a heterogeneous food bolus from the oral cavity (as opposed to the removal of the entire mouthful) in a manner that parallels the gustatory and ingestive behavior of other vertebrates (e.g., fish). This suggests a functionality for taste localization that might help maximize energy intake.

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Poster 48

Taste- and odor-induced facial expressions of young healthy volunteers in solitude and facing a mirror. JACOB E. STEINER and GAD COHEN, *Dept. of Oral Biology, Hebrew Univ. Hadassah School of Dent. Med., P.O.Box 12272, Jerusalem 91120, Israel.*

Twenty six volunteer students (mean age 23 y.; 17 m., 9 f.) were exposed to three intraoral and three nasal stimuli in two sessions, sitting in a quiet, ventilated, airconditioned room: a) in solitude; b) facing a mirror. The two testing-sessions were one week apart randomly alternating for each testee. Examinees were requested to label verbally and estimate the hedonics of each of the six stimuli, using a 100 mm visual analog scale (VAS). Simultaneously the stimulus-induced facial responses were videorecorded, using a camera in full view. Obtained videosequences were rated by two independent evaluators, in terms of like/dislike using the VAS; in addition evaluators had also to identify 15 typical facial features signaling like/dislike. Intraoral stimuli were: 10 ml samples of distilled water, 0.3 M sucrose and 0.007 M quinine HC1. Nasal stimuli included an odorless vial and vials containing chocolate-flavor or mercaptane. All 6 stimuli were presented in individual random orders. The aim of the study was to assess whether viewing and observing one's own stimulus-induced facial displays would influence subjective hedonic estimates or reflectory facial-displays. Results reveal that verbal labeling and hedonic self estimates were almost identical under the two test-conditions. Ratings of facial displays under the two conditions were also most similar. Under both conditions self estimates were found as hedonically slightly more discriminative, than facial responses. It can, therefore, be concluded that observing and being aware of one's own stimulus-induced facial responses (mirror) did not influence either psychophysical hedonic estimates or pleasure-displeasure, as reflected by expressive facial reactivity.

Poster 49

Sensory correlates of beer ingestion. DOREEN P. HAMEL and RICHARD D. MATTES, *Foods and Nutrition, Purdue University, West Lafayette, IN 47907*. hameld@cfs.purdue.edu.

Factors influencing beer consumption are poorly characterized. This project evaluated the association between beer consumption and sensory responses to beer constituents. Fifty adults, 25 males and 25 females, were divided into 3 user categories: 17 rare drinkers ($\leq$12 oz/mo), 17 casual drinkers (>12oz/ mo but <12 oz/day), and 16 heavy drinkers ($\geq$12 oz/day). Sensory tests included taste, olfactory and irritation detection thresholds for ETOH and a taste detection threshold for tetralone. Intensity ratings for sucrose, NaCl, citric acid, and quinine HCl were obtained after adaptive rinsing with CO_2 water, non-alcoholic beer and beer. Five beers with IBU's of 8.5 to 38 were rated for preference and bitter intensity. Three 24-hr diet recalls were also obtained. Thresholds for ETOH were 1.43 ± 0.11%v/v (taste), $3.63\pm 1.15\times10^{-4}$%v/v (olfaction), and $1.09\pm1.95\times10^{-3}$%v/v (irritation). Thus, of the sensory qualities assessed, humans are most sensitive to the odor of ETOH. At threshold, ETOH is more bitter than any other "basic" taste quality ($p<0.005$). Oral cavity adaptation to beer led to a suppression of the bitterness of quinine HCl relative to adaptation with CO_2 water or non-alcoholic beer. Males had higher ETOH taste thresholds than females ($p=0.021$) and showed increasing bitterness ratings with IBU ($p<0.001$). Females gave comparable ratings to all samples. In the combined sample, the highest hedonic rating was given to the beer with an IBU=23 and the lowest to the beer with an IBU=38 ($p=0.003$). A similar pattern was noted in both genders, but only females showed significant differences ($p=0.011$). There was a significant association between total ETOH consumption and hedonic responses to four of the five test beers. An association was noted between ETOH ingestion and energy obtained from predominantly bitter items ($r=0.39$, $p=0.005$). Controlling for ETOH ingestion, heavy consumers derived more energy from bitter items than casual drinkers ($p=0.025$). The latter findings suggest sensory responsiveness to and hedonic ratings for ETOH and beer are related to exposure to bitter notes in the diet.

Poster 50

The effects of preloads on sensory specific satiety and cephalic phase saliva production in humans. SARAH K. VOISARD-KIRKMEYER and RICHARD D. MATTES. *Department of Food and Nutrition, Purdue University, West Lafayette, IN 47907*. sarah_kirkmeyer@b-f.com.

When food is consumed, the palatability of that food decreases along with willingness to consume the item, a phenomenon referred to as Sensory Specific Satiety (SSS). A better understanding of the specificity of SSS response is needed to determine its dietary implications. In addition, the cephalic phase salivary response reportedly varies with exposure to specific foods. Prior exposure and palatability of foods have been shown to alter the salivary response. To explore the specificity of these responses isoenergetic loads of peanuts (macronutrient control), chestnuts (macronutrient control), peanut butter (rheology control), almonds (tree nut), chocolate (sensory control), as well as pickles (matched on weight), rice cakes (matched on volume) and no load were given at one week intervals. The preloads of peanuts, peanut butter and chocolate produced a SSS response only for the actual preload food. The other preloads did not elicit any SSS response. None of the preloads produced a SSS response for classes of foods with similar macronutrient content or sensory properties. Only the preloads of pickles, peanuts and chocolate were found to produce heightened cephalic phase salivary flow. No statistically significant correlation was observed between the SSS and cephalic phase responses. The preload properties responsible for these differential responses warrant further study as they may influence food selections and nutrient digestion.

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Poster 51

Effects of product information on product perception. JOS MOJET & ADRIAAN P.W. KOLE. *TNO Nutrition and Food Research Institute, Zeist, the Netherlands*. mojet@voeding.tno.nl.

Consumer research has shown that a discrepancy between product information and actual product performance can influence hedonic responses towards products. It is known that non-sensory cues may influence taste/flavor judgments. Generally, taste judgments of actual products in combination with product information will tend to deviate from blind scores in the direction of the expectations based on the information ('assimilation effect'). Occasionally it has been found that'combined' judgments deviate from blind scores in the opposite direction away from the expectations ('contrast effect'). Referring to the'Assimilation-Contrast' theory we suggest that assimilative shifts occur within a range of acceptable discrepancies between product expectations and actual product performance. When the discrepancy falls outside the acceptable range, contrastive shifts occur.

Generic labels (based on fat percentages:'full','half full','low' fat), and a taste claim ('mild') were combined with the same actual generic yoghurts in a study, in which blind, expectational, and combined taste evaluations were obtained for hedonic and sensory attributes.

The preliminary results show predominantly assimilative shifts in taste judgments, which is in agreement with most results reported in literature. However, also instances of contrastive shifts were found. In fact, it appears that the contrastive shifts are robust and strong for'negative' taste attributes. Apparently, response shifts are different for'negative' attributes (contrastive shifts) compared to'positive' attributes (assimilative shifts). This might be due to tighter ranges of acceptance for'negative' attributes.

Poster 52

Spatial discrimination of NaSaccharin and NaGlutamate tastes on the different sides of anterior tongue. PAUL A.S. BRESLIN[1], DAVID B.T. MCMAHON[1,2], HIROKI SHIKATA[1,3], AND JEANNINE F. DELWICHE[1], [1]*Monell Chem. Senses Ctr, Phila., PA, 19104*, [2]*Dept. of Neurosci., Univ. Pitt, Pitt., PA 15260*, [3]*Japan Tobacco Inc., Yokohama 227, Japan*. breslin@monell.org

We have previously demonstrated that people can localize a punctate gustatory stimulus on the lingual epithelium in the absence of discriminative tactile cues (*Chem. Sens.*, 22: 650-651, 1997). We have shown further that subjects can discriminate two tastes, NaCl and citric acid, with some difficulty, when presented simultaneously on left and right sides of anterior tongue (*Chem. Sens.*, 22: 792, 1997). The localization of tastes could be based upon gustatory intensity, or quality cues, or both. When citric acid and NaCl were presented together, localization was based initially upon intensity. In the absence of intensity cues localization was very difficult by qualitative cues alone, but possible none-the-less. The difficulty in discriminating between these two may reside in the qualitative similarity between citric acid and NaCl when at equal intensities and presented to the anterior tongue. The purpose of this study was to determine whether humans can localize with accuracy two compounds that differ substantially in taste quality, NaSaccharin (Sac) and NaGlutamate (MSG), using only their qualitative cues. In this experiment, subjects always received 100mM MSG on either the left or right tip of the tongue while various concentrations (0.1 - 10mM) of Sac were placed simultaneously on the other side and subjects were requested to answer on which side they felt an umami taste. MSG was repeatedly presented alone at the beginning of each session on either side of the tongue to give subjects a lateralized umami search image. All four subjects were able to locate the position of the umami stimulus almost perfectly across all Sac concentrations. For three of the subjects, performance dropped to ~80-90% correct at the lowest concentration of Sac. The MSG (100mM) was found to be roughly equi-intense with the middle Sac concentration (1mM). These results strongly suggest that taste localization can occur strictly as a function of gustatory quality information in the absence of discriminative intensity cues.

Paul Breslin is a Morley R. Kare Fellow. This research is supported by NIH grant R29 DC02995 (PASB).

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Putative human pheromones function as state "modulators" rather than behavior "releasers". SUMA JACOB, MARTHA MC CLINTOCK, *Committee on Neurobiology, University of Chicago, Chicago, IL 60637.* sj11@midway.uchicago.edu.

Perfumers have hoped to identify a single chemical compound that will trigger a specific behavioral response, such as sexual attraction or a sense of relaxation and well being that directly enhances social interactions. Although much work has been limited to such a view, it is very unlikely that human pheromones will be an aphrodisiac chemical signal bearing an irresistible or urgent message. Since human behavior is rarely determined by a single signal and strongly depends on the social context, we hypothesized that any effects of chemical signals were more likely to be (1) modulatory, (2) depend on the state of the recipient and (3) affected by contextual cues in the environment. We have examined the effects of two highly acclaimed chemicals and compared their effects to carrier controls with a strong or weak odor (clove oil or propylene glycol). By conducting double-blind, repeated measures experiments within a controlled setting, we have determined that 4,16-androstadien-3-one fails to make women feel more relaxed, self-assured, open or friendly and 1,3,5(10)16-estratetraen-3-ol fails to do the same for men. However, both these chemical signals do modulate human mood and psychophysiological states within specific environmental and hormonal contexts. Although these chemosignals effect psychological state without conscious detection as an odor, future work is necessary to determine whether they also satisfy other aspects of the classic definition of a pheromone.

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People's reactions to age and gender effects on odors. DENISE CHEN AND JEANNETTE HAVILAND. *Psy. Dept., Rutgers University, Livingston Campus, New Brunswick, NJ 08903.* xdc@eden.rutgers.edu.

Olfactory communication is less understood in humans than in animals. Studies have looked at kin recognition and gender discrimination for the evidence of odor identifications, and have looked at menstrual synchronization and synthetic potentially biologically significant compounds for the evidence of odor influences. People have been shown to discriminate between young adult men and women based on odor intensity cues. Natural adult body odors may subtly affect women's reproductive cycles while artificial odors have been shown to affect the moods and memories of both men and women. However, more studies are needed on the social and psychological implications of odor identification and influences. Since odors work on different levels and affect people both consciously and subconsciously, people may show different levels of odor discrimination abilities depending on whether they are asked to directly categorize the odors, or respond to the odors indirectly. Questions of whether people can explicitly discriminate between age and gender on the one level, and of whether people can be implicitly affected by natural body odors that vary in age and gender on the other level, are the focus of this present paper. Odors were collected from an equal number of male and female children, young adults, older adults, and their homes (as controls). In a series of double-blind studies, college students were assigned to the above odor conditions, evaluated their moods both before and after the odor exposure, and rank-ordered all target odors for their intensity, pleasantness, gender, age, and other characteristics respectively. Supporting existing findings, odor rankings are largely a function of odor intensity, suggesting that when directly evaluating odors, people rely on most salient cues. However, mood scores suggest that people do discriminate between odors similar in intensity. Subjects reported most negative moods when exposed to odors of young college men but least negative moods when exposed to odors of older women, although both were ranked highest in intensity. Further results and implications will be discussed.

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An exploration of verbalizations associated with olfactory and visual stimuli. T. L. WHITE[1,2], S. VAN TOLLER[2]. [1]*Clinical Olfactory Research Center at the SUNY Health Science Center, Syracuse, NY.,* [2]*University of Warwick, Coventry, UK*

Since poor labels can lead to a deterioration of performance on an olfactory recognition task, it may be possible to account for the memory performance difference between visual and olfactory stimuli through level of verbalization. Perhaps, under the pressure of time in a memory experiment, both types of stimuli may be imperfectly verbalized. In that case, the qualitative aspects of the label may become important in later recognition tasks, since accurate performance through verbal translation depends upon the same label being applied on more than one occasion. An experiment was performed to evaluate the ease of applying the same label to the same stimulus for both line drawings and odors. In this experiment, twenty subjects were randomly assigned to one of two groups, one exposed to visual stimuli and one to olfactory stimuli. Each subject was presented with 90 trials, which consisted of 10 stimuli presented 9 times each in a random order. During the inter-stimulus interval, subjects were asked to verbally generate a single label for the stimulus which would "help to remember it later". In the event that a label could not be generated, subjects were told to use the word "Pass." Responses were transformed to a measure of information transmitted for each subject in each group. Average information transmitted for the line drawings (2.74 bits) was significantly (t-test, $t=6.08$, ,$df=18$, $p<0.01$) greater than for the odorants (1.61 bits). This result demonstrated that people were much more accurate and reliable at naming the line drawings than the odours. The discrepancy in verbalization between the two types of stimuli suggests that the differential recognition performance in previous literature may be accounted for (at least in part) by differential verbalization.

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Cognitive development and odor categorization. CHRISTINE JEHL and CLAIRE MURPHY. *Center For Lifespan Human Senses, San Diego State Univ., San Diego, CA 92120.* FAX: (619) 594-3773

Although the semantic network is deteriorated in Alzheimer's disease patients, these patients typically show some sparing of ability for recalling objects or items from inanimate categories whereas they show poor semantic memory for animate items (Daum et al., 1996). Thus, despite semantic impairment, it seems that some components of the semantic network are spared from the disease. In children, the ability to recall items from these two groups of categories has been examined (Carey, 1995). It has been shown that children best name the animate categories, specifically animals (McKenna et al., 1994).

In the present study, we tested the hypothesis that as cognition develops in children, so does their semantic network for odors, and what is acquired first is best spared from the impact of aging. We reasonably assumed that, in children, the semantic networks for animate categories develop priorly to those for inanimate items, suggesting that the former networks may be stronger and thus best spared in dementia.

A total of 102 children and adolescents were tested for their abilities to identify a set of 33 common odors. The odors were distributed into 2 groups of categories : animate versus inanimate. Identification scores were compared for these categories using two-way repeated measures ANOVAs. The scores were significantly improved with increased age for odors from both categories : animate ($F(2,99)=41.83$, $p<.0001$) and inanimate ($F(2,99)=43.39$, $p<.0001$). For the 3 age-groups (5-7 yrs, 8-11 yrs, 12-16 yrs), scores of correct identification responses were significantly better for the inanimate category than for the animate one ($p=.0001$). We speculate that this finding, contradictory with what has been found for the verbal modality, may reflect children's increased experience with odors associated with edible items. Future research should replicate such findings for the olfactory modality in Alzheimer's disease patients to establish cross-modality differences in the cognitive process of categorization.

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Normative data for olfactory event-related potentials. CLAIRE MURPHY[1,2,3], CHARLIE D. MORGAN[1,2], SPENCER WETTER[1], MARK W. GEISLER[3], JAMES W. COVINGTON[1], DENNARD W. ELLISON[3], AND JOHN M. POLICH[4] [1]*San Diego State University, 6363 Alvarado Ct., Suite 101, San Diego, CA 92120-4913* FAX:619 594-3773, cmurphy@sunstroke.sdsu.edu., [2]*SDSU/ UCSD Joint Doctoral Program,* [3]*University of California, School of Medicine, San Diego, CA 92103,* [4]*The Scripps Research Institute, La Jolla, CA 92037*

Unlike for other clinical usages of the event-related potential (e.g., brain stem auditory potentials for the assessment of auditory function), normative data for the olfactory event-related potential have been unavailable. The objective of the present project was to establish normative data across the human lifespan for olfactory event-related potentials with a given set of parameters. Participants were 120 persons from six age groups (20, 30, 40, 50, 60 and 70 years of age), with equal numbers of males and females, screened for nasal health* and dementia. The odor stimulus was amyl acetate, presented at nasal temperature in a humidified airstream delivered by an air-dilution olfactometer at a constant flow rate, at 60 sec ISI, in a single-stimulus paradigm. Participants rated perceived intensity, thus generating a P3. OERPs were recorded at Fz, Cz, and Pz of the International 10/20 system, amplified and averaged over trials. Grand averages were computed separately for males and females at each decade. Amplitudes and latencies of N1, P2, N2, and P3 were analyzed. Processing speed decreased at a constant rate over decades for sensory (N1 latency) as well as cognitive (P3 latency) components. The P3 measure of attentional resource allocation and cognitive processing of odor showed a sharp decline in amplitude in the 50s which remained relatively stable thereafter. Such normative data will be useful in research on olfactory function and in clinical assessment of olfactory functional status.

*We thank Dr. Terence M. Davidson for nasal examinations. Supported by NIH grant DC02064 (CM).

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Cerebral processing of olfactory perception and recognition memory in humans. IVANKA SAVIC[1,2], BALAZS GULYAS[1], MARIA LARSSON[3]. [1]*Div. of Human Brain Research, Dept. Neuroscience, and* [2]*Dept. of Neurology,* [3]*Section of Psychology, Stockholm Gerontology Research Center, Dept. of Clinical Neuroscience and Familiy Medicine, Division of Geriatric Medicine, Karolinska Institute, Stockholm, Sweden.*

Introduction: Animal data and lesional studies in humans suggest that olfactory stimuli are processed by limbic structures. However, the exact areas implicated in the human olfactory system have not yet been definitively identified by functional criteria., especially when the lateralization and the higher olfactory functions are concerned.

Methods: Changes in regional cerebral blood flow (rCBF) were measured with positron emission tomography (PET) and 15-O buthanol in nine healthy females during monorhimal smelling of single odors, and tests of odor recognition memory. Significant changes ($p<0.05$) were calculated using cluster analysis (minimal size 0,8 cc) and an implementation of the general linear model ($t>2.8$).

Results: Monorhinal smelling of single odors activiated anterior cingulate and right oribitfrontal cortex independently on the side of presentation. Odor stimulation via the left nostril caused, in addition, activation of the left insula. During tests of olfactory recognition memory, the activation was more widespread and included right orbitofrontal cortex, bilateral pyriform and insular cortices with a right sided predominance, right temporal neocortex, right prefrontal cortex, right thalamus and left cerebellum.

Conclusions: In contrast to what has been suggested earlier, cortex is during monorhinal smelling not activiated ipsilaterally, but bilaterally witha right sided predominance. The activation pattern during odor memory is compatible with the current theory on episodic memory retrieval, and suggests a distinction between two networks: Those subserving the retrieval mode (here represented by the prefrontal and lateral temporal cortices, and cerebellum), and others subserving ecphory. Ecphory is regarded as modality specific and was here represented by the orbitofrontal pyriform and insular cortices.

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The effects of phasic odor administration on continuous performance. DAVID G. ELMES, TYLER S. LORIG, JULIE A. MARKHAM, AND W. KEN THEUS. *Department of Psychology, Washington and Lee University, Lexington, VA 24450.* delmes@wlu.edu

Eighteen subjects (9 male, 9 female) were tested to determine the effects of odors on different cognitive tasks. Previous research has shown that odors, under some conditions, can distract subjects and impair performance on verbal tasks. The present experiment was a replication and extention of those findings and examined the similarity of odor distractors to informational auditory distractors such as animal sounds. The distracting stimuli were odors (butanol 4% or perfume 100%) or tones (500 Hz or 1000Hz) or animal sounds (dog bark or crow call) and were presented for 0.5 s. Administration of each distractor was synchronized with inspiration (monitored via respiration belt) and each distractor was delivered 2 s prior to an imperative stimulus to which the subject responded. Three types of imperative stimuli (verbal, numeric, or spatial) were presented on a video monitor. The verbal stimuli were nouns such as "house" or "rat." Numeric stimuli were 3 digit numbers and the spatial stimuli were circles of differing sizes. Subjects were required to judge whether the current stimulus was larger than the most recent and indicate their decision by a button press. Latency and accuracy data were collected for each class of imperative stimulus. A blank (no distractor) condition was also included to establish a response baseline. Results of the analysis indicated no differences between individual pairs of odors, tones or sounds so these data were combined and analyzed in a 3(task type) X 3(distractor type) X 2 (gender) analysis of variance. Reaction time differed only among task types but the results of the analysis of accuracy indicated an interaction of all three sources ($F(4,64)=3.452$, $p=0.013$). This interaction was due to a large decline in the accuracy of females when evaluating verbal stimuli and distracted by the odor stimuli. Results support previous findings indicating a specific interaction between odors and verbal stimuli but suggest the effect is much greater in females than males.

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Theoretical computations of odorant uptake in the human nose. KEYVAN KEYHANI[1], PETER W. SCHERER[2], and MAXWELL M. MOZELL[3], [1]*School of Chemical Engineering, Georgia Institute of Technology, Atlanta, GA 30332-0100,* [2]*Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104-6392,* [3]*Department of Physiology, SUNY Health Sciences Center at Syracuse, Syracuse, NY 13210,* keyvan@mucus.seas.upenn.edu.

Quantitative determination of odorant uptake in the nasal cavity is needed for a better understanding of olfactory mechanisms. In this study, we numerically simulated transport of inspired odorant molecules in a human nose using an anatomically correct finite element model. We computed odorant fluxes on the olfactory surface as a function of flow rate, diffusivity in air and mucus, solubility in mucus at the nasal walls, and mucus layer thickness. Total olfactory flux, that is highly correlated with perceived odor intensity, was shown to always increase with an increase in flow rate at a constant inlet concentration. However, with increase in flow rate, fractional uptake, i.e., total olfactory flux normalized by inlet convective flux, decreased for poorly soluble odorants, while it increased for highly soluble odorants. Total olfactory flux decreased approximately exponentially with time after cessation of airflow, with a time constant of less than 0.5 s for most odorants, implying a rapid decrease in perceived odor intensity. Patterns of olfactory flux, that are believed to carry information concerning odor identity, were shown to be dependent on physicochemical properties of inhaled molecules. There was an overall decrease in flux from the inferior towards the superior regions of the olfactory surface for all odorants, however, the flux patterns became more uniform, i.e., the steepness of the flux gradients across the olfactory surface decreased, as the mucosal solubility of odorants decreased. Different odorants generated discernibly different uptake patterns across the olfactory surface that may contribute to encoding odor quality. The model predictions were in agreement with various psychophysical and electrophysiological studies of olfaction.

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Poster 61

Differential odor information coding in early and late mitral/tufted cell spiking. K.M. DORRIES AND J.S. KAUER, *Dept. of Neuroscience, Tufts Medical School, Boston, MA 02111*, kdorries@opal.tufts.edu.

Olfactory bulb mitral/tufted cells (MTs) respond to odorants with complex temporal patterns of spiking. We have previously examined tiger salamander (*Ambystoma tigrinum*) MT spiking patterns using a single odorant-pulse paradigm, and found that effects of concentration differ for early versus late spike bursts. Under natural conditions, however, stimuli arrive as a pulse-train generated by sniffing, and responses to successive odorant pulses overlap in time. To examine these more natural spiking patterns, we recorded from 25 salamander MTs, comparing responses to single pulses and "artificial sniffing" pulse-train stimuli over a range of concentrations. MTs commonly responded to pulse-trains with spike bursts tracking the temporal pattern of stimulation, though responses to successive pulses often varied as they were modified by excitation and suppression elicited by earlier pulses. Bursts could be characterized as either "on" or "off" according to when they commenced in each odorant pulse cycle in the train. "On" bursts were associated with single pulse-elicited early spiking, and, like early spiking, generally occurred at higher concentrations. "Off" bursts resulted from spiking following suppression, and occurred at low concentrations when suppression duration was short enough for late spikes to occur prior to the onset of the subsequent odorant pulse. Thus, early and late bursts likely arise from different synaptic inputs, and accordingly may carry different information about a stimulus. Consequently, two different odorants may elicit spike bursts in the same MT, but the significance of those bursts could be very different if they are early in response to one odorant and late in response to the other. Our results indicate that characterization of MT odorant response profiles must include not only which odorants excite and inhibit a cell, but also whether spiking elicited by each odorant occurs early or late relative to stimulus pulses.

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Poster 62

Dynamic mapping of odor-elicited response in rat olfactory bulb by functional magnetic resonance imaging. XIAOJIN YANG[1], RENCO RENKEN[2], FAHMEED HYDER[3], MOHAMEED SIDEEK[3], CHARLES A. GREER[3], GORDON M. SHEPHERD[4] and ROBERT G. SHULMAN[3], *[1]Department of Chemistry, [3]Department of MB&B, Sections of [3]Neurosurgery and [4]Neurobiology (School of Medicine), Yale University;2Department of Chemistry, University of Groningen, the Netherlands.* yang@mrcbs.med.yale.edu

Functional magnetic resonance imaging (fMRI) provides a completely non-invasive method for mapping brain activation, based on activation-induced changes that are blood-oxygenation level dependent (BOLD). Using fMRI, we have previously observed spatially localized odor-elicited activation ($p<0.001$) in the olfactory bulb (OB) of urethane-anesthetized rats (International Symposium on Olfaction and Taste XII and Association for Chemoreception Sciences XIX, 1997, p.66). In the present study with iso-amyl acetate odor (10^{-2} dilution of saturated vapor), the laminar distribution of the odor-elicited BOLD activation in the OB was delineated, and the dynamic changes of the activation during prolonged odor exposure were revealed. The *in vivo* T_1-weighted anatomical MRI (in-plane resolution = 110μm×110μm, slice thickness = 1 mm) clearly delineated the laminar structure within the OB, and the BOLD activation by fMRI (in-plane resolution = 220μm×220μm, slice thickness = 1 mm) was centered at the glomerular layer and highly localized to the outer layers of the OB. Analysis of the activation pattern at a temporal resolution down to 30s in the OB revealed that the spatial pattern of the activation was stable during 5 minutes of prolonged exposure, but varied periodically during 27 minutes of prolonged exposure. Furthermore, over prolonged periods of odor exposure (27 minutes), we saw no evidence of odor adaptation in term of long-lasting suppression of the odor-elicited BOLD signal, suggesting that olfactory adaptation may largely take place in higher centers of the olfactory pathway. These results have demonstrated that non-invasive fMRI has the spatial resolution to resolve the olfactory activation in the individual layers of the OB, and the temporal resolution to reveal the dynamic changes of olfactory activation within tens of seconds.

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Poster 63

Olfactory nerve induced long lasting depolarizations in mitral cells of the rat olfactory bulb. GREG C. CARLSON, MATTHEW ENNIS, MICHAEL T. SHIPLEY and ASAF KELLER. *Dept. Anatomy & Neurobiology, Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201.* gcarlson@umaryland.edu

The first step in processing olfactory information takes place at the synapse between the olfactory nerve (ON) and its post-synaptic targets in the olfactory bulb. To understand the mechanisms that regulate ON inputs we are studying postsynaptic responses in mitral cells. These are recorded from both the somata and dendrites of mitral cells in an *in vitro* slice preparation of the rat main olfactory bulb, using the whole-cell patch method. Constant with previous reports, we find that a single stimulus to the ON generates a long-lasting (>500 ms) depolarization (LLD) in mitral cells. Spontaneous LLDs with similar amplitude and decay time-constants also occur, suggesting that both are generated by a common mechanism. Both the spontaneous and evoked LLDs persist in the presence of the NMDA receptor antagonist AP-5 (50-100 μM), but are suppressed in CNQX(20 μM), an AMPA/kainate receptor antagonist. We have examined whether these LLDs are due to membrane properties intrinsic to the mitral cells, or generated by atypical synaptic mechanisms. Analysis of the relationship between membrane potential and the amplitude of the LLDs suggests that their reversal potential is near 0 mV. Both evoked and spontaneous LLDs are resistant to hyperpolarizing current injection at the soma or dendrites, as well as to intracellular dialysis with the Na^+ channel blocker QX-314 (10 mM) and superfusion of the slice with high divalent cation solutions. These data suggest that the LLDs reflect primarily synaptic mechanisms. This synaptic phenomenon may be generated at the ON to mitral- tufted cell synapse, by polysynaptic inputs, or recurrent excitation among mitral cell dendrites. Ongoing experiments are designed to distinguish among these possibilities.

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Poster 64

Current-source density analysis in the rat olfactory bulb: evidence for autoexcitation in the apical dendrites of mitral/tufted cells. V. ARONIADOU-ANDERJASKA, M. ENNIS & M. T. SHIPLEY, *Dept. Anat. & Neurobiol., Univ. Maryland Sch. Med., Baltimore, MD, 21201.* vanderja@umaryland.edu.

Laminar field potential (FP) profiles evoked by olfactory nerve (ON) or mitral cell layer (MCL) stimulation in olfactory bulb slices from 16-22 d-old rats, were subjected to one-dimensional current-source density analysis. Single ON shocks evoked: (1) a prolonged (³ 400 msec) sink (S1) in the glomerular layer (GL) which reversed in the external plexiform layer (EPL), suggesting it is produced in mitral/tufted (M/T) cell apical dendrites, and (2) a brief sink (S2) in the EPL with corresponding sources in the internal plexiform and granule cell layers, suggesting it is produced in granule cell dendrites. The AMPA receptor antagonist CNQX (10 μM) reduced the early phase of S1, blocked S2, and revealed a small, prolonged sink at the location of the S2 in the EPL. Reduction of Mg^{++}, in CNQX-containing medium, enhanced both the CNQX-resistant component of S1 and the prolonged EPL sink. The EPL sink reversed below the MCL suggesting its generation by granule cells. The NMDA receptor antagonist AP5 (50 μM) reversibly blocked the CNQX-resistant FPs in all layers. Single MCL shocks evoked synaptic currents in granule cells, and a small sink (s1) in the GL. CNQX decreased s1, and nearly blocked the granule cell currents. Reduction of Mg^{++}, in CNQX, enhanced the CNQX-resistant component of s1, which reversed in the EPL suggesting it is produced in M/T cell apical dendrites. Low Mg^{++} also revealed CNQX-resistant currents in granule cells. Combined CNQX and AP5 blocked both the M/T and granule cell sinks, and revealed a low amplitude source in the EPL, with a corresponding sink at and below the MCL. These currents were blocked by the $GABA_A$ receptor antagonist bicuculline methchloride (BMCl, 10 μM). CNQX, AP5 and BMCl combined, or Ca^{++} free media, blocked all synaptic activity. BMCl, in standard media, enhanced the amplitude and duration of s1; this was reversibly reduced by AP5.

ON activation produces prolonged excitation (S1) of M/T cell apical dendrites via AMPA and NMDA receptors, providing opportunity for modulation and integration of sensory input in the GL. Granule cells respond to input from M/T cell lateral dendrites via AMPA and NMDA receptors; however, NMDA receptors do not contribute significantly to this response in physiological Mg^{++} concentrations. The GL sink (s1) evoked by MCL stimulation suggests that glutamate released from M/T cell apical dendrites excites the same or neighboring dendrites of these cells.

Support: PHS grants DC03195, DC00347, DC02588 & NS36940.

Poster 65

Trans-ACPD sensitive metabotropic glutamate receptors reduces glutamatergic transmission from mitral/tufted to granule cells. K.J. CIOMBOR, V. ARONIADOU-ANDERJASKA, M. ENNIS AND M.T. SHIPLEY. *Dept. Anat. & Neurobiol, Prog. Neurosci., Univ. Maryland Sch. Med., Baltimore, MD 21201.* kciombor@umaryland.edu.

Metabotropic glutamate receptors (MGluRs) are located on mitral/tufted, juxtaglomerular and granule cells. Here, we investigated the actions of the MGluR agonist ACPD in olfactory bulb slices from 22-29 day-old rats. Field potentials were simultaneously recorded in the glomerular layer (GL) and external plexiform layer (EPL), while shocks were alternately applied to the olfactory nerve (ON) and mitral cell layer (MCL). The ON-evoked GL field potential represents synaptic activation of mitral/tufted cell apical dendrites mediated by AMPA and NMDA receptors. The EPL field potential evoked by ON or MCL shocks is produced by granule cell responses to input from mitral/tufted cell lateral dendrites, and is mediated primarily by AMPA receptors. The MCL-evoked GL field potential reflects currents generated in the apical dendrites of mitral/tufted cells, probably due to autoexcitation, and is also influenced by granule cell currents.

Bath application of 25-50 μM of the MGluR agonist trans-ACPD significantly decreased (50%) the EPL field potential evoked by either ON and MCL shocks, whereas the GL field potentials were largely unaffected. 200 μM ACPD blocked the EPL field potentials and reduced the GL field potential. Extracellular recordings from single mitral cells showed that 25-50 μM ACPD increased spontaneous activity (187% of control), and enhanced or did not alter ON-evoked activity. All effects of ACPD were completely reversible.

These results indicate that glutamate, acting on MGluRs located on either mitral/tufted and/or granule cells, modulates the dendrodendritic interaction between these cells. MGluRs may decrease GABA release from granule cells, possibly by inhibiting Ca^{2+} influx, in these cells, resulting in disinhibition of mitral cells. Alternatively, MGluRs on mitral cells may decrease glutamate release from mitral cell lateral dendrites, thereby decreasing excitation of granule cells. This would also disinhibit mitral cells. Both of these potential sites of action are possible. In either case, the present results demonstrate that ACPD-sensitive MGluRs decrease granule cell feedback to mitral cells and increase mitral cell excitability. This should increase mitral cell responses to ON input. Experiments are in progress to identify the site(s) and receptor subtype(s) mediating the actions of ACPD.

Support: PHS grants DC03195, DC00347, DC02588 and NS36940.

Poster 66

Intrinsic activation of NMDA receptors influences main olfactory bulb (MOB) mitral cell excitability. P.M. HEYWARD, M. ENNIS, G.C. CARLSON A. KELLER & M.T. SHIPLEY, *Dept. Anatomy and Neurobiology, Program in Neuroscience, University of Maryland, Baltimore MD 21201.* pheyward@umaryland.edu

Mitral cells are the principal output cells of the MOB. Mitral cells recorded in slice preparations can be classified as monostable (30%) and bistable (70%). Monostable mitral cells maintain a single resting membrane potential (-50 mV), whereas bistable mitral cells oscillate spontaneously between two discrete membrane potentials: (1) a stable subthreshold potential (-60mV); (2) a relatively depolarized potential, perithreshold for spike generation (-50 mV). Here we report on an intrinsic excitatory synaptic influence on mitral cell spontaneous activity, and on responses of the two classes of cell to olfactory nerve stimulation (ON).

Mitral somata were visualized in horizontal slices of adult rat MOB by oblique illumination; whole-cell recordings were made during bipolar stimulation of the ON. Monostable cells responded to ON shocks with bursts of spikes. The NMDA receptor antagonist AP5 (50 μM) limited their response to a single short-latency spike. A full burst response could be restored by depolarizing current injection. Their spontaneous activity was unaffected by AP5. Bistable mitral cell responses to ON shocks depended on existing membrane potential. At subthreshold potentials, single spikes were elicited at long and variable latency, preceded by depolarization to the perithreshold potential. At the perithreshold potential, ON elicited single or multiple spikes at short latency. AP5 reduced or abolished spontaneous depolarization to the perithreshold potential in bistable cells, increasing duration at the subthreshold potential. AP5 limited responses to ON shocks to a single short-latency spike. Current-injection restored neither spontaneous depolarizations to the perithreshold potential, nor responsiveness to ON input.

We conclude that the intrinsic activity of mitral cells is influenced by activation of NMDA receptors. NMDA receptor activation participates in responses to ON input, and may also influence mitral cell excitability, through local neuronal activity.

Support: PHS grants DC03195, DC00347, DC02588 and NS36940.

Poster 67

Examining the roles of nitric oxide synthase and soluble guanylyl cyclase in the development of the antennae and antennal lobes of *Manduca sexta.* A. NIGHORN, N.J. GIBSON, and J.G. HILDEBRAND, *Arizona Research Laboratories Division of Neurobiology, University of Arizona, Tucson, AZ 85721.* nighorn@manduca.neurobio.arizona.edu

Nitric Oxide stimulation of soluble guanylyl cyclase has been suggested to play a role in mediating the development of many different olfactory systems. We have cloned the *Manduca sexta* nitric oxide synthase (MsNOS), and both the alpha and beta subunits of soluble guanylyl cyclase (MsGCα1 and MsGCβ1). To better understand the role that these molecules play in the development of the *Manduca sexta* olfactory system, we have begun to characterize the expression of these genes throughout the development of the antennae and antennal lobes. Using Northern blot analyses we find that MsNOS is expressed at a high level early in development of both the antennae and antennal lobe. Its highest expression occurs at the time at which the incoming sensory afferents from the antennae are entering the developing antennal lobe and beginning the formation of glomeruli. After peaking at this early developmental stage MsNOS levels decline in the antennal lobe and are only barely detectable in the adult. MsGCα1 and MsGCβ1 levels mirror each other as expected with a slow steady increase in expression level throughout the development of both the antennae and the antennal lobes. Given the expression patterns described, the NO/cGMP pathway appears to play an important role early in the development of the antennal lobe. We are currently performing in-situ hybridization analyses to determine the identities of the cells expressing MsNOS, MsGCα1, and MsGCβ1 during development so that we can better understand the potential roles of the NO/ cGMP system in the development of the olfactory system of *Manduca sexta.*

We gratefully acknowledge the support of the NSF : IBN9604536 to A.N., and NIH NS28495 to J.G.H

Poster 68

Adult olfactory cyclic nucleotide-gated channel-1 (OCNC-1)-deficient mice display altered biochemistry and morphology in olfactory bulb. [1]HARRIET BAKER, [2]DIANA M. CUMMINGS, [1]LINDA FRANZEN, [3]STEVEN D. MUNGER, [3]RANDALL R. REED, [2]FRANK L. MARGOLIS, [1]*Cornell Univ. Med. Coll. at Burke Med. Res. Inst., White Plains, NY 10605*, [2]*Univ. Maryland, Baltimore Sch. Med., Baltimore, MD 21201*, [3]*Howard Hughes Med. Inst., Johns Hopkins Univ., Baltimore, MD.* 21205. habaker@med.cornell.edu

The OCNC-1 is hypothesized to play a major role in transducing odor information in olfactory receptor neurons. Neonatal mice deficient (KO) for these channels are characterized by lack of an electroolfactogram (EOG). The current studies investigated the consequences of OCNC-1 deficiency and the resulting sensory deprivation on morphological and biochemical maturation of the olfactory bulb. In contrast to olfactory bulbs from wild type mice, those from the KO were dramatically reduced in size. Tyrosine hydroxylase (TH) immunostaining in periglomerular dopamine neurons showed a degree of reduction similar to that produced by unilateral naris closure. An exception was that on both posteromedial and posterolateral aspects of the olfactory bulb a single glomerulus displayed normal TH expression. In agreement with previous studies, cFos immunoreactivity was reduced in parallel with TH in periglomerular neurons. In contrast, olfactory marker protein staining appeared normal with the exception of ectopic fibers in the external plexiform layer in a pattern reminiscent of that observed during development. PAX 6 immunoreactivity appeared normal in the periglomerular region of the olfactory bulb, but many more migrating cells were observed in the forebrain. These studies suggest that sensory activity gated through the OCNC-1 is critical to gene expression in and development of the olfactory bulb. These data also suggest that a small number of glomeruli receive sensory information by a mechanism that does not require this channel.

Supported by NIH Grants AG09686 (HB) DC03112 (FM) & HHMI (RRR)

Poster 69

Characterization of patch-clamp properties of mitral and periglomerular cells in the accessory olfactory bulb of the rat, *Rattus norvegicus*. GREGORY V. GOLDMAKHER and ROBERT L. MOSS, *Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75235.* goldmakh@utsw.swmed.edu

Isolated mitral and periglomerular (PG) cells from the rat AOB were studied using the whole-cell perforated patch in the voltage-clamp configuration to characterize their voltage and ligand-gated currents.

The first studies were aimed at characterizing the voltage-gated currents of PG and mitral cells. Cells were stepped from a holding potential of -70 mV to a range of voltages from -80 to +80 mV in 20 mV steps. Both PG (n=22) and mitral (n=25) cells exhibited currents which resembled the sodium and potassium currents seen in other CNS neurons and which could be blocked by TTX and TEA, respectively. Mitral cells had larger peak currents than those seen in PG cells, and mitral cell Na^+ currents activated at more negative voltages (mitral -39 mV ±3, PG -26 mV ±3). These data indicate that both of these cell types have electrically excitable membranes, and that mitral and PG cells respond in quantitatively different ways to changes in membrane voltage.

The ongoing second set of studies is investigating the responses of PG and mitral cells to two substances prominently found in neuronal terminals in this region. Immunohistochemical studies have shown that the VN nerve terminals contain glutamate, mitral cells contain glutamate, and PG cells contain GABA.

Recordings from mitral cells show that mitral cells respond to glutamate with currents which reverse around +20 mV. Earlier work has shown that mitral cells respond to GABA with i.p.s.cs. Four of seven PG cells tested with GABA responded with a current which reversed at +5 mV ±5. Of 5 PG cells tested with glutamate, two have responded with a current which reverses at +12 mV ±4.

Taken together, these results indicate that the VNO axon terminals may be excitatory to both mitral and PG cells. Mitral cells may be excitatory to PG cells, and PG cells may be excitatory to other PG cells and inhibitory to mitral cells. This leads to a hypothesis of neuronal interaction in which PG cells act as signal dampers and lateral inhibitors in the AOB.

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Poster 70

Single-cell PCR detection of cyclic nucleotide-gated channels in cultured olfactory bulb neurons. P. A. KINGSTON[1], G. M. SHEPHERD[1], C. J. BARNSTABLE[1], and F. ZUFALL[2], [1]*Section of Neurobiology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510*, and [2]*Anatomy and Neurobiology, University of Maryland, 685 West Baltimore, Baltimore, MD 21201.* kingston@pantheon.yale.edu.

Recent evidence indicates that cyclic nucleotide-gated (CNG) channels, which mediate sensory transduction and adaptation in ORNs, regulate activity in CNS neurons as well. We are exploring the potential roles of CNG channels in the brain using the olfactory bulb as a model.

Previous work has demonstrated the presence of mRNA encoding distinct CNG channel subunits in the adult rat olfactory bulb by RT-PCR. In situ hybridization experiments performed with probes targeting CNG channel transcripts labeled cell bodies in the glomerular, external plexiform, mitral cell, and granule layers of the bulb. Counterstaining with bisbenzimide, a non-specific nuclear stain, reveals some cell bodies unlabeled by the probes, indicating that CNG channels are expressed by a fraction of cells in the bulb.

To study CNG channel function in olfactory bulb neurons, we have established primary cultures from embryonic (E21) rat olfactory bulbs in which we can identify subsets of cells by morphology and with immunocytochemical tools, including antibodies to CNG channel subunits. We have now combined analysis of CNG channel gene expression with electrophysiological recordings from single, visually identified neurons in culture. Whole-cell patch-clamp recordings have identified currents generated by cyclic nucleotides and their analogs in a fraction of the neurons tested. These currents exhibit current-voltage relationships, ionic permeability, and pharmacological properties consistent with CNG channels. PCR with nested primers specific for the olfactory CNG channel principal subunit produced fragments from cDNA harvested from these neurons. Neurons that did not exhibit currents in response to cyclic nucleotides lacked detectable CNG channel mRNA, while primers for β-actin did yield PCR products, confirming the integrity of the cDNA samples.

From this evidence we conclude that central neurons express functional CNG channels, and that the olfactory bulb offers a suitable preparation for exploring the role of CNG channels in regulating neuronal activity. Further work is necessary to pursue the hypothesis that CNG channels may control membrane excitability and intracellular calcium levels in neurons throughout the CNS.

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Poster 71

A Ca^{2+}-permeable, Na^+-gated cation channel from neurons in the lobster olfactory lobe. ASLBEK B. ZHAINAZAROV[1], BARRY W. ACHE[1,2], [1]*Whitney Lab. and* [2]*Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32610.* FAX: (904) 461-4008.

We describe a non-selective cation channel specifically activated by intracellular Na^+ from projection neurons and local interneurons in the lobster olfactory lobe. Na^+ reversibly activates the channel in a concentration-dependent manner recorded in cell-free patches taken from the soma of either type of central neuron. The channel is half-maximally activated by 65 mM intracellular Na^+ at -60 mV and 28 mM +40 mV. The open probability (P_o) of the channel activated by 60 mM Na^+ increases with membrane depolarization, with a half-effect ($V_{1/2}$) at +4 mV and a slope (k) of 38 mV. Increasing the Na^+ concentration shifts the voltage-dependency curve of the P_o to the left along voltage axis without significantly changing its steepness. At 210 mM Na^+, the $V_{1/2}$ is -58 mV and the k is 39 mV. The channel conductance is 105 pS in symmetrical Na^+ (210 mM) containing 10 nM Ca^{2+}, but only 32 pS in extracellular solution containing physiological concentrations of Ca^{2+} (13.6 mM) and Mg^{2+} (9.8 mM), indicating that extracellular divalent cations partially block the channel. The channel is reversibly inhibited by both Ca^{2+} (IC_{50}: 320 μM at -60 mV and 364 μM at +40 mV) and Mg^{2+} (IC_{50}: 271 μM at -60 mV and 258 μM at +40 mV) on the intracellular side of the membrane. The channel is permeable to alkali metal cations with a permeability ratio of $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. Divalent cations also permeate the channel: $Ca^{2+} > Mn^{2+} > Sr^{2+} > Mg^{2+} > Ba^{2+} > Na^+$. The channel is reversibly inhibited by W7 (IC_{50}=18 μM) and trifluoperazine (IC_{50}=13 μM), which reduce P_o without affecting the single-channel current amplitude. We hypothesize that the channel generates the depolarizing plateau potentials observed in some of the central neurons and plays a crucial role in generating rhythmic oscillations in the lobster olfactory CNS.

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Poster 72

Timing of odor-evoked impulse bursts and oscillatory electrical activity in lateral protocerebral neurons of the crayfish central olfactory pathway. DE FOREST MELLON and JIANHUA CANG, *Dept of Biology, Univ of Virginia, Charlottesville VA 22903.* FAX: (804) 982-5626.

Enigmatic oscillatory electrical activity is present in the central olfactory pathways of all animals so far examined. In the freshwater crayfish *Procambarus clarkii,* ongoing, synchronized periodic depolarizations at a frequency of 0.5-1.0 Hz are exhibited by lateral protocerebral interneurons (LPIs) of the hemi-ellipsoid body, which are targets of olfactory midbrain projection neurons. In response to stimulation of the antennular olfactory receptor neurons (ORNs), impulse bursts occur in many LPIs, with a latency of 1-5 seconds following stimulus onset. These bursts are a composite response of the LPIs to excitatory synaptic potentials generated by the projection neuron terminals and the periodic depolarizations imposed upon the LPIs by local, unidentified lateral protocerebral circuits (Mellon, DeF., 1997, Neurosci. Abst. 23:1569). Strong simulation of the antennules, either with odorants or brief electrical shocks, generates multiples impulse bursts in some LPIs, and these appear to be synchronized with the oscillatory activity (eg, Mellon, DeF & Alones, VE, 1997, J. Comp. Physiol. 181:205).Because impulse bursts may carry more transynaptic information than single spikes (Lisman, JE, 1997, Trends Neurosci, 20:38) synchronization of bursts in certain subpopulations of LPIs through the distributed periodic depolarizations carries the potential for establishing associative changes in postsynaptic targets. We are using dual intracellular recordings from LPI pairs to examine the timing of impulse bursts among individuals of the LPI population and to assess the role of the oscillatory activity in synchronizing bursts in different LPIs. In addition we are currently searching for protocerebral targets of the LPI axon terminals, in order to assess the role of impulse burst activity in generating postsynaptic electrical changes.

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Poster 73

Web accessible methods for organizing whole cell neuronal models. JASON S. MIRSKY[1], MICHAEL HINES[2], PRAKASH M. NADKARNI[3], MATTHEW D. HEALY[3], PERRY L. MILLER[3] and GORDON M. SHEPHERD[1], [1]*Section of Neurobiology,* [2]*Department of Computer Science,* [3]*Center for Medical Informatics, Yale University School of Medicine, New Haven, CT 06510.* jason.mirsky@yale.edu.

Computational modelling is an increasingly critical part of analyzing function in the olfactory system and other brain systems. Typically, models of cells are published in a journal, but the electronic version of the model may only be available through correspondence with the author. In addition, it is an arduous task to find related models in the literature. Finally, differing representations of the models make them difficult to compare directly. The sequence community faced similar problems. Their solution has been to require that published sequences be simultaneously distributed to the major sequence databases, thus allowing for organization of these large datasets. However, there are no widely used databases of neuronal models. We seek to provide an analogous solution for neuronal models by composing modelling extensions to NeuronDB.

NeuronDB (http://senselab.med.yale.edu/neurondb) tracks membrane properties of various neuronal types (AChemS 97). Since cellular models are composed of models of these membrane properties, it makes intuitive sense to allow these models to be searchable based on the properties that compose them and the cell types which they model. Since NeuronDB already provides the functionality of being able to search for membrane properties within and across neurons, tagging the models with the cell they represent in NeuronDB allows the database to retrieve models of interest after performing membrane property searches. By associating the models with the data they are based on, it is possible to rapidly retrieve and compare models of cells. The infrastructure to download a model built in NEURON and launch it automatically from the database via the web has been developed. We also plan to make the modelling extensions compatible with models coded in GENESIS and flexible enough to handle other modelling environments. We will illustrate with examples from the olfactory system.

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Poster 74

Noise analysis of spatio-temporal information processing in a computer simulation of the olfactory bulb. JOEL WHITE, *Neuroscience Dept., Tufts Med. School, Boston, MA, 02111.* jwhite@opal.tufts.edu

We previously described a new analytical method applied to temporal data from an array of artificial chemical sensors (White et al., 1997, Chem.Senses, 22: 821). The method incorporates a computer model of the olfactory bulb, and shows good vapor recognition over a range of input amplitudes. These results suggest that the method may be useful for processing sensor data in an artificial nose. As is the case for standard computational neural networks often used in artificial noses, however, the performance of this system is dependent on model parameters. Rather than optimizing the parameters for specific sensor data sets, an alternative approach is to use noise analysis and stimulus reconstruction to investigate parameter effects on spatio-temporal processing. Input data with spectral properties similar to sensor data are created using Gaussian noise. These input data are processed by the olfactory bulb model to produce simulated mitral cell spike output patterns. As with the actual sensor data, model outputs elicited by the Gaussian inputs consist of spatio-temporal patterns of brief spike bursts. Many input-output pairs can be generated, providing sufficient replicates for statistical analysis. From a large number of input-output pairs, the mean effective stimulus (i.e., the first Wiener kernel) that gives rise to each type of spike burst is determined. These kernels can then be used to reconstruct the input from a given spike output pattern. Spike burst statistics, individual kernel properties, and reconstructed input data can all be examined to evaluate the effects of changing model parameters. Using this method, an analysis of lateral inhibition by periglomerular and granule cells has begun. In addition to the practical application toward development of an artificial nose, this analysis may also provide insight into the effects of lateral inhibition or other physiological mechanisms on odorant information processing by the biological olfactory bulb.

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Poster 75

Responses of single olfactory bulb neurons to structurally similar and diverse amino acid odorants in the channel catfish. WILLIAM T. MAGEE and JOHN CAPRIO, *Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803.* FAX: (504) 388-2597

The discovery that odorant stimulation produces characteristic patterns of olfactory bulbar glomerular activity has led to the hypothesis that the glomerulus is the fundamental unit of odor discrimination and to reinvestigations of the odorant specificity of individual mitral cells. Evidence by Mori and colleagues (Mori, K. *Curr Opin Neurobiol* 5:467-474, 1995) indicated that the rabbit mitral/tufted cell (M/T) is activated by a range of odorant molecules with closely related structures; however, these results are discrepant with those of previous studies and to a recent report that M/T responses in olfactory bulb of the rat responded to a broad spectrum of odors (Motokizawa, F. *Exp Brain Res 112*:24-34, 1996).

The present study examines the specificity of individual olfactory bulbar (OB) neurons (putative mitral cells) to behaviorally relevant amino acids with systematic alterations in molecular structure (side chain length, structural isomers, stereo isomers and position of functional group0 in the channel catfish, *Ictalurus punctatus.* Preliminary analyses indicate that some bulbar neurons respond to amino acids possessing widely different structural properties (e.g. arginine and alanine), while others distinguish among amino acids which are structurally similar (e.g. arginine and homoarginine). These results are consistent with optical imaging studies of the zebrafish olfactory bulb which indicate that many glomerular modules are activated by structurally diverse amino acids while others show a more narrow response profile (Friedrich, R.W. & Korsching, S.I. *Neuron 18*:737-752, 1997)

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Poster 76

Host-plant odor processing by antennal lobe projection neurons in female *Manduca sexta.* JANE ROCHE KING, THOMAS A. CHRISTENSEN, and JOHN G. HILDEBRAND, *ARLDN, U. of AZ., Tucson, AZ 85721*, jrking@neurobio.arizona.edu.

Primary olfactory centers in most vertebrates and invertebrates characteristically exhibit glomeruli, but the functional significance of these neuropil structures is uncertain. In the male sphinx moth, *Manduca sexta*, certain sexually dimorphic glomeruli in the antennal lobe (AL) are odotopically organized to process information about individual components of the female sex pheromone. It is not known, however, if the "ordinary" glomeruli found in both sexes are also odotopically organized. Recently, two sexually dimorphic glomeruli unique to the female AL have been identified (Roessler et al., in press), but the functional significance of these glomeruli, designated the large female specific glomeruli (LFGs), is unknown. We have begun to characterize individual uniglomerular projection neurons (PNs) of the female ALs. Because these insects use olfactory information to locate hostplants for oviposition, we study responses of individual PNs to identified components of the head-space volatiles emitted by hostplants.

Neurites of individual AL neurons were impaled with microelectrodes in the neuropil, and we recorded physiological responses to antennal stimulation with various odorants, including whole tomato leaf, methyl salicylate, *trans*-2-hexenal and linalool. Cells were then stained intracellularly by Lucifer Yellow injection to reveal morphological features, including the spatial "address" of the glomerulus innervated by each neuron. Some PNs were generalist, while other PNs responded selectively to particular odorants. Using 3-D reconstructions of female AL glomeruli, we have shown that PNs innervating the same glomerulus display very similar response profiles to the range of odors tested thus far. For example, evidence obtained from neurons innervating the lateral LFG suggests that it is specific for terpenes. These data support the idea that individual glomeruli are discrete processing units and suggest that AL glomeruli of both male and female moths are organized odotopically.

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Poster 77

Temporal structure of pheromone plumes: simultaneous recordings from electroantennograms and single projection neurons in the antennal lobes of male moths. NEIL J. VICKERS and THOMAS A. CHRISTENSEN, *ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.* vicksterr@neurobio.arizona.edu.

An odor plume emanating from the everted pheromone gland of a calling female moth consists of strands of odor (filaments) interspersed with clean air. The heterogeneous distribution of odor, in both space and time, is important to male moths orienting upwind, as evidenced by their characteristic flight behaviors. Males cease upwind progress toward a point-source in a homogenous cloud of pheromone and enter into crosswind, casting flight, a behavior normally associated with odor loss. Similarly, a plume created by pulses of air through pheromone-bearing cartridges will not sustain upwind flight and source location by male moths if delivered below a threshold frequency of four pulses per second.

Clearly, the temporal structure of this odor plume plays a critical role in shaping male behavioral responses. In the current study, male *Heliothis virescens* were exposed to pheromone plumes created by a point-source of odor within a miniature wind tunnel to mimic the temporal structure of stimulation that a male moth might encounter during upwind flight to a calling female. An electroantennogram (EAG) was used to monitor the occurrence of a pheromone filament striking the antenna. Activity from a single physiologically-identified pheromone-specific projection neuron (PN) in the ipsilateral antennal lobe (AL) was recorded simultaneously. Physical parameters such as distance to the odor source and windspeed were varied to determine their effects on plume structure, as measured by both EAG and PN recordings. These records were analyzed to assess the correlation between EAG activity and the intracellular PN responses. Where possible, PNs were also stained with a fluorescent dye to reveal details of their morphological structure within the macroglomerular complex of the male AL.

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Poster 78

Effects of preovulatory and ovulatory pheromones in goldfish olfactory bulb mitral and ruffed cells. SAMER NASSER, MARION GLOGER, H. PETER ZIPPEL, *Physiol. Inst. der Universität, Humboldtallee 23, 37073 Göttingen, Germany.* FAX: +49-551-395923.

Single unit activity from both types of relay neurons was simultaneously recorded with tungsten microelectrodes (for details: see Zippel and Wilcke, this vol.). In electro-olfactogram (EOG) highly effective, and lesser effective preovulatory Steroidal pheromones (Sorensen et al., J. Comp. Physiol. A 1990, 166, 373-383) were investigated: highly effective: 17,20ß-Dihydroxyprogesterone, 10^{-9}-10^{-11}M; 17,20ß-21-Trihydroxyprogesterone, 10^{-9}-10^{-11}M and lesser effective: 4-Pregnen-20α-ol-3-one, 10^{-7}-10^{-9}M, 4-Pregnen-20ß-ol-3-one, 10^{-7}-10^{-9}M; 17α, 20α Dihydroxyprogesterol, 10^{-7}-10^{-9}M, and Androsten-dione, 10^{-7}-10^{-9}M. Furthermore the effectiveness of two ovulatory pheromones: Prosta-glandin $F_{2\alpha}$, 10^{-7}-10^{-11}M, and 15Keto-prostaglandin $F_{2\alpha}$, 10^{-7}-10^{-11}M was investigated in comparison to two amino acids (Arg 10^{-7}M, Pro 10^{-7}M) representatives of important food stimuli. Olfac-tory bulb relay neurons frequently respond to a comparatively great number of olfactory stimuli: amino acids, preovulatory and ovulatory stimuli. Contrasting interactions between MC and RC frequently were recorded during stimulus app-lication. In contrast to EOG recording (Sorensen et al. 1990) application of highly effective and lesser effective pheromone stimuli resulted in less contrasting responses in olfactory bulb relay neuron. The EOG is a slow (DC) potential change recorded in teleosts in response to chemical stimulation and is suggested to be the population average to receptor potentials responsible for the initiation of neural impulses. From the present recordings from olfactory bulb relay neurons, however, the EOG obviously is not an excellent indicator of olfactory organ sensitivity and specificity to odorants in fishes.

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Poster 79

Goldfish olfactory bulb relay neurons respond during epithelial application of a probable alarm pheromone. H. PETER ZIPPEL, SUSANNE WILCKE, *Physiol. Inst. der Universität, Humboldtallee 23, 37073 Göttingen, Germany.* FAX: +49 551-395923.

The fish alarm pheromone system is characterized by distinctive epidermal club cells, that contain the alarm pheromone, probably Hypoxanthine-3(N)-oxide. Physiological responses from both types relay neurons (mitral cells=(MC), ruffed cells=(RC)) were recorded extracellularly and simultaneously in the plexiform layer using a single tungsten microelectrode (A-M 5770; 10-12 MΩ). Stimuli (Hypoxanthine-3(N)-oxide, 10^{-7}-10^{-11}M; Hypoxanthine, 10^{-7}-10^{-9}M; a preovulatory pheromone: 17,20ß-dihydroxy-4-pregnen-3-one, 10^{-9}M; an ovulatory pheromone: Prostanglandin $F_{2\alpha}$, 10^{-7}-10^{-9}M; a food stimulus: Arg 10^{-7}-10^{-9}M; and a control stimulus: Hydrocortisone 10^{-9}M) were respectively applied for 15s to the olfactory epithelium. During the 180s interstimulus intervals a laminary water current of similar velocity ($1ml \cdot s^{-1}$) was applied. During interstimulus intervals MC responded with higher, and frequently burs-like impulse rates. The impulse rates of RC were low, and each RC potential triggred a summed granule cell (GC) potential. During stimulation excitation of MC resulted in simultaneous inhibition of RC, and inhibition of MC in excitation of RC. Even the lowest concentration resulted in significant and contrasting inter-actions in relay neurons. In contrast to EOG recordings application of the probable alarm pheromone (which had no recordable effect in EOG; Sorensen, pers. comm.) resulted in a similar effectiveness as the preovulatory and the ovulatory pheromones, and the amino acid. Hypoxanthine was a slightly lesser effective stimulus and application of the control stimulus. Hydrocortisone rareley resulted in recordable effects.

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Poster 80

In vivo optical recording of electrically stimulated neuronal activity in the mouse olfactory bulb. TARIK K. ALKASAB, JOHN S. KAUER. *Dept. of Neuroscience, Tufts University School of Medicine, Boston, MA 02111*, talkasab@opal.tufts.edu.

A current hypothesis of olfactory information processing suggests that odor information is partly represented in spatio-temporal patterns of activity in the olfactory bulb (OB) and that glomeruli may act as functional units in this representation. To provide data in support of this hypothesis, it is crucial to measure activity across a population of glomeruli over time. Optical imaging using voltage-sensitive dyes has been used in vertebrate preparations to explore the distributed nature of olfactory processing, but has not been applied to the intact OB of the mouse. Data from such experiments should be valuable for relating odor representation to the molecular organization of the peripheral olfactory system. We have begun imaging experiments in the intact mouse OB using voltage-sensitive styryl dyes, which have been shown to stain effectively the glomerular neuropil. When the dorsal surface of the adult mouse olfactory bulb is stained with the dyes RH-795 or RH-414, glomeruli can be resolved with a CCD camera and a standard fluorescence microscope. Electrical stimulation of the olfactory nerve bundles in the dorsal epithelium results in detectable changes in fluorescence that are not observed in the absence of stimuli. Specifically, brief decreases in fluorescence lasting less than 50 ms are observed immediately following the stimulus. Responses from glomerular regions can be resolved, and these structures appear to respond differentially to electrical stimuli. We conclude that our apparatus is capable of detecting changes in neuronal activity in the intact mouse OB, and may be able to record odor-evoked activity in many glomerular elements simultaneously.

Supported by grants from ONR, NIH, and DARPA.

Poster 81

Effect of WGA lectin on odor detection and neuronal processing of odor stimuli in the rat olfactory bulb. ALEXANDRA KIRNER[1], RAIMUND APFELBACH[1], AND ERNEST POLAK[2], [1]*Department of Zoology, University of Tübingen, 72076 Tübingen, Germany,* [2]*Department of Chemistry, University of Warwick, Coventry, UK.* FAX: 01149-7071-294634.

Lectins, carbohydrate-binding proteins, have been used on many occasions to study the different carbohydrate residues on cell surfaces. In several histochemical studies lectins were used to label olfactory receptor cells (Stewart and Touloukian, 1996; Menco, 1992, 1994). In the present study we investigated the effect of the lectin wheat germ agglutinin (WGA) on 1) odor detection in the living rat and 2) c-fos immunoreactivity in the rat olfactory bulb after odor stimulation.

Behavioral data were collected using an olfactory Skinner-box, described by Slotnick and Schoonover (1984), where the animals had to distinguish between the odorant ethyl acetate (EA, 6×10^{-5} vol.% s.v.p.) and clean air. Before WGA treatment (WGA concentration 4 mg/ml in Ringer solution) rats reached a performance level of 97% correct responses (cr). Ringer treatment without WGA had no effect on EA detection ability (96% cr). WGA reduced the odor detection ability to 55% cr, which corresponds to perchance performance. In the histochemical part of the study we compared the number of c-fos positive periglomerular cells in the olfactory bulbs of animals which were treated with: 1) Ringer solution before odor EA stimulation (1 vol.% s.v.p.), 2) WGA solution (4 mg/ml in Ringer solution) before odor stimulation, and 3) Ringer solution without odor stimulation. In odor exposed animals periglomerular cells in selected areas - surrounding specific glomeruli - demonstrated Fos activity. In WGA treated animals we found far less staining than in the animals without WGA. Periglomerular cells in the WGA animals never surrounded individual glomeruli. Control animals, which were kept in clean air showed only few, single Fos positive cells. The immunhistochemical results support the behavioral findings suggesting that one or more types of olfactory receptor cells that are involved in EA recognition are affected by WGA.

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Olfactory discrimination ability of human subjects for aliphatic alcohols. MATTHIAS LASKA and SABINE TROLP, *Department of Medical Psychology, University of Munich Medical School, D-80336 Munich, Germany.* Laska@imp.med.uni-muenchen.de

There is common agreement that odor discrimination begins with differential interaction of odor molecules with different types of olfactory receptors. In order to understand how the olfactory system actually achieves odor discrimination it is therefore clearly important to establish which properties of an odor molecule are functional in determining the degree of interaction with a given receptor, and concomitantly in determining its perceived odor quality. One useful means to assess possible correlations between odor quality and molecular properties is to test the discriminability of structurally related odorants.

We have tested the ability of human subjects to distinguish between members of a homologous series of aliphatic alcohols (ethanol to n-octanol). In a forced-choice triangular test procedure 20 subjects were presented with all 21 binary combinations of the 7 stimuli and asked to identify the bottle containing the odd stimulus. Testing was repeated in 9 more sessions each 1-3 days apart, enabling 10 judgements per stimulus pair and panelist to be collected.

We found a) that as a group, the subjects performed significantly above chance level in all tasks but one (n-heptanol versus n-octanol) and thus were clearly able to discriminate between most of the odor pairs presented, b) marked interindividual differences in discrimination performance, ranging from subjects who were able to significantly distinguish between all 21 odor pairs to subjects who failed to do so with 1/3 of the tasks, c) that odor pairs which differed by only one carbon atom (ÆC1) were significantly more difficult to discriminate than odor pairs which differed by two or more carbon atoms, and odor pairs differing by two carbon atoms (ÆC2) were significantly more difficult to distinguish compared to odor pairs which differed by three or more carbon atoms (ÆC3 to ÆC6), and d) that with only few exceptions the odor pairs within a ÆC group did not differ significantly from each other in their degree of discriminability.

Thus, we found a highly significant negative correlation between discrimination performance and structural similarity in terms of differences in carbon chain length of the alcohols. This suggests that carbon chain length may be one of presumably several determinants of the interaction between stimulus molecule and receptor, and thus may be a molecular property affecting odor quality of aliphatic alcohols.

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Voltage-sensitive dye recording of odor elicited oscillations in the turtle olfactory bulb. YING-WAN LAM, LAWRENCE B. COHEN, JING FANG, AND MICHAL ZOCHOWSKI, *Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06520.* ywlam@minerva.yale.edu.

Odorant elicited oscillations in local field potential recordings from vertebrate olfactory bulbs were first recorded 50 years ago in mammals (Adrian, 1950) and 25 years ago in turtles (Beuerman, 1975). Because voltage-sensitive dye signals have better spatial resolution than local field potential measurements, we made optical measurements of the response to several odorants in an *in vivo* turtle preparation. *Terrapine carolina* and *T. ornata* were anesthetized by submersion in ice and a craniotomy was performed over the olfactory bulb. A 0.01-0.2 mg/ml solution of the voltage-sensitive styryl dye, RH414 (Grinvald et al, 1994), was placed on the bulb. Using an olfactometer (Kauer and Moulton, 1974), we applied 500 msec odorant pulses at 0.3%, 1.7%, and 10% of saturation. We measured the optical signals with a 464 element diode array using a 4.5X objective; the area of the object plane imaged onto each detector was $166 \times 166\ \mu m^2$.

The population responses to odors had two components; a wide-spread and long-lasting (seconds) depolarization that was centered more caudally and a more rostrally localized oscillation at 10-15 Hz that appeared after a delay 0.1-1.0 seconds. The oscillations were not stationary waves; they propagated in a rostral to caudal direction. In some preparations, the oscillations were more complex, consisting of two propagating processes.

We measured oscillations in response to amyl acetate, cineole, and pyridine. Preliminary analyses indicate that the propagation direction, the frequency of the oscillation, and the area of the bulb involved in the oscillations was similar for all three odors. However, in many preparations the envelope of the oscillations and the time course of the slow component differed for different odors. Thus, some parameters of these population signals differentiate among the odorants while other aspects are similar.

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Spatial patterns of c-fos expression in the rat olfactory bulb in response to stimulation with two different odors. TOBIAS KRAUTER, ALEXANDRA KIRNER, AND RAIMUND APFELBACH, *Department of Zoology, University of Tübingen, 72076 Tübingen, Germany.* FAX: 01149-7071-294634.

The proto-oncogene c-fos belongs to a large class of immediate early genes, which are induced rapidly by sensory stimuli. The protein product of the c-fos gene, Fos, can be used to identify neurons activated by a variety of stimuli. In this study we investigated odor induced mapping patterns of Fos immunoreactivity in the rat olfactory bulb after stimulation with the odorants ethyl acetate and propionic acid. Odor concentration was 1 vol.% of saturated vapor pressure.

Fos immunoreactive cells were distributed in restricted regions of the glomerular layer where they surround individual glomeruli. A great number of granule cells expressed Fos. Labeled cells were also found in the external plexiform layer and more rarely in the mitral cell layer. Control animals which were exposed to clean air only showed only few single Fos immunoreactive cells in the glomerular layer and considerable less staining in the external plexiform, mitral cell and granule cell layer compared to odor exposed animals.

The distribution of Fos-labeled periglomerular cells in the glomerular layer differed for the two odors used. Propionic acid showed three distinct foci of Fos-positve cells in the glomerular layer. One focus was localized dorsomedial in the rostral third of the bulb, one was found mediolateral in the medial third and the third focus was mediomedial in the caudal part of the bulb. Ethyl acetate produced a more extended staining of periglomerular cells compared to propionic acid. In the glomerular layer of ethyl acetate stimulated animals, Fos positive cells were found in large areas, with fewer stained cells in the ventrolateral apart. There was no staining of periglomerular cells in the rostral third of the bulb.

The present results demonstrate that stimulation with different odors leads to different, however, odor specific Fos staining patterns in the olfactory bulb.

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Context-dependent odor responses reveal dual GABA-dependent spike codes in single olfactory projection neurons. T.A. CHRISTENSEN, B.R. WALDROP, and J.G. HILDEBRAND, *ARLDN, Univ. Of Arizona, Tucson, AZ 85721.* tc@neurobio.arizona.edu

Classical and contemporary studies of olfaction have focussed on the role of the glomerulus in processing information about the molecular qualities of odors, but changes in the physical properties of airborne odorants also affect behavior, and thus influence the brain's perception of olfactory input. In moths, continuous and intermittent stimulation with the same odor evoke two distinct flight behaviors, but the neural basis of this differential response is unknown. Here we show that certain glomerular projection neurons (PNs) exhibit context-dependent odor responses that are shaped in several ways by a bicuculline-sensitive GABA receptor. Pharmacological dissection of PN responses suggests that bicuculline blocks a $GABA_A$ -type chloride channel/receptor on the dendrites of PNs, and this receptor plays a critical role in shaping glomerular output. While the firing patterns in PNs are strongly dependent on GABAergic inhibition, they are also modulated by the temporal pattern of the odor stimulus itself. Brief, repetitive stimulus pulses evoke fast inhibitory potentials followed by discrete bursts of firing that are time-locked to the stimulus pattern. In contrast, the response to a single, prolonged pulse with the same odor is a series of slow oscillations underlying irregular firing; a pattern more indicative of a temporal spike code. Bicuculline destroys the timing of both response types, suggesting that a $GABA_A$ -type receptor underlies both coding mechanisms. These results provide the first evidence that glomerular output neurons could employ more than one coding scheme to represent a single olfactory stimulus. Moreover, these output neurons integrate information about chemical identity as well as information about changes in the physical environment bearing the odor stimulus. When combined with the behavioral evidence, these data suggest that context-dependent odor responses evoke different perceptions in the brain that provide the animal with important information about the temporal variations that occur in natural odor plumes.

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Dynamic models of receptor transduction and olfactory coding in the insect antennal lobes. WAYNE M. GETZ, and ANTOINE LUTZ, *Division of Insect Biology, University of California at Berkeley, CA 94720-3112.* getz@nature.berkeleyedu.

Considerable progress still needs to be made in understanding the dynamic aspects of olfactory coding at the peripheral and antennae lobe levels in insects before a clear picture emerges of what features constitute an olfactory code in the firing patterns in the relay neurons that project from the antennal lobes to the higher centers of the brain. Here we present a chemical kinetic model of the receptor transducti on processes that help explain some of the transient features of receptor neuron response. We also present a discrete-time dynamic network model of the basic olfactory circuitry in the insect antennal lobe and define criteria, or indices, for assessing how well odors of the same quality at different concentrations arc perceived as being the same, and how well odors of different qualities are discriminated by this network. We analyze how various model parameters-activation function, decay (memory), delay, selected synapse weight, and self-activation rate parameters--affect quality and discrimination performance indices. Finally we demonstrate throul,,,b. simulations of the model that olfactory coding may be confined to the initial transient response of the antenna] lobe relay neurons in cases where the equilibrium behavior of the projection neurons is stimulus independent, or that coding may be equilibrium dependent, We compare the performance of our model to data that has been obtained from earlier studies on cockroach olfactory sensory neuron and antennal lobe projection. neuron responses to pulsatile stimuli.

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Mapping the molecular receptive range of individual olfactory cilia by high-resolution calcium imaging. FRANK ZUFALL[1,3], CHARLES A. GREER[1,2], GORDON M. SHEPHERD[1], TRESE LEINDERS-ZUFALL[1,3], *[1]Section of Neurobiology and [2]Department of Neurosurgery, Yale University, New Haven, CT 06510, [3]Department of Anatomy and Neurobiology, University of Maryland, Baltimore, MD 21201,* fzufa001@umaryland.edu.

The initial molecular steps leading to olfactory perception occur in very thin olfactory cilia when odor ligands bind to putative odor receptors, but very little is still known about the functional properties of the receptor proteins. We have devised a novel method using laser scanning confocal microscopy by which calcium signals in single cilia from olfactory receptor neurons (ORNs) can be resolved both spatially and temporally. This provides an optical indicator that can be used as a real-time monitor of events associated with odor detection at the site of primary transduction. Here we have used this approach to begin to define the molecular receptive range of individual cilia of salamander ORNs in response to a series of odor ligands. One of the aims was to test whether all cilia of a given ORN are uniform in their odor sensitivity and thus produce uniform response spectra or whether they respond differentially to different odor ligands. We applied brief pulses of six different odorants (acetophenone, n-amylacetate, iso-amylacetate, cineole, citralva, and ethyl-butyrate, all at 300 μM) to the cilia and also stimulated each cell with the phosphodiesterase inhibitor IBMX (300 μM) to confirm that they were alive and had an intact cyclic nucleotide second messenger system. Out of 13 cells tested each one responded with a robust ciliary calcium signal to IBMX as described previously (Leinders-Zufall et al., 1997, *J. Neurosci.* 17, 4136). In contrast the probability of eliciting odor responses was rather low: 7 cells showed no odor responses, 3 cell responded only to one ligand, 2 cells to two, and 1 cell to three ligands. In no case did we detect an identical response spectrum. In sharp contrast to the variability seen between cells, there was virtually no difference between the ciliary odor spectra for a single cell. These results demonstrate that olfactory cilia control both selectivity and sensitivity of the olfactory system. Furthermore, they indicate that all cilia of a given ORN are functionally uniform in their capacity to detect odor molecules; this means that they contain the same odor receptor or receptor subset.

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Location of olfactory receptors in olfactory epithelium and bulb correlates with potential odor-discriminating receptor residues. MICHAEL S. SINGER and GORDON M. SHEPHERD, *Sect. Neurobiology, Yale Univ. School of Medicine, New Haven, CT 06510.* FAX: (203) 785-6990.

In situ hybridization has shown that each olfactory receptor (OR) subtype tends to be expressed in only one of four characteristic zones in the olfactory epithelium (OE). However, the functional significance of these zones remains unclear. We used correlated mutation analysis (CMA) to test OR sequences for systematic amino acid residue differences across the different zones. This analysis was carried out with mouse sequences from Sullivan et al. (1996). In parallel, we tested ORs across different samples from the olfactory bulb (OB), using rat sequences from Singer et al. (1997). The analysis yielded 17 residues correlated with location in OE or OB. Remarkably, these residues were clustered in the predicted odor-binding pocket, in transmembrane domains TM3, TM4 and TM5. The residues were also clearly clustered with respect to secondary and a-helical structure. They included four residues identified by previous molecular models: 101, 159, 203, and 207. Residue 159 may be particularly important due the predominance of histidines at this site, which in the mouse data set were restricted to zone 1. Based on the hypothesis that the correlated residues determine the OR affinity for different odor ligands, the systematic differences across OE zones and OB locations may provide insight into how odors are mapped in the olfactory pathway.

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Mechanisms underlying odor response desensitization of olfactory receptor neurons. MINGHONG MA[1], TRESE LEINDERS-ZUFALL[1,2], GORDON M. SHEPHERD[1], FRANK ZUFALL[1,2], *[1]Section of Neurobiology, Yale Univ., New Haven, CT 06510, [2]Department of Anatomy and Neurobiology, Univ. of Maryland, Baltimore, MD 21201.* FAX: (203) 785-6990

During sustained stimulation of olfactory receptor neurons (ORNs) the odor response is transient and adapts rapidly to a lower level, a process that is termed desensitization. Biochemical experiments have pointed to the possibility that phosphorylation of odor receptors by various protein kinases may underlie this form of odor adaptation while electro-physiological data suggested that modulation of cyclic nucleotide-gated (CNG) channels by Ca^{2+}/calmodulin is the main step in this process. These contradictory results required a reassessment of the mechanisms mediating odor response desensitization. Here we used perforated patch recording from isolated salamander ORNs. We found that sustained stimulation with 8-sec odor pulses induced transient responses under both current and voltage clamp. Both the decay time constant and the extent of desensitization depended on the stimulus strength.Complete recovery from the reduced responsiveness induced by a second pulse following a control one occurred after 30 sec. When two different odors (cineole and n-amyl acetate) were tested in a single cell, both odors induced very similar effects of desensitization. These two odors also caused cross-adaptation, i.e., exposure to cineole diminished the response to n-amyl acetate and vice versa. We then further investigated the potential mechanisms underlying desensitization. Interestingly, odor receptor activation was not required to induce complete response desensitization. The same effect could also be elicited by activating the cyclic nucleotide second messenger system with the phosphodiesterase inhibitor IBMX. Furthermore, we found that Ca^{2+} entry through CNG channels played a critical role in desensitization because removal of extracellular Ca^{2+} eliminated all desensitization and also delayed the deactivation phase of the response following the end of an odor pulse. The results indicate that down-stream mechanisms in the cAMP pathway are sufficient to cause odor desensitization in salamander and that Ca^{2+} entry through CNG channels is one of the main steps for this process. Further experiments are underway to test whether modulation of CNG channels alone mediates desensitization or whether phosphodiesterase and/or adenylate cyclase are also involved in this phenomenon.

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Genetic Dissection of the Chemoresponse Hyperpolarization in *Paramecium.* ROBIN R. PRESTON[1], WADE E. BELL, JUDITH L. VAN HOUTEN, *The Allegheny University of the Health Sciences, Philadelphia, PA 19129* and University of Vermont, Department of Biology, Burlington, VT 05405.* jvanhout@zoo.uvm.edu.

Paramecia respond to attractant stimuli with fast, smooth swimming that is characteristic of hyperpolarization. Direct membrane potential recordings confirm that cells in stimuli show a sustained hyperpolarization. Interestingly, there are t least 3 chemosensory transduction pathways that result in hyperpolarization, all accomplished by different ionic mechanisms, but with the same behavioral results. We have begun to examine the conductances that result from the application and removal of two attractant stimuli: biotin and acetate. The following is consistent with the conductances that we observe: Application of biotin initiates a small outward conductance consistent with the activation of the calcium pump current, which we believe is involved in 2 of the 3 chemosensory transduction pathways. Removal of biotin results in a larger inward conductance, probably a calcium conductance. Acetate application initiates a transient, small inward calcium conductance, which activates a larger, transient outward K conductance, and a sustained outward conductance probably due to the activation of the calcium pump. Removal of acetate results in a small inward calcium conductance.

To test these ideas, we have examined the behavioral response of normal cells and mutants, which have specific defects in membrane conductances. The results of the behavioral tests are consistent with a calcium pump conductance in biotin, and activation of a calcium channel with the removal of the stimulus. The results also support a K conductance in acetate, but the I_K does not appear to be the calcium/calmodulin-activated K conductance. These results have suggested which mutants and conditions will be useful in further voltage clamp studies.

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The use of human olfactory receptor neurons to study the biochemical basis of bipolar disorder. GEORGE GOMEZ[1], CHANG-GYU HAHN[2], RICHARD JOSSIASEN[2], ETIAN FRIEDMAN[2], LOUIS D. LOWRY[1,3], DIEGO RESTREPO[4] AND NANCY E. RAWSON[1]. *[1]Monell Chemical Senses Center, Philadelphia, PA; [2]Allegheny University of the Health Sciences, Norristown, PA; [3]Thomas Jefferson University, Philadelphia, PA, [4]University of Colorado Health Sciences Center, Denver, CO*

Psychiatric patients that are diagnosed with bipolar (manic-depressive) disorders are often treated with lithium salts. Studies showing that lithium interferes with IP_3 metabolism (causing a long-term activation of various cellular messengers such as calcium and protein kinase C) have used varieties of non-neuronal cells. Human olfactory neurons (HONs) provide a unique advantage for the study of the neural basis of psychiatric disorders and cellular effects of drugs used to treat them because they are neurons that can be obtained via biopsy from healthy and clinical populations, enabling differences in neuronal metabolism to be studied. Since HONs respond to certain odorants with an increase in IP_3 production that ultimately leads to changes in intracellular calcium, we tested isolated HONs with a mixture of these odorants (Mix B) in the presence and absence of 1 mM lithium chloride and studied cell responses with calcium imaging techniques. About 11% of the cells obtained from volunteers from the general population responded to Mix B; the odor responsiveness of cells from these subjects was not affected by exposure to lithium. In cells from bipolar subjects, 50% of the HONs that did not respond to Mix B upon the first application responded to Mix B in the presence of lithium chloride. These data demonstrate the potential of this approach to yield insight into the etiology of bipolar disorder and suggest that one mode of action for lithium may be through the modulation of neuronal signalling pathways involving calcium and/or IP_3.

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Cloning of a large number of putative murine olfactory receptors, and functional expression in HEK 293 cells. DIETMAR KRAUTWURST[1], KING-WAI YAU[2], RANDALL R. REED[1], *[1]Dept. of Mol. Biol. & Genetics, [2]Dept. of Neurosci., Howard Hughes Med. Inst., Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.* FAX: (410) 614-0827.

Despite the identification of putative olfactory receptors as members of a large multigene family, very few of these receptors have been functionally expressed and their responsiveness to specific odorants demonstrated. To address this question, we have heterologously expressed about 100 out of perhaps 500 cloned murine olfactory receptor chimeras and screened them for responses to individual odorants. Receptor chimeras were constructed from mouse olfactory epithelium cDNA by PCR using degenerate primers to conserved sequences in transmembrane regions II and VII of olfactory receptors. PCR fragments were cloned into a CMV promotor driven expression vector cassette between the N terminus/TM region I and the C terminus of a known mouse olfactory receptor (M4). The chimeric constructs were tagged with an N terminal 20 aa peptide fragment of rhodopsin (rho.tag) and transiently transfected into HEK 293 cells. Using confocal microscopy, we observed immunofluorescence at, or near, the plasma membrane after staining with a monoclonal antibody against the rho.tag epitope.

In control experiments, the β2 adrenergic or the *C. elegans* odr10 receptor chimera in the same construct was coexpressed with $G_{\alpha 15,16}$. These G proteins promiscuously couple 7TM receptors to phosphoinositide metabolism. Ratiometric Ca^{2+} imaging with FURA2 has shown a transient increase in intracellular Ca^{2+} when the transfected cells were exposed to 10 μM isoproterenol or diacetyl, respectively.

Using the same coexpression strategy combined with Ca^{2+} imaging, 9 pools each with 8 olfactory receptor chimeras were screened against 25 individual odorants. Responsive pools were then split up into single receptor chimeras and the screening repeated. Three receptors were identified which responded specifically to 10 μM (-)citronellal, carvone and limonene, respectively. In addition, we have found that the expression of the mouse homolog of the I7 receptor, either in the above chimeric construct or as a full length protein, was sufficient to mediate Ca^{2+}-transients specifically induced by heptanal (10 μM).

Further screening and functional expression will help identifying additional receptors and their odorants.

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Are the olfactory receptors also guidance receptors? PAUL FEINSTEIN, CHEN ZHENG, ANNE VASSALLI AND PETER MOMBAERTS. *The Rockefeller University, 1230 York Avenue, New York, NY 10021.* feinstp@rockvax.rockefeller.edu.

A large family of genes, discovered by Buck and Axel, is believed to encode odorant receptors in the mouse. We refer to these genes as olfactory receptor genes. Olfactory sensory neurons that express a particular olfactory receptor gene project their axons to two glomeruli in each olfactory bulb, out of a possible choice of 1,800. The location of these glomeruli is stereotyped, suggesting that the developmental mechanisms underlying their formation are hardwired. A logical speculation is that the olfactory receptors are involved in the guidance process that establishes the precise connections between the epithelium and the bulb. Mombaerts et al. have provided genetic evidence in the mouse that indicate that the olfactory receptor is a determinant of the guidance process, but it cannot be the only determinant (Cell 87, 675-686, 1996).

We have followed up on these observations. We have developed improved methods to visualize axonal projections and to study the consequences of genetic alterations in the coding sequence of an olfactory receptor on the targets of axonal projections in the bulb. We have carried out a series of gene targeting experiments on a duo of highly homologous olfactory receptors, M71 and M72. The results strongly suggest that the M71 and M72 gene products are involved in axon guidance. It remains to be shown that these two proteins are also receptors for odorants, by identifying if expression of these molecules correlates tightly with a particular odorant response profile.

In sum, our findings further support the notion that the olfactory receptors are bifunctional, as receptors for odorants and as receptors in the guidance machinery.

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Poster 95

Suppression of taste responses in the nucleus of the solitary tract of the rat by brief pulses of tastants, CHRISTIAN H. LEMON, CHRISTIAN REICH AND PATRICIA M. DI LORENZO, *Department of Psychology, State University of New York at Binghamton, Binghamton, NY 13902-6000.*

Previous studies of the physiology of cells in the nucleus of the solitary tract (NTS) have suggested that electrophysiological responses to taste stimuli are the result of complex interactions of excitation and inhibition. The specific role of inhibition in the elaboration of taste responses and the construction of response profiles of taste-responsive cells, however, is unknown. To study this issue, electrophysiologicial responses were recorded from single NTS units in urethane-anesthetized rats. Taste stimuli consisted of sapid solutions of NaCl (.1 M), HCl (.01 M), sucrose (.5 M), and quinine HCl (.01 M). Initially, each tastant was presented individually. Next, each tastant (test stimulus) was presented for 3 sec following a brief (100 msec to 1 sec) prepulse of one of the four taste stimuli. The interval between the prepulse and the test stimulus varied between 100 msec and 10 sec. Finally, the response to each test stimulus was recorded following adaptation of the tongue to each of the other tastants. Preliminary analyses suggest that brief pulses of taste stimuli can suppress subsequent taste responses such that the shorter the interstimulus interval the more profound the suppression. It is unlikely that this effect is due to mixture suppression because 1) the effect is not symmetrical with respect to the order of presentation of taste stimuli and 2) the response to the prepulse returns to baseline prior to the beginning of the response to the test stimulus. Further analyses will focus on the comparison of this effect with the effects of adaptation.

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Poster 96

Opioid modulation of gustatory responses in the solitary nucleus: Electrophysiological and immunhistochemical evidence. DAVID V. SMITH, CHENG-SHU LI, and BARRY J. DAVIS, *Dept. Anatomy & Neurobiology and Program in Neuroscience, Univ. Maryland School of Medicine, Baltimore, MD 21201.* dvsmith@umaryland.edu.

Previous experiments have demonstrated intrinsic met-enkephalin-expressing neurons within the gustatory portion of the nucleus of the solitary tract (NST) of the hamster. It has also been shown that systemic morphine administration inhibits gustatory responses in the rat parabrachial nuclei. These data suggest that opioids could play a modulatory role in taste information processing in the NST. In the present experiments, we recorded from taste-responsive cells in the hamster NST following met-enkephalin (ENK) administration and examined the distribution of both μ- and δ-opioid receptors immuno- histochemically. Single units in the hamster NST were recorded extracellularly and ENK was delivered into the vicinity of the recorded cell using a multibarrel pipette assembly. The tips of the injection pipettes were 120 μm dorsal to the tip of the recording electrode. Once a cell was identified as a taste-responsive neuron, its response profile was determined by stimulation of the tongue with 0.032 M sucrose, 0.032 M NaCl, 0.0032 M citric acid, and 0.032 M QHCl. About 30 nl of 0.1 mM, 10 mM or 50 mM ENK were pressure injected into the vicinity of the recorded cell while a repetitive anodal current (20 μA, 0.1 Hz) was applied to the anterior tongue. Preliminary findings show that the responses of 8 of 33 cells (24%) were inhibited by ENK in a dose-dependent fashion; 5 of these were N-best. Further experiments are underway to evaluate the role of ENK on the responses to chemical stimulation of the tongue. Immunohistochemical localization showed that both fibers and cells expressing the δ-opioid receptor were present in the rostral portion of the NST, coincident with the location of taste-responsive neurons. In addition, the rostral NST was heavily invested with fibers immunoreactive to the μ-opioid receptor antiserum. These data show that opioids play a role in modulating taste activity in the NST.

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Poster 97

Short-term synaptic plasticity in the primary gustatory nucleus in goldfish. C.A. SMERASKI[1], T.V. DUNWIDDIE[2], L.H. DIAO[2] AND T.E. FINGER[1], [1]*Department of Cellular and Structural Biology and* [2]*Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262,* cynthia.smeraski@uchsc.edu.

The gustatory portion of the nucleus of the solitary tract in mammals is equivalent to superficial cell layers in the vagal lobe of the goldfish. Electrical stimulation of the vagal nerve root evokes two negative postsynaptic population responses (N2 and N3) in an *in vitro* slice preparation of the lobe. Previous physiological studies suggest that both NMDA and non-NMDA (AMPA/kainate) receptors mediate evoked responses in the sensory layers. Under certain conditions repetitive stimulation of the nerve results in short term potentiation of these responses. These experiments were undertaken to test the relative contribution of NMDA and non-NMDA receptors in this facilitation.

Vagal lobe slices were superfused in oxygenated artificial CSF; fascicles of the vagus nerve (X) were stimulated every 60-90 s with a train of 5 pulses (3-20 V,15 ms apart) for 5-6 m periods using bipolar electrodes. Synaptic facilitation of the population responses depended upon the recording site and stimulus strength. The extracellular recording electrode was positioned within the sensory layers of the vagal lobe so as to maximize the amplitude of the second population response (N3) evoked from the second, third, or fourth pulse of a train. Unlike the N3 population response, the earlier N2 population response did not always exhibit facilitation following repeated stimulation. As reported previously, repeated stimulation can produce depression of the N2 response. Facilitation of the N3 response peak was dramatically reduced by application of the NMDA antagonist, APV (50-100 μM), indicating that NMDA receptors play a major role in facilitation. Repeated activation of non-NMDA receptors appears to facilitate the N3 synaptic response by removing the voltage-dependent Mg^{++} block of NMDA receptors. This also suggests that the NMDA and non-NMDA receptors are colocalized. Depression of the N2 response was not rescued by APV application. In paired-pulse paradigms (interstimulus intervals of 60 ms), APV also did not affect the presumed polysynaptic late potentials observed under GABA blockade by bicuculline (50 μM). NMDA receptors at the synapses of primary gustatory afferents may act to augment synaptic responses.

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Poster 98

Corticofugal modulation of taste responses in the hamster solitary nucleus: Inhibition by GABAergic mechanisms. CHENG-SHU LI and DAVID V. SMITH, *Dept. Anatomy & Neurobiology and Program in Neuroscience, Univ. Maryland School of Medicine, Baltimore, MD 21201.* cli@umaryland.edu.

The rostral portion of the nucleus of the solitary tract (NST) receives gustatory afferent fibers from the VIIth, IXth and Xth nerves and descending fibers from forebrain targets of the gustatory system, including the insular cortex. Previous studies have demonstrated that γ-aminobutyric acid (GABA) inhibits many taste-responsive cells within the NST, where 60% of the cells are maintained under tonic GABAergic inhibition. In the present study we investigated the effects of cortical stimulation on the responses of gustatory neurons in the NST. A three-barrel glass pipette assembly was used to record single-unit activity in response to gustatory stimulation and to apply the $GABA_A$ antagonist bicuculline methiodide (BICM) or physiological saline into the vicinity of an NST cell. Taste stimuli were 0.032 M sucrose (S), 0.032 M NaCl (N), 0.00032 M citric acid (H), and 0.032 M quinine hydrochloride (Q), presented to the anterior tongue. The responses of 50 NST cells were recorded extracellularly and each was classified as S-, N-, H-, or Q-best. The ipsilateral insular cortex was stimulated both electrically (0.5 mA, 100 Hz, 0.2 ms) and chemically (10 mM DL-homocysteic acid) while the spontaneous activity of each cell was recorded. The activity of 34% of the cells ($n = 17$) was modulated by cortical stimulation: 8 cells were inhibited and 9 were excited. The effects of cortical stimulation were tested in the presence of BICM microinjected into the vicinity of the NST neuron, which blocked the cortical-induced inhibition but had no effect on the excitatory response. Microinjection of saline had no effect. Although the excitatory effects were distributed across S- ($n = 4$), N- ($n = 3$) and H-best ($n = 2$) neurons, the inhibitory effects of cortical stimulation were significantly more common ($n = 7$ of 8) in N-best cells ($X^2 = 5.956$, $df = 1$, $p < .05$). These data suggest that corticofugal input to the NST may differentially modulate gustatory afferent activity.

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Poster 99

Intravenous glucose infusions selectively suppress responses of sugar-sensitive taste cells in the rat NST. BARBARA K. GIZA, STUART A. MCCAUGHEY, COURTNEY L. SCOTT, LIN ZHANG, THOMAS R. SCOTT *Department of Psychology and Program in Neuroscience, University of Delaware, Newark, DE 19716.* FAX: 302-831-3645.

When a rat is administered an intravenous load of 0.5 g/kg glucose, multiunit responses of taste cells in the nucleus of the solitary tract (NST) decline sharply for 8-10 min, then recover gradually through the next hour and beyond. In parallel, the rat's perception of glucose intensity is reduced. The issue addressed here is which cells are responsible for this effect. We monitored the responses of 37 single neurons in the rat NST to four basic stimuli (1.00 M glucose, 0.10 M NaCl, 0.03 M HCl, 0.01 M QHCl) before and after iv glucose infusions. Across all cells, the decline to glucose as a taste stimulus during successive 20-min post-infusion periods was 19%, 34%, and 18%. In accord with the multiunit data, smaller declines were observed in responses to NaCl and HCl, while those to QHCl were unchanged. Cells could be divided into 3 subgroups according to their pre-infusion response profiles: sodium-, acid-, and sugar-oriented. The impact of the iv load on glucose responses was carried almost totally by neurons of the sugar subgroup. Mean discharge rate (spikes/s) to glucose among the 32 cells of the other groups declined from 9.6 to 9.5 (1%) in the first 20 min following the iv load, while that of the 5 cells in the sugar subtype decreased from 20.1 to 5.5 (73%). Responses to other taste stimuli in sugar-sensitive cells did not decrease significantly. Thus the effect of iv glucose was to reduce taste responsiveness primarily to glucose, and the effect was carried by the sugar subtype of neuron. These data offer support for a gustatory channel crucial to the afferent code for sugars.

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Poster 100

Early postnatal changes in the dendritic morphology of salt-sensitive neurons in the rodent nucleus of the solitary tract (NST). YU-ZHI LIU[1], JEFFERY MASSEY[1], LAURA SCHWEITZER[2] AND WILLIAM E. RENEHAN[1], [1]*Division of Gastroenterology, Henry Ford Health System, Detroit, MI and* [2]*Department of Anatomical Sci. and Neurobiol., University of Louisville School of Medicine.* wrenehan@ix.netcom.com. FAX:313-876-9487.

We demonstrated recently (Renehan et al., 1997, Dev. Brain Res. 102:231-246) that NST neurons that responded to NaCl, KCl and NH_4Cl in animals that were four weeks old (postnatal day [PND] 22-28) were larger and had a higher density of dendritic spines and swellings than adult neurons with similar response properties. We suggested that these results were consistent with data presented by Bao et al. (Dev. Brain Res., 1995, 86:143-154) and supported the hypothesis that some gustatory neurons exhibit a period of dendritic remodeling during postnatal life, though the precise timetable of this remodeling was not clear. The present study builds on these prior investigations by including neurons from animals that were three weeks old (PND 15-21). We found that the PND 15-21 neurons that responded to the three salts in our taste array (n = 8) had fewer dendritic branch points (13.9 ± 2.7 vs. 27.5 ± 3.8; t=1.9, d.f.=32, p<0.01), fewer dendritic branches (31.3 ± 5.6 vs. 59.1 ± 7.7; t=1.95, d.f.=32, p<0.01), a lesser density of dendritic spines (0.003 ± 0.001 vs. 0.009 ± 0.001 spines/micron; t=2.7, d.f.=32, p=0.01) and a lower density of dendritic swellings (0.03 ± 0.007 vs. 0.06 ± 0.005 swellings/micron; t=3.1, d.f.=32, p<0.01) than cells with similar response properties (n = 26) in PND 22-28 animals. These data indicate that the dendrites of salt-sensitive NST neurons become more'complex' between the third and fourth week of postnatal life. When viewed in the context of our previous work, this period of increasing dendritic complexity would appear to be followed by a period of dendritic remodeling that may serve to refine the neurons' response to selected tastants.

Supported by NIH grant DC 01074.

Poster 101

Immunohistochemical localization of $GABA_A$ receptors in the rostral nucleus of the solitary tract of the adult rat. WENDY L. HECK[1], WILLIAM E. RENEHAN[2], and LAURA SCHWEITZER[1], [1]*Dept. of Anat. Sci. and Neurobiol., University of Louisville School of Medicine, Louisville, KY 40292,* [2]*Henry Ford Hospital, Detroit, MI 48202,* wlheckz1@ulkyvm.louisville.edu.

Neurons of the rostral nucleus of the solitary tract (rNST) receive primary afferent taste fibers from branches of cranial nerves VII, IX, and X, and thus form an essential relay in the processing of gustatory information. Previous immunohistochemical studies have demonstrated that neurons within rNST contain the inhibitory neurotransmitter GABA. Electrophysiological studies have shown that GABA receptors in rNST influence the response properties of essentially every cell. This suggests the importance of the inhibitory network in rNST mediating gustatory processing. In this study, the expression and cellular distribution of $GABA_A$ receptors, which are members of the ligand-gated ion channel family that mediate rapid inhibitory chloride currents, was determined in the rNST of the adult rat through the use of immunohistochemistry. The location of immunohistochemical label in the rNST was confirmed using both a myelin and Nissl stain. A monoclonal antibody raised against the β-chain (clone bd 17) of a purified $GABA_A$/benzodiazepine complex was used for the immune reaction. There was both diffuse staining throughout the neuropil of the rNST and patterns of more discrete staining in the nucleus. Linear arrays of label appeared to be associated with dendrites. Somatic immunoreactivity was also present and was predominantly concentrated in the small neurons (20-112 μm^2) of the rostral central subdivision of rNST. The labeling of large neurons

(113-301 μm^2) within this subdivision was more infrequent. Other subdivisions of the rNST had far fewer labeled cell somata. These results suggest that GABA-evoked inhibition in rNST, mediated by the $GABA_A$ receptor subtype, is important to gustatory processing. Our next goal is to determine the distribution of $GABA_B$ receptors and to investigate developmental expression of both receptor types.

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Poster 102

Changes in the distribution of GABA in the developing rat rNST. M. BROWN[1], J. PENG[1], W.E. RENEHAN[2]and, L. SCHWEITZER[1] [1]*Univ. of Louisville School of Medicine, Louisville, KY 40292,* [2]*Henry Ford Hosp., Detroit, MI 48202,* mebrow01@homer.louisville.edu.

GABA (γ-aminobutyric acid) is an inhibitory transmitter in the rNST of the adult rat. However, recent studies of postnatal rats have shown GABA to have a depolarizing, or excitatory effect on some neurons of the developing rNST (Kim et al., AChemS Abst., 1997). During development, GABA has been shown to have a morphogenic role in nerve growth and synapse formation in other brain regions and may serve this role in the rNST as well. Our study investigates the presence of GABA in the developing rNST of the rat. Postnatal day (PND) 1, 5, 10, 15 and 20 and adult (>60) Vibratome-sectioned rat brainstems were embedded in LX 112 and resectioned into 1 μm thick sections, deplasticized and processed using post-embedment immuno-histochemical techniques to visualize GABA using a light microscope. In the adult, somatic staining was observed. Immunoreactive axons and puncta were also seen throughout the neuropil. Labeled puncta were commonly observed apposing unlabeled dendrites, however, very few puncta were observed on labeled or unlabeled somata. In the neonate the amount and distribution of immunoreactivity is totally different. A greater percentage of somata (over 50%) and a higher density of puncta are labeled. Somata at this age are commonly observed to be studded with surrounding GABAergic puncta. This immature pattern of increased label declines somewhat by PND 15, but labeling around somata is still apparent at this age. By PND 20, however, the adult pattern is well established. We have completed a study of the synaptic arrangements of GABAergic terminals in the adult rNST and next plan an electron microscopic developmental study. Preliminary evidence suggests that at PND 20 synaptic types, distributions and post-synaptic targets are like those seen in the adult. In view of the changing physiology and potential developmental role of GABA we expect to see novel synaptic arrangements in the younger animals.

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Poster 103

Functional properties of neurons of the nucleus of the solitary tract following chorda tympani nerve crush and regeneration. MICHAEL A. BARRY, LAWRENCE D. SAVOY *Dept. BioStructure and Function, School of Dental Medicine, Univ. of Conn. Health Center, Farmington, CT 06030-3705.* barry@neuron.uchc.edu

The recovery of the gustatory system is being investigated following severe nerve crush and regeneration of the chorda tympani nerve (CT) in the golden Syrian hamster. The approximately 40% of myelinated CT fibers that regenerate after this procedure are capable of mediating behavioral recovery. At 8 weeks following nerve crush, the regenerated CT whole nerve response is weaker than normal, and in some cases responses to sucrose and water rinses are atypical (Cain et al., 1996). This study investigated the functional properties of single units in the nucleus of the solitary tract (NST) 8 weeks following CT nerve crush and compared them to normal controls. Single NST units were recorded in urethane-anesthetized hamsters, during stimulation of the fungiform taste field with the following solutions: 0.03 & 0.1 M Na acetate, 0.1 & 0.3 M sucrose, 0.1 & 0.3 M KCl, 0.003 M saccharin (hemicalcium salt), a search solution containing the high concentrations of the preceding 4 stimuli, 0.01 M HCl, and the high concentrations of all stimuli mixed with 10 μm amiloride. To date, our analysis of the data suggests that NST units exhibited a full normal range of responsiveness to taste stimuli, following CT regeneration. Some units mirrored the 3 unit types found in the CT nerve (sucrose/saccharin, Na, or KCl/HCl best; Frank et al. 1988), or responded to combinations of these sensitivities. Discovery of all types of functional response is consistent with behavioral recovery. Analyses will examine the hypotheses that NST units show less taste evoked activity, and are less specific than normal following CT regeneration. Less activity is expected because of the reduced CT input, which could also affect relative specificity.

Supported by NIH grant DC00168-16.

Poster 104

The central gustatory pathway: Calbindin-D28k immunoreactivity in the gustatory regions of the nucleus of the solitary tract and parabrachial nuclei in the hamster. BARRY J. DAVIS[1] and JOYDEEP SOM[2], [1]*Dept. Anatomy & Neurobiology and Program in Neuroscience, and* [2]*Div. Otolaryngology: Head & Neck Surgery, Univ. Maryland Sch. Medicine, Baltimore, MD 21201.* bdavis@umaryland.edu.

A potpourri of neurotransmitter candidates have been identified in the gustatory relay nuclei of the brainstem. GABA, substance P, and enkephalin influence the responses of taste neurons in the nucleus of the solitary tract (NST). Such studies have been instrumental in developing a functional circuit diagram of the gustatory NST. We now report that the number of calbindin-D28k (CALB) immunoreactive neurons in the NST and parabrachial nuclei (PbN) greatly exceeds any previously reported specific cell marker. CALB's presence in the central gustatory pathway may be related to Ca^{++} currents and activity driven via glutamatergic gustatory fibers. In the NST, intermingled populations of small and large (mean=122 μm^2; range: 64-183 μm^2) immunoreactive ovoid, elongated and multipolar somata are detected, suggesting several classes of neurons express CALB. Dendritic processes are also well-defined. Unilateral transections of the chorda tympani / lingual nerve produced a 20.4% decrease in the number of CALB-immunoreactive somata ipsilaterally in the NST after a 21-day survival period. The combined transection of the chorda / lingual and glossopharyngeal nerves produced a 29.3% reduction. The gustatory nuclei of the PbN also contain numerous immunoreactive neurons, varying in both somal size (range: 63-332 μm^2) and morphology. The medial nucleus is defined by intensely immunoreactive fibers and terminals, presumably arising from immunoreactive projection neurons of the rostral NST. These findings demonstrate that CALB is associated with heterogeneous populations of neurons involved in the processing of gustatory information. The transection data suggest that CALB expression may be activity-dependent and modulated by gustatory input.

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Poster 105

Double labeling reveals that a subpopulation of rat rostral NST neurons that project to the medial PBN express glutamate immunoreactivity. M.S. KING, J.M. MADDEN and M. KORNBERG, *Biology Dept., Stetson Univ., DeLand, FL 32720.* mking@stetson.edu.

Since the projection from the caudal nucleus of the solitary tract (NST) to the pons may rely upon glutaminergic mechanisms (Jhamandas and Harris, Am. J. Physiol., 263:R324, 1992), it is hypothesized that rostral NST neurons that project to the medial parabrachial nucleus (mPBN) use glutamate as a neurotransmitter. The current study used immunohistochemical and fluorescent tract tracing techniques to determine the pattern of glutamate labeling in the rNST, and if rNST neurons that project to the mPBN express glutamate immunoreactivity.

Red fluorescent microspheres (0.4 μl, Molecular Probes) were stereotaxically injected into the right mPBN in 4 adult male Wistar rats (Hilltop Labs). After a two week transport period, colchicine (100 μg/1 μl) was injected into the fourth ventricle and, the following day, the rats were perfused with 4% paraformaldehyde. Forty micron thick coronal sections were cut and alternate sections were immunohistochemically labeled for glutamate (Chemicon, AB133) using standard immunoperoxidase procedures. Both the immunoperoxidase and fluorescent labels were visualized in the same sections under different light conditions. Labeled neurons were located and counted using a Zeiss Image analysis system.

Glutamate immunoreactive cell bodies, fibers and puncta were located throughout the rNST. Glutamate labeling was not concentrated in any subdivision or rostral/caudal location of the nucleus. Eighty six percent of the retrogradely labeled neurons were located ipsilateral to the injection site, with the majority (61%) of these found within the central subdivision of the rNST. Fourteen percent of the pontine projection neurons were located in the left rNST, contralateral to the injection site. A total of 312 double labeled neurons were identified. Most (68%) of these cells were in the ipsilateral rostral central subdivision. The 312 neurons represent 20% of the retrogradely labeled rNST neurons and about 5% of the glutamate positive perikarya. These data indicate that glutamate immunoreactive neurons are prevalent throughout the rat rNST and that about 20% of the neurons that project to the mPBN are glutamate positive. This suggests that glutamate may be one of the neurotransmitters used to transmit information from the rNST to the mPBN.

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Poster 106

Latency to discriminate between binary mixtures: Prediction of discriminability from an odor space. PAUL M. WISE and WILLIAM S. CAIN, *Dept. of Surgery, University of California at San Diego, La Jolla, CA 92039-0957.* FAX: 619-458-9417.

Subjects make slower same-different judgments to pairs of odors with the same quality (i.e., A vs. A) than to pairs with different qualities (i.e., A vs. B). The distribution of latencies to make A vs. A judgments compared to the distribution of latencies to make A vs. B judgments appears to afford an adequate measure of qualitative similarity using the metric d'. Application to mixtures has given the reasonable conclusion that the odor quality of binary combinations of intensity matched chemicals lies essentially midway between the qualities of the components. To go beyond such demonstrations of descriptive (face) validity to predictive validity, we used a multidimensional odor space based on d's between unmixed components to predict the relationship of one binary mixture to another. Subjects participated in 8 sessions each in which they responded as quickly as possible whether or not successively presented binary mixtures differed in quality. The multidimensional odor space predicted relative discriminability very well, while absolute values implied certain methodological features of the discrimination experiment require further understanding. Corrective feedback is one such feature.

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Poster 107

Reduced habituation is achieved with a ten minute inter-stimulus interval in the olfactory event-related potential. SPENCER WETTER[1] AND CLAIRE MURPHY[1,2], *[1]SDSU Department of Psychology, 6363 Alvarado Ct., Ste. 101, San Diego, CA 92120-4913; [2]UCSD Medical Center, San Diego, CA 92103.* FAX:619-594-3773.

Recent research on the olfactory event-related potential (OERP) using a 60 second inter-stimulus interval (ISI) has revealed a significant decrease in component amplitude after the first trial, with a leveling off for the remaining trials. Studies which manipulate the ISI in olfactory and other modalities demonstrate an association between higher amplitudes and longer ISIs, suggesting that habituation occurs at short time intervals between each stimulus presentation. Habituation produces a decrease in the perceived intensity of the odor and a resulting lowering of OERP component amplitudes. The present study attempted to reduce the effects of habituation by using a ten minute ISI and fewer trials. The olfactory stimulus, amyl acetate, was presented by an olfactometer which has been previously described (Murphy, et al., Chemical Senses, 19, 1994). Airflow, temperature, and humidity were kept constant. Velopharyngeal closure was employed to maintain a consistent airflow inside the nose. OERPs were recorded monopolarly at the Fz, Cz, and Pz electrode sites in ten subjects (five males, five females), for three trials. Brain potentials were amplified and digitized, and a grand average was created for all subjects at each trial. Repeated measures ANOVAs demonstrated no significant reduction in component amplitudes across trials and no significant difference in latencies over trials, indicating no habituation effect at this ISI. In fact, for the second and third trials, the data were in the direction of higher amplitudes, although in this sample size, the effect was not statistically significant. Such an amplitude increase in later trials might suggest that the first presentation may be preparing the brain to better attend to the stimulus. These results indicate that with a ten minute ISI, a complete reduction in habituation can be achieved.

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We wish to acknowledge the helpful comments of John M. Polich

Poster 108

Semantic-free sorting of odor qualities as perceived by pemenone-osmic and allosmic subjects. DAVID A. STEVENS[1], ROBERT J. O'CONNELL[1,2], *[1]Dept. of Psychology, Clark University, Worcester, MA 01610, [2] Dept. of Physiology, University of Massachusetts Medical Center,, Worcester, MA 01655.* dstevens@clarku.edu.

Our current work continues to explore individual differences in odor qualities among subjects grouped by their perception of the modally putrid odorant, pemenone (PEM). Characteristic differences were noted in an earlier study which included a group of putrid odorants. Here we examined a large group of pleasant odors and asked if individual differences in their odor qualities would continue to be related to PEM osmicity. A non-verbal, semantic-free sorting method was used by undergraduate volunteers who sniffed odors from individual tubes, each containing neat samples of one of 18 odorants or a blank. The odorants had modally floral or fruity, woody or spicy, and vegetable-smelling odors. Subjects independently sorted all of the odorants into groups such that each member of a group had the same or a very similar odor quality. The subjects were allowed to form as many groups as they wished. They then sniffed a tube containing 390 μM PEM, rated its intensity on a 9-point scale, and reported its quality. The subjects were then classified as osmic (N=8) if the quality report was putrid (urine-like, sweaty), allosmic (N=16) if it was non-putrid, and anosmic (N=12) if no odor was detected. The frequencies with which each of the different odorants was paired with the others were then used as data for independent multidimensional scaling by MINISSA for each class of subject. The coordinates of the individual three-dimensional solutions were then used as input for joining cluster analyses by STATISTICA®. Although the 3 groups of subjects produced spaces with similar dimensionality, a number of differences were apparent. Perception of phenyl ethyl alcohol (PEA) in particular is different for the 3 PEM osmicity groups. PEA is grouped with florals among PEM osmics, shifts to the woody cluster in allosmics, and is isolated with a fruity odorant by anosmics. Thus, the individual differences characteristic of PEM osmic, allosmic, and anosmic subjects are also apparent when modally pleasant odorants are characterized.

Poster 109

Effect of nasal dilators on olfactory function. D.J. SMITH[1,2], D.E. HORNUNG[1,2], D.B. KURTZ[1], T.L. WHITE[1]. *[1]Clinical Olfactory Research Center at the SUNY Health Science Center, Syracuse, NY., [2]St. Lawrence University, Canton, NY.*

Previous work has demonstrated that subjects wearing nasal dilators rated olfactory stimuli as being more intense as compared with ratings done without nasal expansion. Since preliminary data suggested the presence of a nasal dilator did not influence sniff flow rate, volume, or duration, the increase in intensity ratings was hypothesized to support a perceptual constancy model in olfaction (Chemical Senses 22:177-180, 1997). To test this hypothesis, we compared results on threshold and identification tasks performed with and without nasal dilators. When wearing nasal dilators, subjects had lower butanol thresholds and were able to identify more odorants on a low concentration version of the Odorant Confusion Matrix. As a further test of the hypothesis, we used a pneumotacograph to record sniff flow rate, volume, and duration during the course of detection, intensity rating, and identification tasks. Compared to the undilated controls, all sniff parameters increased when subjects were wearing dilators. These results argue strongly that nasal dilators affect olfactory ability by increasing the number of odorant molecules available to olfactory receptors. Although the present results do not negate the applicability of a size constancy model in olfaction, they do raise the possibility that at least part of the explanation of the perceptual constancy model may be a change in the regional airflow.

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Poster 110

Effect of nasal dilators on olfactory processing: Threshold, intensity, hedonics and sniff patterns. BRYAN RAUDENBUSH and ROBERT A. FRANK, *Department of Psychology, University of Cincinnati, Cincinnati, OH 45221*, raudenbc@email.uc.edu.

The present study assessed the effect of nasal dilators on olfactory thresholds, stimulus intensity and pleasantness ratings, and sniffing patterns. Participants (N=150) completed an odorant detection task, and rated the intensity and pleasantness both with and without Breathe-rite® nasal strips. Changes in air pressure associated with sniffing behavior were measured during the completion of the stimulus rating tasks. Odor detection thresholds for phenylethylaldehyde (PEA) were assessed using a variation of the method of limits. Participants rated the intensity and pleasantness of 15 olfactory stimuli using category rating scales. The nasal dilators significantly decreased odorant detection thresholds. This effect lasts for some time after the strip was removed. While wearing the strips, participants rated the olfactory stimuli as more intense and less pleasant, as opposed to when they were not wearing the strip. They also had lower sniff magnitudes when wearing the strip. The present study demonstrates that nasal dilators can affect perception of odors, and alter the dynamics of sniffing behavior. These findings replicate those reported by Hornung et al. (1997) at ISOT/AChemS last year.

This research was made possible by a grant from CNS, Inc. to the authors.

Poster 111

Odor sensitivity and successful aging. STEVEN NORDIN[1,2], OVE ALMKVIST[3], BIRGITTA BERGLUND[2,4], *[1]Department of Psychology, Umeå University, SE-901 87 Umeå, Sweden, [2]Institute of Environmental Medicine, Karolinska Institute, SE-171 77, Solna, Sweden, [3]Department of Clinical Neuroscience and Family Medicine, Division of Geriatric Medicine, Karolinska Institute, Huddinge University Hospital, SE-141 86 Huddinge, Sweden, [4]Department of Psychology, Stockholm University, SE-106 91 Stockholm, Sweden.* steven.nordin@psy.umu.se.

Previous research suggests large variability in odor sensitivity in the elderly population, and that whereas some elderly, compared to young, demonstrate poor sensitivity, others do in fact show normality. It has previously been suggested that screening for dementia may contribute to smaller age-related impairment in odor sensitivity than that found in the general literature. To shed more light on this issue as well as on the possibility that general medical-health status may be related to odor sensitivity in the elderly, absolute thresholds for pyridine were determined by the method of constant stimuli by means of a dynamic olfactometer. This was conducted in 16 elderly (77Ð87 years) who were successfully aged with respect to medical health (according to neurological examination, blood and serum tests, urinalysis, ECG, EEG, chest radiography, and MRI of brain tissue; none received medication, had a history of brain trauma, brain disease, psychiatric disease, arteriosclerosis, or a family history of dementia) and cognitive ability (according to various neuropsychological tests of general intelligence, verbal ability, visuospatial function, memory, and attention), and in 16 young adults (20Ð25 years) matched for gender and smoking habits.

The results showed that the elderly (105 ppb pyridine) were very similar to the young adults (100 ppb) in their odor thresholds and in their slope of the psychometric detection function. These findings imply that aging, per se, may not necessarily generate any substantial decline in odor detectability, and that previously reported age-related impairment may be due to factors secondary to aging, including general medical-health status and cognitive ability.

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Poster 112

Influence of estradienol and methoxyestateraene on the assessment of the opposite sex, REGINA E. MAIWORM *Department of Psychology II, University of Münster, Fliednerstr. 21, 48149 Münster.*

In a double blind study estradienol, methoxyestateraene or the controls cholesterol or mineral oil were applied to the upper lip of 240 men and women. Before the application a standardized test about the mood was administered. After the application 5 persons (standardized whole body photographs) of the opposite sex were rated on 19 bipolar, seven stepped ratingscales. Each subject rated the 5 persons one by one. After the rating the test about the mood was conducted again. The session was finished by a structured interview. Each subject was tested individually by a same sex experimenter. Physiological parameters (respiration, heart rate, skin conductance) were measured during each session. Under estradienol the men were rated by women as better, harder and more nonchalant than under control. Under the influence of methoxyestateraene the women were rated by men as better, kinder and they were much more liked than under control.

Poster 113

Behavioral effect of androsta-4,16-dien-3-one (androstadienone). LOUIS MONTI-BLOCH[1,2], BERNARD I. GROSSER[1], CLIVE JENNINGS-WHITE[2] AND DAVID L. BERLINER[2]. *[1]Dept. of Psychiatry, Univ. of Utah Salt Lake City, UT 84108, and [2]Pherin Pharmaceuticals , Menlo Park, CA 94025.*

Androstadienone, which occurs naturally in the human skin, produces local stimulation of the female human vomeronasal organ (VNO). Forty clinically normal female human volunteers participated in a double blind randomized parallel study to determine the biological effect resulting from local delivery of this substance to the VNO. A discrete vapor pulse of androstadienone was applied to the right VNO of twenty subjects using a multifunctional miniprobe, with scavenging to prevent contamination of the olfactory system or respiratory system. The other twenty subjects received vapors of control (propylene glycol). All subjects were given a psychometric test before, and 30 minutes after, VNO stimulation. Cardiac frequency, respiratory frequency, electrodermal activity, body temperature and electroencephalograms were also recorded during the experiment. Androstadienone significantly ($p<0.01$) reduced negative affect, while increasing relaxation, well being, and contentment. Concomitant with the psychological changes there was significant reduction of respiratory, and cardiac frequency, decreased skin conductance, increased body temperature, and increased alpha brain-waves. In the control group there were no statistically significant psychological or autonomic nervous system changes.

We conclude that local androstadienone stimulation of the female human VNO, without subjective olfactory sensation, induced behavioral and autonomic nervous system changes that persisted for 30 minutes. Thus, there would appear to be no criteria by which to exclude androstadienone from being accepted as a human pheromone.

Poster 114

Effects of *l*-menthol on sensory ratings and breathing parameters in humans. MARTIN KENDAL-REED[1], JAMES C. WALKER[2] & DONALD W. WARREN[1], *University of North Carolina, CB# 7450, Chapel Hill, NC 27599* [1], *R&D, R.J. Reynolds Tobacco Co., Winston-Salem, NC 27102* [2]. kendalr@email.unc.edu.

Based on evidence that *l*-menthol is an especially potent stimulus for both olfactory receptors and trigeminal thermoreceptors, we measured perceptual and breathing responses of 8 normosmics and 2 anosmics to clean air and a range of concentrations of *l*-menthol: 0.0027, 0.014, 0.069, 0.34, 1.73 and 8.69 ppm. We employed a repeated-measures paradigm, using a computerized air-dilution olfactometer equipped with a pneumotachograph to continuously measure respiration (Kendal-Reed *et al.*, 1998). For normosmics, perceived odor, cooling and nasal irritation were first elevated above control values at 0.069 ppm and rose with progressively higher concentrations. Increases in perceived nasal patency first appeared at 0.34 ppm and were much less than those seen with anosmics. Absence of the olfactory nerve also greatly diminished, but did not eliminate, odor perception and decreased (by ~ 25-fold) sensitivity to the cooling and nasal irritation of *l*-menthol. In contrast to these perceptual results, breathing changes were much greater in the anosmics. Above 0.014 ppm, anosmics exhibited a rise and subsequent fall in inhalation volume and duration as concentration was increased, and both exhalation volume and duration fell monotonically; declines for the highest concentration were 30-50%. With normosmics, some effect on breathing parameters was seen above 0.014 ppm, but the degree of change at each concentration was less than that seen with anosmics. We conclude that: 1. Since olfactory activation may augment or antagonize perceptual and breathing effects of trigeminal stimulation with *l*-menthol, determinations of relative olfactory vs. trigeminal sensitivity will depend on the endpoint(s) used. 2. Breathing changes in anosmic participants are consistent with a trigeminal contribution, in normosmics, to perceived cooling and nasal irritation (and possibly odor) in response to concentrations not perceived by anosmics.

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Poster 115

Do men and women respond differently to repeated olfactory or intranasal trigeminal stimuli? [1,3]THOMAS HUMMEL, [1]ARIEL SOIFFER, [1,2]OLIVER OPATZ, [1]RICHARD L. DOTY [1]*Smell and Taste Center, University of Pennsylvania Medical School, Philadelphia, USA;* [2]*University of Erlangen Medical School, Germany;* [3]*Department of ORL, University of Dresden, Germany.* hummeltc@compuserve.com

Sex differences in olfactory sensitivity have been reported since the late 1800's. Women often outperform men on tests of odor identification, detection and discrimination. Whether women adapt differently to odorous stimuli than men is not known.

Seventeen healthy volunteers participated (9 female, 8 male, mean age 22 years). As established by an odor identification test (UPSIT, score ³38) all subjects had normal olfactory function. ERPs were recorded in response to olfactory (25% v/v phenyl ethyl alcohol, PEA) and trigeminal (44 % v/v CO2) stimuli presented to the left or right side (flow 8 L/min; stimulus duration 200 ms). Stimuli were applied at 4 different intervals (5, 10, 20, and 60s). Amplitudes and latencies of ERP peaks P1, N1, P2, and P3 were measured. Using visual analogue scales subjects also rated stimulus intensity.

When compared to PEA, the slightly more intense CO2 produced larger amplitudes (P1, N1, P3, N1P2, N1P3: $F[1,15]>4.93$, $p<0.043$) and shorter latencies (P1, N1, P2, P3: $F[1,15]>5.66$, $p<0.032$). Responses to the trigeminal and olfactory stimuli changed similarly in relation to repetitive stimulation (interaction "stimulant" by "interval"; amplitudes: $F[3,45]<1.14$, $p>0.344$; latencies: $F[3,45]<2.48$, $p>0.073$; ratings: $F[3,36]=0.86$, $p=0.47$). Both, ratings ($F[3,45]=5.76$, $p=0.003$) and ERP amplitudes P3 and N1P3 ($F[3,45]>7.71$, $p<0.001$) decreased with a shortening of the interval between stimuli. Women had larger ERP amplitudes P3, N1P2, and N1P3 ($F[1,15]>5.39$, $p<0.036$); they also tended to rate intensities higher than men ($F[1,12]=4.03$, $p=0.068$). However, no gender-related difference in relation to repeated stimulation was observed, as indicated by the missing interaction between factors "sex" and "interval" ($F[3,45]<2.38$, $p>0.08$).

These data indicate on a psychophysical and an electrophysiological level that there is no difference between young, healthy men and women in terms of short-term adaptation to suprathreshold chemosensory stimulation.

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Poster 116

Cross-cultural comparison of judgements of odours by Australians and Indonesians. HAE-JIN SONG and GRAHAM A. BELL, *Centre for ChemoSensory Research, The University of New South Wales, Australian Technology Park, Sydney, NSW 1430, Australia.* hj.song@unsw.edu.au.

Cross-cultural studies of odour judgements are important as perceptions of and preferences for certain odours may be culture-specific and underlie differences in food acceptance.

In this study, twenty Australian and twenty Indonesian subjects assessed 25 odour samples (8 typically 'Australian', 13 food and 4 non-food odour samples). Using graphic rating scales, each subject rated each odour sample for the following: overall intensity, overall liking, pungency, familiarity, memory (degree to which the odour evokes memories), feeling (degree to which the odour evokes pleasant feelings) and eating (degree to which the odour makes subjects feel like eating).

The results indicated that Australians liked 4 odours significantly more than Indonesians did. These odours were boronia, forest floor, smoke (3 typically 'Australian' odours) and curry. The Indonesian subjects did not like any of the odours significantly more than the Australians did. Indonesians perceived the intensity of 7 food odours (butter, celery, chilli, coriander, ginger, milk and soy sauce) significantly more strongly than Australians did.

Linear correlation revealed that all food odours used in this study had a significant positive correlation between liking and familiarity, supporting recent findings in other cultures that familiarity "drives" liking of food odours. Also, for all food odours, familiarity correlated with "memory image", "pleasant feeling" and "feel like eating". Familiarity correlated with intensity for 5 of the 25 odours (eucalyptus, celery, curry, milk and orange). Hence, in developing food products for "foreign" markets it may be advantageous to study responses of consumers to prototype food odours and pay particular attention to the degree of familiarity the target consumer has for the odour.

We acknowledge support from Dr Sachiko Saito of the NIBHT, Japan, and the Dragoco Company, Australia.

Poster 117

Gustatory, olfactory and other drivers of preference for 55 samples of Australian food by adult Indonesians of varying age and socio-economic status. GRAHAM A. BELL, HAE-JIN SONG, ALEX LAC, and BEN HE, *Centre for ChemoSensory Research, The University of New South Wales, Sydney, Australia, 1430.* FAX: +61 2 9209 4081.

Indonesia, the world's fifth most populous country (201 Million), has a potential for consumption that makes it an attractive trading partner and importer of processed foods. However, very little is known in food exporting countries about consumer habits and the chemosensory determinants of acceptance of foods.

This study looked at attitudinal and behavioural issues and measured a number of dependent chemosensory variables, including PROP status and olfactory perception and preferences. The major part of the study was the sensory evaluation of 55 Australian foods, made by two panels drawn from two socio-economic strata of Indonesians in the Bandung region of Indonesia. In addition, four focus groups were held, from each socio-economic category and two age groups. A questionnaire on food usage and consumer behaviour was administered to all 100 participants.

The sensory drivers of food acceptance varied with type of food. For example, odour and appearance attributes were most important in determining acceptability of food pastes and sauces, while taste and after-taste were prominent determinants of acceptance in breads and bakery products. The important common determinant of acceptance in most of the 55 products was texture in the mouth. The optimum level of spiciness in a food, as judged by Indonesians, strongly depends on its balance with other tastes, particularly sweetness and sourness.

From the attitudinal data, it was found that taste, quality, freshness and nutrition were the most important drivers of purchase for processed food. Price was more important for younger consumers than for older consumers, while country of origin was more important for older subjects. Brand of, and familiarity with the product were more important for the upper socio-economic group than the lower.

Understanding the role of spice in the Indonesian palate is a challenge to food companies and an important subject for further sensory research.

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We thank Dr. Roestamsjah, and the Director and staff of the Research and Development Centre for Applied Chemistry, LIPI, Bandung, Indonesia.

Poster 118

Can an arousing dose of caffeine potentiate the effectiveness of an odor retrieval cue? RACHEL S. HERZ, *Monell Chemical Senses Center, Philadelphia, PA 19104*. herz@monell.org.

Previous research has shown that a distinctive ambient odor experienced during encoding and retrieval conditions can elevate recall for material learned during encoding. Additionally, anxiety experienced during encoding in the presence of an ambient odor was found to increase recall in the presence of that odor beyond the increase seen without the anxiety manipulation. Based on these findings, it was proposed that heightened emotional arousal experienced during the encoding of an odor with an event in memory will enhance the effectiveness of that odor as a memory cue. Recently, we found that a 5mg/kg dose of caffeine increases arousal but does not affect learning and/or memory. To test whether this dose of caffeine could potentiate an ambient odor as a retrieval cue the following experiment is currently in progress. Fifty subjects are randomly assigned to 5 experimental groups (5 males and 5 females per group). The independent variables are whether subjects receive caffeine or placebo at the encoding and retrieval sessions and whether an ambient odor is present or absent at both sessions. Subjects incidentally learn a list of 16 neutral nouns during encoding. The dependent measure is the number of words recall at the retrieval session one week later. All subjects are low-moderate (M=100 mg/kg caffeine /day) consumers of caffeine and are 24 hr caffeine deprived and 12 hr food deprived before each session. Based on previous findings, it is predicted that subjects who experience caffeine at encoding and either placebo or caffeine at retrieval and who have an ambient odor present at both sessions will recall the most words.

Supported by NIH grant R03 DA10953-01.

Poster 119

Association among perceived gender frequency and familiarity in odor recall. VICKI HARTWELL[1], RUDY QUINOES[1], ANNA BACON[L,2] AND CLAIRE MURPHY[L,2] *[1]San Diego State University, [2]University of California San Diego, CA 6363 Alvarado Court, St #101, San Diego, CA 92120* FAX: 619-594-3773

It is generally known that there is a strong relationship between ty of an odor and ones ability to recall on a recall task. Additionally, it has -been shown that women recall significantly more than men on an odor recall task. We hypothesized that rating of an odor as mascuhne or feminine would influence f ty. The focus of the current study is to assess the relationship between perceived gender of an odor and it's association with f ty. For this study, f ty was operationally defmed as the participants' rated familiarity with the odor stimulus. The components explored were the perceived frequency of use of the odor and perceived femininity or masculinity of the odor stimulus. Forty-eight participants (male, female, young and old) were administered an annotated version of the Cafifomia Odor Learning Test (COLT). Added measures for perceived gender of the odor, f ty with the odor and frequency of exposure to the odor were included. The COLT included measures for short and long delay recall of the stimuh. Perceived gender for odors was rated on a 100 mm Likert scale with 1 representing very masculine and 100 representing very feminine. Familiarity was self reported on a scale from I to 1 0 with I representing least familiar and IO most . Frequency was rated by the participant reporting how many times per month he/she might use the particular odor. Familiarity ratings were positively and significantly.correlated with correct recall responses "for all groups (male, female, young and old). Young and old women and young males showed significant correlations for frequency of exposure and familiarity. Interestingly, young males also showed significant correlation between perceived gender and frequency and between perceived gender and familiarity. These results raise interesting questions regarding the potential contributions of other variables to the ability to recall odors.

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Poster 120

The decline in odor recall and recognition memory ability begins as early as the 50s. MARIO F. DULAY[1] and CLAIRE MURPHY[1,2], *[1]Department of Psychology, San Diego State University, San Diego, CA 92120-4913, [2]University of California at San Diego Medical Center, San Diego, CA, 92108*. mdulay@sunstroke.sdsu.edu.

The ability to recall and recognize odors has been found to decline with age in old adults, while odor memory function in the middle decades of life has been relatively neglected. The present study measured odor memory ability in these middle decades. The goal was to track odor memory ability across the adult life-span to determine at what age odor memory performance starts to decline. Recall and recognition memory for odors were measured in a sample of healthy adults grouped by age decades; 20s, 30s, 40s, 50s, 60s, and 70s. Age differences in odor memory were investigated by using the California Odor Learning Test (COLT); an odor memory test modeled after the California Verbal Learning Test (CVLT). The COLT allows for the successive examination of immediate recall, short and long delay recall, recognition memory, learning strategies, intrusions, and false positives. All subjects were recruited from a pool of San Diego State University students or were participants in an ongoing study at the Life-span Human Senses Lab. Subjects were prescreened for dementia and nasal sinus disease. Results indicate a sharp decline in odor recall and recognition memory performance between the fourth and fifth decade. Significant age-related differences between the 20s/30s age-groups and 50s age-group were found (with the young outperforming the middle aged) for short and long-delay free odor recall performance and odor recognition memory performance. Furthermore, age-related decrement was found between the 20s and 50s age-groups for immediate recall and short and long-delay cued odor recall performance, and a significant increase in false positives and intrusions were also found between the two age-groups. These findings indicate that a significant reduction in odor memory performance begins as early as the fourth decade.

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Poster 121

The recollective experience of odor memory: A comparison to visual memory. MATS J. OLSSON, *Department of Psychology, Uppsala University, S-751 42 Uppsala, Sweden.* mats.olsson@psyk.uu.se.

In two experiments, common odors and two classes of visual stimuli, pictures of common objects and featureless free forms, were compared with regards to the recollective experience using two approaches: one tapping the participants recollection of presentation order of study items, and one directly assessing the participants state of awareness using the remember/know paradigm of Gardiner (1988). In a yes/no recognition experiment, participants decided in which of two consecutive study sessions the target stimuli had been encountered. Overall, recognition of pictures of common objects proved superior to both odors and free forms. Interestingly, correctly recognized pictures of common objects were proportionally more often assigned to the accurate study session than were correctly recognized odors. In a 2AFC recognition experiment, participants judged their state of awareness using three categories: "remember", "know", and "guess". Again, pictures of common objects were the stimulus type most accurately recognized. Pictures of common objects tended to result in larger proportions of remember responses in comparison to odors and free forms. Altogether, the experiments suggest that there is a greater awareness of recollection for pictures of common objects whereas recognition of odors and free forms is more based on feelings of familiarity.

Poster 122

Human psychophysical and neurophysiological measurements on ethanol. R. A. DE WIJK, W.S. CAIN, and G. PILLA-CAMINHA, *Dept. of Surgery (Otolaryngology), Univ. of California, San Diego, La Jolla, CA 92093-0957.* rdewijk@ucsd.edu.

Groups of normosmic subjects (n=15-22) and anosmic subjects (n=4) gave thresholds for the airborne chemosensory properties of ethanol. A new device designed to give thresholds of high reliability and environmental relevance served for control and delivery of the stimulus. The device, made of Teflon and glass, provided a 1.6-liter head space and guaranteed that all inhaled vapor went into the nostrils. The odor threshold measured via the device equaled 0.2 ppm, with a smaller than customary geometric standard deviation of 3.5. The threshold fell below that measured previously with squeeze-bottles by more than two orders of magnitude. Threshold for irritation in the anosmics equaled 3,300 ppm, about 40% of that measured previously. (These values lie below virtually all others for ethyl alcohol in the literature.) Anosmics could localize the nostril of presentation at virtually the same concentration they required for detection. Normosmics could localize at a concentration within 8% of that of anosmics. The geometric standard deviation for localization equaled an extremely low 1.6. Thresholds for eye irritation fell in the vicinity of those for nasal pungency. Measurements of the negative mucosal potential (NMP), a neurophysiological index of pungency, gave a stimulus-response function in a range compatible with the thresholds for pungency and localization. The function began to rise above baseline at concentrations in the vicinity of 3,000 ppm. It grew at about the 1.5 power of concentration, also consistent with exponents seen psychophysically for stimuli that produce sharp pungency. In summary, with attention to critical details of stimulation, thresholds for odor detection apparently go down, both in their means and variability. Under comparable conditions of stimulus control, however, thresholds for irritation vary far less than those for odor. Under the conditions studied here, anosmics showed no disadvantage relative to normosmics in perception of nasal pungency. Finally, the NMP behaved entirely as would be expected of a neurophysiological index of perceived pungency.

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Poster 123

A mass transport model of human olfactory adaptation. PAMELA DALTON And PETER W. SCHERER, *Monell Chemical Senses Center, Philadelphia, PA, 19104 and Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, 19104.* dalton@monell.org.

Occupational or repetitive environmental exposure can lead to extraordinarily persistent olfactory adaptation. Traditional approaches to understanding adaptation have focussed on the role of receptor and central olfactory processes, but it is not clear how these processes can explain very persistent adaptation. We have begun to examine the possible role of peri-receptor mechanisms in explaining long-term adaptation. Peri-receptor mechanisms of adaptation have been largely ignored, in part because of the complex problems of quantification in estimating the actual amounts of odorant available and its rate of uptake, and in modeling the physiological mechanisms that govern its transport, accumulation and clearance.

Recently, a physiological model of odorant transport, accumulation and clearance in the nasal/olfactory region has been developed that can relate the peri-receptor odorant transport events to the perceived response during olfactory adaptation. The model utilizes finite element calculations (Keyhani et al, 1997, *J. Theor. Biol.* 186, 279-301) to describe the flux of odorant onto the olfactory epithelium and assumes that accumulation of odorant within this region is a necessary condition for the development of adaptation. Within this multi-layer region, consisting of the air phase, the mucus phase and the tissue/blood phase, physiological events such as the rate of mucus flow, blood flow and air flow are important parameters of the model. This paper outlines an application of the model to explain two general findings from recent studies of long-term olfactory adaptation, namely, the persistence of a decrease in the initial intensity of an odor and the increased rate of re-adaptation upon subsequent exposures.

Poster 124

Trigeminal impact of odorants assessed with lateralized stimulation. [1,2]JULIA BERG, [1,3]THOMAS HUMMEL, [1]GRACE HUANG, [1]RICHARD L. DOTY *[1]Smell and Taste Center, University of Pennsylvania Medical School, Philadelphia, USA; [2]University of Erlangen Medical School, Germany; [3]Department of ORL, University of Dresden, Germany.* hummeltc@compuserve.com

When lateralized intranasal stimulation is performed, identification of the stimulated nostril is believed to be due to trigeminal chemoreception (Kobal, Van Toller, Hummel, 1989, Experientia 45:130). It is, however, unclear, whether the degree of a chemical's ability to stimulate CNV can be discerned from the proportion of trials on which it can be correctly lateralized. The present study compared the ability of healthy subjects to localize odors with data obtained in a previous study; there, anosmics and healthy subjects ("trigeminal focus group") rated the presence of trigeminal activation (Doty, 1978, Physiol Behav 20: 175). In addition, we investigated odor localization in relation to stimulus intensity, and gender.

Forty volunteers (20 f, 20 m; mean age 24 [range 18-44] years) participated in 4 sessions separated by at least one day. During each session 2 of 8 odorants were tested (vanillin, phenyl ethyl alcohol, geraniol, limonene, methyl salicylate, anethole, linalool, and menthol). The sequence of testing the 8 odorants was randomized across subjects. Each odor was presented 40 times at an interval of 30 s (20 times to the left and right, randomized sequence). Mounted in a hand-held squeezer air from two polyethylene bottles were applied to the left and right nostril of the blindfolded subjects. One bottle contained 20 mL of odorant, the other one was empty. In 20 subjects (10 f, 10 m) 11.1 mL of odorized air were presented to each nostril, the other 20 always received 21.1 mL.

Except for vanillin, geraniol, and methylsalicylate all odors could be localized to a certain degree. The subjects overall ability to localize odorants correlated with odor detection in anosmics (r8=.74, p=0.035) and data from the "trigeminal focus group" (r8=.85, p=0.007). A higher degree of localization was found when larger volumes of odorized air were presented (which also produced higher intensity ratings). In addition, the experiment revealed that women localize odors to a higher degree than men. The results suggest that the degree of trigeminal activation can be quantified by odor localization of the stimulated nostril

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Poster 125

Eyeblink classical conditioning to olfactory stimuli. ANNA W. BACON[1,2,3] and CLAIRE MURPHY[2,3] *[1] SDSU/ UCSD Joint Doctoral Program in Clinical Psychology, San Diego, CA 91210, [2] SDSU, Department of Psychology , [3] UCSD, School of Medicine.* FAX: (619)594-3773

Eyeblink classical conditioning (EBCC) to auditory stimuli has been extensively studied in both animals and humans. This is a simple paradigm in which a puff of air to the eye (called an unconditioned stimulus or US) is paired with a stimulus that, in and of itself, does not generate an eyeblink (called an conditioned stimulus, or CS). The CS and US are paired until the participant blinks in response to the US. The precise timing of the CS and US presentations, in milliseconds, are critical elements in achieving conditioning. Given this need for precision, the possibility of eyeblink conditioning to odors as the US has yet to be investigated in humans. However, the recent development of olfactometers based on previous models of Kobal (1991), provides a unique and exciting opportunity to adequately control precise presentation of an odorant stimulus. The goal of this study was to investigate the possibility of eyeblink classical conditioning to olfactory stimuli in a group of healthy adults ranging in age from 23 to 60 years. A conditioned response was defined as any eyeblink occurring 150 ms after onset of the odorant (CS) and prior to onset of the air puff (US). Participants generated more CRs after 10 trials pairing the CS and US than in the first trial, indicating that conditioning was achieved. Results of this novel study successfully demonstrate that humans can be conditioned to olfactory stimuli in an EBCC paradigm. EBCC to olfactory stimuli may prove to be a useful tool in further understanding the neural substrates of the olfactory pathway, central associative processes, and the relationship between the olfactory system and the central associative processes.

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We thank Charlie D. Morgan for his invaluable technical assistance and Robert E. Clark, Ph.D. for his assistance with methodological development and statistical analysis.

Poster **126**

The effects of aging on the brain's response to natural gas odor: Diminished sensitivity and slower cognitive processing. MICHAEL D. MADOWITZ, MARK W. GEISLER, *San Diego State Univ. and UCSD, 6363 Alvarado Ct., Suite 101, San Diego, CA 92120-4913.* FAX:(619) 594-3773.

Every year hundreds of people are injured or killed due to accidental ignition of natural or liquefied petroleum (LP) gas. A disproportionate number of these victims are elderly, and are unaware of possible danger. Natural gas and LP gas are odorless. For most people the inclusion of a strong odorant provides a sufficient warning of impending threat. As demonstrated by Chalke and Dewhurst (1957), gas odor can be difficult for the elderly to detect, leaving them more prone to injury or fatality due to gas exposure or ignition. However, little is known about cognitive processing of gas odor. Only in recent years has the technology become available to investigate the cognitive processing of olfactory information in the brain using olfactory event-related potentials. Event-related potentials were used in the study of gas odor perception and processing. Hypotheses were: 1) The age of the participants would be directly reflected through differences in N1/P2 amplitudes when stimulated with the ethyl mercaptan odor. 2) The elderly would demonstrate slower cognitive processing of the ethyl mercaptan odor as indicated by longer P3 latencies than those of the youn g.

Brain activity was recorded from Fz,Cz, and Pz in 8 young (mean age 26) and 8 elderly persons (mean age 73), screened for anosmia using the Alcohol Sniff Test and for dementia using the Mini-Mental State Exam. Participants with a history of sinus disease, allergies, or head trauma were excluded. The stimulus, ethyl mercaptan, the odorant most commonly associated with gas odor, was presented using an olfactometer, based on a previous model (Kobal and Hummel, 1988). Subjects were asked to breathe normally, but through the mouth (to maintain odor concentration), to perform a tracking task between trials (to block the production of alpha waves), and to give magnitude estimates of intensity (to produce a cognitive component which manifests itself as heightened activity in the P3 region [Polich, et al, 1994]). Brain activity was amplified, filtered and digitized. Sixteen trials from each participant were averaged. Amplitude and latency for N1/P2, N2 and P3 were subjected to ANOVA. Sensory components (N1/P2) showed a significant decline in amplitude which corresponded to age. In addition, significantly increased P3 latencies in the elderly suggest that even when an older person is able to detect gas odor, he/she is slower to cognitively process that odor. The results pose interesting questions about the safety of our current warning systems for natural and LP gas.

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Poster **127**

Chemosensory function in patients with temporal lobe epilepsy before and after focus resection BIRGIT KETTENMANN[1,2], PARVANEH MOHAMMADIAN[1], ELISABETH PAULI[2], HERMANN STEFAN[2], GERD KOBAL[1], [1]*Department of Experimental and Clinical Pharmacology and Toxicology and Department of Neurology, Univ. of Erlangen-Nürnberg, 91054 Erlangen, Germany.* FAX: +49-9131-856898

Electrophysiological data indicate that processing of chemosensory information in patients suffering from temporal lobe epilepsy is different from normal subjects. The aim of this study was to investigate whether both olfactory and trigeminal stimuli applied either ipsilaterally or contralaterally to the epilpetic focus, before and after epileptic surgery, are processed differently.

Nineteen patients were investigated, 9 of whom suffered from epilepsy with a focus in the left temporal lobe. The remaining 10 patients had a right sided temporal lobe focus. Nasal chemosensory performance was investigated three times (preoperative, 7 days postoperative, and 3 months postoperative) with Sniffin'Sticks. Additionally chemosensory function was assessed three times (preoperative, 7 days postoperative, and 3 months postoperative) by evaluating chemosensory event-related potentials (CSERP). Input from the trigeminal system was examined by the use of CO_2, input from the olfactory system was evaluated using vanillin and hydrogen sulfide as stimuli.

The lobectomy patients showed significant impairement in the performance of odor discrimination but strictly via the nostril ipsilateral to the resected lobe. In patients with right temporal lobe focus a tendency for shortening of the N1 CSERP latencies after surgery was found.

Thus, it is suggested that the use of the three types of temporal lobe resections do not impaire the sense of smell in general but temporal lobe surgery takes some toll on global changes of cognitive capacity of the human brain.

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Genetic sensitivity to 6-*n*-propylthiouracil (PROP) and sensory responses to sugar and fat mixtures. ADAM DREWNOWSKI, SUSAN AHLSTROM HENDERSON, ANNE BARRATT-FORNELL and CLAYTON HANN. *Human Nutrition Program, The University of Michigan, Ann Arbor, MI 48109.*

Genetic sensitivity to 6-*n*-propylthiouracil (PROP) has been reported to be associated with greater sensitivity to both sugar and fat in foods. Subjects were 118 young women from different ethnic backgrounds, mean age 26.9 years and mean body mass index (BMI) 23.4. The women were classified as nontasters (n = 39), medium tasters (n = 48), or supertasters (n = 31) of PROP. Nontasters of PROP had thresholds of 1.8×10^{-4} mol/L PROP or greater, while tasters had thresholds below 1.0×10^{-4} mol/L PROP. PROP tasters were then divided into medium tasters and supertasters, based on the ratio of intensity ratings of 5 suprathreshold PROP solutions relative to NaCl solutions. Supertasters were defined as those with PROP/NaCl ratios of 1.90 or more. The pattern of sensory responses to sweetened dairy products of varying sugar and fat content closely replicated data obtained in our previous studies. Genetic sensitivity to PROP was not associated with enhanced perception or altered hedonic response profiles for this range of 15 sugar/fat mixtures. Separating subjects into "likers" and "dislikers" of sweetened dairy products failed to reveal significant links to PROP taster status in this all-female sample.

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Experience-induced increases in sensitivity for glucose in human glucose-hypogeusics or for fructose in fructose-hypogeusics. SHACHAR EYLAM and LINDA M. KENNEDY, *Neuroscience Program, Biology Department, Clark University, Worcester, MA 01610.* seylam@vax.clarku.edu

Human psychophysical response functions for concentrations under 100 mM are different for fructose and glucose and suggest different taste mechanisms for the two monosaccharides (Kennedy et al, 1997; Eylam & Kennedy, 1998, in press). In our earlier studies, individuals identified as having a reduced sensitivity for one monosaccharide, but not the other, by the shift of one of their response functions showed a sensitivity change when retested. To investigate this phenomenon, *Positive Identification* (*PID*) (the lowest concentration at which the sugar always was judged as sweeter than the water) were determined for each of 32 subjects. Five categories were distinguished according to the means and standard deviations (S.D.) of the population PID lognormal distribution. Then, 92 subjects (mean age 22, range 14-50 years) were screened with the mean, mean+1 S.D. and mean+2 S.D. concentrations of fructose and glucose. They were presented with 3 replications of each sugar concentration paired with water in one session (18 pairs) and asked to indicate the sweeter of the two. Hypogeusics (HPGs) were defined as having a PID for one sugar > 2 S.D. above the mean, while that for the other sugar remained within 1 S.D. of the mean (Eylam & Kennedy, 1998). Of the 12 glucose HPGs (HPG_g) and 4 fructose HPGs (HPG_f) identified, 5 HPG_g and 2 HPG_f were tested in six additional sessions. Five average subjects, whose responses fell in the mean ±1 S.D. range (*Average* range) for both sugars, served as controls. The HPGs showed a gradual increase in sensitivity only to the sugar for which they were initially hypogeusic: the HPG_g exhibited a significant reduction in PIDs to the *Average* range of glucose, but no change in sensitivity to fructose, while the HPG_f showed a corresponding effect with opposite sugars ($p<0.05$, Kramer). No significant change occurred in PIDs of the average subjects for either sugar. Only in the initial test was there a statistically significant difference between glucose sensitivity of the HPG_g or fructose sensitivity for HPG_f and that of the other subjects ($p<0.05$, Tukey-Kramer HSD), but the increases were gradual over time. Since the increase in sensitivity to one sugar occurred without changes in the sensitivity to the other, and without changes in sensitivities of the average subjects for either sugar, an inducible taste mechanism seems more likely than a learning process.

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A molecular model for multicomponent mixtures: Evidence for the existence of a transducer explains why fructose is sweeter than glucose in humans. DANIEL M. ENNIS, *The Institute for Perception, 300 Arboretum Place Suite 430, Richmond, VA 23236.* FAX: (804)-272-8943.

Throughout this century, the Law of Mass Action has played a central role in the development of receptor models in Pharmacology and the Chemical Senses to explain the effects of single substances on biological systems. The development of models that specify a transducer entity are fairly recent and a notable application of this type of model was in the study of β-antagonism. Extensions of these models to mixtures of substances with similar effects (such as sweet tasting compounds or drugs with the same desired effect) is important because:

- Inferences about binding mechanisms (such as the existence of transducers, common or independent receptors and types of binding) can be determined without specifying the function that connects the peripheral events to the percept or biological response other than that it is monotonic,
- Synergistic effects can be predicted and used to determine levels of compounds in mixture that are equally effective to the compounds alone, thus reducing subject exposure to these compounds.

A general molecular model that allows any number of compounds to bind any number of receptors and transducers will be described. Using this model, it will be shown that there is evidence for the existence of a transducer in human sweet taste and the synergistic effect of these two substances on sweet taste will be explained.

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Familiarity modulates neophobia's effects on the sensory evaluation of foods. BRYAN RAUDENBUSH and ROBERT A. FRANK, *Department of Psychology, University of Cincinnati, Cincinnati, OH 45221.* raudenbc@email.uc.edu.

People who avoid new foods (neophobics) and people who approach new foods (neophilics) differ in their responses to olfactory stimuli. Neophobics tend to sniff odors less vigorously, and rate them as less intense and less pleasant. Neophobia's effects on responses to foods are less clear, with some investigators reporting that neophobics tend to negatively evaluate foods, and others finding no effects. The present study assessed the possible role of stimulus familiarity on neophobics' and neophilics' response to foods. Neophobics and neophilics (identified using Pliner and Hobden's (1992) Food Neophobia Scale) sampled and rated a set of foods selected to range from unfamiliar to familiar. Participants judged how willing they were to try the foods, their familiarity with the foods, and how much they expected to like them. They then sampled these foods and made liking ratings based on actual tasting. Next, they indicated how willing they would be to sample the foods again in the future. Differences in the responses of neophobics and neophilics were substantially and significantly modulated by the familiarity of the foods. Responses to the familiar foods were similar for the two groups, while neophobics tended to be much more negative in their evaluations of unfamiliar foods. It is proposed that the responses of neophobics and neophilics will differ when little information about a food is available, and that these differences will diminish as more sensory information about the food is acquired.

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Liminal, "ageusic" taste. TOMAS RADIL[1,2], MARCIA PELCHAT[2], CHARLES J. WYSOCKI[2], [1] *Institute of Physiology, Czech Academy of Sciences, Prague, CZ, 14220,* [2]*Monell Chemical Senses Center, Philadelphia, PA, 19104.* FAX: 215-898-2084.

Previously, we demonstrated that subjects correctly detected weak odors, apparently unaware of them, and, when queried, stated that they were guessing (Radil and Wysocki 1997).

Similar experiments were performed with salty, sweet, sour, and bitter gustatory stimuli, using suitable concentrations of sodium chloride, sucrose, citric acid, and quinine sulfate, respectively. Individual detection- thresholds were first estimated for the taste modality in question, using an ascending, binary, forced-choice procedure. Subsequently, the threshold concentration, five lower-, and two higher-concentrations, each paired with a blank, were presented ten times, in random order. The task of the subjects was to indicate whether the first or second cup in the pair contained the tastant. The subjects rinsed between trials. Additionally, subjects marked on a continuous scale their degree of certainty, from "Absolutely Certain" to "Absolutely Guessing" at the extremes, with no additional labels between. Feedback information was provided on correctness of choice after every trial.

The results were similar to those obtained with olfactory stimuli. At close to threshold concentrations the subjects correctly detected the majority of taste stimuli of different modalities, although they generally reported that they were guessing. Thus, the subjects apparently were "unaware" of the stimuli, even though they respondied at better than chance levels.

The physiological basis and functional role of this "liminal perception" remains unclear.

Radil, T. and Wysocki, C.J. 'Anosmic olfaction' -- a blindsight-like phenomenon in normal subjects. Chemical Senses, 1997, 22, 198.

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Evidence for different olfactory foraging strategies in Antarctic seabirds. GABRIELLE A. NEVITT and KEITH REID, *Section of Neurobiology, Physiology and Behavior, Univ. of California, Davis, CA 95616 and the British Antarctic Survey, High Cross, Madingley Road, Cambridge, CB3 OET, UK.* FAX: 530-752-5582.

Procellariiform seabirds have a well-developed sense of smell, but little information is available about how these birds use olfaction in foraging. We performed detailed studies in sub-Antarctic waters near South Georgia Island to test bird's responses to 3-methyl pyrazene. This aromatic is released by macerated Antarctic krill, a primary prey item for many seabird species in this region. As in previous studies, birds were presented with either scented or 'unscented' vegetable oil slicks. Birds flying upwind into slicks were counted as showing interest in the slicks. At some locations we also compared birds' responses to herring oil, an established olfactory attractant. We found that responses were highly species-specific to pyrazene but not to herring oil. Cape petrels, giant petrels, black-browed albatrosses and white-chinned petrels were significantly more attracted to pyrazene-scented slicks than to control slicks ($P<0.01$-0.05; G-test). Storm-petrels (both Wilson's and black-bellied) and prions showed no significant attraction. In contrast, all these species were significantly attracted to herring oil ($P<0.01$-0.05; G-test). Interestingly, these results contrast with earlier studies investigating species-specific responses to dimethyl sulfide, an aromatic released not by krill, but by the phytoplankton that krill eat. We propose that different species of procellariiform seabirds may have behaviorally distinct olfactory strategies for locating and exploiting patchily distributed prey resources.

Supported by NSF (OPP-96-15061 to GAN) and the British Antarctic Survey.

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Odor plume tracking by the living fossil, *Nautilus pompilius*. JENNIFER A. BASIL[1], ROGER T. HANLON[2], SARAH I. SHEIKH[3], AND JELLE ATEMA[1], [1]*Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA, 02543,* [2]*Marine Resources Center, Marine Biological Laboratory, Woods Hole, MA 02543,* [3]*Department of Biological Sciences, University of Edinburgh, Edinburgh, UK.* basil@bio.bu.edu.

Inferring sensory capabilities and behavior of fossil species is difficult, but Nautilus offers a unique opportunity to examine these kinds of questions experimentally. Nautilus remains in dimly lit waters for most of its life, living in deep waters during the day then vertically migrating up tropical coral reef slopes at night to forage in waters as shallow as 75 m. Its primitive pinhole eye suggests that vision is not the most essential sensory system for foraging as it is for most modern coleoid cephalopods. Nautilus has therefore been referred to as a "smeller and groper". There are anecdotal descriptions of nautiloids using what appears to be chemical detection to locate prey items. We therefore tested animals experimentally in a large flume and found that they were consistently capable of detecting and following turbulent odor plumes of low concentration for at least 10 m against a current (7 cm/s) and that they sampled the plume in three dimensions. When the putative olfactory organs, the rhinophores, were selectively and reversibly lesioned, Nautilus were unable to track the location of distant odor source. Lesioning only the rhinophore prevented all animals from locating the source as well, although they were able to initially detect its presence. Nautilus therefore requires bilateral sensing from its rhinophores to maintain a correct heading to locate a weak signal from a distant odor-producing food source. Such sensitive smelling and groping may have been the essence of the Umwelt of ammonites and belemnites before complex eyes and keen vision evolved in coleoids.

This research was supported by a fellowship from the Grass Foundation to JAB.

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Far Field Chemo-orientation in the American Lobster (*Homarus americanus)*: A Role for Micro-Mechanosensation? FRANK W. GRASSO[1], PAUL F. BEGLANE[2], JENNIFER A. BASIL[1] AND JELLE ATEMA[1] [1]*Boston University Marine Program, MBL, Woods Hole, MA 02543* [2]Department of Biology, Suffolk University, Boston, MA 02114

Ablation studies demonstrate that the American lobster, Homarus americanus, requires both of its lateral antennules to choose the flavored odor source from a pair of turbulent plumes (Devine and Atema 1982). Other studies have suggested that these animals may be using the temporal pattern of patchy odor encounters to locate odor sources (Moore, Sholz and Atema 1991, Basil and Atema 1994). In recent studies we have examined the effects of a) unilateral antennule ablation and b)unilateral chemoreceptor lesion of the lateral antennule in lobsters allowed to freely orient to turbulent odor sources. In timed trials shams and lesioned animals, but not ablates, showed a significant decrease in their emergence from the start box in response to food-flavored compared with unflavored turbulent odor plumes [Shams $\chi^2(1,71)=13.14$ $p<0.01$, Lesions $\chi^2(1,55)=29.94$ $p<0.001$]. Lesioned animals did not differ significantly from shams in their source directed behavior but ablates exhibited significantly fewer source directed responses compared to shams [$\chi^2(1,26) = 5.45$ $p<0.02$]. Because the lesioning procedure is thought to spare mechanoreceptors while lysing the chemoreceptors of the lateral antennule, these results suggest a significant role of mechanosensation in the orientation behavior of the lobster, affecting arousal and possibly a guidance to the odor source.

Supported by NSF Grant IBN-9212650 to JA.

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Hormonal control of response to and secretion of a newt sex pheromone, sodefrin. SAKAE KIKUYAMA[1], FUMIYO TOYODA[2], TAKEO IWATA[1] AND KAZUTOSHI YAMAMOTO[1], [1]*Dept. Biology, Schl. Educ, Waseda Univ., Tokyo 169-50, and* [2]*Dept. Physiol., Nara Med. Univ., Kashihara 634, Japan.* FAX: 81-(3)-3207-9694.

Sodefrin is a female-attracting decapeptide pheromone isolated from the abdominal gland of the cloaca of the male newt, Cynops pyrrhogaster. Preference for sodefrin was found to be completely abolished in the females by bilateral nostril plugging or olfactory nerve sectioning. In the female newts, sodefrin elicited electro-olfactogram (EOG) responses in a dose-dependent fashion. EOG with the highest amplitude was elicited by sodefrin in the lateral part of the ventral olfactory epithelium (lateral nasal sinus). Hormonal effect on the EOG response to sodefrin was studied in the ovariectomized newts. Prolactin (PRL) plus estradiol-treated females exhibited a marked EOG response to sodefrin. Estradiol alone enhanced the EOG response to a lesser extent. The immunoassayable sodefrin content in the abdominal gland was diminished by castration and hypophysectomy. The content was increased markedly in the castrated and hypophysectomized newts after treatment with both testosterone and PRL. Testosterone but not PRL increased the sodefrin content moderately. It was also demonstrated that a combination of PRL and testosterone enhanced sodefrin mRNA levels in the abdominal gland as determined by northern blot analysis using sodefrin cDNA as a probe. The results are indicative of the involvement of PRL and sex steroids in the olfactory responsiveness to and the secretion by the abdominal gland of the female-attracting pheromone.

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The chemosignals causing puberty acceleration in the house mouse: natural stimuli and their structural analogs. MILOS V. NOVOTNY, WEIDONG MA, LUKAS ZIDEK, *Department of Chemistry, Indiana University, Bloomington, Indiana 47405.* FAX: (812)855-8300.

Both the volatile (male-originated) urinary cues and large biomolecules (the so-called major urinary protein, MUP) were previously claimed to be the constituents responsible for enhancement in the uterine weight among the stimulus-exposed female mice. The long-standing controversies about a chemical nature of the puberty-accelerating pheromone now become explainable through a pheromone/MUP complexation phenomenon, with MUP serving as a slow-releaser, or an instant transporter molecule for the actual chemosignals. In our recent experiments, a recombinant MUP (with its biochemical and binding behavior being identical to the natural MUP) was found inactive in the absence of pheromones. In contrast, several testosterone-dependent volatiles (all with a strong affinity to MUP) could enhance significantly the uterine weights when tested as the laboratory-synthesized chemicals in dilute aqueous solutions. Several structural analogs of the biosynthetically (naturally) produced pheromones were also tested in parallel to enhance our understanding of their biological activity and binding to receptive proteins.

Supported by NIH grant DC-02418 (to MVN)

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Initial characterizations of secreted proteins from Asian elephants that bind the sex pheromone, (Z)-7-dodecenyl acetate. L. E. L. RASMUSSEN[1], J. LAZAR[2], D. GREENWOOD[3], L. FENG[2], G. PRESTWICH[2], *[1]Dept. of Chemistry, Oregon Graduate Institute, Beaverton, OR 97006, [2]Dept. of Medicinal Chemistry, University of Utah, Salt Lake City, Utah, [3]Horticulture & Food Research Institute of New Zealand, New Zealand.*

The details of molecular mechanisms by volatile chemical signals trigger physiological and behavioral changes in mammals remain poorly understood. Recently, the elucidation of an essentially single-component female-produced sex pheromone ((Z)-7-dodecenyl acetate) in the Asian elephant, Elephas maximus, allowed us to initiate an in-depth investigation of the proteins involved in production, transport, recognition, and signaling by this ligand. The study is facilitated by the availability of a quantitative, robust bioassay, as well as large amounts of emitter sources (preovulatory urine), transporter media (mucus) and receptive tissue (vomeronasal organ), subdivisible into receptive and non-receptive regions.

We postulate that binding proteins are present in the preovulatory urine that sequester Z7-12:Ac and affect its volatilization rate and that other related proteins are present in the male trunk mucus. These proteins may have key roles in determining the apparently high degree of specificity exhibited toward these signaling molecules. We have synthesized radiolabeled and photoaffinity-labeled pheromone analogues to assist in the identification of these proteins. The bioactivity of the photoactivatable analog, (Z)-7-dodecenyl diazoacetate (Z7-12:Dza) has been documented with two groups of male Asian elephants (n=7). Female elephant urinary proteins were photolabeled with [^{3}H]-Z7-12:Dza, separated by SDS-PAGE, and visualized by fluorography. In addition, separated proteins were electroblotted and N-terminal sequences obtained by Edman degradation. Urinary proteins interacting with the pheromone include albumin and proteins in the lipocalin region. Similarly, male trunk mucus proteins were separated and two lipocalins with >50% sequence identity to bovine OBP (for 18 amino acids) were identified. Preliminary comparisons of respiratory and neuroreceptive vomeronasal regions with ^{3}H-BZDC-P-2 linked-Ins(1,2,4,5)P_4, a photoaffinity analog of Ins(1,4,5)P_3 demonstrated an enrichment the IP_3 receptors in the sensory tissue. Binding specificity and affinity of urinary, mucous and vomeronasal proteins afore 3H-Z7-12:Ac, saturated 3H-Z7-12:Ac and the photoaffinity analogue are in progress. We are especially interested in how these odorant binding proteins compare to insect pheromone binding proteins. In particular, this system allows a comparison of two separately evolved solutions to binding and transducing signals from the same chemical entity.

Supported by NIH grant RO1-DC03320 (to L.E.L.R)

Poster 140

Peptidergic nerve fibers and synaptic vesicle protein-containing nerve fibers in the rat tongue epithelium and taste buds: Are they the same fibers? Complex results. GINA M. NELSON, *Depts of Pathology and Anatomy and Cell Biology, Univ. of Iowa Hospitals and Clinics, Iowa City, IA 52242.* gina-m-nelson@uiowa.edu

Several reports have previously described the presence of peptides and synaptic vesicle proteins in and around the taste buds in rat lingual epithelium. Peptidergic nerve fibers (nf) are primarily perigemmal with some intragemmal nf being immunoreactive (Finger, *Chemical Senses 11:135,86).* NF containing synaptic vesicle proteins are primarily intragemmal with some perigemmal nf being immunoreactive (Nelson & Finger, *Chemical Senses abstr #144, 90*). These two groups of nf are not distinct groups. Nerve fibers with both peptides and synaptic vesicle proteins are present.

The colocalization of peptides and synaptic vesicle proteins was investigated using antibodies directed against synaptophysin, SV2, CGRP, and PGP9.5 using double labeled immunohistochemistry with fluorescent secondary antibodies, in *Sprague dawley* rats. Synaptophysin & PGP9.5: both intragemmal and perigemmal synaptophysin nf are PGP9.5-positive, but there are PGP9.5 positive only fibers. Synaptophysin & CGRP: a few of the intragemmal nf are positive for both, most of the intragemmal nf are synaptophysin only or CGRP only. All perigemmal synaptophysin positive fibers are CGRP-positive. SV2 & CGRP: In both intragemmal and perigemmal nf there are only a few nf with both SV2 and CGRP. Most nf are SV2-only or CGRP-only.

Nerve fibers associated with taste buds can not be classified into broad categories of peptidergic' or'synaptic vesicle containing'. How much the peptide containing nerve fibers overlap with the synaptic vesicle protein containing nerve fibers depends on the location of the nf and which synaptic vesicle protein is in question. In order to investigate various experimental effects of these two groups of nf, the labels must be carefully chosen.

Poster 141

Immunohistochemical analysis of synaptic proteins: (syntaxin, synaptobrevin, synaptic protein, and synaptotagmin) in rat taste buds. MICHAEL E. ROCK, RUIBIAO YANG, HILDEGARD H. CROWLEY and JOHN C. KINNAMON, *Department of Biological Sciences, University of Denver, Denver, CO 80208.* merock@du.edu

We are currently using immunohistochemical techniques to determine the presence and distribution of several synaptic proteins (syntaxin, synaptobrevin, synaptic protein {a 22 kD protein similar in reactivity to SNAP-25} and the glycoprotein synaptotagmin) in taste cells and nerves of the rat circumvallate taste bud (TB).

Descriptions of the synaptic vesicle cycle include the following mechanisms and locations for the above synaptic proteins: syntaxin and synaptic protein are membrane proteins associated with the presynaptic terminal; synaptobrevin [VAMP] is thought to be a membrane protein associated with the synaptic vesicle (SV). These three proteins make up a part of the core complex, which regulates vesicle docking and fusion. Synaptotagmin is a glycoprotein which interacts with syntaxin in a Ca^{2+} dependent manner, possibly a trigger mechanism, for neurotransmitter release from SVs.

We observed strong immunoreactivity (IR) to syntaxin in a subset of taste cells. Intense punctate staining is present in the apical portions of the taste cells. Gustatory nerve fibers are not immunoreactive to syntaxin. Immunoreactivity to anti-rat synaptobrevin-2 [VAMP-2] is present in a large subset of taste cells. VAMP-2 IR is present in both intra- and perigemmal nerves, and strong IR is observed in the nerve plexus beneath the taste buds. Synaptotagmin has somewhat weaker IR, a mottled or granular pattern is present in the cytoplasm of a small subset of taste cells. Staining is present in both intra- and perigemmal nerves. Immunoreactivity to the monoclonal antibody directed against synaptic protein (Sternberger #SMI 82) is present in both intragemmal and perigemmal nerves. This IR is very similar to that of anti-SNAP-25 antibody. Taste cells show little or no IR to synaptic protein. These results confirm the presence of synaptic proteins in taste cells of the circumvallate papilla The significance of those observations is unknown at present, but we speculate some of those synaptic proteins may play a role in the synaptic vesicle cycle.

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Poster 142

Immunocytochemical characterization of SNAP-25 in rat taste buds. RUIBIAO YANG, HILDEGARD H. CROWLEY, MICHAEL E. ROCK and JOHN C. KINNAMON, Department of Biological Sciences, University of Denver, Denver, CO 80208. ryang@du.edu

The process of taste transduction involves the interaction of taste stimuli with the apical microvilli of taste cells, followed by membrane conductance changes, depolarization of the taste cell membrane, and exocytosis of transmitter onto gustatory afferent neurons at presumed synaptic contacts. We hypothesize that taste bud synapses utilize different synaptic proteins than are present in many of the synapses of the central nervous system. One of those synaptic proteins is SNAP-25, a neuron-specific protein localized primarily in axons and axon terminals. This protein has been thought to participate in the fusion of of the synaptic vesicle and presynaptic membranes associated with neurotransmitter release. Cleavage of SNAP-25 with botulinum toxin in developing neurons inhibits axonal growth, prevents synapse formation and induces rapid neuronal death.

Cryostat sections (20 µm thick) from circumvallate taste buds of Sprague-Dawley rats were examined with indirect immunofluorescence using a monoclonal antibody to SNAP-25. Immunoelectron microscopy was performed using DAB to localize SNAP-25 at the cellular level.

Our studies indicate that SNAP-25 immunoreactivity is present in both intragemmal and perigemmal nerve processes, with intense immunoreactivity associated with the nerve plexus located below the basal lamina of the taste bud. No immunoreactivity is present in any of the taste cells. The lack of immunoreactivity in taste cells argues against a role for SNAP-25 in the fusion and exocytosis of synaptic vesicles at afferent synapses in taste buds. The intense staining of the intragemmal nerve fibers may support an efferent function for intragemmal nerve fibers.

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Poster 143

A highly sensitive method for *in situ* hybridization using tyramide-amplification permits cellular resolution of less abundant mRNAs in taste buds. HUI YANG, STEPHEN D. ROPER and NIRUPA CHAUDHARI, Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33136. nchaudha@newssun.med.miami.edu.

In situ hybridization using non-radioactive probes typically allows mRNAs to be detected with improved cellular resolution when compared with radiolabeled probes. However, sensitivity is compromised so that only relatively abundant target mRNAs can be examined. We have re-examined and optimized many of the parameters for conducting *in situ* hybridization and have employed the new Tyramide Signal Amplification (TSA) system (NEN) to achieve high sensitivity as well as cellular resolution. Using this method, we find that signal intensity is enhanced 10 to 50-fold over that achievable without TSA. Hybridization and detection conditions were optimized in rat brain sections using antisense and sense probes for metabotropic glutamate receptor type 4, and in rat lingual sections using probes for gustducin. Signal intensities were compared using long (upto 3kb) RNA probes, tested either intact, or following hydrolysis to a mean size of 0.3kb. We find that if sections are treated with proteinase K before hybridization, permeation of long probes does not appear to be a problem, and indeed, long probes yield stronger signals than do hydrolyzed probes. Secondly, we find that RNase digestion following hybridization is an unecessary step that leads to a substantial reduction of signal intensity. Parallel hybridizations with sense probes confirmed that if high stringency is maintained, these conditions do not lead to non-specific noise. Using this method, we are able to detect mRNA for transducin in taste cells. We find that transducin-positive cells are found at a lower frequency than gustducin-positive cells in circumvallate and foliate taste buds from rat. Signal intensities for transducin mRNA are substantially lower than for gustducin mRNA. Preliminary results imply that both Gα subunits are found at lower concentration in weanling than in adult rats.

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Poster 144

Rat taste cells express two distinct forms of mRNA for the metabotropic glutamate receptor, mGluR4. ALEXEI FEDOROV and NIRUPA CHAUDHARI, *Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33136,* nchaudha@newssun.med.miami.edu.

The metabotropic glutamate receptor type 4 (mGluR4) has been shown, using molecular analyses, to be expressed in rat taste receptor cells (Chaudhari et al., 1996). This raised the question of whether mGluR4 may play a role in the taste transduction of glutamate as an apical taste receptor. Conditioned Taste Aversion experiments have implied that rats detect the taste of glutamate through a receptor that is activated by L-AP4, a ligand known to activate mGluR4. However, glutamate is effective as a taste stimulus at approximately a thousand-fold higher concentrations than at synapses. Thus, it is possible that an altered receptor may be involved. We now present evidence that in taste tissue, mRNA for mGluR4 is found as two distinct forms, one of which is novel, and if translated, should yield a receptor with a dramatically altered binding site for glutamate. 5'RACE (rapid amplification of cDNA ends) reactions were carried out using mRNA from taste tissue to examine the coding sequence of the extracellular N-terminus of mGluR4. Consistently, the 5' end of mGluR4 mRNA from taste tissue was found to be located approximately 1kb downstream of the translational initiation point of brain mGluR4 mRNA. Also, the 5' end of the taste-derived mRNA includes approximately 50bp which is not found in mGluR4 mRNA from brain. RNase Protection Assay was used to verify that this 5'-truncated form is found as a poly(A)RNA in taste tissue and is not an artifact of PCR. Using taste poly(A)RNA, bands were obtained that represent both the "brain-form" and the novel form of mGluR4. The 5'-truncated form is not found in brain RNA and appears to represent a "taste-specific" form. By quantifying the intensities of protected RNA bands, we estimate that approximately equal amounts of the two forms are found in taste tissue. Efforts are underway to obtain expression of the novel truncated mGluR4 to determine if it is a functional form.

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Poster 145

Serotonin-concentrating Taste Cells lack Serotonin-associated Synthetic Enzymes and Transporters. BÄRBEL BÖTTGER and THOMAS E.FINGER, *Rocky Mountain Taste & Smell Ctr. and Dept. Cellular & Structural Biology, Univ. Colorado Health Sci. Ctr., Denver CO 80262. Barbel.Bottger@UCH,SC.edu.*

Some taste cells in most rat taste buds will accumulate serotonin (5-HT) if the animal is injected with 5-hydroxytryptophan, the immediate precursor substance. This has led several investigators to suggest that a specific subset of taste cells has a serotonergic phenotype. The serotonergic phenotype in mast cells, enterochromaffin cells and neurons involves several properties including: 1) presence of tryptophan hydroxylase (TrypOH), 2) an intracellular vesicular monoaminergic transporter (VMAT2), and 3) a membrane-associated specific serotonin transporter (SerT). We obtained commercially-available antisera against these three serotonergic markers to utilize on lingual tissues. Rat tongues were fixed in 4% buffered paraformaldehyde either with or without 0.4% picric acid. Similarly-fixed tissues from brain served as positive controls. In addition, the circumvallate papilla contains numerous mast cells which serve as an intrinsic positive control. Two different TrypOH antisera were utilized. Both produced strong reactivity of mast cells and 5-HT CNS neurons; only one produced reactivity in taste buds. The immunoreactive taste cells observed with this antibody were, however, morphologically different from the type of taste cell that exhibits 5-HT immunoreactivity. Since this immunoreactivity is found with only one of the two TrypOH antisera employed, we believe this to be non-specific reactivity and not attributable to TrypOH. Taste buds also completely lack immunoreactivity to VMAT2 although mast cells and varicose (autonomic) nerve fibers surrounding lingual blood vessels exhibit such immunoreactivity. Similarly, mast cells but not taste cells exhibit SerT-like immunoreactivity. Thus although taste cells may accumulate 5-HT *in vivo,* they do not appear to be serotonergic cells in the same way that neurons, mast cells and enterochromaffin cells are.

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Poster 146

Immunocytochemical Analysis of Serotonin Biosynthesis in Taste Cells. MEGAN E. LITSTER[1,3], LESLIE M. STONE[1,3], THOMAS E. FINGER[2,3], and SUE C.KINNAMON[1,3], [1] *Department of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523,* [2] *Department of Cell and Structural Biology, University of Colorado Health Sciences, Denver, CO 80262,* [3] *The Rocky Mountain Taste and Smell Center, Denver, CO 80262*

Most taste buds have some cells that contain the bioactive amine serotonin (5-HT). However, its role as a neurotransmitter or neuromodulator in taste buds is still debated. In neurons, serotonin biosynthesis involves conversion of tryptophan to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase. The 5-HTP then is converted to serotonin by an aromatic amino acid decarboxylase. In previous studies of mammalian taste buds, serotonin immunoreactivity was observed, but was most obvious in animals preinjected with 5-HTP. This raises the question of whether taste cells are capable of synthesizing 5-HT from circulating tryptophan or whether they require the hydroxylated precursor in order to generate 5-HT.

To determine whether taste buds can synthesize 5-HT from the amino acid precursor, the lingual epithelium was stripped from regions of rat tongue containing fungiform, foliate, and circumvallate papillae. The tissue then was incubated in media containing 500 µM of either the precursor L-tryptophan or the precursor 5-HTP. After 3-24 hrs., the tissue was fixed in 4% paraformaldehyde and sectioned on a cryostat at 10 to 15 µm. Immunocytochemistry was used to detect the presence of the serotonin. Distinct serotonin immunoreactivity was present when the epithelium was incubated with the immediate precursor 5-HTP, but not when incubated with L-tryptophan. These results suggest that taste cells do not contain the enzyme tryptophan hydroxylase which is necessary for the synthesis of serotonin from tryptophan.

This work was funded by grant #DC00244 from the NIDCD

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Cellular expression of α-gustducin and the A blood group antigen in rat fungiform taste buds cross-reinnervated by the IXth nerve. JOYDEEP SOM[1], JOHN D. BOUGHTER, Jr.[2], STEVEN J. ST. JOHN[2], CHENGSI YU[2], ROBERT C. CHRISTY[2], and DAVID V. SMITH[2], [1]*Div. Otolaryngology - Head & Neck Surgery,* [2]*Dept. Anatomy & Neurobiology and Program in Neuroscience, Univ. Maryland School of Medicine, Baltimore, MD 21201.* dvsmith@umaryland.edu.

Although taste buds are trophically dependent on their innervation, cross-reinnervation experiments have shown that their sensitivity is determined by the epithelium. Both the gustatory G-protein, α-gustducin, and the cell-surface carbohydrate, the A blood group antigen, are expressed by significantly fewer fungiform than vallate taste buds in the rat. The proximal portion of the IXth nerve was anastomosed to the distal portion of the chorda tympani (CT) nerve using fibrin glue and animals survived for 12 weeks postoperatively. Control animals had the CT cut and reanastomosed using the same technique, or had the CT avulsed from the bulla and resected to prevent regeneration. Tongues were removed, stained with methylene blue, and the fungiform taste pores were counted on both sides. Tissue from the anterior 5 mm of the tongue was cut into 50-µmsections, which were reacted with monoclonal antibodies against α-gustducin (Santa Cruz) and the human blood group A antigen (Dako). Sections were processed for double-labeling using CY2- and CY3-conjugated secondary antibodies (Jackson Immuno- Research) and viewed on a confocal microscope. Fungiform taste buds on the intact side had a mean of 3.0 α-gustducin- and 0.21 A-containing cells per taste bud. Reinnervation by the CT nerve produced 82% regeneration of the fungiform taste buds, whereas cross-reinnervation by the IXth nerve ranged from no regeneration to 73%. In some rats without taste bud regeneration, electrophysiology showed regeneration of IXth nerve somatosensory fibers. In animals with >50% regeneration of taste pores, there was no significant difference in the numbers of α-gustducin- or A-containing cells, regardless of which nerve innervated the fungiform papillae, indicating that the local epithelium determines their expression.

Supported in part by NIDCD DC00347 (DVS).

Poster 148

Distribution of taste buds in the zebrafish, *Brachiodanio rerio.* YUKO OHKUBO, NORIKO AKIKUSA, KATSUTO SUZUKI, MITSURU TSUJI, TAKAYUKI MARUI, *Dept. Oral Physiol., Ohu Univ. Sch. Dent., Koriyama, Japan 963.* FAX: +81-249-33-7372

Zebrafish are well-known experimental models in developmental biology. Recently, zebrafish are being used in the study of olfaction; however, little is known concerning taste transduction in this species. As a first step, we are studying the distribution of taste buds in this species by scanning and electron microscopy. The total number of taste buds in a 3.7 ± 0.2cm length (n= 5) zebrafish is approximately 2,000, which are distributed in approximately equal numbers on the external facial skin surface and within the oral cavity, including gill regions. The highest density of taste buds occurs on the lips (total number, 155.0 ± 10.1) and on the two pair of barbels [total number 206.4 ± 7.0 (long barbel) and 126.4 ±15.7 (short barbel)]. Taste buds located on the outer facial skin and within the oral cavity differ in appearance. Taste buds on the external surface protrude from the epithelia, whereas those in the oral cavity are sunken. This difference in appearence might be caused by the thickness of the epithelia (external body surface, 20.8 ± 1.2 µm, n=10; oral cavity, 11.2 ±± 0.7 µm, n=10). The height and maximum diameter (ca 21 x 21 µm) of the taste buds in the two regions are similar, but are of smaller dimensions than those observed in other teleosts (i.e. 30-80 µm in height and 20-50 µm in width)*. The reason for the small-sized taste buds in zebrafish may be related to the short body length of this species compared to the other fish species studied.

* Iwai,T.A. , A comparative study of the taste buds in gill rakers and gill arches of teleostean fishes. *Bull. Misaki Mar. Biol. Inst. Kyoto Univ.* 7: 19-34 (1964)

Poster 149

Taste bud development in the zebrafish, *Danio rerio.* ANNE HANSEN[1], KLAUS REUTTER[2], AND ECKART ZEISKE[1], [1]*Zoological Institute and Zoological Museum, University of Hamburg.* FAX: +49-40-4123-3937, D-20146 *Hamburg, Germany,* [2]*Anatomical Institute, University of Tuebingen, D-72074 Tuebingen, Germany.*

The aim of our study was to investigate the development of taste buds (TBs) in zebrafish. Embryos and larvae from our own breeding colony were reared at a temperature of 26.5°C. Scanning (SEM) and transmission (TEM) electron microscopy were used to visualize the formation and growth pattern of TBs. Preceded by the formation of the peripheral olfactory organ and the solitary chemosensory cells (SCCs), the first few taste receptor areas are detectable on the lips of the larvae approx. 5 days after fertilization, corresponding to one to two days after hatching. Open receptor areas within the oropharyngeal cavity appear one to two days earlier and receptor areas on the head appear later during development. Within the oropharyngeal cavity a receptor area is situated on the tip of every gill raker primordium. Additional taste buds - randomly distributed - develop slightly later. On the lips, the receptor areas are also distributed randomly. The onset of receptor area opening may vary a little from one animal to the next. Receptor areas appear either in the nook of three epidermal cells and then usually do not protrude above the epidermal surface. Other receptor areas appear at the suture of two epidermal cells. One of these two epidermal cells then grows around the receptor area covering a little hillock. Only later in development, when the hillock is growing, further epidermal cells contribute to the cover of the TB. As seen in TEM, the first TB cell type to appear are cells with several small villi, followed by cells with one stout villus. Mature TBs - especially those more rostrally located in the oral cavity and those on the lips - have an additional cell type which bears a brush-like apical ending. At first, this cell type is found only on the margin of the taste receptor area. In bigger, more mature TBs, these cells are also evident in the middle of the receptor area. The cells with brush-like endings resemble young SCCs. Studies to determine whether these TB cells and the SCCs have further common features are in progress.

Poster 150

Dual embryonic origins for vertebrate taste receptors: implications for taste bud patterning. LINDA A. BARLOW. *Dept. of Biological Sciences, Univ. of Denver, Denver CO 80208.* lbarlow@du.edu.

In all vertebrates, taste buds are found throughout the oropharynx. These taste buds arise embryonically from the local epithelium which, in axolotls, is derived primarily from endoderm. Taste bud formation from endoderm is independent of nerve contact. In fact, once gastrulation is complete, the oropharyngeal endoderm will give rise to taste buds without further contact with any other embryonic tissues. However, the oropharyngeal epithelium has two embryonic origins; while most of it is derived from endoderm, the most anterior region (including the mouth) is derived from ectoderm. Nonetheless, taste buds are found in both ectodermal and endodermal zones, despite the fact that these two tissues have radically different embryonic histories. This dichotomy raises several questions: 1) Does ectoderm, like endoderm, give rise to taste buds?; 2) If so, is differentiation of taste buds intrinsic to ectoderm, as is the case for endoderm?; or 3) Are inductive signals from the endoderm required for the formation of taste buds in ectoderm?

To test if ectoderm generates taste buds, fate mapping studies were performed where presumptive oral ectoderm from pigmented axolotl embryos was transplanted into albino hosts. At hatching, pigmented cells were found in the mouth and pharynx, either in small clusters or as isolated pigmented cells in otherwise unpigmented epithelia. In addition, pigmented taste receptor cells were present, indicating their ectodermal origin. Thus, like endoderm, ectoderm can give rise to both epithelial cells and taste receptor cells *in vivo*. To test if the ability to make taste buds is an intrinsic feature of ectoderm, the presumptive oral ectoderm was isolated from axolotl embryos and allowed to develop in culture until intact control embryos had taste buds. Ectodermal explants failed to make taste buds *in vitro*, indicating that unlike endoderm, inductive signals are necessary for differentiation of taste buds from ectoderm. We are currently testing if contact with oropharyngeal endoderm is necessary for the formation of taste buds in ectoderm.

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Expression of *Sonic hedgehog* and *Patched* in taste papillae during late mouse development. JOSHUA M. HALL[1,2], KARL ANDERSON[2], JOAN E. HOOPER[2], THOMAS E. FINGER[2], [1]*Medical Scientist Training Program,* [2]*Rocky Mountain Taste & Smell Center, and Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262.* halljosh@york.uchsc.edu

The diffusable signaling molecule Sonic hedgehog (Shh) is implicated in many vertebrate developmental processes, including formation of neural plate, axial patterning in limbs, and organogenesis of lung and tooth. The Shh signal is transduced through the membrane-bound Patched (Ptc) receptor, which itself is transcriptionally activated by Shh signaling. Both Shh and Ptc are expressed within developing fungiform and circumvallate papillae from E12 through E16.5 in mouse embryos, i.e. appearing earlier than the morphological formation of the taste bud-bearing papillae. Both genes are expressed broadly throughout the tongue at the beginning of this time period and subsequently are restricted to regions in and around developing fungiform and circumvallate. *Shh* expression is limited to the lingual epithelium and, later, in fungiform papillae, to the central few cells of the epithelium. However, *Ptc* is expressed in areas surrounding the *Shh*-expressing regions: in underlying mesenchymal cells of the developing papilla and in cells surrounding the central cluster within the papillary epithelium. This suggests that the Shh signal may act early in lingual development to specify papillary morphogenesis or spacing. The present study seeks to determine whether *Shh* and *Ptc* expression extends later in development, into the immediate prenatal and early postnatal periods as well as into adult stages. Ongoing experiments show that both genes are expressed at E18 in a pattern similar to that seen at E16, i.e. where Shh is present centrally with a surround of Ptc in each papilla. Expression of *Shh* and *Ptc* in fungiform and circumvallate papillae during taste bud histogenesis may indicate a role for Shh signaling in this process as well.

Supported by NIH grant DC00244 to TEF)

Poster 152

The differentiation of a subpopulation of fungiform taste buds is independent of innervation in NT5 ko mice. JOSEPH-PASCAL MBIENE[1] and LUIS F. PARADA[2] [1]*Department of Biomedical Sciences - Baylor College of Dentistry- a member of Texas A&M University System, Dallas TX 75266,* [2]*Center for Developmental Biology - The University of Texas Southwestern Medical Center at Dallas, Dallas TX 75235.*

The neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT4/5) act via the TrkB receptor and support survival of primary somatic and visceral sensory neurons. Previous studies have established that BDNF is required for normal development of taste neurons. Discrepancies in different studies as they relate to the role of NT-4/5 in the survival of taste neurons prompted us to analyze the development of taste buds in fungiform papillae of NT-4/5-deficient mice. The tongues were taken from 0, 7, 21 and 60 days old postnatal NT-4/5-deficient mice, prepared for and analyzed with scanning electron microscopy (SEM). Chordalingual innervation was labeled with DiI and analyzed at embryonic days 12, 14 and 16, and postnatal day 0. The distribution and the number of fungiform papillae were normal in mutant mice until postnatal day 21. However, the number of fungiform papillae was reduced in 60 days old postnatal NT-4/5-deficient mice by about 63% compared to wild type mice. The majority of these fungiform papillae (more than 95%) were located in the most rostral quarter of the tongue; only a few (less than 5%) were distributed on the anterior border of the intermolar eminence. Each fungiform papilla in both mutant and wild type had a visible pore. DiI labelings showed that fungiform papillae located in the most rostral quarter of the tongue were not innervated at all the embryonic stages. These data indicate that NT-4/5, but not BDNF, is required for the survival of a subpopulation of neurons that innervate taste buds papillae located in the most rostral quarter of tongue. These results suggest that the differentiation of taste buds in mammals, at least in the rostral quarter of the tongue, is independent of sensory innervation.

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p53, Bax, and Nedd-2 are sequential components of the taste cell death pathway in mice. BRUCE OAKLEY and QUN ZENG, *Dept of Biology, Univ. of Michigan, Ann Arbor, MI48109.* FAX: (734) 647-0884.

Little is known about the mechanisms of cell death during cell turnover in renewing epithelia. The renewing receptor cells in mouse vallate taste buds have an average life-span of 9 days. Using an improved in situ TUNEL assay to detect fragmented DNA, we demonstrated apoptosis in lingual squamous epithelium and in taste buds. Mammalian apoptotic pathways characteristically involve the sequential activation of: a bcl-2 family death factor, a cysteine protease, and the fragmentation of DNA. Consequently, we used immunocytochemical expression patterns to survey possible death factors in the tongue of adult and neonatal mice. p53, a tumor suppressor; Bax, a cell death promoter of the Bcl-2 family; and Nedd-2, an ICE-like cysteine protease, were expressed in 10-15% of intragemmal cells. Some p53-positive taste cells and some Bax-positive taste cells showed the degenerative morphology characteristic of apoptosis. These dying taste cells were probably aged since young taste cells, identified by Brdu labeling, were never Bax-positive. The significant overlap between p53-positive and Bax-positive taste cells in adult and especially in developing mice is consistent with the molecular evidence that p53 is a transcription factor for bax. In turn many Bax-positive cells were positive for Nedd-2. While p53--Bax--Nedd-2 appears to be the constitutive taste cell death sequence, redundancy is typical of cell death pathways. In our research we found that Bax-expression was not diminished by the absence of p53 caused by null mutation. Although the cell death pathway of the lingual squamous epithelium remains unknown, it is clearly different from that of the embedded taste receptor cells. None of the molecular constituents of the taste cell death pathway (p53, Bax, and Nedd-2) was expressed in basal cells or in the squamous epithelial cells that comprise over 99% of the tongue epithelium

Poster 154

The distribution of cell proliferation in the nasal cavity, olfactory epithelium and brain of the larval sea lamprey. HONG N. HUA, BARBARA S. ZIELINSKI, *Dept. of Biological Sciences, Univ. of Windsor, Windsor, Ont. Canada N9B 3P4.* FAX: (519) 971-3609.

In mammals, postnatal neurogenesis occurs in two separate locations: the olfactory epithelium and the subventricular zone of the brain. Cells from these two populations differentiate into neurons within the same circuit: olfactory basal cells, into afferent olfactory sensory neurons, and subventricular cells, into olfactory bulb (OB) inter-neurons (periglomerular cells and granular cells). As part of an ogoing effort to investigate the regulation of the development of the olfactory organ, we have examined the spatial distribution of cell proliferation in a developing vertebrate. The anatomic arrangement of the head in larval lampreys has enabled cells from several tissues to be viewed within single horizontal sections. These include the respiratory epithelium of the the nasal cavity, olfactory epithelium, olfactory nerves and brain. In this study, cell proliferation and the fate of the proliferating cells were examined by 5-bromo-2'-deoxy-uridine (BrdU) immunocytochemistry. BrdU labeling was most abundant in the respiratory epithelium of the nasal cavity. In the olfactory epithelium, BrdU labeled nuclei occured more frequently in the dorsal portion compared to the ventral region. Within the brain, BrdU labeling was consistently heavy in the habenula. In approximately 50% of the injected animals, there were BrdU nuclei along the ventricles, including the OB ventricular layer. By the third day following the injection, OB BrdU cells were observed in the granular layer, and by 14th day, in lateral and dorsal locations within the granular and glomerular layers. Results from this study suggest that in the larval lamprey: 1) the dorsal portion of the olfactory epithelium is expanding, 2) neuronal circuits enlarge in the dorsal/lateral quadrant of the OB, and 3) cells within the OB granular and glomerular layers originate from the ventricular layer.

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Poster 155

Olfactory-bulb deafferentation in the adult zebrafish, *Danio rerio*. CHRISTINE A. BYRD and STEVEN RAY, *Department of Biological Sciences, Western Michigan University, Kalamazoo, MI 49008.* christine.byrd@wmich.edu.

The influence of the olfactory organ on maintenance of olfactory-bulb structure was examined in zebrafish. This fish provides us with a model in which the olfactory organ is easily accessible for removal, the animals easily survive the surgery, and the olfactory bulbs are small enough to allow rigorous analysis of the resulting effects. We performed unilateral olfactory-organ ablations on anesthetized adult zebrafish using a small-vessel cautery iron (following protocols approved by the WMU Institutional Animal Care and Use Committee). Fish were allowed to survive for 1, 3, or 6 weeks following the procedure. Analysis of deafferented animals revealed that most, if not all, of the olfactory organ was missing on the ablated side.

The morphology of the olfactory bulb was affected notably by the removal of its primary afferent innervation. At all of the survival times the deafferented bulb appeared significantly smaller at the gross level, and our preliminary volume measurements show that there was a definite decrease in bulb size when compared to the contralateral bulb. The olfactory nerve layer was absent or diminished depending on the extent of the ablation. We are analyzing if other bulb layers also were affected. Tyrosine hydroxylase expression, as revealed by immunohistochemistry, was decreased noticeably on the ablated side. This decrease appears to be due to fewer cells expressing tyrosine hydroxylase, but we are examining this phenomenon further. In conclusion, we have shown that the olfactory organ is important in the preservation of normal olfactory-bulb anatomy and neurochemistry in adult zebrafish.

Supported by WMU and NIH-NIDCD

Poster 156

Pax-6 expression in the olfactory bulb of *Xenopus laevis* following olfactory nerve lesions. LESLIE GEE and GAIL D. BURD, *Department of Molecular and Cellular Biology, University of Arizona Tucson, AZ 85721.* FAX: (520) 621-3709

The transcription factor, Pax-6, is critical for proper nose and eye development in a wide variety of animals. In the African clawed frog, *Xenopus laevis,* Pax-6 is expressed in the olfactory bulb as well as other regions of the central nervous system and may play a role in the formation and differentiation of olfactory bulb neurons. We know from previous work that innervation of the olfactory bulb by olfactory receptor cell axons is necessary for the development of the normal number of neurons. The goal of the current experiment was to determine whether the absence of this innervation following olfactory nerve lesions results in a loss of Pax-6 expression in the developing olfactory bulb. Bilateral olfactory nerve lesions and sham lesions were performed on tadpoles during late larval development (stage 58), and the animals were allowed to survive for either 6 days or 21 days. Immunocytochemistry (using an antibody donated by R. Reed) and *in situ* hybridization were used to analyze Pax-6 protein and MRNA products, respectively. The expression patterns for the Pax-6 protein were similar in both the lesioned and control animals at both 6 and 21 days. Expression patterns of the Pax-6 MRNA also did not differ between lesion and control animals for either time period. These findings were observed in spite of the apparent decrease in neuron number in the deafferented olfactory bulbs. The results show that the olfactory nerve lesion did not affect Pax-6 expression. The lack of change in expression patterns indicate that Pax-6 is either only expressed in the stable population of fully mature neurons or Pax-6 expression in maturing neurons stops before lesion induced cell death occurs. Future studies will focus on determining the expression pattern of Pax-6 in the life cycle of olfactory bulb neurons.

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Poster 157

How does a mammalian olfactory glomerulus form? HELEN TRELOAR, ANGELA PURCELL and CHARLES GREER, *Dept. Neurosurgery, Yale University School of Medicine, New Haven, CT 06510,* Helen.Treloar@yale.edu

The very early embryonic events when olfactory receptor neurons (ORNs) first contact the presumptive olfactory bulb (OB) have been partially described (Pellier and Astic,'94[1]; Gong and Shipley,'95[2]; Treloar et al.,'96[3]), however the events occurring in later embryogenesis remain largely unexplored (Valverde et al.,'92[4]). Previous studies have shown that ORN axons are restricted to the presumptive nerve fiber layer (NFL) of the OB at E16 (Treloar et al.,'96[3]). To characterize ORN axonal growth into the developing OB and glomerular formation we have used confocal microscopy and double label immunofluoresence to examine the interactions that occur between ORN axons and mitral/tufted (M/T) cell dendrites. Double labeling of ORN axons and M/T cell dendrites was performed using GAP-43 to label immature axons and MAP-2 to label dendrites. At E17 GAP-43 staining was similar to that reported at E16. The majority of axons were restricted to the NFL which is directly apposed to a thick mesh of MAP-2 stained dendrites. A very small number of axons were observed leaving the NFL and projecting at right angles into the dense dendritic zone, forming a fringe-like projection. This fringe-like projection continued through E19, with the number of interdigitating processes increasing. M/T cell dendrites remained closely apposed to the NFL, forming a thick layer just deep to the NFL. From E20 onwards, the ORN axons in the dendritic zone were observed to coalesce into bundles of neuropil, forming glomerular-like structures. These protoglomeruli appeared fused to the NFL but showed distinct separation from neighbors. The M/T cell dendrites, however, did not similarly coalesce, rather they continued to form layer deep to the NFL and were found within and between glomeruli. In contrast to E17-E19, the dendritic zone appeared to separate into 2 distinct zones. The outermost zone (presumptive GL) is densely populated by dendritic processes and contains the protoglomeruli and a lightly populated inner zone (presumptive EPL) which extends to the mitral cell layer. In neonates, glomeruli are morphologically distinct; they have separated from the NFL and M/T cell dendritic arbors remain broad but have coalesced to a few neighboring glomeruli. These data suggest that ORN axons extend deeper into the OB and initiate glomerular formation.

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[1]Cell Tiss. Res. 275:587-598. [2]Neuron 14:91-101. [3]J. Neurobiol 31:41-55. [4]Neurosci. 49:255-275.

Poster 158

Localization of Nurr-1 and NGFI-B in the adult mouse olfactory bulb. NIAN LIU, LINDA FRANZEN, HARRIET BAKER, *Cornell Univ. Med. Coll. at the Burke Med. Res. Inst., White Plains, NY 10605.* habaker@med.cornell.edu.

Nurr-1, an orphan nuclear receptor, has been colocalized with tyrosine hydroxylase (TH) in the rodent substantia nigra (SN) (Science, Zetterstrom et al. 276:248). The loss of TH expression in the SN of Nurr-1 deficient mice indicated an important role for this receptor in TH gene regulation in SN dopaminergic neurons. TH in the periglomerular dopamine neurons of the rodent olfactory bulb displays sensory activity-dependent regulation that also might require the presence of Nurr-1. To determine if Nurr-1 is involved in TH expression in periglomerular neurons of the mouse olfactory bulb the present study examined their colocalization using *in situ* hybridization with oligonucleotide probes. Relatively weak Nurr-1 expression was found in the granule cell layer, but not in the glomerular layer. As previously reported, Nurr-1 message could be demonstrated in SN of the midbrain. The strongest labelling occurred, not in dopamine neurons, but in the deep layers of the frontal cortex. NGFI-B, a Nurr-1-related orphan nuclear receptor, also was present in the granule cell layer of the olfactory bulb. In contrast to Nurr-1, NGFI-B was absent from the SN. These data demonstrate that there is no correlation between expression of the orphan receptors, either Nurr-1 or NGFI-B, and TH in the olfactory bulb and suggest that these receptors are not involved in TH gene regulation in this brain region.

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Poster 159

Reduced immunoreactivity of low affinity receptors for nerve growth factors, $p75^{NGFR}$, in the olfactory bulbs of aged rats. KHOA D. TRAN[1], GREGORY S. SMUTZER[1,2], IGOR L. KRATSKIN[1], LLOYD HASTINGS[1], and RICHARD L. DOTY[1], [1]*Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery,* [2]*Institute for Human Gene Therapy, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.* FAX: (215) 349-5266.

It is known that olfactory loss occurs in diseases such as Alzheimer's and schizophrenia, as well as in normal aging. Neurotrophins have been demonstrated to interact with low affinity nerve growth factor (NGF) receptors $p75^{NGFR}$ and exert trophic effects on survival and regeneration of sensory neurons. In adult rat olfactory bulbs, the glomeruli normally exhibit high level of immunoreactivity to $p75^{NGFR}$ which directly mediates binding of NGF and brain-derived neurotrophic factor (BDNF). However, little is known about the expression of $p75^{NGFR}$ in olfactory bulbs of aged rats. This study addressed the question as to whether the olfactory bulb $p75^{NGFR}$ expression varies with senescence. Rats 3 months, 18 months, and 23 months of age were transcardially perfused with saline and 4% paraformaldehyde. The olfactory bulbs were removed, fixed, and paraffin-embedded. Seven-μ thick sections were immunohistochemically stained with mouse monoclonal anti-$p75^{NGFR}$ antibody using the ABC method. We found a slight decrease in $p75^{NGFR}$ immunoreactivity, which was well visualized in the glomerular layers, in the 3-month-old compared to the 18-month-old rats. However, the $p75^{NGFR}$ immunoreactivity was dramatically diminished in the 23-month-old rats. There was no evidence of increased $p75^{NGFR}$ immunoreactivity in the olfactory nerve layers with age, which would suggest neurotrophin-dependent olfactory nerve regeneration to compensate for age-related olfactory neuron loss. Our results are consistent with reduced number of mature olfactory neurons projecting to the glomeruli in aged rats.

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Poster 160

Estrogen increases GnRH content in nervus terminalis of *Xenopus laevis*. CELESTE R. WIRSIG-WIECHMANN[1] and CHARLOTTE E. LEE[2], [1]*Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 and* [2]*Division of Endocrinology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215.* celeste-wirsig@ouhsc.edu.

The nervus terminalis (NT) is a gonadotropin-releasing hormone (GnRH) containing neural plexus in the nasal cavity. Although the precise function of the NT unknown, evidence suggests that the NT may modulate olfactory function. Little is known about factors that influence GnRH production and secretion from the NT. Since estrogen is known to have effects on GnRH neurons in the hypothalamus, we hypothesized that similar regulatory mechanisms may exist for neurons of the NT. To test this hypothesis, female *Xenopus laevis* were subjected to ovariectomy only, ovariectomy plus estrogen administration, estrogen administration only or none of these procedures. Whole heads were sectioned horizontally and processed with standard immunocytochemical procedures to identify GnRH immunoreactive structures in the nasal cavity and brain. The GnRH neuronal cell bodies in the nasal cavity and brain were quantified visually, and computer assisted densitometric analysis was used to calculate GnRH fiber densities. The number of GnRH neural cell bodies did not differ significantly between treatments. However, fiber densities were significantly decreased by ovariectomy, while fiber densities were significantly increased by estrogen administration. We conclude that nervus terminalis GnRH neurons are under similar hormonal controls as those GnRH neurons in the hypothalamus. Whether estrogen influences nervus terminalis neurons directly, or indirectly as occurs in the hypothalamus, will be the subject of further study.

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Poster 161

Cloning of a gene encoding adenylate cyclase from vomeronasal organ of garter snakes. WEIMIN LIU*, DALTON WANG*, JINMING LIU#, PING CHEN* AND MIMI HALPERN# *Departments of Biochemistry* and Neuronal and Behavioral Science#, SUNY Health Science Center at Brookly, Brooklyn, New York 11203.*

We previously reported (Luo et al., 1994) that ES20-receptor binding activates phosphoinositide (PI) turnover resulting in an increase in IP3 which in turn mobilizes intracellularly stored calcium in the vomeronasal sensory epithelium of garter snakes (Wang et al., 1997). We also found (Wang et al., 1997) that the activity of adenylate cyclase (AC) in the VN organ is very sensitive to Ca^{2+} regulation. A 250 bp fragment of adenylate cyclase type VI (AC-VI) was obtained from brain cDNA of garter snake by RT-PCR with degenerate primers. The 250 bp fragments were amplified, cloned and sequenced. Both Northern blot and RNase protection assays revealed that the VNO and brain contained higher abundance of AC type VI than the main olfactory epithelium. A 3.8 kb cDNA was then cloned from the vomeronasal cDNA library of garter snakes and sequenced. The 5' cDNA was obtained by means of 5' RACE PCR and sequenced. We have sucessfully cloned a 5198-nucleotide cDNA from VNO of garter snakes containing an open reading frame encoding 1150 amino acids of AC-VI protein. The vomeronasal AC is referred as AC_{VN}. AC_{VN} shows a high degree of homology with type VI AC of rat, mouse or human. In situ hybridization with Dig-labeled cRNA demonstrated that AC_{VN} mRNA was abundant in vomeronasal organ of garter snakes.

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Poster 162

Chemosignal Transduction in the Vomeronasal Organ of Garter Snakes: Chemoattractant-receptor-mediated Phosphorylation of p35 and p20. JINMING LIU#, PING CHEN*, DALTON WANG* AND MIMI HALPERN# *Departments of Biochemistry* and Neuronal and Behavioral Science#, SUNY Health Science Center at Brooklyn, Brooklyn, New York 11203.* FAX: (718)270-3316

We previously reported (Luo et al., 1994) that the binding of chemoattractant ES20 to its receptors activates phosphoinositide (PI) turnover resulting in an increase in the level of IP_3 which in turn mobilizes intracellularly sequestered calcium in vomeronasal (VN) sensory epithelium (Wang et al., 1997). During PI turnover, both IP_3 and DAG are generated and the latter is known to activate protein kinase C which catalyzes the phosphorylation of certain protein substrates. We have investigated the phosphorylation of cytosolic proteins mediated by ES20 in the VN sensory epithelium. The binding of ES20 to its receptors resulted in phosphorylation of two cytosolic proteins having molecular masses of 35 kDa (p35) and 20 kDa (p20), and the extent of phosphorylation was increased in an ES20 concentration-dependent manner. The phosphorylation kinetics of these two proteins were rather different. The phosphorylation of p20 attained a maximum value within one minute and then decreased with time, whereas that of p35 increased gradually with no apparent decrease. These proteins served as phosphoryl acceptors from either GTP- or ATP-donors. The phosphorylated p20 protein reacted positively only with antibodies specific against phosphotyrosine residues, whereas phosphorylated p35 reacted positively with antibodies specific against either phosphoserine, phosphothreonine or phosphotyrosine residues.

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Poster 163

G protein-mediated protein phosphorylation in mouse vomeronasal organ.
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The vomeronasal organ (VNO) provides the detection and transduction machinery necessary for pheromonal signaling in terrestrial vertebrates. Studies have indicated that G proteins are involved in pheromone signal transduction pathways in VN cells. In the present study, a specific activator of G proteins, GTP-γ-S was used to investigate the presence of a G protein-mediated phosphorylation process in mouse VN cells. By using a quantitative phosphorylation assay, we observed that *in vitro* incubation of crude cell membranes prepared from female mouse VNO with 100 μM GTP-γ-S resulted in a significant increase of overall protein phosphorylation content as compared to basal levels. This process was both time and dose dependent. As a control, the cytosolic fraction of VN cells, which lacks G proteins, did not show phosphorylation changes after incubation with GTP-γ-S. Furthermore, we observed that the phosphorylation process induced by GTP-γ-S was inhibited by H-89 and bisindolylmaleimide I, selective inhibitors of PKA and PKC respectively. This result was further confirmed by using SDS-PAGE in combination with autoradiography. In addition, possible G proteins involved signal transduction pathways in VNO were investigated by using Western blot assays. Overall, the results are consistent with previous findings that activation of G proteins in VNO can increase second messenger cAMP and IP_3 levels in VN cells, and suggest that PKA and PKC are the key kinases responsible for the protein phosphorylation that may serve as the downstream process to modulate pheromonal signaling.

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Poster 164

Basal firing patterns of vomeronasal receptor neurons in the female mouse.
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Vomeronasal receptor neurons (VNRNs) detect pheromone molecules present in the lumen of the vomeronasal organ (VNO) and transduce these chemical signals into electrical events. To date, the electrophysiological properties of mouse VNRNs have been studied almost exclusively by patching on to dissociated cells. A more intact preparation would be useful for the study of spontaneous activity and for relating activity pattern to location within the VNO. In the present study, a reliable method of producing VNO slices viable for more than 5 hrs was developed and used to characterize basal firing patterns of VNRNs.

Female mice 25-53 days old were rapidly decapitated and the entire bony capsule with the VNO inside was removed. The capsule was glued to a groove carved in a paraffin block which had been previously glued to a Vibratome chuck. Cross sections of 400 μm were cut in cold, oxygenated Tyrode's solution. Selected sections were stored at room temperature in oxygenated medium before being placed into a slice chamber and superfused with Tyrode's at 32 °C. Conventional extracellular recording techniques were used to record from VNRNs. A total of 30 cells were recorded from 15 experiments. Analysis was restricted to the last 15 cells recorded on a chart paper to assess long-term fluctuations in activity. Single units were held up to 88.8 min with 6/15 cells held for > 30 min. Most cells exhibited fluctuations in firing rate, but two patterns became apparent: 1. prolonged low frequency firing (0-3.3Hz for 6.3 to 15.6 min) and 2. intermediate to prolonged bursting (6.7-19.2 Hz for 0.5-12.6 min). At least one period of bursting activity was observed in 10 cells, and 3 cells displayed more than one bursting period. Adding the periods of bursting for each of these 3 cells revealed remarkably similar total burst times (21.0, 22.4, and 21.1 min). The observed fluctuations in spontaneous activity indicate that unique testing strategies may be required to accurately assess the effects of pheromones on the firing rate of VNRNs.

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Poster 165

Expression of nitric oxide synthase, protein inhibitor of neuronal nitric oxide synthase, and guanylyl cyclase in rat vomeronasal organ.
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Nitric oxide (NO) and carbon monoxide (CO) are synthesized by nitric oxide synthase (NOS) and heme oxygenase (HO) respectively. NO and CO are thought to act as cellular messengers in many tissues via mediation by guanylyl cyclase (GC). To determine whether the mRNAs encoding NOS, HO and GC are present in the rat vomeronasal organ, tissue was obtained free of bone and cartilage from four animals and combined. Total RNA was extracted, treated with DNase I to remove any chromosomal DNA contamination, and reverse transcribed to yield cDNA. PCR amplification of cDNA was performed using primer pairs based on published sequences. Bands of the expected size were gel-isolated, subcloned into pGEM-T Easy (Promega) and the inserts sequenced. A PCR product (638 bp) for NOS mRNA was obtained and a BLAST search indicated that its sequence had the highest similarity with that of rat neuronal NOS. Recent studies in other cellular systems have identified a small protein (89 amino acids), named PIN (Protein Inhibitor of neuronal NOS), that may be an endogenous inhibitor of neuronal NOS. PCR amplification of PIN from vomeronasal tissue yielded a product (267 bp) that had 100% identity with rat brain PIN. Amplification of GC yielded a PCR product (330 bp) whose sequence had the highest similarity with that of rat brain cytoplasmic GC. No evidence was obtained for the expression of heme oxygenase in vomeronasal tissue. These results raise the possibility that nitric oxide (but perhaps not carbon monoxide) has a functional role in the rat vomeronasal organ. *In situ* hybridization experiments are in progress to determine the localization of NOS, PIN and GC within the vomeronasal organ.

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Poster 166

Induction of c-fos gene expression by 17β-estradiol in mouse vomeronasal organ.
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Estrogen has long been known to play critical roles in female reproductive functions, such as puberty acceleration, estrous cyclicity, and mating behavior. These particular functions are also modulated by the vomeronasal organ (VNO) through detection and processing of chemical cues broadly defined as pheromones. The discovery of estrogen receptors in the VNO raised the possibility that the steroid hormone may affect the activity of VN neurons. Therefore, the effect of estrogen on cellular activity in the VNO was measured by using Northern blot assays for c-fos gene expression. In vitro incubation of VN tissue with 17β-estradiol caused an increase in c-fos mRNA expression. 17β-estradiol induced a larger increase in c-fos gene expression than 17α-estradiol, which indicates that the effect is stereoselective. Furthermore the estrogen receptor antagonist ICI 182,780 only partially blocked the increase in c-fos mRNA expression in VN tissue induced by 17β-estradiol, and 17β-estradiol remained effective in VN tissue of estrogen receptor α knock- out animals. These results indicate the existence of estrogen receptor β in the VNO. This is the first report that estrogen can activate the expression of the cellular proto-oncogene in cells of the VNO. The data suggest that estrogen might be involved in the modulation of VNO function in vertebrates.

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Poster 167

Proliferation density in the rat vomeronasal organ during postnatal development. ELKE WEILER, MARY A. MCCULLOCH AND ALBERT I. FARBMAN. *Northwestern University, Dept. of Neurobiology & Physiology, Evanston, IL 60208-3520, USA.* afarbman@nwu.edu

Proliferation in the vomeronasal organ (VNO) is seen throughout life of a vertebrate. In the rat, body weight and size continuously increase during postnatal development at least up to one year. Proliferation might therefore be related to growth in VNO size and/or replacement.

We used the BrdU-method to label dividing cells in the sensory epithelium of the VNO and counted proliferating cells in 10 μm paraffin sections of male and female Sprague-Dawley rats aged P1 (birth) to P666. The number of labeled cells was counted separately in the basal, intermediate and apical compartments and the VNO divided into quadrants: the two marginal zones, where the sensory epithelium is adjacent to the non-sensory epithelium, and two central zones.

The proliferation density (number of labeled cells/section) decreases dramatically during early postnatal development, P1 (115 cells), P11 (47 cells), P21 (27 cells), P40 (12 cells), P66-P333 (10 cells), P400-666 (8.5 cells), although the area in a section increases. In addition, the distribution of the proliferating cells changes. From a nearly even distribution in the quadrants in newborns, proliferating cells become concentrated in patches at the margins in adult rats. Further, apically labeled cells (very likely supporting cell nuclei) decrease in frequency more, proportionally to the total labeled cells.

These complex changes in proliferation density, spatial and cell-type distribution suggest that in early postnatal development the proliferation is due to rapid growth. In adults, a pool of proliferating cells remains in the margins for further growth whereas the proliferating population in the central part may reflect a pool for replacement.

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Poster 168

Lectin histochemistry in the regenerating vomeronasal epithelium. JUNKO YOSHIDA-MATSUOKA[1], RICHARD M. COSTANZO[2]. MASUMI ICHIKAWA [1], *[1]Department of Anatomy and Embryology, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo 183, Japan, [2]Department of Physiology Virginia Commonwealth University, Richmond, VA 23298-0551.* jun@tmin.acjp

Receptor cells undergo continuous replacement in both the olfactory and vomeronasal systems. In a previous study we observed that transaction of the vomeronasal nerves resulted in a degeneration of the receptor cells. The number of cells decreased to a minimum during the first 6 days after nerve transaction and then increased with additional recovery time. To investigate qualitative changes among degenerating and regenerating cells, a histochemical study was done in the vomeronasal sensory epithelium using two lectins, *Bandeiraea simplicifolia lectin* I (BSL-1) and *Vicia villosa* agglutinin (VVA) after a left nerve transaction. It has been reported that BSL-1 which recognize N-acetylgalactosamine and D-galactose and VVA which recognize N-acetylgalactosamine have a high affinity for the vomeronasal organ, especially to vomeronasal receptor cells and vomeronasal axons. Male SD rats were examined at postoperative recovery times of 2,6,10,15, and 21 days. BSL-1 and VVA was observed bound to the luminal surface of the vomeronasal sensory epithelium at recovery day 2 on the control (right sensory epithelium) and operated (left sensory epithelium) sides. Similar staining patterns were observed at days 6,10,15 and 21. The present study reveals that the reactivities to BSL-1 and VVA in the vomeronasal organ were not affected by the nerve transaction procedure. These results suggest that N-acetylgalactosamine and D-galactose residues do not change in the degenerating and regenerating vomeronasal epithelium and that supporting cells may have affinities to BSL-1 and VVA.

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Poster 169

Interactions between lectin and sugar on the vomeronasal epithelium measured with the atomic force microscope. TOSHIYA OSADA[1], SHINICHIRO TAKEZAWA[1], ARIMICHI ITOH[1], HIDEO ARAKAWA[1], MASUMI ICHIKAWA[2], SAKAE KIKUYAMA[3], AND ATSUSHI IKAI[1], *[1]Department of Biological Sciences, Tokyo Institute of Technology, Yokohama, Japan, [2]Anatomy and Embryology, Tokyo Metropolitan Institute for Neuroscience,Tokyo,Japan. [3]Departmen of Biology, School of Education, Waseda University.* tosada@bio.titech.ac.jp

We have examined the distribution of the glycoconjugates on the sections of the rat vomeronasal organ with an atomic force microscopy (AFM) and also examined the adhesive force between the sugar chain and its specific lectin. AFM gives physical properties of the sample surface as well as its topography in liquid by measuring interactions between the scanning probe and sample surface. AFM tips were modified with lectin, vicia villosa agglutinin(VVA) which recognizes N-Acetyl-D-galactosamine(GalNAc). FITC labeled VVA was reported to stain the luminal surface of vomeronasal sensory epithelium. When the modified tip scan the luminal surface, the adhesive forces were observed. The result of force mapping indicated that GalNAc was enriched in apical one-third and basal one-third of microvilli. The adhesion force was inhibited by adding GalNAc to the scanning solution. The adhesion force was not observed when the tip scanned on the neuronal layer on the section where no FITC-lectin staining was observed. These results suggest that the adhesion force observed here is related to the binding force between the sugar and the lectin. This method may be useful for examining the interaction between pheromone and its receptor and the distribution of the receptors on the luminal surface.

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Poster 170

The effects of nicotine receptor blockers on trigeminal nerve responses to nicotine. HESSAMEDIN ALIMOHAMMADI, WAYNE L. SILVER, *Department of Biology, Wake Forest University, Winston-Salem, NC 27109.* FAX: (336) 758-6008.

In order to learn about the mechanisms involved in trigeminal chemoreception and to determine which specific receptors may be involved, we are studying the effects of nicotinic receptor (nAChR) blockers on trigeminal nerve responses to nicotine. The peripheral receptors of the trigeminal system in the nasal cavity are presumed to be free nerve endings arising from Aδ and C fibers. These fibers appear to be scattered throughout the respiratory epithelium and arise from the nasopalatine and ethmoid branches of the trigeminal nerve.

We obtained multiunit neural recordings from the ethmoid nerve of Sprague-Dawley rats in response to nicotine. Vapor phase nicotine (12.5 ppm) was delivered to the rats' nares via an air-dilution olfactometer. The magnitude of the response to nicotine decreased after the administration of the nAChR blockers dihydro-β-erythroidine hydrobromide (DHBE) and mecamylamine hydrochloride. DHBE is a competitive nicotinic receptor antagonist specific for the $\alpha4\beta2$ receptor subtype and mecamylamine is known to be specific for the $\alpha3\beta4$ and $\alpha4\beta2$ receptor subtypes.

This research sheds more light on the mechanism of trigeminal stimulation by demonstrating a causal link between possible receptor proteins and the perception of a known trigeminal stimulant. We are also studying other trigeminal stimulants in addition to nicotine in order to increase our understanding of the mechanisms involved in trigeminal stimulation and the perception of irritation.

Poster 171

Discrimination of R- and S-nicotine by the trigeminal nerve. B. RENNER[1], F. MEINDORFNER[1], M. KAEGLER[2], N. THUERAUF[3], A. BAROCKA[3] AND G. KOBAL[1], [1] *Institute of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, D-91054 Erlangen, Germany,* [2] *Institute for Biological Research, D-51149 Cologne, Germany,* [3] *Department of Psychiatry, University of Erlangen-Nuremberg, D-91054 Erlangen, Germany.* FAX: +49 9131/85 6898.

The aim of this study was to investigate whether nasal trigeminal receptors are able to discriminate between nicotine stereoisomers.

Twenty male Wistar rats (weight 300 - 500 g) were anaesthetised with urethane and artificially ventilated. Stimuli of either S- or R-nicotine vapour (stimulus duration 1350 ms) were delivered to the nasal mucosa using an apparatus that allows specific stimulation without concomitant thermal or mechanical alteration of local conditions. This was achieved by embedding the nicotine stimuli in a stream of humidified nitrogen (relative humidity 80%) of constant flow and temperature (500 ml/min and 36°C, respectively). In order to obtain extracellular single cell recordings from the ipsilateral Gasserian ganglion, the right hemisphere of the brain was removed. Stimuli of R- and S-nicotine were applied randomly at different concentrations. Gaseous CO_2 was used as a control stimulus of trigeminal receptors (concentrations of 20, 40, 60, 80, 100% v/v). In an additional experiment hexamethonium was topically administered to the nasal mucosa before stimulation with S-nicotine or CO_2.

Trigeminal responses were clearly different for the nicotine enantiomers. Specifically, the threshold for R-nicotine was significantly higher than that for S-nicotine. Furthermore, the responses to S-nicotine but not to CO_2 were suppressed by hexamethonium indicating the involvement of acetylcholine receptors in the peripheral processing of nociception.

In conclusion, by means of single cell recordings from the Gasserian ganglion, we were able to demonstrate that nasal trigeminal receptors can distinguish between nicotine enantiomers.

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Poster 172

Isolation and neurophysiological characterization of a sensory irritant compound from *Zanthoxylum*. IGOR MEZINE and BRUCE BRYANT. *Monell Chemical Senses Center, Philadelphia, PA 19104.* FAX: (215) 898-2084.

Chemically-induced sensory irritation arises from the excitation of subsets of sensory neurons and pathways, not all of which have been identified, much less characterized. We have isolated and confirmed the structure of a major pungent principle (N-(2'-methyl-2'-hydroxypropyl)-dodeca-2E,6Z,8E,10E-tetraenamide; hydroxy-α-sanshool (HOαS)) from the fruit of *Zanthoxylum* spp. Unlike capsaicin (CAP), which gives rise to sensations ranging from warming to burning and pain, this compound elicited distinctly different sensations described as tingling and buzzing. Extracellular single-unit trigeminal nerve recordings from rats indicate that HOαS activated previously silent high threshold receptors (nociceptors), and provoked increases in ongoing activity in low threshold thermoreceptive and low-threshold tactile neurons. In addition, HOαS sensitized these latter tactile neurons to become sensitive to cooling, and cool receptive neurons to become sensitive to mechanical stimuli. Moreover, treatment with HOαS also induced sensitivity to noxious cooling (8°C). The all-*trans* isomer of HO-α-S and its saturated analogue were inactive in sensory and neural assays. This suggests, unlike the case of CAP, that the presence of a conjugated system of double bonds in a certain configuration is required for activity. Together these findings suggest that other, important non-CAP mechanism(s) exist in sensory neurons and may play an important role in chemesthesis.

Poster 173

Responses of CO_2-sensitive trigeminal primary afferents of the nasal cavity recorded from the gasserian ganglion in the rat. N. THUERAUF[2], B. RENNER[1], A. BAROCKA[2] and G. KOBAL[1], [1]*Institute of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nürnberg, Erlangen, Germany, 91054,* [2]*Department of Psychiatry, University of Erlangen-Nürnberg, Erlangen, Germany, 91054.* snthuerauf@aol.com.

We developed a method to record from trigeminal primary afferents of the Gasserian ganglion in the rat. Using the trigeminal irritant CO_2 a cellband of CO_2-sensitive cells of 3x1 mm dimension could be located in the Gasserian ganglion. For chemical stimulation a Kobal-olfactometer was used allowing the stimulation of the nasal cavity without simultaneous activation of mechano- or thermosensors in the nasal mucosa. In order to characterize the type of primary afferent stimulated by CO_2 we (1) measured the conductance velocity following electrical stimulation, (2) tested the response to mechanical stimulation, (3) the responses following application of capsaicin and mustard oil into the nasal cavity, and (4) calculated the maximum peak frequency for responses elicited by CO_2. We investigated the influence of stimulus concentration (20, 40, 60, 80, 100 v/v $C0_2$; stimulus duration: 1s) and stimulus duration (1, 2, 3, 4 s; stimulus concentration: 80 v/v CO_2) on CO_2-sensitive primary afferents using pseudorandomized sequences of stimuli. The interstimulus interval was 120 s for both stimulus sequences. Our experiments revealed that short and non-repetetive CO_2-stimuli activate exclusively primary afferents with conductance velocities in the range of ~8-22 m, peak frequencies in the range of 300 to 900 Hz. The CO_2-sensitive primary afferents responded to mechanical stimulation and to chemical stimulation with capsaicin and mustard oil. These data are in accordance with the selective activation of A-delta-fibers by short and non-repetetive CO_2-stimuli.

Poster 174

Role of biotin-binding proteins in *Paramecium* chemoresponse. WADE E. BELL and JUDITH L. VAN HOUTEN, *Dept. of Biology, Univ. of Vermont, Burlington, VT 05405.* wbell@zoo.uvm.edu.

Paramecium tetraurelia are attracted to a number of bacterial metabolites allowing for localization of bacterial populations on which to feed. We have shown that biotin is a strong attractant of *P.tetraurelia* in behavioral assays. Whole cell recordings of *P. tetraurelia* exposed to biotin show a hyperpolarization consistent with a positive chemoresponse. Radiolabelled binding assays using displacement of ^{3}H-biotin from whole cells with biotin indicate that a saturable and specific binding component exists consistent with that of a biotin receptor. The K_d value for biotin displacement, 0.4 mM, is in agreement with the value for half-maximal behavioral and electrophysiological response of 0.3mM. Diaminobiotin, a structural analog that lacks the keto group in the ureido ring, displaces ^{3}H-biotin with the same kinetics as the parent compound, but this analog does not act as an attractant. Diaminobiotin also interferes with the behavioral response to biotin suggesting it acts as an antagonist.

Behavioral assays using antisera raised against *P. tetraurelia* cell surface proteins, freed by activation of an endogenous phospholipase-C, indicate that a GPI anchored protein may play a role in biotin chemoresponse. Exposure of cells to the antisera for 30 minutes prior to the assay significantly reduced chemoresponse to biotin. The response of cells treated with antibody to the attractant ammonia was unaffected. Affinity chromatography employing both phospholipase cleaved and Triton X-114 permeablized proteins is being conducted to identify candidates for a putative biotin chemoreceptor.

Poster 175

Calmodulin binding domain of the calcium pump: role in chemoresponse in paramecium. JUNJI YANO, VILLA RAKOCHY, JUDITH L. VAN HOUTEN, Department of Biology, University of Vermont, Burlington, VT 05405, jyano@zoo.uvm.edu.

We have correlative evidence for participation of a calmodulin-regulated calcium pump in two chemoresponse pathways in Paramecium. To test indirectly for the role of the calcium pump, we have down-regulated calmodulin using antisense technology and found that attraction to glutamate and acetate is affected. Glutamate and acetate are representative stimuli of 2 different chemosensory transduction pathways, which we believe utilize the calcium pump. Chemoresponse to NH_4Cl is not affected by calmodulin down-regulation. NH_4Cl is a stimulus of a 3rd pathway, which we predict does not involve the calcium pump. To more directly test for pump function, we have sub-cloned the auto-inhibitory domain of the pump. This domain inhibits the pump unless it is phosphorylated or bound with calmodulin, hence its name "calmodulin-binding-domain" (CBD). The CBD has been cloned into an expression vector, and the vector is microinjected into paramecia for paromomycin selection. Homogenates of transformed cells are examined on Western blots to determine whether the CBD (»10 kD) is expressed before they are used in assays of chemoresponse swimming behavior. The transformed cells show altered speed and frequency of turning in both glutamate and acetate stimuli and, as predicted, show normal motility in NH_4Cl. T-maze assays are consistent with abnormal chemoresponse of transformed cells in glutamate, but attraction to acetate is variable, but often in normal ranges. In order to better examine and quantify the over-expression of CBD, we have added 3 myc tags in order to detect it in homogenates more effectively. Additionally, we will examine myc tagged mutant forms of CBD.

We have generated 2 mutant forms of CBD, with the two serines of the PKA phosphorylation site changed to 2 alanines or to 2 glutamates. The ala mutant CBD binds calmodulin, but the glutamate mutant does not, as expected. We are now poised to use the mutant forms in over-expression in Paramecium.

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Neurotrophin effects on neurite extension from cultured explants of embryonic rat geniculate, petrosal and trigeminal ganglia. C. M. MISTRETTA[1], Z. XU[1] and D. K. MACCALLUM[2], *School of Dentistry[1] and Medical School[2], University of Michigan, Ann Arbor, MI 48109.* chmist@c.imap.itd.umich.edu.

To establish neural connections in the taste system, neurites must first grow from appropriate sensory ganglia to gustatory papillae on the developing tongue. Among the molecules that influence ganglion cell differentiation and neurite extension are the neurotrophins. However, neurotrophin roles and specificity of action have not been determined for the ganglia that innervate the tongue, the geniculate, trigeminal and petrosal, during the period of target innervation. We compared effects of brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin–4 (NT4) and neurotrophin-3 (NT3) on neurite extension from geniculate, trigeminal and petrosal ganglia in rat embryos at 16 days of gestation. At day 16 gustatory papillae are well developed and extensively innervated, but not morphologically mature. Pregnant rat dams were anesthetized, embryos were removed and ganglia dissected. Ganglion explants were placed on a bovine corneal endothelial cell matrix that had been previously deposited on glass coverslips. Ganglia were cultured in DMEM/F12 plus 1% fetal bovine serum and 2% B-27 supplement (Gibco), with and without exogenous BDNF (1, 10, 50 and 100 ng/ml), NGF (10, 50 ng/ml), NT4 (10, 50 ng/ml) and NT3 (10, 50 ng/ml), all from Alomone Labs. After 30 hours, cultures were fixed and immunoreacted with antibodies to neurofilaments or PGP 9.5. Based on numbers of neurites, explants were scored as having none, few, some, or extensive outgrowth. For both geniculate and petrosal ganglia, order of neurotrophin effectiveness was BDNF≥NT4>NT3>NGF. Although quantitative effects of BDNF and NT4 were similar, neurite outgrowth was distinctly fasciculated in the presence of NT4. NGF elicited little outgrowth from the geniculate ganglion. In contrast, for the trigeminal, NGF>NT3>NT4>BDNF. Addition of the tyrosine receptor kinase inhibitor, K252a, or a neutralizing antibody to BDNF, blocked neurite outgrowth from ganglia. Results indicate a clear difference in neurotrophin dependence for neurite outgrowth from ganglia that will innervate taste buds (geniculate and petrosal) versus the trigeminal ganglion, which will innervate taste papillae, but not taste buds.

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Early molecular events in gustatory papilla development are independent of nerve fibers. CHRISTOPHER A. NOSRAT, DON K. MACCALLUM, CHARLOTTE M. MISTRETTA. *Department of Neuroscience, Karolinska Institute, S-171 77, Stockholm, Sweden and Schools of Medicine and Dentistry, University of Michigan, Ann Arbor, MI 48109.*

Recent data suggest that the local epithelium, from which taste buds develop, is prespecialized to produce brain derived neurotrophic factor (BDNF) prior to the arrival of gustatory nerve fibers. Whether this prespecialization is due to the presence of other nerve fibers in the target area or is an intrinsic programming of the local epithelium has not been resolved. We used an in vitro model that eliminates the influence from nerve fibers on lingual and gustatory epithelium, and studied BDNF mRNA expression in developing fungiform papillae.

Rat tongues at gestational days 13 and 14 were dissected from anesthetized embryos and maintained in organ culture. At embryonic day 13 in vivo the rat tongue first appears as a series of tissue swellings and no papillae have formed. At day 14, gustatory papillae have just begun to form. After 2, 3 or 6 days in culture, in situ hybridization was used to determine whether BDNF mRNA was expressed in restricted tongue regions, in the absence of intact sensory innervation. Whether tongue cultures were begun at day 13 or 14, after 2 and 3 days in culture fungiform papillae developed, as reported previously, and the superior surface epithelium of the papillae contained BDNF mRNA labeling. However, in 6 day cultures, fungiform papillae were not well maintained and labeling was less distinct and more widespread in the lingual epithelium.

In conclusion, we suggest that initial BDNF mRNA expression in the epithelium of taste papillae is not dependent on intact sensory innervation. However, both sustained morphogenesis of papillae and continued localization of BDNF mRNA to gustatory epithelium presumably require innervation. Therefore, we propose that sensory innervation is important in continuing to maintain the early morphogenic and molecular phenotypes of fungiform papillae.

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The temporal-spatial developmental pattern of olfactory neurogenesis is influenced by the ecdysteroid hormones during metamorphosis in the moth *Manduca sexta*. R. G. VOGT[1], and M.-D. FRANCO[1,2], [1]*Dept. Biological Sciences, Univ. of South Carolina, Columbia, SC 29208,* [2]*Dept. Molec. Cell Biology, Univ. of Arizona, Tucson AZ 85721.*

We are studying the action of steroid hormones on the regulation of olfactory neurogenesis during metamorphosis. The process of metamorphosis changes an animal from a non-reproductive juvenile to a reproductive adult. Classically studied in amphibians and insects (esp. lepidoptera), metamorphosis is an extreme example of sexual maturation. Among the most studied olfactory systems undergoing metamorphosis is the adult antenna of the sphinx moth *Manduca sexta*. A classic example of postembryonic development, the adult antenna derives from imaginal disc tissue of the larva (Sanes & Hildebrand, 1976[a]); the disc extends to the adult size at pupation, and forms into the adult antenna during the ~18 day pupal period (adult development). Ecdysteroids regulate many developmental events in *M. sexta*; during adult development hormone levels rise early and fall late. The cells of an olfactory sensillum include several sensory neurons plus three supporting cells; all are thought to derive from a sensory mother cell (SMC) by division soon after pupation.

Our studies revealed two periods of DNA replication during early adult development, based on BrdU incorporation. During the first three days, neurogenesis occurs in a wave progressing inward from the proximal and distal edges of each of 80 repeat annuli (segments). Following this period DNA replication occurs again, corresponding to endoreplicative events in specific classes of sensilla. Culturing neurogenic tissue in the presence of ecdysteroid hormone advances the neurogenic pattern suggesting endogenously rising levels of hormone are involved in the temporal regulation of this process. Expression analysis of ecdysteroid receptors support this role. Ecdysteroids coordinate birth of olfactory neurons (this study), as well as development of their glial targets (Oland & Tolbert 1996[b]), supporting the view that the formation olfactory circuits are under significant control of the primary neurons.

([a]*Dev. Biol.*51,282; [b]*J. Neurobiology* 30,92.)

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Patterns of expression of the GDNF receptor complex support different signalling mechanisms in the olfactory neuroepithelium and bulb. MICHAEL E. BUCKLAND AND ANNE M. CUNNINGHAM. *Neurobiology Program, Garvan Institute of Medical Research, St Vincent's Hospital, Darlinghurst, NSW 2010, AUSTRALIA.* FAX: 61 2 92958281.

Glial cell line-derived neurotrophic factor (GDNF) is a potent trophic agent for midbrain dopaminergic neurones, spinal motoneurones and other neuronal populations. GDNF signals through a receptor complex comprised of the product of the c-ret protooncogene (RET) and a GPI-linked co-receptor GDNF-α. Recently, a second co-receptor protein GDNF-β was described in the nervous system and peripheral tissues (Widenfalk et al. 1997). Our study aimed to colocalize the members of the GDNF receptor complex in the olfactory neuroepithelium (ON) and olfactory bulb. Using triple labelling immunofluorescent confocal microscopy we examined the expression of GDNF, GDNFR-α and RET in the rat ON and found mature olfactory receptor neurons (ORNs) expressed all three receptor components. In olfactory bulb, GDNF immunoreactivity was present in mitral and tufted cells, the glomeruli and some periglomerular and granule cells. GDNFR-α distribution was similar with most marked localization to the olfactory nerve fibres and the periglomerular cells. In contrast, RET was avidly expressed by fibres in all layers but most densely in the granule cell and internal plexiform layers.

Our results suggest that ORNs are GDNF responsive and this is likely due to autocrine production. In addition, as ORNs coexpress all three components of the receptor complex, it is likely that GDNF signalling occurs via GDNF-α and RET. In contrast, in olfactory bulb, GDNFR-α is expressed at sites significantly distant from RET immunoreactive fibres, an observation also reported in other CNS regions (Nosrat et al. 1997; Trupp et al. 1997), implicating other RET and GDNFR-α related molecules at these sites. GDNFR-β is an obvious candidate as the accessory co-receptor protein and this would be consistent with the recent report of GDNFR-β mRNA in the mitral cell and internal plexiform layers of the bulb (Widenfalk et al, 1997). Novel RET-related molecules have not yet been identified. Our findings firmly implicate the GDNF family of ligands and receptor components with roles in the olfactory system and provide further evidence for multiple mechanisms of GDNF trophism in different neuronal populations.

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Widenfalk et al. (1997) *J. Neurosci.* 17: 8506-8519.
Nosrat et al. (1997) *Exp. Brain Res.* 115: 410-412.
Trupp et al. (1997) *J. Neurosci.* 17: 3554-3567.

Poster 183

Gustatory function following third molar extraction. D.M. SHAFER[1], M.E. FISCHER[2], J.F. GENT[3], M.E. FRANK[3], *Depts. of [1]Oral Maxillofacial Surgery, [2]Orthodontics and [3]BioStructure and Function, UConn Health Center, Farmington, CT 06030.* dshafer@nso.uchc.edu.

The extraction of third molars is a common surgical procedure. One risk associated with third molar extraction (TME) is damage to peripheral somatosensory and chemosensory nerves. Previous data suggest taste function is affected (Mott et al., 1994). The purpose of the present study was to assess the frequency, location, severity, and time course of sensory changes following TME. Seventeen patients (6 males, 11 females; mean age 21.2 yrs. ± 3.7 sd) who had all four third molars extracted participated in the study. Psychophysical tests performed included whole mouth and spatial tests of taste function, and tests of somatosensory function. Tests were administered before surgery and at 1 and 6 mos. post surgery. At 1 mo. post TME perceived intensities of the 3 strongest solutions sampled in the whole mouth test were reduced, on average, by 10% for NaCl (0.1 - 1.0 M), citric acid (3.2 mM - 0.032 M), and quinine.HCl (0.1 - 1.0 mM), but were unaffected for sucrose. On spatial testing (highest concentration from the whole mouth test) ratings for the anterior and posterior lateral surfaces of the tongue and soft palate were also reduced ($p < 0.05$). Tactile spatial acuity thresholds measured with J.V.P. Domes on the anterior surface of the tongue were increased ($p < 0.09$), but pressure detection thresholds measured with monofilaments were unaffected. By 6 mo. post TME, perceived whole-mouth intensity recovered for NaCl and quinine.HCl, but not for citric acid. Perceived taste intensity for restricted lingual areas and somatosensory spatial acuity did not recover by 6 mos. either. In conclusion, the results are consistent with a partial sensory loss with incomplete recovery at 6 mo. post TME.

Reference. Mott AE, Shafer DM, Miller D, Banki M, Norton L. (1994) Chem Senses 19:526-527.

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Poster 184

Sweet taste and dietary intake in normal pregnancy and pregnancy complicated by Gestational Diabetes Mellitus (GDM). BEVERLY J. TEPPER[1], ANNIE C. SELDNER[1], LONE C. STEINMANN[1], LOUIS B. AMOROSA[2], *Dept. of Food Science, Rutgers University, New Brunswick, NJ 08901 and Dept. of Medicine, Robert Wood Johnson University Hospital, New Brunswick, NJ 08903.* tepper@taste-test.rutgers.edu.

Changes in sweet taste are well known in Type 1 and Type 2 diabetes, but no data exist in women with gestational diabetes mellitus (GDM). The purpose of this study was to document changes in sweet taste and dietary intake associated with GDM that are distinct from those of normal pregnancy and to determine whether these changes persist after delivery. Subjects were 25 pregnant women recently diagnosed with GDM, 30 pregnant women without GDM and 12 non-pregnant controls. Pregnant women were tested at 28-32 wk gestational age and re-tested at 3 mo post partum. Subjects evaluated strawberry milks varying in sucrose (0%-10%) and fat content (0%-10%) and glucose solutions (0.01M-0.16M). They also completed a 1-d dietary recall and a food-frequency questionnaire. Women with GDM showed no change in liking for the strawberry milks at either test session. Pregnant women without GDM showed a decrease in liking for the sweetest milk samples that returned to control levels at post-partum ($p \leq 0.01$). Neither pregnancy nor GDM had reliable effects on mean ratings of the glucose solutions. However, plasma glucose concentrations in women with GDM were correlated with increased liking for the glucose solutions ($r=+0.67$; $p \leq 0.001$) and also higher consumption of fruit and fruit juices ($r=+0.46$; $p \leq 0.02$). These results suggest that GDM is associated with a sustained preference for sweet taste late in pregnancy. These data have important implications for the dietary management of GDM since elevated sweet preference could interfere with efforts to achieve glycemic control and increase vulnerability to excess weight gain from sweet, high-calorie foods.

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Poster 185

Gustatory function in patients complaining of oral burning. B.K. FORMAKER, J.F. GENT, & M.E. FRANK, *Dept. of BioStructure & Function, The Univ. of Conn. Health Center, Farmington, CT 06030.* brad@neuron.uchc.edu

We examined the results of our whole mouth taste test in a group of 60 Taste and Smell Clinic (TASC) patients (46 females, 14 males; mean age ± SE: 56 ± 2 yrs.) whose presenting chemosensory symptoms included oral burning. Compared to 52 age- and sex-comparable controls, patient intensity responses were significantly lower for 0.32 and 1.0 M sucrose (20 and 10%, respectively; $p < .01$). No group differences were found for the NaCl, citric acid or quinine.HCl (QHCl) concentration series.

Because burning mouth syndrome is an oral pain disorder that primarily affects post-menopausal women, we partitioned the data into separate analyses for women and men. Among the women, intensity ratings for 0.1, 0.32 and 1.0 M sucrose were reliably lower for patients than controls (18, 27 and 16%, respectively; $p < .05$). Interestingly, intensity ratings for 0.32 and 1.0 M NaCl were also less for women patients than controls (13 and 9%, respectively). No group differences were evident among the men.

In addition, 41 of the 60 patients were asked if they had active oral burn at the time of testing (Formaker et al., 1997). Average intensity ratings for 0.32 and 1.0 M sucrose were lower (28% and 16%, respectively) in 33 patients with active oral burn compared to 8 without burn ($p < .05$).

These whole mouth taste test results imply that gustatory function is compromised in women complaining of oral burning. Intensity perception deficits were significant for sweet and salty stimuli while sour and bitter stimuli were unaffected.

Reference: Formaker BK, Mott AE & Frank ME. (1997). Chem Senses, 22: 681.

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Poster 186

Human salt-sensitivity: Diagnosis by sensory indices. RICHARD D. MATTES, *Department of Foods and Nutrition, Purdue University, W. Lafayette, IN 47907.* mattesr@cfs.purdue.edu.

Individuals whose blood pressure is responsive to manipulations of dietary sodium are termed "salt-sensitive" (SS). This project aimed to identify sensory-based, rapid screening indices of SS since traditional methods (i.e., 5mmHg change of mean arterial blood pressure following one week of low salt intake versus one week of high salt intake) are not practical for mass screening. Sixty-five individuals were tested for NaCl intensity scaling and hedonics, susceptibility of salt taste suppression by amiloride, resting and stimulated salivary flow and sodium content, natriuretic response to a saline challenge and insulin resistance. Initial findings indicate that only 54% of subjects were classified reliably. Among those, SS subjects rated the taste intensity of 0.2M Nacl following pretreatment with 0.5mM amiloride as a 5.00±1.21 (9-pt scale) whereas SI subjects rated it as a 2.75±0.59 after. Values for the 0.7M NaCl after the same pretreatment were 6.38±0.90 versus 3.87±0.69 for SS and SI subjects, respectively. Resting and stimulated salivary sodium were 6.87±1.64mM/l and 5.55±0.59 mM/l for SS individuals and 3.60±1.20 mM/l and 7.02±1.02 mM/l for SI individuals. Over the first hour after the salt challenge, SS individuals have elevated (12-15%) osmolality values compared to SI individuals. However, over the next 40 minutes, the SS individuals excrete urine lower (6-10%) in osmolality than the SI individuals. Baseline mean±SE plasma glucose levels in the SS and SI subjects were (1.22.0±0.51 versus 0.63±0.25 ng/ml). Peak insulin values are higher among the SS (3.58+0.63 versus 2.08+0.27 ng/ml) yet plasma glucose levels are comparable suggestive of heightened insulin resistance. Analyses are now underway to determine the predictive value of these group differences.

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Poster 187

A new easy, portable test for the screening of gustatory function: „Tasties". G. AHNE[1], A. ERRAS[1], T. HUMMEL[2], AND G. KOBAL[1], *[1] Institute of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, D-91054 Erlangen, Germany, [2]Department of Otorhinolaryngology, University of Dresden, D-01307 Dresden, Germany.* FAX: +49 9131/85 6898.

The aim of this study was to develop a simple test for screening of gustatory function in a clinical setting.

The substances sucrose (sweet taste), citric acid (sour), sodium chloride (salty), and caffeine (bitter) were presented as tablets (diameter 4 mm) similar to common sweetener tablets. This approach avoids disadvantages of aqueous taste solutions e.g. short shelf life or microbial contamination. For quantitative assessment of gustatory function 6 different dosages (dilutions in 50 % steps) of each taste substance were used whereby the highest dosage could be easily detected (sucrose: 30 mg, citric acid: 3 mg, sodium chloride: 2 mg, caffeine: 2 mg) and the lowest was within the range of threshholds.

We tested 101 healthy volunteers (44 male, 57 female; mean age 47 years). Twenty-eight tablets (6 different dosages of the 4 basic tastes plus 4 placebos) were placed on the subjects tongue in a randomised order; the interval was approximately 30 sec. A forced choice task was used for taste identification. The whole test took approximately 15-20 minutes. In order to evaluate the within-subject test-retest reliability, sessions were repeated after 1 week. Results were compared with those obtained by means of a conventional three drop forced choice procedure (ascending method).

The results of the new gustatory test were significantly correlated with the those obtained with the three drop forced choice procedure.

In conclusion, this new test is easy to use, can be self-administered, requires little time and has a long shelf-life. It appears to be suited for routine clinical assessment of gustatory function.

Poster 188

Gustatory event-related potentials in healthy controls and patients with hypogeusia or ageusia. [1,2]ALEXANDRA GENOW, [1,3]Thomas Hummel, [1]HANS KROGER, [1]RITU BAGLA, [1]DOUGLAS C. BIGELOW *[1]Smell & Taste, University of Pennsylvania, Philadelphia, USA; [2]University of Erlangen Medical School, Germany; [3]Dept. of ORL, University of Dresden, Germany.* hummeltc@compuserve.com

Gustatory event-related potentials (ERPs) can be recorded in response to stimulation of the tongue with the vapor phase of acetic acid. This study investigated these responses regarding gender-related differences, their relation to stimulus intensity and psychophysical measures of taste function, and the test-retest reliability.

Sixteen healthy volunteers (9 f, 7 m, mean age 24 years) participated in two sessions separated by at least one day. As established by a pipette test of each tongue quadrant all subjects had normal gustatory function. To record ERPs, vapor phase acetic acid was brought onto the left or right anterior portion of the tongue; stimuli were embedded in a constantly flowing airstream (70 and 100 % v/v acetic acid; flow 8 L/min; stimulus duration 250 ms, interval 24 s). Amplitudes and latencies of ERP peaks P1, N1, P2, and P3 were measured. Subjects rated the stimulus intensity using visual analogue scales. Lateralized sour thresholds (citric acid; ascending limits; anterior third of tongue) were established by means of a double forced-choice filter-paper method.

Amplitudes P1 and N1 were largest at fronto-central recording sites while amplitudes P2 and P3 had a more parietal distribution. Women had significantly larger P1N1 amplitudes and shorter latencies P1, N1, and P2 than men ($F[1,10]>5.31$, $p<0.045$). Intensity-related differences were found for amplitudes P3, and N1P2, and for latencies P1, and N1 ($F[1,10]>5.17$, $p<0.047$). The subjects' ratings also differenciated between intensities; other than with ERPs no gender-related differences were found. None of the measures demonstrated differences between the left and right tongue. Test-retest reliability was highest for the higher stimulus concentration, and, as a rule, highest coefficients of correlation were found for latencies of ERP peaks P1 and N1 ($.62<r<.75$). Preliminary investigations in patients with hypogeusia or ageusia indicated the potential usefulness of gustatory ERPs in the diagnostic process, especially with regard to medico-legal cases.

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Poster 189

Effect of medications used by HIV-infected patients on the sense of taste. SUSAN S. SCHIFFMAN, MARK S. SUGGS, JENNIFER ZERVAKIS, ALISON E. HEALD. *Departments of Psychiatry and Medicine. Duke University Medical School. Durham, NC 27710.* FAX: (919) 684-8449.

Taste dysfunction has been reported in HIV affected individuals but the cause of this dysfunction is not well understood. Data from previous studies suggest that taste disorders worsen with the number of medications taken and with progression of the disease. Statistical analysis of 2,801 patients with HIV-1 infection found that patients with AIDS used an average of 7.1 drugs per month (Fogelman et al., J. Acquir Immune Defic. Syndr. 7:1057-1063;1994; 14% of these patients used more than 10 concomitant medications. Patients with AIDS-related complex (ARC) or who were asymptomatic used 3.1 and 2.7 per month, respectively. Antiretroviral drugs, protease inhibitors, and drugs for Pneumocystis pneumonia were evaluated in humans and a gerbil model for their effect on taste responses. Drugs were applied topically to the tongue to assess their effect on taste perception at the periphery. Extensive research has shown that drugs are secreted into the saliva, and salivary concentrations of many drugs are high enough to exert adverse effects on taste sensations either by modifying taste transduction mechanisms or by producing a taste of their own. In humans, zidovudine (AZT) decreased salty ratings of KCl and the burning sensations of capsaicin. Lamivudine decreased intensity ratings for KCl, salty ratings of $CaCl_2$, intensity, hot, and burning ratings for capsaicin, and intensity ratings for WS-3 (a menthol-like compound). Didanosine (DDI) decreased salty ratings for KCl, intensity ratings for $CaCl_2$, and burning ratings for WS-3. Saquinavir (Invirase) decreased intensity and salty ratings for NaCl and increased intensity ratings for citric acid and capsaicin. Indinavir (Crixivan) decreased intensity ratings for citric acid, intensity and metallic ratings for $FeSO_4$, and cooling ratings for WS-3. Trimethoprim had no effect on taste. Responses from gerbils had similarities and differences with human psychophysical results.

Poster **190**

The anterior temporal lobe and gustatory processing in humans. DANA SMALL, MARILYN JONES-GOTMAN, ROBERT ZATORRE, and MICHAEL PETRIDES, *Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4.* FAX: 514-398-1388.

The anterior temporal lobe (ATL) has been implicated in several aspects of gustatory processing including stimulus recognition, conditioned taste aversion learning, and hedonic analysis (Small et al., 1997, Yamamoto et al., 1994; Scott et al., 1993). To further elucidate the role of the ATL in gustatory processing in humans, patients undergoing either a right or a left ATL resection for the treatment of intractable epilepsy performed intensity estimations of 6 concentrations each of sucrose and quinine, as well as 6 saturations of grey, which served as a control. The task involved a memory component in that Ss were told to estimate intensity in relation to previously presented stimuli. Intensity estimates made by the patient groups were compared to estimates made by a group of healthy control subjects. The left ATL group did not differ from the control group on any measure. In contrast, the correlations between intensity estimates and concentration were significantly lower for the right ATL compared to the control group. The right ATL group also showed a significantly steeper slope compared to the control group for quinine but not for sucrose, suggesting that the right ATL patients perceived the quinine to be more intense than control subjects. These results are consistent with our previous study in suggesting asymmetrical involvement favoring the right ATL in gustatory processing (Small et al., 1997). Furthermore, they suggest that this region may play a role in taste learning and memory for hedonically significant tastes in humans.

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Scott et al., (1993). *J Neurophysiol,* **56,** 1810-1820.
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Yamamot o et al.,(1994). *Behav Brain Res,* **65,** 689-700.

Poster **191**

Odor identification as a function of retrieval support in normal aging and early Alzheimer's disease. MARIA LARSSON[1,2], HELÉNE SEMB3, BENGT WINBLAD[1,2], KAARINA AMBERLA[2], AND LARS-OLOF WAHLUND[2] *[1] Section of Psychology, Stockholm Gerontology Research Center, and [2] Department of Clinical Neuroscience and Family Medicine, Division of Geriatric Medicine, Karolinska Institute, [3]Psychiatric Department, Danderyd Hospital, Sweden.* maria.larsson@cnsf.ki.se.

Olfactory sensitivity and odor identification were examined in persons in an early stage of Alzheimer's disease (AD). The main aims were to investigate (a) whether mild AD is associated with a lower olfactory sensitivity, (b) odor identification performance as a function of retrieval support, and (c) the relationships between global cognitive functioning (MMSE scores), and performance in the olfactory tests. The results indicated that AD patients had an intact olfactory sensitivity, but performed poorer in the odor identification test as compared with age-matched healthy controls. Both groups benefitted positively from retrieval cues in odor identification, and the magnitude of the performance gains was equally large in both AD patients and healthy controls. The finding that AD patients did not benefit selectively from retrieval support in identification, suggests that a degradation of olfactory knowledge plays an important role for the observed odor identification deficits in mild AD. MMSE and identification performance showed a positive relationship, whereas MMSE and olfactory sensitivity were unrelated. Taken together, these findings suggest that the olfactory impairment observed in mild AD may stem from lesions in cortical structures rather than from changes in peripheral structures.

Poster **192**

Relationship between subjective and objective indices of nasal irritant sensitivity among seasonal allergic rhinitic and non-rhinitic subjects. DENNIS SHUSTERMAN, MARY ALICE MURPHY, and JOHN BALMES, *University of California, San Francisco, CA 94143,* dennis@itsa.ucsf.edu.

In an effort to explore variations in nasal irritant sensitivity, we compared the subjective and objective responses of seasonal allergic rhinitic subjects (SAR) and non-rhinitic subjects (NR) to a 15-minute controlled exposure to chlorine gas (Cl_2, at 0.5 ppm) vs. filtered air. Exposures took place via nasal mask within a climate-controlled chamber regulated at 22 $\pm$ 1° C and 40 $\pm$ 3% relative humidity. SAR subjects were studied outside of their allergy season, and the air and Cl_2 exposures occurred a week apart. The study employed equal numbers of SAR and NR subjects (8 each), and was counterbalanced with respect to subject gender and order-of-exposure (i.e., air or chlorine first). An "ETS+vasomotor rhinitis" scale (subjective upper respiratory tract reactivity to environmental tobacco smoke, changes in temperature and humidity, bright lights, perfumes, cleaning products, and ingestion of spicy foods) was also administered pre-exposure. On three occasions (before exposure, at the conclusion of the exposure period, and 15 minutes post-exposure), subjects had their nasal airway resistance (NAR) measured, and were asked to rate sensations (odor, nasal irritation, nasal congestion, rhinorrhea, post-nasal drip, and headache) on a 5-point verbal descriptor scale. By repeated measures ANOVA, pooled SAR + NR subjects showed significant increases in their odor and nasal irritation ratings at the end of exposure ($p < 0.001$ and 0.01, respectively), and in nasal congestion 15 minutes post-exposure ($p < 0.05$). Although SAR subjects objectively congested more than NR subjects, the differences in symptom intensity ratings between the two groups were not statistically significant. ETS+ vasomotor rhinitis scores were only weakly predictive of odor and irritation ratings, and were even less so for objective changes in NAR, particularly after controlling for nasal allergies. Within the limitations of our sample, allergic rhinitis status was the best single predictor of objective response to irritant provocation, with subjective response differing little by rhinitis status. Self-reported reactivity to common environmental irritants did not add substantially to the predictive power of rhinitis status for either subjective or objective endpoints.

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Poster **193**

The natural history of smell dysfunctions secondary to upper respiratory infection (URI). B.J. COWART[1,2], I.M. YOUNG[2], E.K. VARGA[1] and L.D. LOWRY[2], *[1]Monell Chemical Senses Center, Philadelphia, PA 19104; [2]Department of Otolaryngology—Head & Neck Surgery, Thomas Jefferson University, Philadelphia, PA 19107.* cowart@monell.org.

There have been conflicting reports concerning spontaneous recovery in olfactory disorders precipitated by URIs, with some indicating it is extremely rare, and others that the majority of these patients improve over time. At the Monell-Jefferson Taste & Smell Clinic, we have conducted follow-up interviews with 85 patients whose olfactory losses were related to nasal/sinus disease (NSD), 48 with head trauma related loss, 72 whose loss was of unknown etiology and 94 with URI-related loss (these represent our major etiologic categories). Slightly over 50% of the URI patients reported a general improvement in their symptoms, which is the highest percent in any of these categories (improvement was also reported by 35.3% of the NSD patients, 25% of the trauma patients and 30.6% of those with idiopathic losses). Moreover, in addition to those URI patients reporting general improvement, 11.7% reported their absolute olfactory sensitivity had improved, but that they had begun to experience odor quality distortions or pre-existing ones had worsened; this was rarely reported by patients in any other category. Finally, we have been able to re-test, on one or more occasions, 24 patients with URI-related loss. Five (20.8%) showed improvement on at least two of our three primary olfactory tests (phenethyl alcohol and pyridine thresholds and odor identification), and an additional 10 (41.7%) showed substantial improvement, shifting diagnostic categories. In fact, in the latter group, 9 produced olfactory test scores on re-test that fell within normal limits. We have also documented two cases of URI patients moving from a state of complete or virtual anosmia to one of dysosmia. This transition is often reported by patients, but to our knowledge has not been demonstrated psychophysically. One of these patients eventually returned to normal function (two years after the onset of her dysfunction), as documented in a second re-test.

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Poster 194

Olfactory function and allergic rhinitis. A. J. APTER[1], J. F. GENT[2], M. E. FRANK[2], *Departments of [1]Medicine and [2]BioStructure and Function, UConn Health Center, Farmington, CT 06030.* apter@nso.uchc.edu.

The association between allergic rhinitis and olfactory function (Apter et al., 1995) was further explored with our 60 most recent Taste and Smell Clinic (TASC) patients with positive allergy tests and presenting complaints of olfactory dysfunction. The 30 patients without chronic sinusitis or nasal polyps (Group 1[G1]); the 14 with chronic sinusitis but without polyps (G2); and the 16 with nasal polyps with or without chronic sinusitis (G3) differed in mean composite olfactory score ($p < 0.0001$). The average scores were highest for G1 (3.11 ± 0.42); intermediate for G2 (1.29 ± 0.49); and lowest for G3 (0.52 ± 0.28). Self-reported duration of olfactory problems also differed ($p < 0.017$). The average duration was 2.19 ± 0.44 yrs. for G1; 5.02 ± 1.98 yrs. for G2 and 7.64 ± 2.25 yrs. for G3.

We compared the TASC G1 (12 males, 18 females) to 30 patients (10 males, 20 females) presenting to the Allergy Clinic (AC). TASC patients were older (51.7 yrs. $\pm$ 13.1 sd) than AC patients (39.5 yrs. $\pm$ 10.0 sd) ($p < 0.0001$). TASC patients were severely hyposmic compared to the normosmic AC patients (mean composite olfactory score = 6.00 ± 0.22) ($p < 0.0001$), although the 9 AC patients who complained of olfactory dysfunction were hyposmic (5.67 ± 0.43). Compared to AC patients, TASC patients reported a greater incidence of olfactory distortions (14 compared to 3 out of 30 patients, $p < 0.002$) and olfactory loss associated with upper respiratory infections (17 compared to 2, $p < 0.0001$). The olfactory function of TASC patients with allergic rhinitis appears to be part of a continuum: they suffer less severe olfactory loss of shorter duration than TASC patients with more severe nasal-sinus disease, but more olfactory loss than AC patients.

Reference. Apter AJ, Mott AE, Frank ME, and Clive JM. (1995) Ann Allergy Asthma Immunol 75:311-316.

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Poster 195

Multiple olfactory measures in *SCHIZOPHRENIA*. CLAUDIA RUPP[1], JOSEF ILMBERGER[2], HARALD OBERBAUER[1], ARNOLD SCHOLZ[3], CAROLINE WANKO[1] and HARTMANN HINTERHUBER[1], *[1]Department of Psychiatry, Univ.-Clinics of Innsbruck, Innsbruck, Austria, [2]Clinic for Physical Medicine, Klinikum Großhadern, Ludwig-Maximilians University of Munich, Munich, Germany, [3]Department of Otorhinolaryngology, Univ.-Clinics of Innsbruck, Innsbruck, Austria,* claudia.rupp@uibk.ac.at.

It is well established that schizophrenia like many other neuropsychiatric disorders is accompanied by a reduced odor identification ability. This identification impairment is seen early in the illness, and appears unrelated to neuroleptic use, smoking history, olfactory hallucinations and illness severity.

However no study has yet examined unilaterally multiple types of olfactory measures. Multiple unilateral olfactory evaluation was performed by thirty male schizophrenic patients and thirty healthy male controls using the Munich Olfaction Test (olfactory threshold, odor discrimination, intensity discrimination, odor recognition and non-semantic associations of odors: hedonics, familiarity, edibility, intensity) and a simple odor identification task with everyday odors.

Our data confirm previous findings of olfactory identification deficits in patients with schizophrenia. Furthermore, schizophrenic patients differed significantly from healthy controls in their non-verbal olfactory discrimination ability. No differences were found in the phenyl-ethyl-alcohol thresholds, the olfactory recognition performance, the intensity discrimination ability and most of the non-semantic associations of odors. Contrary to expectations, ratings for pleasantness of the odors were higher in schizophrenic patients compared to the controls. No clear laterality differences between schizophrenics and controls were seen.

Our results suggest that there is no generalized dysfunction in olfactory processing in schizophrenia. These selective olfactory deficits most likely reflect specific cognitive deficits and/or specific dysfunctional olfactory brain regions. Future research including modern neuroimaging methods should address this issue and may help elucidate the specific dysfunctions underlying olfactory identification deficits in neuropsychiatric disorders.

Poster 196

Autobiographical memory in patients with right hemisphere damage: Olfactory and verbal probes. PAUL J. MOBERG[1,2], RICHARD N. MAHR[1], STEVEN E.. ARNOLD[1], HENRY RIORDAN[1], RICHARD L. DOTY[2], *[1]Department of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA 19104, [2]Department of Otorhinolaryngology: Head and Neck Surgery, Univ. of Pennsylvania, Philadelphia, PA 19104.* FAX: (215) 662-7903.

This study investigated the ability of right hemisphere damaged (RHD) patients and matched healthy controls to recall autobiographical material in response to olfactory and verbal cues differing in emotional valence. A modified Crovitz paradigm was used in which subjects were asked to recall a specific episode from their own life that related to a cue odor or word. Presentation of olfactory and verbal cue conditions were counterbalanced across subjects to avoid position effects. All patient and control responses to stimuli were recorded verbatim and transcribed. Identifying information was removed from these transcripts and four independent raters graded responses for emotionality of content and specificity on a 1-10 Likert scale. Results indicated that RHD patients recalled less specific and less emotional memories than controls across both verbal and olfactory cue conditions. In contrast to the pattern seen in controls of more emotional and specific memories in response to odor cues, RHD patients showed a marked deficit in the ability to recall autobiographical memories in response to odor cues. Within the RHD group, response ratings differed significantly between the word and odor cue conditions, with memories produced to verbal probes being rated more emotional and specific. For word cues, emotional words tended to elicit significantly greater emotional response from the RHD patients than did non-emotional words. In contrast, ratings of the RHD patient's responses to the odors did not differ between emotional and non-emotional smells.

RHD patients exhibit significant deficits in their ability to generate autobiographical material to odor cues. This stands in contrast to healthy controls, where odors elicited more emotional and specific autobiographical memories. These findings support a greater right-hemisphere predominance for odor processing, and that odors are more sensitive to the resulting disruption in autobiographical memory seen in RHD.

Poster 197

Olfactory symptom and test analysis in chronic rhinosinusitis patients. CHAN RHYOO, BIRGITTA E. MOYLAN, DONALD A. LEOPOLD, *Dept. of Otolaryngology-Head and Neck Surgery, Johns Hopkins Univ., Baltimore MD 21287.* FAX: 410-550-2064.

Patients with chronic rhinosinusitis often complain of decreased olfactory ability. In these patients, the relationship between olfactory symptoms, unilateral olfactory test results, and symptom scores is unknown. Using patients enrolled in the Johns Hopkins Chronic Rhinosinusitis Study, we were able to study these relationships.

The study population included 159 patients who had been diagnosed with chronic rhinosinusitis and had signs of thickened mucosa on nasal endoscopy and /or CT scanning. Olfactory testing on this group was performed uninasally using the 3-odorant scratch and sniff identification test (Sensonics, Inc., Haddon Heights NJ). All patients were given a symptom questionnaire that included assessments of olfactory ability and general rhinosinusitis symptoms.

Analysis of the data shows no significant relationship between the chronic rhinosinusitis symptom scores and the odorant identification test ($p=0.45$). As expected there is a relationship between the olfactory symptom score and the smell test score ($p=0.0001$). This relationship was also present when the smell symptoms were analysed against the right ($p=0.0001$), left ($p=0.0001$), and best ($p=0.0001$) nostril olfactory test scores. There was also a relationship between smell symptoms and the olfactory test results by handedness(right ; $p<0.005$, left ; $p<0.005$) and there was little variability between the left and right sides($p=0.42$).

The lack of correlation between general rhinosinustits symptoms and olfactory test scores suggests that at least as a large group, olfactory performance will not predict the level of symptom severity. It also suggests that the pathophysiology producing the nasal and sinus symptoms is not associated with olfactory function. In previous studies, we (DAL) have suggested that the left nostril is more important in general odorant perception in humans. These data would suggest that there is no apparent "favorite" side, and that the nostrils function independently.

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Poster 198

Clinical application of the Alcohol Sniff Test in HIV+ and HIV- patients with nasal sinus disease. CHRISTINA R. SCHLOTFELDT[1], MARK W. GEISLER[2], TERENCE M. DAVIDSON[2], AND CLAIRE MURPHY[1,2], *[1]Psych. Dept., San Diego State Univ., CA 92120, [2]Head and Neck Surgery, Univ. of California Medical Center, San Diego, CA 92103.* FAX: (619) 594-3773

The Alcohol Sniff Test (AST) allows for rapid clinical evaluation of olfaction, and has been shown to reliably discriminate between groups exhibiting normal olfactory ability (normosmia), decreased olfactory ability (hyposmia), and complete olfactory loss (anosmia) (Davidson and Murphy, Arch. of Otolaryngol. Head Neck Surg., Vol. 123, June 1997). The present study attempted to extend the clinical application of the AST and determine its ability to discriminate between groups of HIV+ patients with nasal sinus disease, HIV- patients with nasal sinus disease, and healthy controls. Participants were 71 patients presenting to the UCSD Nasal Dysfunction Clinic with nasal or sinus complaints (N = 21 HIV+, 50 HIV-) and 50 healthy controls participating in a continuing study of olfactory function across the lifespan. All subjects were administered the AST as part of a complete olfactory analysis, including nasal endoscopy. The AST is administered to the patient with eyes closed and breathing at a normal rate using a 70% Isopropyl Alcohol pad. The pad is opened to expose 0.5 cm, and beginning at a distance of 30 cm from the nose, the pad is advanced at a rate of 1 cm per expiration until the alcohol odor is detected. The threshold is the distance in cm averaged across five trials. An analysis of variance indicated that the AST thresholds of HIV+ sinusitis patients were significantly lower (less sensitive) than HIV- sinusitis patients, and the AST thresholds of both sinusitis groups were significantly lower (less sensitive) than the healthy controls, $p < .03$, in all cases. Thus, the results show that the AST can reliably discriminate between sinusitis patients of HIV+ or HIV- status, and distinguish sinusitis patients from healthy controls. Therefore, it is suggested that the AST be integrated into the routine cranial nerve examination of HIV+ patients to aid in the diagnosis of nasal sinus disease.

Supported by NIH grant #AG04085 to CM and DC 00032 to TMD.

Poster 199

The relationship between the alcohol sniff test and sensory olfactory event-related potentials: validation of a psychophysical test. CHRISTINA B. MIDDLETON[1], MARK W. GEISLER[2], TERENCE M. DAVIDSON[2], AND CLAIRE MURPHY[1,2], *[1]Psych. Dept., San Diego State Univ., CA 92120, [2]Head and Neck Surgery, Univ. of California Medical Center, San Diego, CA 92103.* FAX: (619) 594-3773

Traditional evaluations of olfactory functioning involve psychophysical threshold and odor identification tests that can be timely to administer and can require extensive testing materials or cost. The need for a quick, cost efficient, and reliable olfactory test that measures gross sensory functioning led to the development of the Alcohol Sniff Test (AST) (Davidson and Murphy, Arch Otolaryngol Head Neck Surg/vol 123, June 1997). The purpose of the present study was to validate the utility of the AST by correlating it with the sensory components (N1/P2) of the olfactory event-related potential (OERP). Eighty participants (40 male and 40 female) comprising six age decades from 20 to 70 years of age were given the AST and OERPs. The AST was administered using a standard 70% Isopropyl Alcohol pad which was raised one centimeter per expiration beginning at a distance of 30 centimeters and ending when the subject indicated detection of the odor. For the OERP recordings, an olfactometer delivered the odor stimulus, amyl acetate, with an inter-stimulus interval of 60 seconds. Event-related potentials were collected via gold-plated electrodes affixed to the Fz, Cz, and Pz electrode sites. Results indicated that performance on the AST correlated positively with sensory inter-peak amplitudes, and negatively with sensory latencies of the OERP, indicating that the better the ability to detect the alcohol odor the greater the sensory amplitudes and the shorter the sensory latencies. Further, the relationship between the AST and OERP amplitudes was more pronounced for males than females, while OERP latencies were consistent for both genders. This effect was found across the age decades. An analysis of variance on AST performance for gender by age decade indicated no difference for gender, a main effect for age decade, and no interaction. The main effect for age decade showed a precipitous drop between the 40 and 50 year decades. The present findings indicate that the AST is a valid and useful sensory test of olfactory functioning.

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Poster 200

Rapid screening of olfactory function in Down's Syndrome. CANDI L. FREED[1], ANDREA M. DALVE-ENDRES[1], TERENCE M. DAVIDSON[2], and CLAIRE MURPHY[1,2], *[1]Department of Psychology, San Diego State University, 6363 Alvarado Ct., Suite 101, San Diego, CA 92120-4913.* FAX:(619)594-3773, *[2]University of California San Diego Medical Center, San Diego, CA 92103.*

Olfactory thresholds are typically not included in a cranial nerve examination. The Alcohol Sniff Test (AST) was developed as a clinically useful, rapid screening test for olfactory function (Davidson & Murphy [1997]. *Archives of Otolaryngology - Head and Neck Surgery*, 123, 591). It has been shown to correlate well with the standard n-butyl alcohol test of olfactory threshold and recent studies have shown that the AST is a reliable test of olfactory capability in children. Down's Syndrome is a common type of mental retardation in which the individual eventually develops neuropathological abnormalities that result in decreased olfactory function. The goal of the present study was to examine the validity of the AST when used with children and to detect the olfactory deficits of children with Down's Syndrome. The participants were 61 children from the ages of 5-18 yrs. To begin the test, a metric ruler was extended downward from the child's nose and held in place. A standard 70% isopropyl alcohol pad was placed 30 cm below the nose. With each exhalation the pad was moved upward 1 cm until the child reported detection. The children were instructed to keep their eyes and mouths closed and breathe normally during testing. At this level of concentration, the alcohol pad does not elicit a trigeminal response in a normal person. Five trials were performed with an interval of 45 s between each trial to avoid adaptation. The mean of the 5 trials was used as the threshold score. Results indicated a significant difference between the normal children and the children with Down's Syndrome, $F(1,59) = 11.62$, $p < .01$. These results suggest that the AST is a valid instrument when applied to children and is effective in detecting the documented olfactory deficits in children with Down's Syndrome.

Supported by NIH grant AG08203-10 to CM

Poster 201

Law students defeat the UPSIT: dissimulated olfactory dysfunction. ALAN R. HIRSCH[1], JASON GRUSS[2], *[1]Smell & Taste Treatment and Research Foundation, Chicago, IL 60611, [2]University of Michigan, Ann Arbor, MI.* FAX: (312) 649-0458.

Physicians are often called upon to judge the credibility of trauma victims' claims of loss of olfactory sensitivity; often financial interests are involved. Because of the potential for dissimulating chemosensory dysfunction, clinicians need reliable criteria for diagnoses. To determine whether highly educated subjects could successfully dissimulate olfactory dysfunction in a commonly used standardized test, the University of Pennsylvania Smell Identification Test (UPSIT), was administered twice to each of 62 second and third year law students. Students were instructed in the first test to feign smell loss to the best of their ability, and in the second, to perform the test honestly. Of the students with normal olfaction (as shown in the second test) (n=34), 57% scored within the UPSIT's published guidelines for malingering, 47% were able to successfully malinger, scoring in the category of true smell loss. Of the students with real olfactory deficits (as shown in the second test) (n=28), 61% were successful at exaggerating their existing olfactory deficit, without being identified by the UPSIT as malingering, while only 39% scored as malingerers in the published UPSIT guidelines. For various reasons, highly educated persons may be better able to dissimulate than those with less formal schooling. Where malingering seems a possibility, clinicians must use various strategies, for instance, repeat procedures, parallel tests, and psychological evaluation, to differentiate malingering from true olfactory deficits.

The authors would like to acknowledge Loyola Law School and Judge Stephen Schiller, Circuit Court of Cook County, Illinois for their participation and support.

Poster 202

What a tangled web we weave: malingering, anosmia, and the odorant confusion matrix. D.B. KURTZ, D.E. HORNUNG, T.L. WHITE, E.B. BELKNAP. *Clinical Olfactory Research Center at the SUNY Health Science Center, Syracuse, NY.*

Most of the smell clinics in the United States have been asked, on occasion, to render a professional opinion regarding the nature of a patient's olfactory loss for purposes of litigation. Although it is easy to simply report test scores, the fundamental question remains: Is the smell loss genuine? To cope with this problem, the UPSIT includes a "malingering scale", which allows detection of malingering based on statistical inference, as well as the cheating strategies of 148 people asked to feign a loss (Doty et al., 1984). However, in this day of generous compensation for physical loss, patients (and their lawyers) may be highly motivated to develop ways to avoid appearing to malinger. Since the OCM (Wright, 1987) is a longer and more complex than the UPSIT, it was hypothesized that this test would be less amenable to successful malingering. Further, since the potential benefit of the OCM does not lie solely in interpretation of percent correct, it was hypothesized that people attempting to malinger would demonstrate a unique and identifiable pattern of responses. Accordingly, the OCM was administered to two groups of normosmic individuals, both of which were asked to feign a total olfactory loss. The second group was also "coached" with the instruction that ten percent of the queries should be answered correctly. Responses from these two malingering groups were compared to results obtained from the clinical testing of people who were anosmic from head injury or who were congenitally anosmic. Based on the previously described response patterns of anosmic patients for odorants and irritants (Hornung, Kurtz, & Youngentob, 1993) we were able to create an *a priori* decision criteria which identified true anosmic patients and, by exclusion, those subjects who were feigning an olfactory loss.

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Poster 203

Fluorescence imaging of membrane vesicle recycling as a probe for stimulus activation of taste receptor cells *in situ*. M. MUZZVIMAN[1] AND JOHN H. TEETER[1,2]. [1]*Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104* ,[2]*University of Pennsylvania, Philadelphia, PA 19104*. FAX: (215) 898-2084.

Localized synaptic membrane vesicle recycling has been visualized in presynaptic neurons using the fluorescent membrane probe, FM 1-43 (Betz and Bewick, 1992, Science 255:200; Ryan et al., 1993, Neuron 11:713). We have adapted this technique for use with a taste bud slice preparation in order to identify not only which taste cells are activated by particular taste stimuli, but which cells actually release neurotransmitter. The fluorescent stryl dyes used to examine vesicle recycling (e.g. FM 2-10, FM 1-43, FM 1-84) emit in the range from 0.5 to 0.6 μm when in a lipophilic environment such as the plasma membrane, but have a negligible fluorescence in aqueous solution. By exposing taste cells in the slice preparation to a 10 μM solution of dye while inducing release of neurotransmitter, either by application of a 100 μM L-arginine or L-alanine or by general depolarization with a high potassium solution, internalized recycling vesicles are stained. Non-specific staining washes away with removal of the dye. Destaining is accomplished by inducing exocytosis of synaptic vesicles with the excitatory taste stimuli or high potassium solution. By subtraction of fluorescence images before and after stimulation with an excitatory stimulus, the cells that responded to the simulation with neurotransmitter release are visualized. Using this technique we have recorded synaptic release induced by L-arginine. This type of analysis will permit differentiation of taste cells functionally connected to afferent nerve fibers or other taste cells from those simply expressing particular types of receptors.

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Poster 204

ATP-mediated suppression of outward potassium current carried by a novel voltage-gated potassium channel expressed in taste buds of channel catfish, *Ictalurus punctatus*. JOHN H. TEETER[1,2], RALPH B. PUCHALSKI[1,3] [1]*Monell Chemical Senses Center, Philadelphia, PA 19104;* [2]*Department of Physiology,* [3]*Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104*. FAX: 215-898-2084.

To further elucidate how taste stimuli are processed in the channel catfish, *I. punctatus*, we have cloned, as reported previously, a novel voltage-gated potassium channel that is, within the taste tissue, expressed specifically in the taste buds. Unlike all other previously cloned voltage-gated potassium channels, the new channel (KvATP1) has a consensus site for ATP binding in the carboxyl terminus. To determine whether the channel activity is modulated by ATP, we used patch clamp analysis and two-electrode voltage clamp recording from *Xenopus* oocytes injected with complementary RNA encoding KvATP1.

Macroscopic currents are reversibly suppressed by ATP, ATPγS, a hydrolysis resistant analog of ATP, and AMP-PNP, a nonhydrolyzable analog. We propose that ATP and its analogs are binding directly to the channel protein to suppress outward potassium currents. It is unlikely that ATP's primary effect on the channel is mediated by a mechanism involving phosphorylation since AMP-PNP is not a substrate for protein kinases and thiophosphorylated residues are poor substrates for protein phosphatases.

Poster 205

Inhibition of K^+ channels by fatty acids may represent a common mechanism for the chemoreception of fat in both pre- and post-ingestive targets. I. KIM, L. LIU AND T.A. GILBERTSON. *Pennington Biomedical Research Center, LSU, Baton Rouge, LA 70808-4124.* kimi@mhs.pbrc.edu.

Recently we performed experiments demonstrating that *cis*-polyunsaturated fatty acids (PUFAs) inhibit delayed rectifying K^+ (DRK) channels in taste cells (Gilbertson et al., *Am. J. Physiol.* 272:C1203, 1997), that the DRK channel is likely a Shaker Kv1.5-like channel (Liu et al., *this meeting*) and that the sensitivity of taste cells to PUFAs was inversely correlated with dietary fat preference (Liu et al., Soc. Neurosci. Abs. 23:1036, 1997). To determine if inhibition of DRK channels by PUFAs is a common chemosensory mechanism for sensing dietary fat, we have begun experiments to look at the effects of PUFAs in various taste buds and at several fat-sensitive post-ingestive targets in rats. PUFAs inhibit DRK channels in taste cells from the fungiform, foliate and vallate papillae, as well as those from the soft palate in a similar fashion involving a Kv1.5-like channel. We next looked for the presence of Kv1.5 channels at several post-ingestive targets. Protein from duodenal enterocytes, hepatocytes and pancreatic ß cells, which are known to be responsive to dietary fat, were run on Western blots and probed with an anti-Kv1.5 antibody. All these tissues, as well as taste cells, contained a ~66 kD protein that labeled with the anti-Kv1.5 antibody. Presence of this protein was confirmed using immunocytochemical techniques on these tissues. Patch clamp recordings from a pancreatic ß cell line have shown that PUFAs inhibit a DRK channel in these cells. These preliminary results suggest that a PUFA-mediated inhibition of a Shaker Kv1.5-like channel may be a universal mechanism by which a number of pre- and post-ingestive sites detect dietary fat.

Supported in part by grant DC02507 and a grant from Novartis Pharmaceutical Corp.(T.A.G.)

Poster 206

Identification of a Shaker Kv1.5-like K^+ channel in taste cells: The primary target for fatty acid inhibition. L. LIU[1], I. KIM[1], S. HU[2], S. WANG[2], H. ZHANG[1] AND T.A. GILBERTSON[1]. [1]*Pennington Biomedical Research Center, LSU, Baton Rouge, LA 70808-4124 and* [2]*Novartis Pharmaceutical Corp., Summit, NJ 07901-1398.* liul@mhs.pbrc.edu.

We have used patch clamp recording, Western blotting and immunocytochemistry to identify the subtype of fatty acid-sensitive K^+ channel in rat taste cells. The properties of the K^+ current in taste receptor cells (TRCs), including activation and inactivation kinetics, use-dependent inactivation and tail currents, were consistent with cloned and expressed Kv1.5 channels. Agents known to inhibit Kv1.5 channels, such as terfenadine, bupivacaine, flecainide and quinidine, all inhibited K^+ currents in TRCs, while compounds that do not inhibit Kv1.5 channels, namely charybdotoxin, dendrotoxin and MCD peptide, had no effect. Polyclonal antibodies raised against the Kv1.5 C-terminus significantly inhibited K^+ currents when applied intracellularly, while antigen absorbed antibody did not. Anti-Kv1.5 antibody labeled a ~66 kD band in protein isolated from taste buds, which was absent in taste bud-free lingual epithelium. This band was also found in cardiac myocytes, which are known to contain a fatty acid sensitive Kv1.5 channel (Kim et al. *J. Physiol.* 484:643, 1995). Immunocytochemistry revealed that the majority of taste receptor cells, as well as cardiac myocytes, labels with the anti-Kv1.5 antibody. Immunocytochemistry and Western blotting also revealed the presence of a Shab Kv2.1 channel in taste cells. Our results indicated, however, that this channel was likely a minor component of the total delayed rectifying K^+ current. Moreover, anti-Kv2.1 antibodies did not significantly inhibit K^+ currents in TRCs. Taken together, it appears that a Shaker Kv1.5-like channel is the major delayed rectifying K^+ channel in rat taste cells and thus is likely the target for fatty acid inhibition.

Supported in part by NIH NIDCD grant DC02507 and a grant from Novartis Pharmaceutical Corp. to T.A.G.

Poster 207

Isolation and biophysical characterization of an ion channel taste receptor for l-arginine from channel catfish. W. GROSVENOR[1]; YU. A. KAULIN[1]; A.I. SPIELMAN[1,2]; D.L. KALINOSKI[1]; J.H. TEETER[1,3] and J.G. BRAND[1,3,4]. [1]*Monell Chem. Senses Ctr, Phila., PA;* [2]*NYU College of Dentistry, NY, NY;* [3]*U of PA, Phila. PA;* [4]*VA Med. Ctr., Phila. PA.* brand@monell.org

Taste receptors for amino acids on the channel catfish, *I. punctatus*, have been characterized both neurophysiologically and biochemically. The high affinity ion channel-type receptor for the taste stimulus, L-arginine (L-Arg), has been extensively studied in our laboratory. The ability of the lectin, *Ricinus communis agglutinin* (RCA), to block L-Arg binding to this receptor lead us to use lectin affinity chromatography to partially purify this receptor. Solubilized proteins from barbel membranes were passed through an agarose-bound RCA-I column and bound proteins were eluted with D-galactose. When the eluted protein was reconstituted into lipid bilayers, an L-Arg-stimulated conductance increase of between 50 and 80 pS was observed which could be inhibited by D-Arg. Electrophoresis of the eluted protein under reducing conditions (4-20% Bio-Rad Ready gels) showed a distinct band at 83 kD, from which polyclonal antibodies were developed in guinea pig. These recognized specific sites on the apical region of barbel taste buds (Chem. Senses 22:692). Further purification was achieved by passing the RCA-eluant through Sephacryl S300HR. The initial fraction contained L-Arg stimulated activity. SDS PAGE of this peak revealed protein of 83 kD, recognized by the antibodies. 2-D gels of electroeluted 83 kD protein revealed 2 adjacent protein spots. Native IEF gels of the protein showed peptides to have pI's of 7.5-7.8. An additional 1-3 peptide bands were observed. Channel activity was recovered from the Sephacryl column, and parameters such as channel size, ion selectivity and agonist activity were compared with similar parameters of the native receptor (Kumazawa et al., submitted).

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Poster 208

Is PKA involved in sweet taste transduction? BRIAN VARKEVISSER AND SUE C. KINNAMON, *Dept. of Anatomy & Neurobiology, Colorado State Univ., Ft. Collins, CO 80523 and Rocky Mountain Taste and Smell Center, Univ. of Colorado Health Sciences Center, Denver, CO 80262,* sckinna@lamar.colostate.edu

Sugars and synthetic sweeteners have been shown to activate different second messenger pathways in taste cells. Synthetic sweeteners activate phospholipase C (PLC) producing IP_3 and diacylglycerol, while sugars activate adenylyl cyclase producing cAMP (Bernhardt et al., J. Physiol. 490:325-336, 1996). There is also evidence for a role of Protein Kinase C (PKC), a downstream target of PLC activation, in the transduction of synthetic sweeteners (Varkevisser et al.,Chem. Senses, 22,6:815, 1997). We have previously shown that cAMP blocks a resting K^+ current in sweet-sensitive taste cells (Cummings et al., J. Neurophysiol. 75:1256-1263, 1996). Given that PKC is involved in the synthetic sweetener pathway, it is likely that sugar transduction involves activation of PKA by cAMP. In this study we used the loose-patch clamp technique for recording from taste buds *in situ* to investigate the role of PKA in sweet taste transduction. Hamster fungiform taste buds were tested for their response to sucrose (200μ) and the synthetic sweetener NC-01 (200μm) in the presence and absence of the cell permeable PKA inhibitor H89 (10 and 19μM). In all cases (n = 6), H89 caused a two fold increase in response to both sucrose and NC-01. These data suggest that sucrose does not utilize PKA in eliciting action potentials. Instead, PKA may play another other role in sweet taste transduction, such as contributing to adaptation.

Supported by NIDCD 00244, and a minority fellowship to B. V.

Poster 209

Bitter transduction of dextromethorphan: modulation by cAMP and membrane excitability. TATSUYA OGURA[1,3], SANDRA L. NELSON[2], and SUE C. KINNAMON [1,3], [1]Department of Anatomy and *Neurobiology, Colorado State University, Fort Collins, CO 80523,* [2]*Health Care Research Center, The Procter & Gamble Company, Mason, OH 45040,* [3]*The Rocky Mountain Taste and Smell Center, Denver, CO* 80262. tocura@lamar.colostate.edu.

A variety of substances with different chemical structures elicits a bitter taste. Recent studies have shown that several different transduction mechanisms are used for bitter taste (for review, Kinnamon & Margolskee, Current Opinion in Neurobiology 6:506-513, 1996). We have found that the bitter substance denatonium (DN) increases intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) in mudpuppy taste cells by Ca^{2+} release from intracellular stores, resulting in increase of voltage-gated outward currents and membrane hyperpolarization. The Ca^{2+} responses are not significantly modified by intracellular cAMP levels (0-ura et al., J. Neurosci. 17:3580-3587, 1997). In contrast, the bitter antitussive dextromethorphan (DEX) also releases Ca^{2+} from intracellular stores, but via a different intracehular pathway (Ogura et al., ISOT XII, 1997). In the present study, we tested whether the Ca^{2+} response to DEX is affected by intracellular cAMP levels, and whether DEX affects volta-egated currents and resting membrane potentials. $[Ca^{2+}]_i$ was measured in isolated single taste cells using the Ca^{2+} sensitive-dye, fura-2. In saline containing 8cpt-cAMP (1 mM) and IBMX (100 μM), Ca^{2+} responses to DEX (2.5 mN4) were significantly potentiated. H-89 (10 gM), a CANVkinase inhibitor, inhibited the potentiation, suggesting that intracenular cAMP levels modulate Ca^{2+} responses to DEX by phosphorylation. In whole-cell patch recording, bath-applied DEX blocked voltage-gated Na^+, K^+ and Ca^{2+} currents. In current clamp mode, DEX depolarized resting membrane potentials. These data suggest that DEX and DN appear to use different pathways for transduction in mudpuppy taste cells.

Supported by NTH grants DC00244, DC00766 and The Procter & Gamble Company to S.C.K.

Poster 210

Blocking the bitter taste of pharmaceuticals. RICHARD A. MCGREGOR[1], STEPHEN A. GRAVINA[1], and LUIS RUIZ-AVILA[2], *[1]Linguagen Corp., 100 Delawanna Ave, Clifton, NJ 07015, [2]Almirall Prodesfarma Cardener 64, Barcelona, Spain.* FAX: 973-591-5145.

The ability to detect bitter compounds protects against ingestion of pharmaceutically active and/or poisonous compounds. Most oral dosage pharmaceuticals are bitter and hence we are studying guanine nucleotide binding regulatory protein (G protein) activation by bitter compounds *in vivo* and *in vitro* to develop bitterness blockers. Taste receptor cells contain the taste receptor cell specific G protein gustducin and the closely related transducin. Gustducin has been implicated in transduction of compounds that humans consider sweet or bitter.

Several bitter compounds were presented to wild type and gustducin knockout mice. Gustofacial responses indicated that gustducin was required for responsiveness to several bitter compounds including denatonium.

We used the trypsin sensitivity assay to follow G protein activation in response to bitter compounds. Bovine circumvallate membranes were mixed with transducin from bovine retina (to substitute for gustducin). Activated transducin yields a 32 kDa tryptic fragment while nonactivated transducin yields a 25 kDa fragment. Activated vs nonactivated transducin was quantified by SDS PAGE and immunoblotting. Denatonium and other bitter tasting compounds were tested and it was found that those compounds dependent on α gustducin in the *in vivo* studies activated transducin in the trypsin digest assay.

The trypsin digest assay was used to assay compounds for their potential to block activation of transducin by bitter tasting compounds and hence mask bitter taste. It was found that some compounds were capable of inhibiting the activation of transducin by bitter tasting compounds. This approach has tremendous potential for the pharmaceutical industry as it is amenable to high throughput analysis of large libraries of compounds leading to the discovery of bitterness blockers of pharmaceutically active compounds.

Poster 211

Gustducin/transducin activation assay: an *in vitro* analytical method to detect bitter tastants. DING MING[1], LUIS RUIZ-AVILA [2] and ROBERT F. MARGOLSKEE[3], *[1]R&D, Pepsi Cola Company, 100 Stevens Ave., Valhalla, NY 10595 and [2] Almirall Prodesfarma Cardener 64, Barcelona, Spain and [3] Howard Hughes Medical Institute, Department of Physiology and Biophysics, The Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, USA,* dming@pepsi.com

Sensory analysis consists of the evaluation of three characteristics: flavor (taste quality), potency (concentration/response function) and the temporal profile (time/intensity function). The standard method of measuring these characteristics is psychophysical evaluation by a human taste panel. Recently, electrophysiological approaches have been shown to be particularly useful in temporal profile measurement. However, neither psycophysics nor electrophysiology are amenable to screening large numbers of samples rapidly, and these other methods require relatively large amounts of the tastants. Gustducin is a taste specific G protein that couples in vitro with taste receptors and second messenger regulating enzymes. We have developed an *in vitro* biochemical assay to identify bitter tastants by measuring the activation of gustducin and/or transducin in the presence of taste receptor cell membranes, the activated G proteins can be visualized by limited trypsin digestion, SDS-PAGE and immunoblotting with the appropriate antibody. This method can be used to identify bitter tastants, and determine their concentration/response function. The rapid throughput and microsample handling capability of this assay make it an ideal method for screening for bitterness inhibitors or enhancers. Initial experiments indicate that this *in vitro* assay correlates well with behavioral and electrophysiological evaluations.

Poster 212

Responses of the hamster chorda tympani nerve to caffeine/sucrose mixtures. BRUCE I. MACKINNON, BRADLEY K. FORMAKER, THOMAS P. HETTINGER & MARION E. FRANK. *Dept. of BioStructure and Function, University of Connecticut Health Center, Farmington, CT 06030.* bmackinn@neuron.uchc.edu.

Hamster (*Mesocricetus auratus*) whole nerve, steady-state, chorda tympani (CT) responses to sucrose are reduced in the presence of quinine (Formaker and Frank, 1996). Like quinine, caffeine is avoided by hamsters in one and two-bottle taste tests vs. water, but at much higher concentrations than caffeine affects humans. Even though humans perceive both quinine and caffeine as bitter, taste aversion tests indicate that in hamsters these compounds are distinct perceptual entities (Bouverat et al., 1997). Furthermore, quinine stimulates the hamster CT, but caffeine does not. In order to determine possible caffeine inhibition of sucrose responses, we measured whole-nerve CT responses to binary mixtures of sucrose with caffeine. The anterior tongue was stimulated with a concentration series of sucrose (30-300 mM), caffeine (3-30 mM) or binary combinations of these stimuli. NH_4Cl (0.5 M) was periodically applied as a standard and responses relative to the standard were analyzed. Preliminary results indicate that caffeine has no consistent effect on either steady-state or transient responses to sucrose. In summary, caffeine does not have the same taste quality as quinine, and in contrast to quinine, caffeine neither excites nor inhibits fungiform taste-receptor cells in hamsters.

References: Formaker BK, Frank ME. (1996) Brain Research 727:79-90. Bouverat BP, MacKinnon BI, Frank ME. (1997) Chem Senses 22(6):649.

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Poster 213

Effect of lysine on afferent activity of the hepatic branch of the vagus nerve in normal and lysine deficient rat. TORU MIMURA[1], AKIRA NIIJIMA[2], and KUNIO TORII[1], *[1]Basic Research Laboratory, Ajinomoto Co., Inc., Kawasaki, Japan 210.* FAX:+81-44-244-9617, *[2]Department of Physiology, Niigata University, Niigata, Japan 951.*

It has been reported that a deficiency of an essential amino acid in the diet causes a change of taste preference towards solution or diet that favors the deficient amino acid. For example, lysine, an essential amino acid with bitter taste, was most preferred to quantitatively ingest during lysine deficiency in choice paradigm of solutions containing various L-amino acids. There is a possibility that lysine in the circulating blood plasma can be sensed by a peripheral lysine sensor activated due to prevention from unpleasant symptom evoked by ingestion of lysine deficient diet. In addition, the existence of amino acid sensors, including ones for lysine in the hepatoportal region, was investigated using adult male Wistar rat with or without lysine deficiency. It was mentioned that these lysine sensors send information on the concentration of lysine in the portal venous blood to the central nervous system through hepatic vagus afferents. This report deals with sensory signals from hypothesized hepatoportal lysine sensors in the normal and lysine deficient rat.

Normal and lysine deficient male rats (Wistar strain) weighing approximately 300g were used. To develop the lysine deficient rats, L-lysine deficient food (formulated as previously reported) was supplied for 7-10 days. Food, but not water, was withdrawn at least 6 hours before the beginning of each experiment. The hepatic vagus nerve filament of each rats, anesthetized with urethane (1g/kg BW, i.p.), was isolated and the responses were recorded by electrodes before and after ingestion of amino acid solution into the portal vein by cannulation. Results show that in the L-lysine deficient rat the sensitivity to L-lysine increased 100 fold from that observed in the normal animal. D-lysine, L-alanine and L-leucine sensitivities did not change. This increase in L-lysine sensitivity may accelerate selective intake behavior for L-lysine in food as well as water to aid recovery from a L-lysine deficient state.

Poster 214

Conditioned taste aversions to a corn oil and sucrose emulsion in rats with parabrachial nucleus lesions. PATRICK L. SMITH[1], PATRICIA SUE GRIGSON[2], RALPH NORGREN[2] & JAMES C. SMITH[1], *Department of Psychology, The Florida State University, Tallahassee, FL[1] and Dept. of Behavioral Sciences, College ofMedicine, Penn State University, Hershey, PA[2]* FAX: (850)644-7739.

In our laboratory we have studied sensory qualities of a mixture of corn oil and sucrose solution by conditioning a LiCl-induced aversion to the emulsion and noting the generalization of the aversion to one or the other constituents. The emulsion is prepared by blending 16 parts of corn oil and 84 parts of a 0.25 M sucrose solution with Tween 80. When ingestion of this emulsion is followed by an intraperitoneal (IP) injection of LiCl, the ensuing conditioned taste aversion (CTA) generalizes exclusively to fat rather than to sucrose. This dissociation indicates that fat is the more salient feature of the emulsion. The present research is designed to identify the sensory character of the corn oil (i.e., to determine whether the corn oil is a gustatory or a trigeminal stimulus). It has been shown that rats with lesions of the parabrachial nucleus (PBN) fail to learn a CTA to a gustatory cue (Spector et al., 1992, Reilly et al., 1993) but can readily acquire an aversion using trigeminal input associated with ingestion of a weak solution of capsaicin (Grigson, et al., 1998). In the present experiment we attempted to condition an aversion to the sugar/fat emulsion in SHAM rats and in rats with ibotenic acid PBN lesions (PBNX). If the fat/sucrose mixture is merely a gustatory stimulus, then rats with PBN lesions will fail to acquire the aversion. On the other hand, if the emulsion is a trigeminal stimulus, then PBNX rats should have no difficulty learning the aversion. 16 male, Sprague-Dawley rats were given 10 minutes access to the sugar/fat emulsion followed by an IP injection of either saline (n=5 PBNX, n=3 SHAM) or 0.6 M LiCl (0.005 ml/g bw; n=5 PBNX, n=3 SHAM). The results showed that, while the SHAM rats demonstrated a strong aversion to both the emulsion and to fat, rats with bilateral lesions of the PBN failed to show any signs of acquiring a CTA. This finding demonstrates that the fat/sucrose emulsion has sensory characteristics that are more similar to an appetitive gustatory, rather than to an aversive, trigeminal, stimulus.

Poster 215

Methylxanthines differ in their ability to desensitize a bitter-sensitive taste receptor cell through prolonged dietary exposure. JOHN I. GLENDINNING and SONYA ENSSLEN, *Department of Biological Science, Barnard College, Columbia University, New York, NY 10027* FAX: (212) 854-7491.

There is increasing evidence that the responsiveness of chemoreceptors to their ligands is *not* fixed. For example, mere exposure to a stimulus for several hours or days can profoundly alter the responsiveness of a chemoreceptor to the same stimulus (and in some cases to novel ones). We examined how prolonged dietary exposure to methylxanthines modulates the response properties of a bitter-sensitive taste receptor cell (TRC) in *Manduca* caterpillars. This TRC (henceforth, lateral deterrent TRC) occurs in the lateral styloconic sensillum and responds to a variety of compounds that humans characterize as bitter, including methylxanthines (caffeine and theophylline), phenolic glycosides (salicin and helicin) and aristolochic acid. We focused on the effects of dietary exposure to methylxanthines because previous research demonstrated that 2 days of exposure to a caffeine-containing diet desensitized the lateral deterrent TRC to caffeine. We asked (1) whether dietary exposure to caffeine desensitizes the lateral deterrent TRC to caffeine exclusively, or whether the phenomenon generalizes to other bitter compounds, and (2) whether dietary exposure to other methylxanthines (*e.g.,* theophylline and IBMX) produces a similar desensitization phenomenon. To this end, we made extracellular recordings from each caterpillar's lateral deterrent TRC, both before and after dietary exposure to caffeine, theophylline or IBMX. We found that dietary exposure to caffeine desensitized the lateral deterrent TRC to caffeine, salicin and helicin, but not theophylline or aristolochic acid. Dietary exposure to theophylline desensitized the lateral deterrent TRC to theophylline, salicin, helicin and aristolochic acid, but not caffeine. Finally, dietary exposure to IBMX failed to desensitize the lateral deterrent TRC to any of the compounds tested. Our results demonstrate that caffeine-induced desensitization generalizes to some but not all ligands of the lateral deterrent TRC, suggesting that this phenomenon involves selective desensitization of specific transduction pathways within the lateral deterrent TRC. In addition, our results reveal that that methylxanthines differ both in their ability to induce the desensitization phenomenon, and in the type of desensitization they produce.

Supported by NIH grant DC-02416.

Poster 216

Electrophysiological evidence for cross-talk between two transduction pathways within a bitter-sensitive taste receptor cell. CAROLINE R. TADROS and JOHN I. GLENDINNING, *Department of Biological Science, Barnard College, Columbia University, New York, NY 10027* FAX: (212) 854-7491.

In a previous study (*J. Neurophys* 78: 734-745; 1997), we provided electrophysiological evidence for two transduction pathways within a bitter-sensitive taste receptor cell in *Manduca* caterpillars. Here, we asked whether cross-talk occurs between these two transduction pathways while they are activated simultaneously. To this end, we used the tip recording method to make extracellular recordings. Our bitter stimuli were caffeine and aristolochic acid (AA); we chose these particular compounds because they each stimulate a different transduction pathway. We could readily distinguish excitatory responses by each pathway based on their distinctive temporal pattern of firing: the caffeine-activated pathway produces a tonic firing pattern that reaches its maximal firing rate within the 100 ms, whereas the AA-activated pathway produces a firing pattern that accelerates with time, reaching its maximal firing rate after 600-900 ms. When we stimulated both pathways with low concentrations of caffeine and AA, we observed constructive cross-talk; i.e., the temporal pattern of firing was the average of both pathways and the firing rate was greater than that with either compound alone. In contrast, when we stimulated both pathways with high concentrations of each compound, we observed a destructive form of cross-talk in which the caffeine-activated pathway appeared to inhibit the AA-activated pathway; i.e., we observed a tonic pattern of firing with no additivity, suggesting the caffeine-activated pathway alone generated the spiking pattern. Our results provide clear evidence for cross-talk between the transduction pathways, and indicate that the nature of the cross-talk is concentration-dependent and in some cases asymmetrical. This cross-talk may facilitate detection of potentially toxic compounds at low concentrations, but impede detection of the same compounds at high concentrations.

Supported by NIH grant DC-02416 and a grant to Barnard College from the Howard Hughes Medical Institute.

Poster 217

The effect of impermeant anions on Na^+ chemoreception and water transport by the toad skin. S. D. HILLYARD, K. VS. HOFF AND P. A. SULLIVAN. *Dept Biology, University of Nevada, Las Vegas, NV 89154.* hillyard @ccmail.nevada.edu.

Amphibians do not drink. Instead they absorb water by osmosis across their water permeable skin. Toads in the genus Bufo have a specialized region of their ventral skin that is more permeable to water and this region is pressed to moist surfaces when dehydrated toads are expressing hydration behavior. *Bufo punctatus* dehydrated by 10% of their hydrated weight consistently demonstrated hydration behavior for an average of 800 $\pm$ 62 seconds during a 15 minute (900 sec) rehydration period on water saturated surfaces but hydration behavior was drastically reduced to 42 $\pm$ 9 seconds when toads were placed on surfaces moistened with 250 mM NaCl. If toads were placed on 250 mM Na gluconate, hydration time was increased over six-fold to 280 $\pm$ 63 seconds. Thus, toads appear less sensitive to concentrated Na^+ solutions having a less permeant anion even when they have the same Na^+ concentration and osmolality as NaCl solutions. The larger gluconate anion was more effective in prolonging hydration time than were smaller anions such as sulfate and phosphate. These results may be analagous to the "anion paradox" in mammalian tongue where reduced intensity is similarly observed for Na^+ taste transduction in the presence of less permeant anions.

Dehydrated toads placed on surfaces moistened with dilute (50 mM) NaCl show longer periods of hydration behavior than do toads placed on deionized water. When immersed in 50 mM NaCl solutions, toads are able to rehydrate more rapidly than from water alone indicating that toads are able to couple Na^+ and water transport across their skin to increase the rate of rehydration. Dehydrated toads immersed in 50 mM Na gluconate solutions rehydrated more slowly than did toads in 50 mM NaCl. Thus, the coupling of Na^+ and water transport across the toad skin requires a permeant anion.

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Poster 218

Changes in apical sodium channel number and efficiency contribute to Na^+ taste response development in rat. S.J. HENDRICKS[1], R.E. STEWART[1,2], G.L. HECK[3], J.A. DeSIMONE[3], D.L. HILL[1], *[1]Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22903, [2]Dept. of Psychology, Washington and Lee Univ., Lexington, VA 24450, [3]Dept. of Physiology, Med. Coll. of Virginia/Virginia Commonwealth Univ., Richmond, VA 23298.* FAX: (804) 982-4785.

Chorda tympani (CT) taste responses to sodium stimuli increase during the first several weeks of postnatal development in rat. This developmental increase in sodium sensitivity may be due to a progressive addition of functional amiloride sensitive sodium channels to the taste cell apical membrane. We combined whole CT recording with simultaneous in vivo lingual voltage clamp to explore changes in channel function that occur during the postnatal development of sodium responses. As expected, rats aged 10 to 14 days show reduced NaCl CT responses and response voltage sensitivity when compared to adults. Kinetic analyses reveal a three-fold increase in the efficiency of apical amiloride sensitive sodium channels that occurs between 10 to 14 and 19 to 23 days postnatal. In addition, the relative density of functional apical sodium channels nearly doubles between 19 to 23 days and 29 to 31 days of age. By 29 to 31 days postnatal, CT responses to sodium stimuli approach adult magnitude. Preliminary analyses indicate that CT responses to sodium gluconate show similar age related increases in amplitude and voltage sensitivity. Therefore, the increase in CT taste responses to sodium stimuli observed during development can be explained by two mechanisms: 1) an increase in the efficiency; and 2) an increase in the number of sodium channels in the apical domain of taste cell membranes.

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Poster 219

Greater superficial petrosal responses and terminal field morphology are not altered by developmental sodium restriction in rats. SUZANNE I. SOLLARS and DAVID L. HILL, *Department of Psychology, Univ. of Virginia, Charlottesville, VA 22903,* sis2n@virginia.edu.

Developmental sodium restriction results in major alterations of chorda tympani nerve (CT) neurophysiology and nucleus of the solitary tract (NTS) terminal field morphology. Specifically, adult rats that have been maintained on sodium-deficient diet from embryonic day 3 have decreased CT responses to sodium stimuli that are reflective of minimal sensitivity to the sodium channel blocker amiloride. Additionally, CT terminal fields in the NTS of rats sodium restricted during development are greatly expanded relative to controls. In the present study, we examined neural responses and NTS terminal field morphology of the greater superficial petrosal nerve (GSP) in rats fed a low-sodium diet throughout development. In control rats, the GSP is highly sensitive to sodium stimuli and has terminal fields in the NTS which overlap those of the CT. Five rats maintained on low sodium (0.03% NaCl) chow since 3 days postconception were tested as adults. Following chloral hydrate anesthesia, the GSP was exposed in the tympanic bulla, placed on a platinum recording electrode, and integrated responses were obtained to a series of stimuli presented to the palate. Following a stimulation series in which water served as the rinse, a series of stimuli was presented in 100 μM amiloride. Responses were expressed relative to a 0.5 M NH_4Cl response standard. The GSP was highly responsive to NaCl, sodium acetate (NaAc), NH_4Cl and sucrose, with NaCl and NaAc responses strongly suppressed by amiloride. There were no significant differences in the responses between restricted and control rats. Additionally, the GSPs of five restricted and five control rats were labeled with biotinylated dextran and terminal field volumes were measured. There were no significant differences in terminal field size between the two groups. Thus, the profound effect of developmental sodium restriction noted for the CT does not occur for the GSP. This finding is particularly interesting because of the multiple similarities in anatomy and physiology between the CT and GSP. Furthermore, the results suggest that these two similar components of the gustatory system undergo distinct developmental processes and have different susceptibilities to environmental influences.

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Poster 220

The influence of perinatal NaCl intake on chorda tympani responses to NaCl, KCl, and Q-HCl with and without amiloride in rats. DAVID W. PITTMAN AND ROBERT J. CONTRERAS, *Program in Neuroscience, Department of Psychology, The Florida State University, Tallahassee, FL 32306-1270,* pittman@psy.fsu.edu.

Elevated maternal dietary sodium produces a long-term increase in NaCl preference in adult offspring, while restriction of maternal dietary sodium produces both a long-term decrease in NaCl preference and a long-term increase in the fluid intake of adult offspring. This experiment examined the responsiveness of the chorda tympani nerve (CT) for long-term effects due to perinatal dietary sodium exposure. Dams were placed on 1 of 3 sodium dietary conditions, Basal (0.1% NaCl), Intermediate (1% NaCl) and High (3% NaCl) chow at least 1 week prior to mating and were maintained on these diets throughout pregnancy and lactation. The offspring were weaned on P21 but had ad lib access to dietary chow maintaining their respective salt level until P30. On P30 all rats were placed on a 1% NaCl diet for the remainder of the experiment. Whole nerve electrophysiological recordings were obtained from the CT of adult rats during stimulation of the anterior tongue by increasing concentration series of NaCl, KCl (75, 150, 300, 450, 555, and 750 mM), and Quinine-HCl (3, 15, 30 mM) with and without 100 μM amiloride in the solution. Each stimulus was presented for 10 s with at least a 90 s water rinse between each stimulation. The area ($volts^2$) under the integrated signal (time constant = 100 ms) was measured for 10 s following response onset. The relative response magnitudes of NaCl, KCl, and Q-HCl did not differ between dietary groups. Amiloride produced similar robust inhibition of the NaCl response for all three dietary groups (30% inhibition). There was a lesser amiloride inhibition of KCl with a similar magnitude across all three groups (10% inhibition). There was no amiloride inhibition of Q-HCl in any of the three dietary groups. Based on the preliminary results, it appears that perinatal salt exposure does not produce long-term changes in the CT responsiveness to NaCl, KCl, or Q-HCl.

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Poster 221

The cellular basis of osmotic effects on the chorda tympani response of rats to salt stimuli. VIJAY LYALL[1]. JANET K. TAYLOR[1], GERARD L. HECK[1], GEORGE M. FELDMAN[1,2], JOHN A. DeSIMONE[1], *[1]Department of Physiology, Virginia Commonwealth University, Richmond, VA 23298-0551, [2]McGuire Veteran's Affairs Medical Center, 1201 Broad Rock Road, Richmond, VA 23249.* FAX: (804) 828-7382.

Although taste receptor cells (TRCs) can experience wide variation in osmotic pressure, osmotic effects *per se* on the chorda tympani (CT) response to taste stimuli have not been extensively studied. Mannitol alone is a poor stimulus of the rat CT, but when 150 mM NaCl (good stimulus) was dissolved in 300 mM mannitol and applied to the rat tongue, an increase in both the phasic and tonic parts of the CT response occurred relative to 150 mM NaCl in water. Mannitol dissolved in 20 mM KCl caused the lingual transepithelial resistance to increase relative to 20 mM KCl alone. In other transporting epithelia the resistance-increase was correlated with an osmotically-induced decrease in cell volume. To probe for distinct fast and slow temporal correlates of the CT response at the cellular level we used conventional imaging to measure osmotically-induced changes in isolated TRC axis-length. Volume changes were also assessed by confocal laser microscopy using the volume marker fluoroprobe calcein. The relationship between cell volume and calcein fluorescence was linear. An increase from 320 to 640 mOsM induced a rapid decrease in cell volume which was followed by a slower spontaneous increase in cell size (regulatory volume increase; RVI). A decrease in osmolarity from 320 to 120 mOsM induced an increase in cell volume which was followed by a spontaneous decrease in cell size (regulatory volume decrease, RVD). RVI was, in some cases, accompanied by activation of Na^+ and Cl^- -independent membrane mechanisms that alkalinized the TRCs. RVD was accompanied by activation of an anion pathway in TRC membranes that facilitated the transport of fluorescent dyes (calcein and BCECF) from cell interior to the extracellular medium and was also Na^+ and Cl^--independent. We conclude that changes in the phasic part of the CT response may reflect a rapid osmotically-induced perturbation of TRC volume, whereas, the changes in the slower CT adaptation rate may be governed by the slower RVI and RVD phases of TRC volume recovery.

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Poster 222

Taste responses in geniculate ganglion neurons that innervate lingual receptors in rats. ROBERT F. LUNDY, JR. and ROBERT J. CONTRERAS, *Department of Psychology, Florida State University, Tallahassee, Fl 32306-1270.* lundy@psy.fsu.edu

The responses of geniculate ganglion neurons to four prototypical taste stimuli and three monovalent salts were studied using extracellular recording techniques to learn about taste-quality coding in the chorda tympani nerve. Specifically, neurons that synapse with taste receptor cells in fungiform papilla on the dorsum of the tongue were investigated. We have analyzed the neurophysiological responses from 16 neurons to 0.5 M sucrose, 0.1 M NaCl, 0.01 M HCl, 0.02 M QHCl and 0.075 M and 0.3 M concentrations of NaCl, KCl, and NH_4Cl at 35ûC. The present results are in agreement with prior single neuron studies in this species showing that geniculate neurons may be classified into fiber types on the basis of their responses to sucrose, NaCl, HCl, and QHCl.

Cluster analysis revealed three groups of neurons that have been described previously; sucrose-sensitive (S-units, n=3), NaCl-sensitive (N-units, n=4), and HCl-sensitive (H-units, n-4) neurons. The S- and N-units were by far the most selective, responding nearly exclusively to sucrose or NaCl, respectively. Potassium chloride and NH_4Cl apparently were ineffective stimuli for S- and N-units. However, one N-unit responded to both concentrations of NH_4Cl. H-units responded best to HCl, but also responded well to NaCl and QHCl. Moreover, H-units were sensitive to the three salts and responded in a concentration dependent manner. A fourth group of neurons (X-units, n=5), revealed by cluster analysis, responded best to NaCl, but also responded well to HCl and QHCl. The three salts elicited robust responses in X-units, however, the response rate was independent of salt concentration. Although X- and H-units did not respond differentially to the three salts, the response rate of X-units to 0.075 M NaCl and KCl was more than two-fold greater than that of the H-units. In summary, it appears that the chorda tympani nerve responds selectively to the presence of compounds with physiological import (S- & N-units). Due to the broad sensitivity of H-units, it is unlikely that input from these neurons alone could signal the presence of a particular compound. Finally, it may be that an additional group of neurons exists in the chorda tympani nerve that has gone undetected by prior studies (X-units).

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Poster 223

Intrinsic membrane properties of neurons in the rat petrosal ganglion innervating the posterior tongue. TOMOSHIGE KOGA, and ROBERT M. BRADLEY. *School of Dentistry, Univ. Michigan, Ann Arbor, MI 48109-1078.* FAX: (734) 764-7406.

Afferent fibers innervating taste and other receptors on the posterior tongue travel in the glossopharyngeal nerve with cell bodies in the petrosal ganglion. While investigators have studied the biophysical properties of petrosal ganglion neurons innervating the carotid body, little is known about petrosal ganglion neurons that innervate receptors on the tongue. We have therefore studied the biophysical properties of acutely isolated neurons from the petrosal ganglion that exclusively innervate the tongue. Under brief anesthesia, Fluorogold was injected into the tongue in the region of the circumvallate papilla in 14 rats. After a suitable time for the label to transport to the cell bodies, the ganglion was removed and the cells dissociated using enzymatic digestion and trituration. Whole cell recordings were performed on 62 neurons and the basic characteristics of the action potential and repetitive discharge characteristics measured by current injection pulses. The isolated neurons had a mean diameter of 28.5 ± 4.7 μm; a resting membrane potential of -58 ± 9 mV; an action potentials amplitude of 99 ± 14 mV; and a membrane time constant of 35.1 ± 10.8 ms. Two groups of neurons could be distinguished based on their action potentials. One group (n = 19) had a deflection or hump on the descending limb of the action potential and the other group (n = 43) had narrow spikes. There was no significant difference in the other basic biophysical properties between the neurons in the two groups. About two-thirds of the neurons (44/62) responded with a train of action potentials to a 100 ms depolarizing current pulse, while the rest only responded with a single spike. The mean spike threshold of the repetitively firing neurons was significantly lower than that of neurons with a single spike (Student t-test). Thus, petrosal ganglion neurons innervating the tongue have heterogeneous biophysical characteristics which may relate to the different types of sensory receptors that they innervate.

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The generalizability of capsaicin sensitization and desensitization. JOHN PRESCOTT, *Sensory Science Research Centre, University of Otago, Dunedin, New Zealand.* john.prescott@stonebow.otago.ac.nz.

In humans, repeated oral stimulation using filter papers impregnated with the irritant capsaicin produces sensitization or desensitization, depending on the temporal spacing of the stimuli. Research on other irritants, eg. zingerone, using whole mouth rinses has shown desensitization when stimulus spacing that produces sensitization with capsaicin filter papers is used. While this may reflect different physiological responses to the irritants, there is a need to clarify that differing methodologies are not responsible for this discrepancy and to determine the generalisability of sensitization or desensitization. Exp 1 compared responses to 3 ppm capsaicin filter papers and whole mouth rinses, and a 0.6 ppm capsaicin rinse which was equal to initial intensity to the 3 ppm capsaicin filter paper. Both 3 ppm conditions showed sensitization over 10 samples at 1 min intervals while the 0.6 ppm condition failed to show sensitization. Following a 10 min hiatus, all 3 conditions showed desensitization. Thus, capsaicin sensitization and desensitization occur irrespective of method of delivery, suggesting that failure to find sensitization with other irritants may reflect different receptor activity or sub-optimal timing of stimulus delivery.

In Exp 2, the extent to which capsaicin sensitization and desensitization could be demonstrated during food consumption was examined. Subjects rated burn intensity during consumption of 2 foods - tomato soup containing 7 ppm capsaicin and *chili con carne* containing 9 ppm capsaicin. Both foods were consumed under 2 conditions: external timing, so that each of 10 samples of 7 ml was consumed every 30 secs; and self paced consumption of the samples to simulate normal eating. In both conditions, consumption of the initial samples was followed by a 10 min hiatus, after which a final sample was consumed and rated. These data showed evidence of only slight sensitization during food consumption during timed eating and none during self-paced eating. In both conditions and foods, however, desensitization was clearly evident following the hiatus. Together with patterns of high inter-individual variability during initial series of stimuli in both experiments, these data show that desensitization is a much more robust phenomenon than sensitization across different conditions of stimulation.

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Human response to capsaicin in oil and water based carriers. HARRY LAWLESS, CAROLINE HARTONO AND SUSANA HERNANDEZ, *Department of Food Science, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853.* htl1@cornell.edu.

The study of oral chemesthesis has often used the chili pepper compound capsaicin in model systems that are water based. However, many foods and some hot pepper condiments are oil based or high in lipid content. The purpose of these studies was to examine psychophysical response to capsaicin in oil and water based model systems. Thresholds were measured among 23 individuals using an ascending forced choice method of limits. Detection thresholds in oil were 10.8 mg/L while thresholds in water were 0.28 mg/L. Suprathreshold scaling functions were measured in corn and soybean oils and in water systems using an emulsifier (polysorbate) or alcohol to solvate the capsaicin in preparation of stock solutions. A horizontal labeled magnitude scale was used. In the water-based systems, capsaicin ranged from weak to very strong ratings over a range of 0.3 to 10 mg/L while the same perceptual range was achieved in oil over a range of 10 to 316 mg/L. Nonconsumers of hot/spicy foods generally had lower thresholds and higher suprathreshold responses. Both threshold and suprathreshold measures differed by a factor of approximately 30:1 for oil vs. water concentrations needed to evoke the same level of response. The results are consistent with a simple partitioning of the lipophilic capsaicin molecule from oil based systems into the aqueous peri-receptor medium of about 30 to one.

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Right-hemisphere preponderance of responses to painful CO_2 stimulation of the human nasal mucosa. BIRGIT KETTENMANN[2], KARIN PORTIN[1], VEIKKO JOUSMÄKI[1], GERD KOBAL[2], RIITTA HARI[1], [1]*Brain Research Unit, Low Temperature Lab., Helsinki Univ. of Technology, 02150 Espoo, Finland and* [2]*Dept. of Experim. and Clinic. Pharmacology and Toxicology, Univ. of Erlangen-Nürnberg, 91054 Erlangen, Germany.* FAX: +49-9131-856898.

We recorded whole-scalp (Neuromag-122™) cerebral magnetic fields from healthy adults to painful CO_2 pulses (duration 200 ms, concentration 65-90%), led to the left or right nostril once every 20 or 30 s. CO_2 selctively activates A-delta and C-fibres of the trigeminal nerve. The stimuli were embedded in a continuous airflow (140 ml/s, 36.5°C, relative humidity 80%) to prevent alterations in the mechanical and thermal conditions of the nasal mucosa. The recording passband was 0.03-90 Hz and 16 single responses were averaged per run.

Five out of the 9 subjects showed replicable and artefact-free responses 280-400 ms after stimulus onset. The main responses to originated close to the second somatosensory cortex, SII, most frequently in the right hemisphere, and also in the rolandic areas, mostly on the left. The signals were considerably stronger over the right than the left frontotemporal region, with a right-to-left ratio of 2.3 for areal mean signal amplitudes calculated across 16 channels, for both left and right nostril stimuli. Air puffs delivered to the nasal mucosa resulted in a trend for right-hemisphere dominant responses, but responses to air puff stimulation of the lip and the forehead were symmetric (1).

The right-hemisphere dominance of the SII responses may be associated with the painful, and thus unpleasant, nature of the CO_2 stimulus, thereby suggesting involvement of the right hemisphere in emotional/motivational aspects of trigeminal pain, in agreement with the role of the trigeminal pathways as a general respiratory warning system.

(1) Hari et al. (1997) Pain, 72, 145-151.

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Olfactory and trigeminal detection of 1-butanol and 2-heptanone singly and in binary mixtures. J. ENRIQUE COMETTO-MUÑIZ[1], WILLIAM S. CAIN[1], MICHAEL H. ABRAHAM[2], and RACHEL KUMARSINGH[2], [1]*Department of Surgery (Otolaryngology), University of California, San Diego, La Jolla, CA 92093-0957,* [2]*Department of Chemistry, University College London, London WC1H OAJ, UK.* ecometto@ucsd.edu.

Using a group of 4 normosmics and 4 anosmics and a two-alternative forced-choice procedure with presentation of increasing concentrations, we have measured sensory detectability functions for 1-butanol and 2-heptanone. The sensory endpoints studied included odor (in normosmics), nasal pungency (in anosmics), and eye irritation (in both groups). All detectability functions were approximately ogival. Odor functions for both chemicals, compared with the respective nasal pungency functions, were displaced towards lower concentrations by about three orders of magnitude. Over the lineal range of the curves, odor functions for the two substances had a slope of 0.5 whereas pungency and eye irritation functions had a slope of 0.7. Eye irritation functions were virtually the same in anosmics and normosmics. Based on the detectability functions for the single chemicals we prepared binary mixtures where the compounds were present at concentrations producing 20, 40, 60, or 80 % detection above chance. Next, detectability functions were measured again for the single substances and for the mixtures. The results showed sensory agonism between the two chemicals for all three modalities. Overall, the two trigeminal responses depicted a higher degree of agonism than the olfactory response, and in various cases reached the ceiling (100 % detection). The present approach, combined with chemical modeling and applied to the systematic study of additional binary and higher order mixtures, shows promise as a tool to elucidate the rules of sensory interaction among odorants and among irritants at perithreshold levels.

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Quality coding of intranasal irritation. CHARLES J. WYSOCKI and ANDREA RIBIER, *Monell Chemical Senses Center, Philadelphia, PA, 19104.* wysocki@monell.org.

Odorants that are not intranasal irritants cannot be lateralized during monorhinal stimulation. Using lateralization as a criterion, an irritation threshold for an odorant/irritant stimulus can be obtained. We have determined that pre-exposing the nasal cavity to concentrations of the stimulus that exceed the lateralization threshold elevates the threshold for that stimulus, i.e., chemesthetic adaptation occurs. As is true for other sensory modalities, we reasoned that irritants that utilize the same chemesthetic channel as the one experiencing adaptation should show cross-adaptation, i.e., their lateralization thresholds should also be elevated. Furthermore, if more than one quality of intranasal irritation exists, then the thresholds for irritants that utilize an independent channel should be unaffected by the prior adaptation. Lateralization tests with acetic acid, butanol and eugenol revealed that each compound could produce self-adaptation, i.e., exposure to the compound at a concentration that exceeded it's lateralization-threshold level just prior to each lateralization test elevated the threshold. Importantly, exposures to supra-threshold levels of acetic acid also elevated the lateralization threshold for butanol, and vice versa: Cross-adaptation occurred. Repeated exposures to eugenol, which typically eliminated any irritation from it (eugenol can act as a topical anesthetic) did not affect chemesthetic sensitivity to butanol. Exposures to supra-threshold concentrations of butanol did, however, elevate lateralization thresholds for toluene, but did not affect the thresholds for menthol. These results suggest at least two and possibly three chemesthetic modalities, one mediating acetic acid, butanol and toluene (other work suggests that this channel also responds to carbon dioxide), one mediating eugenol, and one mediating menthol (at present we cannot eliminate the possibility that the eugenol and menthol may utilize the same channel). Quantifying the number of chemesthetic qualities and exploring their characteristics is the goal of this research.

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Poster 229

Glutamate and betaine activate opposing conductances in individual squid olfactory receptor neurons. JONATHAN P. DANACEAU AND MARY T. LUCERO, *Department of Physiology, University of Utah, Salt Lake City, UT 84108.* jonathan.danaceau@m.cc.utah.edu.

Betaine is an organic osmolyte that has been shown to be a behaviorally repellant odorant for cephalopods (squid, cuttlefish and octopus) [1]. Our previous studies have shown that betaine activates a Cl^--selective current that hyperpolarizes the olfactory receptor neurons (ORNs) of the squid *Lolliguncula brevis* [2]. In the present study, we made perforated-patch voltage-clamp recordings of ORNs isolated from *Lolliguncula brevis*, and found that glutamate activates a Na^+-selective current. Subsequent current-clamp recordings showed that glutamate application resulted in relatively large membrane depolarizations of 20 to 30 mV. Both glutamate and betaine responses could be elicited from the same cell. In 7 of 13 cells that responded to glutamate, betaine activated a Cl^- current. Further studies will examine the effects of co-application of betaine and glutamate on ORN excitability. Our studies in squid add to the growing list of organisms in which both excitatory and inhibitory responses can occur in an individual ORN. The presence of more than one receptor and effector system may enable integration and coding of odor mixtures at the level of primary sensory neurons.

This work was supported by NIH grant DC02587-04

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Poster 230

Dopamine D_2 receptors mediate the dopaminergic modulation of the hyperpolarization-activated current, I_h, in rat olfactory receptor neurons. GRICELLY VARGAS and MARY T. LUCERO, *Department of Physiology, University of Utah, Salt Lake City, UT 84108.* Gricelly.Vargas@m.cc.utah.edu.

The hyperpolarization-activated conductance, I_h, is a mixed K^+-Na^+ inward rectifying current, important in setting the resting membrane potential, and in controlling cell excitability and neuronal firing patterns. In rat olfactory receptor neurons (ORNs), I_h is modulated by dopamine (DA), which reversibly shifts I_h voltage dependence of activation to more hyperpolarized potentials, and decreases I_h peak current amplitude.[1] These effects of DA on I_h are consistent with a dopaminergic-mediated decrease in intracellular cAMP levels. Since DA inhibits adenylyl cyclase activity by activation of a DA D_2 receptor in rat ORNs[2], we used whole-cell patch clamp techniques and specific DA D_2 receptor agonists to test if D_2 receptor activation and subsequent decrease in cAMP mediate the actions of DA on I_h. Application of D_2 receptor agonists (20 μM bromocriptine or 20 μM quinpirole) to rat ORNs produced a decrease in I_h peak current amplitude without a change in the voltage dependence of I_h activation. However, a higher concentration of quinpirole (50 μM) produced a decrease in I_h ranging from 100% to 50% reduction and a reversible hyperpolarizing shift in the voltage dependence of I_h activation. To test if a cAMP-dependent phosphorylation event is involved in this modulation, the catalytic subunit of protein kinase A (PKA) was added to the internal solution. Internal perfusion of PKA (100-400 U/mL) produced no change in the voltage dependence of activation of I_h. Further experiments will determine if phosphorylation is involved in the reduction of I_h peak current amplitude. These data demonstrate that activation of DA D_2 receptors mediates the effects of dopamine on I_h in rat ORNs, and represent the first evidence for a functional role of D_2 receptors present in rat ORNs.

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Poster 231

Amino acids elicit increases and decreases in intracellular calcium in *Necturus* olfactory receptor neurons. RONA J. DELAY[1], TATSUYA OGURA[2], VINCENT E. DIONNE[3] [1]*Department of Cell & Structural Biology, University of Colorado Health Science Center, Denver, CO 80262.* [2]*Department of Anatomy & Neurobiology, Colorado State University, Ft. Collins, CO 80523 and* [3]*Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543*

Amino acids, which are potent olfactory stimuli in many aquaticanimals, elicit excitatory and inhibitory electrical responses in olfactory receptor neurons (ORN) from mudpuppies,*Necturus maculosus* (Dionne, 1992). We have also reported that amino acids can elicit changes in intracellular calcium concentration ($[Ca]_i$) in these same cells (Delay, et al 1997). In preparation for correlating the electrical and calcium responses, we have used calcium imaging techniques to examine the calcium response further. Our studies were conducted with the calcium-sensitive fluorescent indicator, fura-2AM, which is cell permeable. Isolated ORNs, loaded with fura-2, respond repeatedly to stimuli, and could be studied for over one hour. Only intact ORNs with cilia (but without a long axon) were studied. Stimuli were dissolved individually at neutral pH in bath saline and applied rapidly at concentrations from 10 μM to 10 mM. The stimuli included l-amino acids (ala, arg, asp, gln, his, leu, met, pro, ser, tyr, val, and taurine) and a potent phosphodiesterase inhibitor (IBMX) that can increase intracellular cAMP concentrations. Only about one-third of the ORNs showed an increase in $[Ca]_i$ in response to IBMX, suggesting that stimulation of cAMP-responsive second messenger pathways may underlie only a portion of the chemosensory responses from these cells. Most of the amino acids elicited increases in $[Ca]_i$ with two notable exceptions: histidine and proline both produced consistent *decreases* in calcium concentration. Interestingly, those ORNs that responded to histidine or proline with a decrease in $[Ca]_i$ responded to IBMX with an increase in calcium. This indicates that the transduction of these two amino acids is not mediated by stimulation of adenylyl cyclase. The result suggests that there may be more than one transduction pathway in *Necturus* ORNs, or that olfactory transduction may be mediated by differential regulation of cyclic nucleotide levels., Experiments are ongoing to determine the second messenger pathway mediating the decrease in $[Ca]_i$ produced by histidine and proline.

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Poster 232

Odorants suppress a delayed rectifier conductance in rat olfactory neurons. FRITZ W. LISCHKA[1], JOHN H. TEETER[1,2] and DIEGO RESTREPO[3], [1]*Monell Chemical Senses Center,* [2]*Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104 and* [3]*Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262.* lischka@monell.org.

The response of rat olfactory receptor neurons (ORNs) to odors was examined by recording membrane current under perforated patch conditions. Cells were stimulated with odorant mixtures one second after a step change in membrane potential from a holding potential of −60 mV to potentials in the range from −120 to +80 mV. We find that rat ORNs display two predominant types of responses. Thirty percent of the cells responded to odorants with activation of a cyclic nucleotide-gated nonspecific cation conductance. In contrast, in 55% of the ORNs, stimulation with odorants inhibited a component of the depolarization-activated outward current. This component was identified as a delayed rectifier K+ conductance (IKo) on the basis of ion substitution and pharmacological experiments. The effect of odorants on IKo was specific (only certain odorants inhibited IKo in each ORN), and there was a significant latency between arrival of odorants to the cell and the onset of suppression. These results indicate that many rat ORNs respond to odorants with suppression of a delayed rectifier K+ current. Suppression of IKo would be expected to shift the cell into a prolonged excitable state, enhancing depolarization via second messenger-mediated pathways.

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Poster 233

Patch-clamp recordings from identified chemosensory neurons of the nematode *Caenorhabditis elegans*. T. NICKELL[1] , R.Y.K. PUN[2] , and S.J. KLEENE[1] , [1]*Department of Cell Biology, Neurobiology, and Anatomy,* [2] *Department of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH 45267.* tom@syrano.acb.uc.edu.

In the soil nematode *C. elegans*, two pairs of chemosensory neurons are responsible for detection of different identified sets of odorants. In one of these neurons (AWA), response to the odorant diacetyl requires a known seven-transmembrane-domain receptor. Because matching of a specific odorant to its receptor protein and chemosensory neuron are possible in C. elegans, it offers a unique opportunity to study chemosensory transduction. Due to the small size of C. elegans (1 mm in length), routine electrical recording from its identified neurons has not previously been achieved.

Nematodes in which the AWA neurons express green fluorescent protein (GFP) were the gift of Cori Bargmann. Approximately 50 adult worms were transferred from a culture plate to a drop of Dent's saline. These worms were then individually transferred to a second drop of Dent's saline plus sucrose and carbachol. Next, this drop was pipetted onto a glass microscope coverslip cooled to near 0oC. Under 60x magnification, worms were cut with a #11 scalpel blade at about the level of the grinder. In about 10% of these "heads", the GFP-labeled AWA neurons were intact and exposed. Recordings were made from the AWA neurons using blunt-tipped pipettes under 40x water-immersion optics and slow perfusion. Under whole-cell perforated-patch recording, voltage-activated fast inward currents were not seen. This is in agreement with genetic data showing the absence of homologs of the Hodgkin-Huxley sodium channel in the genome of C. elegans and with electrophysiological data in other nematodes. However, voltage-activated outward currents were found that resembled the outward rectifiers of other excitable membranes.

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Poster 234

A GABA-induced chloride current in the soma of lobster olfactory receptor neurons. RICHARD E. DOOLIN[1,2], RAINER HOEGG[1], ASLBEK B. ZHAINAZAROV[1], BARRY W. ACHE[1,2,3], *[1]Whitney Lab., and Depts. of [2]Neuroscience and [3]Zoology, Univ. of Florida, St. Augustine, FL 32086.* FAX: (904) 461-4008.

Whole-cell patch-clamping cultured lobster olfactory receptor neurons while superfusing the cells with GABA (100 μM) evoked an inward current at -60 mV in 35 of 46 cells tested. The reversal potential (E_r) of the current was insensitive to changes in $[K^+]_I$, but changing $[Cl^-]_I$ from 30 to 210 mM shifted E_r from -53.0±5.4 to -14.0±0.4 (S.D.) mV (n=3), indicating that the current was carried primarily by chloride. The current was reversibly blocked by picrotoxin (n=11), but not by the $GABA_A$ receptor antagonist bicuculline methiodide (n=8), nor by the $GABA_B$ receptor agonist baclofen (n=3). GABA directly activated a chloride-selective channel in outside-out patches excised from the somata (n=3). The unitary current had a pharmacological profile similar to that of the whole-cell current. Applying GABA to the outer dendrites of the cells *in situ* as a potential odor depolarized 12 of 20 cells tested. The GABA-evoked depolarization was insensitive to picrotoxin, indicating that the somatic GABA receptor cannot account for dendritic sensitivity to GABA. Applying GABA to the soma of the cells *in situ* reversibly suppressed the spontaneous discharge in 2 of 15 cells. Applying GABA to acutely dissociated somata elicited a current in 5 of 6 cells tested, suggesting that access of the ligand to the somata was experimentally limited *in situ*. The E_r of the GABA-evoked current in acutely dissociated cells was -50 mV, near but slightly positive to the calculated E_r for chloride (-72 mV). We propose that some, possibly most, lobster olfactory receptor cells express a GABA-gated chloride channel that reduces the output of the cell. Most of the cells express a somatic, picrotoxin insensitive, histamine (HA)-gated chloride channel that also suppresses output (PNAS,89:8137,1989), suggesting that the cells may be regulated by GABA and HA.

Supported by the NIDCD (DC01655).

Poster 235

Calcium regulation of second messenger signaling in lobster olfactory receptor neurons. GERHARD REICH[1], INGRID BOEKHOFF[2], HEINZ BREER[2], BARRY ACHE[1,3]. *[1]Whitney Laboratory and [3]Depts. Zoology and Neuroscience, Univ. Florida, St. Augustine, FL 32086 USA, and [2]Inst. Zoophysiology, Univ. Stuttgart-Hohenheim, 7000 Stuttgart 70, FRG.* FAX: (904) 461-4008.

Odors stimulate levels of two olfactory second messengers, inositol 1,4,5-trisphosphate (IP_3) and adenosine 3', 5'-cyclic monophosphate (cAMP) in the outer dendrites of lobster olfactory receptor neurons *in vitro* (*J Neurosci.* 14:3304-09, 1994). Here, we determine the influence of free Ca^{2+} on odor-induced changes in these signals. Odor-induced increases in cAMP were strongly suppressed in a concentration-dependent manner by increased free Ca^{2+}. Ca^{2+}-dependent changes in cAMP production also occurred when adenylyl cyclase was stimulated with forskolin, when G-proteins were activated with GTPγS, and in the presence of various phosphodiesterase inhibitors, suggesting that the cyclase is the target of calcium's action. Type III adenylate cyclases are known to be down-regulated by free Ca^{2+} *in vivo* (e.g., *J Bio Chem* 270:21480-86, 1995). Western blot analysis reveals that the outer dendritic membranes express an adenylate cyclase III-immunoreactive protein running at 120 KD that is olfactory organ-specific. Free Ca^{2+} had a substantially smaller effect on odor-induced levels of IP_3 under the same experimental conditions. These results indicate a potentially important biochemical pathway for crosstalk between the two transduction pathways in lobster olfactory receptor cells.

Supported by the NIDCD (award DC01655 to BA) and the DFG (award Br 712/10-3 to HB).

Poster 236

Properties of cyclic-nucleotide-gated currents and odor responses in salamander olfactory receptor neurons after olfactory nerve transection. TRESE LEINDERS-ZUFALL[1], W. KARL KAFITZ[2], CHARLES A. GREER[2], *[1]Department of Anatomy and Neurobiology, University of Maryland, Baltimore, MD 21201, [2]Department of Neurosurgery, Yale University, New Haven, CT 06510,* tlein001@umaryland.edu.

Cyclic nucleotide-gated ion (CNG) channels play a key role in the transduction and adaptation of odor signals in olfactory receptor neurons (ORNs). Recent progress has suggested that the CNG channels are not only critical components of the sensory transduction cascade in ORNs but that they may also play important roles during development. To begin to explore this hypothesis, we have examined excitable properties and second messenger cascades in immature salamander ORNs.

To obtain a homogeneous population of immature ORNs we performed unilateral olfactory nerve transections. Immunocytochemistry for GAP-43 and Giemsa staining demonstrated that virtually the entire mature ORN population had undergone retrograde degeneration by day 10 after the nerve transection. Using the perforated patch-clamp technique, recordings were then made from newly developing ORNs dissociated from the epithelium at day 13, 26, 48 or 61 following nerve transection. The preliminary data demonstrate that there are important physiological differences between mature and developing ORNs with respect to the expression of various ion channels. At day 13 and 26 we detected virtually no Na^+ and Ca^{2+} currents. In contrast, these ORNs had inward currents through CNG channels that could be stimulated by pulses of the phosphodiesterase inhibitor IBMX. This indicates that both CNG channels as well as some components of the second messenger pathways were present by this early stage, before Na^+ and Ca^{2+} channels were detected. Interestingly, these cells did not respond to any of the odor mixtures, each of which included different odor ligands, suggesting that odor receptors might not be present. At day 48 and day 61 the ORNs contained a rich repertoire of voltage-gated currents including large Na^+ and Ca^{2+} currents, as well as CNG channel currents. Odor responses were consistently observed in these ORNs. The data suggest that during an early phase of ORN development, although the cells are not yet responsive to odors and lack Na^+ and Ca^{2+} channels, CNG channels could serve to mediate graded membrane potentials and Ca^{2+} entry into the cells. Future work will analyze the functions of such Ca^{2+} signals in ORN development.

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Poster 237

Detection of KCl responses in cultured rat olfactory receptor neurons using a voltage sensitive dye assay. NANCY L. KOSTER and SARAH K. PIXLEY, *Dept. of Cell Biology, Neurobiology, and Anatomy, Univ. of Cincinnati, Cincinnati, OH 45267.* nancy.koster@uc.edu.

Voltage sensitive dye (VSD) assays allow detection of membrane potential changes and could be used to examine the individual responses of several olfactory receptor neurons (ORNs) simultaneously. A multicellular functional assay is an important goal because individual ORNs vary in their responses to a given odor. Using a VSD assay with a culture system in which new ORNs are generated will allow us to ask questions about differentiation related changes in an ORN's ability to respond to odors.

ORN containing cultures were grown on glass coverslips. Immunostaining for neuron specific tubulin (NST) and refinding cells previously shown to be labeled with Di8ANEPPS (Molecular Probes) indicated that the VSD labeled most, if not all, ORNs. The cultures were stained with 1 μM Di8ANEPPS for one hour and then placed in a laminar flow perfusion chamber. The chamber was perfused with a 5 mM KCl salt solution (140 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES). Use of an additional perfusion setup (Warner Instruments) permitted rapid application of stimuli to small regions. The dye intensity changes were viewed with a confocal microscope. Changing from a 5 to 145 mM KCl salt solution (osmolarity maintained; estimated 85 mV depolarization) resulted in a decrease in dye intensity. The intensity decrease for an individual cell was visible without averaging across time or trials. Next, we will try to detect the smaller voltage changes that would be expected from an odor response. Then, we will use the assay to examine odor responses. Our ability to immunostain after the VSD experiment and refind specific cells will allow us to stage the responding ORNs as either mature (Olfactory Marker Protein +, antibody provided by Dr. F. Margolis) or immature (NST+/OMP-).

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Poster 238

Functional imaging of mamalian olfactory receptor neurons *in vitro*. E. LANCASTER, A.C. PUCHE, M. PYRSKI, F.L. MARGOLIS, F. ZUFALL, M.T. SHIPLEY, A. KELLER. *Dept. Anatomy & Neurobiology, Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201.* elancast@umaryland.edu.

The biophysics underlying responses of olfactory receptor neurons (ORNs) to odorant stimulation is a key determinant in odor detection. Previous studies focused on analyzing properties of dissociated ORNs. However, ORN functions are likely modulated by their local microenvironment. For this purpose, we have developed an epithelium-bulb organotypic slice preparation *(Shipley and Puche, Soc. Neurosci. Abst. 1997, 23:740).* Horizontal slices containing both the olfactory epithelium and olfactory bulbs are harvested from early postnatal mice, and maintained in serum-free conditions. ORNs in these cultures have morphological and neurochemical characteristics of mature ORNs *in vivo*, and maintain functional connections with the olfactory bulb.

We are using imaging of voltage and Ca^{2+}-sensitive dye signals to study olfactory signal detection and transduction in this preparation. Unlike dissociated cells, ORNs in organotypic slices do not take up Ca^{2+}-sensitive dyes. We have therefore developed two approaches to load ORNs with these indicators. In one approach, mild, transient hyperosmotic shock precedes the incubation of the cultures in fluo-3 AM. This results in labeling of a large population of ORNs, which respond to agonist stimulation with characteristic Ca^{2+} transients. The second approach is to retrogradely label ORNs following injections of Ca^{2+} or voltage indicators into the olfactory bulb. The advantage of this approach is that it selectively labels mature ORNs that project to the bulb. Functional imaging will be combined with whole-cell patch clamp recordings from ORNs. Together such approaches wil permit the study of signal detection and transduction mechanisms in mammalian ORNs, under conditions that closely resemble their *in vivo* state.

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Poster 239

Membrane-permeant cyclic nucleotides and a nitric oxide donor activate potassium efflux in olfactory nerve axons of the garfish, *Lepisosteus platostomus*. GEORGE R. KRACKE, ELLA D. SPEICHINGER, JUSTIN S. OGDEN, ROBIN K. SHAON, *Dept. Of Anesthesiology, University of Missouri, Columbia, MO 65212.* hckracg@muccmail.missouri.edu

Cyclic nucleotide-gated channels in the cilia of olfactory receptor neurons play an integral role in the odor transduction process. These non-selective cation channels, directly activated by intracellular cAMP or cGMP, are also present on the dendrites and somata of olfactory receptor neurons. In order to provide initial evidence for the presence of these channels in the axons of these cells, we used the garfish olfactory nerve to test for the presence of cyclic nucleotide-activated K fluxes. The nerves were removed from animals, mounted on wire frames, and incubated in a bicarbonate-buffered Ringers solution containing either ^{42}K or ^{86}Rb, a K analog. A flow-through system was used to determine the rate coefficient of isotope efflux in isotope-free, low divalent cation, bicarbonate-buffered perfusate at 28°C. In control experiments, K efflux rate coefficient was 0.020 $min.^{-1}$ over the 30 min. efflux period. Exposure of nerves to 1 mM cAMP or 1 mM cGMP had no significant effect on K efflux. However, the membrane-permeant cyclic nucleotide analogs, 8Br-cGMP at 0.1 and 1 mM, and 8CPT-cAMP at 1 mM, reversibly increased efflux by 112, 133, and 89%, respectively. Since nitric oxide can also activate cyclic nucleotide-gated channels, we tested the NO donor, S-nitroso-cysteine. At 0.1 mM it increased K efflux by 25%. We conclude that membrane-permeant cyclic nucleotides and an NO donor activate K efflux in garfish olfactory nerve axons. These findings provide initial evidence that cyclic nucleotide-gated channels are present in vertebrate olfactory receptor nerve axons.

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Poster 240

Single-cell cDNA RDA: identifying differences in gene expression between two individual lobster olfactory neurons. ALEXANDER A. GIMELBRANT, SANDRA J. KUHLMAN, TIMOTHY S. MCCLINTOCK, *Department of Physiology, University of Kentucky Medical Center, Lexington, KY 40536.* aagime0@pop.uky.edu.

In recent years, PCR-based methods have been developed for the identification of differences between two cDNA pools (Hubank M, Schatz DG, 1994. Nucleic Acids Res 22: 5640), as well as methods for generating representative cDNA from single cells (Dulac C., Axel R, 1995. Cell 83: 195). We combined these approaches to identify mRNA differences between individual olfactory receptor neurons from the American lobster. cDNA representational difference analysis (cDNA RDA) was performed using cDNA prepared from individual neurons. The resulting difference products numbered from 8 to 12 per subtraction. They were specific to respective single-cell cDNA pools and to the lobster genome, indicating the validity of the strategy. Individual difference products hybridize to less than 0.05% of the clones in olfactory organ cDNA library.

The work was supported by NIH awards DC02366 and DC02736 to T.S. McClintock.

Poster 241

Single cell analysis of odorant receptor gene expression in olfactory receptor neurons. KATHRYN F. MEDLER, HANG N. TRAN, RICHARD C. BRUCH, *Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803.* kmedler@unix1.sncc.lsu.edu.

The identification of the multigene odorant receptor family has provided the opportunity to investigate the expression patterns of the receptors at the molecular level. It has been suggested that an individual olfactory receptor neuron expresses a single, or at most a few, odorant receptor proteins. However, electrophysiological data in various species suggests that individual receptor neurons are often responsive to multiple stimuli. In addition, in channel catfish (*Ictalarus punctatus*), ligand binding experiments showed that odorant receptor sites for amino acids were relatively specific in their ligand binding characteristics. In an attempt to reconcile these observations, we investigated odorant receptor gene expression in catfish olfactory receptor neurons by single cell PCR to determine the number of receptor genes that were expressed in individual neurons. Three criteria were used to determine whether single neurons expressed one or multiple receptors: digestion of PCR products with frequent cutting endonucleases, DNA sequence analysis and genomic Southern blotting. Of the 19 individual neurons analyzed, most cells expressed 2-4 receptors per cell. A small number of cells expressed a single receptor gene. No other odorant receptors were amplified using a variety of additional receptor PCR primers. Since the catfish taste system is also responsive to amino acids, we investigated odorant receptor expression in taste tissue. Multiple odorant receptor genes were amplified from barbel and from isolated taste buds. These results suggest that odorant receptor gene expression is heterogeneous in olfactory receptor neurons and that similar receptors may be involved in taste reception in this organism.

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Poster 242

Topographical analysis of odorant receptor expression in channel catfish olfactory epithelium. MICHELE L. SCHAEFER, KARL ANDERSON, THOMAS E. FINGER, DIEGO RESTREPO, *Neuroscience Training Program,, Dept. of Cell and Structural Biology, Univ. of Colorado HSC, Denver, CO 80262.*

Analysis of the spatial arrangement of receptor expression in the olfactory epithelium (OE) has revealed similarities and differences among species. Rat, mouse, and zebrafish show regionalized expression of olfactory receptors (ORs). In contrast, catfish ORs are reported to be distributed in a random, non-zonal pattern (Ngai J. et al., 1993). We wanted to test whether the apparent random distribution of catfish ORs might be attributed to technical issues in previous studies. Ngai et al utilized full length cDNA probes which conceivably may have hybridized to more than one OR. We constructed a highly specific probe against the untranslated region of an OR, and performed whole-mount *in situ* hybridization. The probe against the untranslated region of #8.2 OR had a nearly identical staining pattern as did the entire cDNA probe. By using the highly divergent untranslated region as a probe, we eliminated the ambiguity of possible cross-reactivity within and between subfamily members. Whole-mount hybridization signals from opposite faces of the same and adjacent lamellae do not show any coincident pattern, confirming the whole-mount lamellar reconstructions by Ngai. The results using three probes (#1, #202, #8.2) suggested that ORs are randomly distributed throughout the OE, suggesting that a zonal distribution is not necessary for olfactory function.

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Poster 243

Spatial organization of olfactory receptor neurons on the antenna of the cabbage looper moth, *Trichoplusia ni.* ALAN J. GRANT[1,2,3] and ROBERT J. O'CONNELL[1,3]. [1]*Worcester Foundation for Biomedical Research, Shrewsbury, MA 01545,* [2]*American Biophysics Corporation, East Greenwich, RI 02818,* [3]*University of Massachusetts Medical School, Worcester, MA 01655.* ajgrant@edgenet.net.

The cabbage looper moth is of significant economic importance because its larval stage causes considerable damage to many crusiferous crop plants. Adult male moths orient to females for mating using a blend of chemicals that are released from specialized abdominal glands in females. Neurons sensitive to the female-produced pheromones are located in specialized sensilla on the male antenna. Electrophysiological responses to stimulation with each of six "behaviorally relevant" compounds were recorded from receptor neurons within sensilla on the antennae of male cabbage looper moths. We searched for receptor neurons responsive to each of these compounds and noted their spatial distribution on the antenna. Receptor neurons were found that responded specifically to stimulation with (Z)-7-dodecen-1-ol acetate (Z-7,12:AC), (Z)-7-tetradecen-1-ol acetate (Z-7,14:AC), (Z)-9-tetradecen-1-ol acetate (Z-9,14:AC) and (Z)-7-dodecen-1-ol (Z-7,12:OH). Specialized receptor neurons were not found for the remaining three pheromone blend components; (doceden-1-ol acetate (12:AC), (Z)-5-dodecen-1-ol acetate (Z-5,12:AC) and 11-dodecen-1-ol acetate (11,12:AC). However a new class of sensillum containing a pair of neurons unresponsive to all of the cabbage looper pheromone components was encountered.

The specialized sensilla are spatially organized along the antenna. For example, sensilla containing Z-7,12:AC-sensitive neurons are preferentially located on the proximal half of the antennal flagellum. In addition to this distribution along the length of the antenna, a pattern across individual flagellar subsegments is described. Sensilla containing neurons sensitive to Z-9,14:AC were found exclusively on the lateral margins of the individual flagellar subsegments.

Poster 244

Distribution of responses to aromatic compounds in the rat olfactory epithelium. JOHN W. SCOTT and TRACY BRIERLEY. *Department of Cell Biology, Emory University, Atlanta, Georgia 30322.* Johns@cellbio.emory.edu.

Electroolfactograms (EOGs) from rat olfactory epithelium have shown differential distribution of responses to odors with certain functional groups. We previously reported that these differences were particularly large for some aromatic compounds. In freshly killed rat preparations where the olfactory epithelium was directly exposed to odor stimulation, we investigated the response profile for a series of aromatic compounds in which a double bond between oxygen and carbon was placed at different positions. Eight recording electrodes were placed along the dorsal edge of the fourth endoturbinate bone. Electrode position was recorded photographically. Odors were presented in a concentration series generally varying from a dilution of $3 \cdot 10^{-2}$ to $1 \cdot 10^{-1}$. The distribution of responses was evaluated statistically by multiple polynomial regression using the position along the turbinate and odor concentration as variables. The distance between the double bonded oxygen and the benzene ring had a profound effect on the distribution of responses. Dorsal responses were greater for benzaldehyde, methyl benzoate, propyl benzoate, and acetophenone, in which the double bond is next to the ring. These odors had steep, statistically significant dorsal-to-ventral response gradients. The gradient was significantly less steep for hydrocinnamaldehyde, phenyl acetate, and other compounds where the double bonded oxygen is removed from the ring by one or more atoms. The presence of a single bonded oxygen near the ring, as in anisole, does not produce a strong dorsal-to-ventral response gradient. These results indicate that many receptors in the
dorsal part of the epithelium, possibly corresponding to the most dorsal expression zone, have a particular sensitivity to charged molecules and to the configuration of those charges.

Supported by DC-00113 to J.W.S.

Poster 245

Differences in odor response of open vs. intact rat olfactory epithelial preparations. PAMELA E. SCOTT-JOHNSON[1,2], DAPHNE BLAKLEY[1], and JOHN W. SCOTT[1]. *Department of Cell Biology, Emory University, Atlanta, Georgia 30322*[1] *and Department of Psychology, Spelman College, Atlanta, Georgia 30314*[2]. FAX: (404)215-7863.

The electroolfactogram (EOG) recordings from our laboratory have shown a distribution of odor sensitivity that to some degree parallels the receptor gene expression zones that have been described by *in situ* hybridization. Those experiments were performed with an opened nasal cavity preparation in which odors were directly applied to the receptor sheet on the medial edges of the turbinate bones. In the present series of experiments we have reinvestigated the response to odors in freshly killed rats with intact nasal cavities by drawing air through the nose by suction and penetrating the epithelium with microelectrodes. This preparation allowed us to record responses from the olfactory epithelium lining the lateral recesses of the nose. We have compared these results to recordings from the opened preparation. According to studies with *in situ* hybridization, we should have been able to sample equivalent receptor populations with both approaches. We found that the differences between the responses were greater for the intact preparation than for the open preparation. For example, while the responses to a homologous series of aliphatic aldehydes were very similar for the dorsal and ventral parts of the epithelium in an open preparation, the patterns for the medial and lateral regions of the intact preparation were distinctly different. For the intact preparations, the longer chain aldehydes gave smaller responses in the lateral parts of the epithelium. In the intact preparation the differences between hydrocarbon compounds and more charged compounds were consistently greater than for the open preparation. It is not yet clear whether these differences arise from intrinsic properties of the epithelium or from odor sorption along the airflow path. These results indicate that one should be cautious in interpreting the data from the opened olfactory epithelium preparation.

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Poster 246

Patterns of olfactory receptor neuron projections are similar during development and after recovery from peripheral deafferentation. DIANA M. CUMMINGS, and FRANK L. MARGOLIS, *Dept. of Anat. and Neurobiol., Univ. of Maryland School of Medicine, Baltimore, MD 21201.* FAX: (410) 706-2512.

The rodent olfactory epithelium (OE) is unique in its ability to regenerate and reinnervate the bulb after deafferentation. We examined the pattern of olfactory receptor neuron (ORN) axon reinnervation into the bulb of H-OMP-LacZ-6 transgenic mice following chemical deafferentation. H-OMP-LacZ-6 mice were created using a transgene containing a truncated OMP promoter fused to the LacZ gene (Walters et al., J. Neurosci. Res., 43). In this strain, LacZ expression is limited to a subset of ORNs that are bilaterally distributed in the epithelium and project to a few glomeruli in the ventral region of the olfactory bulb (Treloar et al., J. Comp. Neurol., 367). In order to address the question of whether ORN axons preserve their topographic organization when they reinnervate the bulb, we lesioned the OE in adult H-OMP-LacZ-6 mice via intranasal irrigation with Triton X-100 and examined the distribution of §-galactosidase immunoreactive (§-gal-ir) and X-gal stained processes after short (10-day) and long (6-8 week) recovery times. At 10 days after the lesion, immunostaining for olfactory marker protein and §-gal was drastically reduced in the bulb. After 6-8 weeks of recovery, the pattern of §-gal-ir and X-gal staining was similar to the pattern found in young animals. Interestingly, the expression of LacZ is dramatically reduced in older H-OMP-LacZ-6 mice. However, when the epithelia of older mice are lesioned and allowed to regenerate, the pattern of §-gal-ir and X-gal staining mimics that found in younger animals. These data suggest that the pattern of bulb innervation that is present during development can be reinstated after Triton X-100 lesioning of the OE and bulb reinnervation. Current studies are exploring possible reasons for the down regulation of §-gal-ir ORNs in older H-OMP-LacZ-6 mice.

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Poster 247

Transplantation of olfactory epithelium containing genetically labeled olfactory receptor subtype, P2 neurons. ERIC H. HOLBROOK and RICHARD M. COSTANZO, *Department of Physiology, Virginia Commonwealth University, Medical College of Virginia Campus, Richmond, Virginia 23298-0551.*

The unique ability of the olfactory epithelium to continually replace olfactory sensory neurons is dependent upon its basal cell population. It has been shown in several labs that basal cells give rise to multiple cell types comprising the olfactory epithelium including sensory neurons. However, the degree of multi-potentiality these cells possess or the role local environment plays in their differentiation is not known. To help answer these questions, we used a genetically altered strain of mice (P2-IRES-tau-lacZ) developed in the laboratory of Richard Axel (Mombaerts et al., Cell 87:675-686, 1996). In this strain, the P2 receptor subtype can be identified based on the production of beta-galactosidase. P2 receptor cells are expressed within the olfactory epithelium in a reproducible zonal pattern. We transplanted olfactory epithelium taken from within and outside of the P2 zone into the brains of wild type C57/BL6 mice. We then allowed the animals to recover for various lengths of time. Transplants were then examined for the presence of P2 expressing neurons. This method allowed us to address several questions: 1) Will the basal cells that once gave rise to P2 type olfactory sensory neurons continue to produce P2 neurons in a novel brain environment? 2) Will any basal cells taken from outside the P2 zone start producing neurons that express P2 receptor subtypes in the new environment? 3) What type of connections will P2 axons make in this new environment? Answers to these questions will help determine the multi-potentiality of olfactory basal cells and the role they may play in the future repair of CNS lesions.

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Poster 248

Glial cells may be involved in axonal sorting in the developing olfactory system of *Manduca sexta*. LYNNE A. OLAND, MARK R. HIGGINS, LESLIE P. TOLBERT, *ARL Division of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.* FAX:(520) 621-8282.

In most sensory systems, primary afferent axons form a more or less continuous map of the peripheral end organ on the CNS; in olfactory systems, in contrast, particular molecular attributes of odorant molecules are detected by receptor neurons sparsely distributed over wide areas of the olfactory sensory epithelium, and these neurons project to particular discrete "glomeruli" in the CNS. Except on a very coarse level in mammals, the glomeruli are not arrayed in a pattern that reflects the distribution pattern of the receptor neurons in the epithelial sheet. Therefore, olfactory systems face a different challenge in their development: axons from dispersed sensory neurons must find each other and find targets without a simple topographic guidance system.

We previously have reported that a subset of developing olfactory receptor axons in the moth *Manduca sexta* express *Manduca* fasciclin II, a cell-adhesion molecule, and that, just before they enter the antennal lobe, receptor axons undergo dramatic reorganization in a discrete zone filled with glial cells. In this zone, they shed the neighbor relationships established in the sensory epithelium and establish relationships with axons that have common glomerular targets and therefore presumably share common odor specificities. Further study with the confocal microscope has revealed that before entering the sorting zone, fas II-positive and fas II-negative receptor axons are intermixed, whereas the fascicles that emerge from the sorting zone contain either axons that are generally positive for fas II or no axons that express fas II. The possibility of a functional relationship between the glia and axons was explored by electron microscopy of unlabeled material. As early-arriving receptor axons course through the sorting zone, their growth cones show a high tendency to travel next to the glial processes that permeate this zone. We currently are using glia-deficient animals to examine the importance of the glia in axon sorting.

[NIH 20040 to LPT.]

Poster 249

A novel family of ancient vertebrate odorant receptors in the lamprey *Lampetra fluviatilis*. LAURENCE DRYER[1], ANNA BERGHARD[2], [1]*Department of Biology and Biochemistry, University of Houston, Houston, TX, 77204-5513*, [2]*Department of Cell and Molecular Biology, Umeå University, Umeå, Sweden, S-901 87, Ldryer@bayou.uh.edu.*

We have isolated and characterized an ancient family of vertebrate odorant receptor (OR) genes from the olfactory epithelium of the lamprey *Lampetra fluviatilis*. This the most ancient vertebrate OR gene family isolated to date.

Although overall sequence analysis reveals striking homology to biogenic amine receptors, some conserved sequence motifs are characteristic of modern ORs. In situ hybridization analysis reveals that lamprey ORs are exclusively expressed in the lamprey olfactory epithelium. There is no zonal expression pattern similar to that observed in modern vertebrates. Instead, lamprey OR gene expression appears to be stochastic through the olfactory epithelium. Southern blot analysis reveals that these genes can be grouped into small subfamilies.

The nature of lamprey OR genes suggests common ancestry between modern ORs and other G protein-coupled receptors. These results suggest that modern ORs appeared after the first vertebrate radiation and that they have evolved slowly since their emergence.

Supported by the Swedish Medical Research Council, M. Bergvall and A. Wiberg Foundations, and NS32748.

Poster 250

Sequencing of olfactory receptor pseudogenes in the tiger salamander, *Ambystoma tigrinum*, and laminar analysis of mRNA expression within the olfactory epithelium by *in situ* hybridization. JAMES E. MARCHAND*, JOHN S. KAUER**, *Anesthesia Research* and Neuroscience**, Tufts University School of Medicine, Boston, MA 02111.* FAX: (617) 636-6738.

Olfactory receptor pseudogenes have been reported previously from cDNA cloned from human and rodent tissue, although *in situ* hybridization (ISH) analyses of pseudogene mRNA expression in olfactory epithelium have not been reported. Twenty-one unique odorant receptors were cloned by PCR using degenerate oligo primers derived from conserved regions of rodent olfactory receptors and sequenced on forward and reverse strands. ISH was performed on epithelial sections using ^{33}P-UTP labeled RNA probes, and the distribution and density of labeled cells within layers of the olfactory epithelium quantified.

Two clones, 1F9 and 1F10, each contain a frameshift mutation and two stop codons, indicating that they are pseudogenes and that, if translated, would result in a protein with only a partial olfactory receptor specific sequence; 6 other clones exhibited full length sequences characteristic of olfactory receptors. ISH analysis yielded ^{33}P-labeled cells for all eight clones, each with characteristic topographical and laminar distributions. Clones 1F9 and 1F10 exhibited a density of 1.5 and 1.8 cells/section, respectively, with a mean laminar distribution, with respect to the basal lamina, of 33.3 um and 49.8 um, respectively. The 6 complete receptor clones exhibited densities ranging from 4.6 to 49.8 cells/section (15.3± 7.3, mean±SE), and laminar depths ranging from 85 um to 132 um from the basal lamina (101±8.3). The difference in laminar distribution between the pseudogenes and full length genes is statistically significant ($p<0.01$), demonstrating that pseudogenes are expressed in cells that are located more basally in the epithelium.

These data suggest that expression of a complete, functionally active olfactory receptor protein may be necessary for normal division and migration of olfactory receptor neurons within the olfactory epithelium.

Supported by NIDCD grant (JSK)

Poster 251

Olfactory specific genes in *Xenopus laevis*. MARIO MEZLER, PATRICIA RÖSSLER, SIDONIE KONZELMANN, JÖRG FLEISCHER, JOACHIM FREITAG, HEINZ BREER *University Stuttgart-Hohenheim, Institute of Physiology, 70593 Stuttgart, Germany.* FAX:(711) 459-3726.

The complex functional organization of the vertebrate olfactory system requires subtle mechanisms for the coordinated expression of tissue- and cell-specific genes determining the specificity of chemosensory neurons. To approach the underlying events, the onset, the temporal and the spatial expression of olfactory specific genes (olfactory marker proteins [OMP], olfactory receptors) was determined in the developing olfactory system of *Xenopus laevis*. The early tadpoles start out with a simple olfactory placode (stage 23), which is transformed during development into the complex adult structure, consisting of the vomeronasal organ (VNO) and the main chamber, which is itself subdivided into the lateral (LD) and the medial diverticulum (MD). Earlier studies have shown that the adult animal has two classes of genes encoding distinct olfactory receptor types: fish-like (class I) and mammalian-like (class II) receptors. Each class appears to be spatially expressed in one of the chambers; class I receptors in the water-filled LD, and class II receptors in the air-filled MD. Using semiquantitative RT-PCR and *in situ* hybridization mature olfactory neurons are first observed at stage 32, corresponding to about two days after fertilization as indicated by the onset of OMP expression. At the same stage, expression of class I receptors but not of class II receptors was observed. Class II receptors, which are supposed to detect airborne odorants, were first detected at stage 49, about 12 days after fertilization. Monitoring the spatial distribution of cells expressing the distinct receptor types during the divergence of LD and MD in later tadpoles, it was found that a regional segregated expression pattern for class I and class II receptors already occurs before the two compartments are separated.

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Expression odorant binding proteins and olfactory receptors. HEINZ BREER, JÜRGEN KRIEGER, HANS KIEFER*, JOHANNES NOE, Dietrich Löbel, *University Stuttgart-Hohenheim, Inst. Physiology,, *Inst. Microbiology, 70593 Stuttgart, Germany.* FAX: (711) 459-3726

The olfactory system recognizes and discriminates myriads of odorants of diverse molecular structure. This task is supposed to be accomplished by odorant binding proteins and a large array of seven-transmembrane domain receptors encoded by a multigene family. To evaluate their binding properties, two distinct OBP-subtypes of the rat were expressed as N-terminal His-tagged fusion proteins in E.coli, thus allowing an efficient purification. Based on gel chromatography and CD-spectroscopy analysis the recombinant OBP subtypes seem to share several structural features with other members of the lipocalin family. Approaches to elucidate whether heterologous expressed OBPs in fact interact with odorous compounds revealed that OBP1 specifically binds [^{3}H]-2-isobutyl-3-methoxypyrazine whereas OBP2 did not. In contrast, displacement experiments revealed that fatty acids with appropriate chain length are efficient ligands for OBP2. These results indicate that rat OBPs display distinct ligand specificity. Employing the baculovirus/Sf9 cell system it was found that receptor proteins can be expressed at high levels. Stimulating receptor-expressing Sf9 cells with a mixture of numerous odorous compounds elicited a significant and dose dependent second messenger response, which was never observed in control cells. Assaying a large panel of odorous compounds, including representatives of different odor classes and compounds of different chemical classes revealed that distinct receptor subtypes respond to certain odorants but not to others. Graded responses to only a subset of odorants indicate that the heterologously expressed receptor types have a selective but relatively broad ligand specificity. The easily manipulated bacterial system was employed to produce olfactory receptor proteins in large quantities. It was solubilized from inclusion bodies and upon reconstitution in liposomes displayed specific interaction with odor ligands.

Supported by the Deutsche Forschungsgemeinschaft

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Characterization of the expression pattern of the newly identified odorant receptor gene OR-Z6. M. M. PYRSKI, Z. XU [1], S. MOUSSAVI [1], E. WALTERS [2], F. L. MARGOLIS [1], *Dept. Anat. & Neurobiol., Sch. Med., UMAB, Baltimore, MD21201[1], Dept. Biochem. & Mol. Biol., Med. Coll., Howard University, Washington, DC20059[2]*. mpyrski@umaryland.edu.

The goal of this study is to characterize the tissue-specific expression pattern of the recently identified odorant receptor gene OR-Z6 in the mouse olfactory epithelium and bulbs. OR-Z6 was initially discovered on the background of the transgenic mouse line H-lacZ6 (Walters et al., 1996) that was generated to study the function of a specific promoter element in the olfactory marker protein (OMP) gene. The expression pattern of the reporter gene β-galactosidase (OMP-lacZ construct) in olfactory tissues from H-lacZ6 mice closely resembles those of some members of the odorant receptor gene family. Investigation of the genomic region that corresponds to the transgene integration site led to the discovery of a new gene whose deduced amino acid sequence shares many similarities with members of the odorant receptor gene family. For example, the open reading frame of OR-Z6 is present as a single exon that encodes a typical G-protein-coupled, seven transmembrane protein. In addition, the first and third extracellular loops of OR-Z6 are short (7 and 15 residues, respectively), whereas the second extracellular loop is long (37 residues). Furthermore, OR-Z6 contains several conserved amino acid sequence domains and single residues that are located at identical positions when compared to the protein sequence of other odorant receptors. Moreover, our preliminary RT-PCR experiments using gene-specific primers and total RNA from mouse olfactory epithelium, main olfactory bulb, and liver demonstrate that OR-Z6 mRNA is only expressed in olfactory tissues. Taken together these findings strongly suggest that OR-Z6 encodes a new member of the large family of putative odorant receptor genes.

Our current interest is now focused on characterizing the expression patterns of OR-Z6 and lacZ mRNAs in mouse olfactory tissues to determine if their expression patterns are congruent in olfactory tissues from H-lacZ6 mice, indicating coordinate regulation.

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Making the connection from odor molecule to olfactory receptor: A Bioinformatics approach. EMMANOUIL SKOUFOS [1,2], PRAKASH M. NAKARDI[1], PERRY L. MILLER[1] and GORDON M. SHEPHERD[2]. [1]*Center for Medical Informatics and* [2]*Section of Neurobiology, Yale University School of Medicine New Haven, CT 06520 USA.* FAX: (203) 785-6990

Olfactory receptors (ORs) are seven transmembrane domain, G-protein coupled receptors and comprise the largest eukaryotic gene family, numbering about one thousand different genes in mouse. It is believed that their ligands are the odor molecules, however information on the ligand specificity of ORs is still very limited. This task is further complicated by the large number and the close relations among the receptors, by the large number and heterogeneity of the ligands, and by the hypothesis that a particular receptor may interact with a variety of different ligands in variable degrees of affinity. In addition, the difficulties in developing heterologous expression systems for the ORs has further complicated the task of linking particular odor molecules to specific receptors due to lack of experimental data. To aid the cloning, sequencing and classification of the ORs, the olfactory receptor database (ORDB) has been created. To help understand the odor molecules and their complexity as well as to classify their diverse effects in various experimental settings a new database (OdorDB, html pilot at: http://paella.med.yale.edu/~odordb) is under development. OdorDB, in addition to a listing of odor molecules, will contain models of their chemical structures, detailed information about the physiological and neurobiological effects of each, and the tissues and organisms in which these effects are exhibited, linked to the bibliography sources where these effects are demonstrated. Several ways to link OdorDB and ORDB based on data on specific receptor-ligand interactions will be presented. This linkage can be of aid to experimentalists in identifying potential odor molecule targets for particular receptors in further studies.

Supported in part by NIDCD, NASA and NIMH (Human Brain Project).

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Molecular cloning of a g-protein coupled receptor kinase from american lobster olfactory organ. FUQIANG XU, AND TIMOTHY S. MCCLINTOCK, *Department of Physiology, University of Kentucky College of Medicine, Lexington, Kentucky, 40536.* FAX: 606-323-1070

Signal termination in olfaction, as in the other G-protein coupled receptor mediated processes, is initiated by phosphorylation of the activated receptors. One of the kinases involved is a G-protein coupled receptor kinase (GRK). We have isolated cDNA clones for a GRK from a cDNA library made from the olfactory organ of the American lobster. Homology analysis shows that this putative lobster GRK belongs to the GRK2 subfamily. It has 78% amino acid identity with *Drosophila* GPRK1 (GRK2 subfamily), 72% with *C. elegans* GRK, and 71% with mammalian GRK3, which is highly expressed in olfactory epithelium and necessary for olfactory signal termination. The lobster GRK and the previously isolated $G_{\alpha s}$, $G_{\alpha q}$, G_{β} subunits, and PLC-β are putative components of the lobster olfactory transduction pathways. We are investigating their involvement and roles in olfactory signal transduction.

The research was supported by NIDCD award DC02366 to T.S. McClintock.

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Identification and characterization of a novel tissue-specific transcriptional activating element in the olfactory mucosa-predominant cytochrome P450 *CYP2A3* gene. XINXIN DING[1,2], JIANHUA ZHANG[1], [1]*Wadsworth Center, New York State Department of Health,* [2]*School of Public Health, State University of New York at Albany, NY 12201.* xding@wadsworth.org.

YP2A3, a cytochrome P450 monooxygenase highly active toward many olfactory toxicants as well as endogenous compounds, such as sex steroids and odorants, is expressed abundantly in rat olfactory mucosa and, in trace amounts, in the lung, but not in any other tissues examined. To understand the mechanisms that dictate the olfactory mucosa-selective expression of this and other cytochrome P450 genes, we have examined the 5'-flanking region of the *CYP2A3* gene. DNase I footprinting analysis revealed a single protected region in a 300-bp probe (-255 to +58) with nuclear extracts from olfactory mucosa, but not from liver, lung, kidney, or brain. The tissue-specific binding was confirmed by gel-shift analysis. The core sequence of the binding site, named NPTA (nasal-predominant transcriptional activating) element, was identified and found to be essential for transcriptional activity of the *CYP2A3* promoter *in vitro*. NPTA-binding proteins were detectable at day 1 and were much more abundant at day 8 than at day 60 after birth. Furthermore, the levels of the binding proteins decreased dramatically during chemically-induced degeneration of the olfactory epithelium, paralleling the disappearance of olfactory microsomal CYP2A3 protein, and rebounded to higher-than-pretreatment levels during recovery. Thus, we have identified a novel transcriptional activating element which may play a crucial role in the tissue-selective expression of the *CYP2A3* gene in the olfactory mucosa.

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Induction of stress proteins in olfactory epithelial supporting cells by odorants. VIRGINIA MCM. CARR and A.I. FARBMAN, *Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208-3520.* FAX: (847) 491-2867.

We have described a subpopulation of rat olfactory receptor neurons (ORNs) that is reactive with Mab 2A4, generated to the human 70 kD heat shock protein (HSP70), provided by R.Morimoto, Northwestern Univ.).[1] In rats Mab 2A4 shows reactivity to both the inducible HSP70 and the constitutively expressed HSC70 members of the HSP70 family. To test the possibility that 2A4 ORN immunoreactivity (IR) might reflect responses to environmental odors, the olfactory epithelia (OE) of adult Sprague Dawley rats were monitored for any changes in 2A4(+)ORN patterns following addition to cage bedding of odorants in the form of 1.0 ml commercially available essential oil extracts. Odors tested included peppermint, spearmint, lemon, lavender, anise, cinnamon, and clove. No changes in 2A4(+)ORN IR patterns were observed, indicating that ORN HSP70 expression is not likely to be odorant-related. However, for each odor tested except clove, a strong induction of 2A4 IR occurred in the supranuclear, Golgi region of the nonneuronal supporting cells (SCs). XIt was "dose"-dependent and followed classical stress protein response temporal patterns. Similar responses were obtained with antibodies to ubiquitin (representing a very different stress response), HSP90, and HSP25. However, α-HSP25 IR was prominent throughout the SC cytoplasm rather than the supranuclear region alone. Responses were also observed with antibodies directed specifically to HSP70 and to HSC70 (Santa Cruz). In contrast, clove odor (as either the essential oil extract or eugenol) failed to elicit stress protein expression, even when 4 times the normal odorant volume was used. Experiments testing a wider odorant array (i.e., less "pleasant") are underway.

1. Carr et al., J. Comp. Neurol. 348:150,'94).

Poster 258

Expression of retinoic acid receptor mRNA isoforms in rat olfactory mucosa after bulbectomy. DAVID B. CONLEY, ALAN M. ROBINSON, ROBERT C. KERN, DIMITRI Z. PITOVSKI, *Department of Otolaryngology, Northwestern University Medical School, Chicago, IL 60611*. FAX: 312-503-1616.

Retinoids play an integral role in the differentiation of many epithelial tissues. Effects of retinoic acids are mediated by nuclear retinoic acid receptors (RARs) which bind to their cognate ligands. Subsequent binding of the ligand – receptor complex to retinoic response elements modulates gene expression. Three types of RARs have been described, α, β, and γ, each of which are expressed in multiple isoforms. These isoforms, produced by alternative mRNA splicing, exhibit age and tissue dependent differential expression.

In this study, we utilized the technique of RT-PCR to compare the expression of RAR α and γ mRNA isoforms in bulbectomized and control olfactory mucosa. Comparisons were also made to rat brain and lung. Specific isoforms were chosen for study based on their differential expression in other epithelial tissues. Results demonstrate a differential expression of these isoforms in the olfactory mucosa after bulbectomy verses control. This study suggests that retinoids play a significant role in olfactory mucosal hemostasis.

Poster 259

Conduction velocity of olfactory receptor neurons in the omp-null mutant mouse. EDWIN R. GRIFF[1], FRANK L. MARGOLIS[2], MATTHEW ENNIS[2] & MICHAEL T. SHIPLEY[2], [1]*Dept. Biological Sciences, Univ. Cincinnati;* [2]*Dept. Anat. & Neurobiol., Univ. Maryland Sch. Med.* Edwin.Griff@UC.EDU.

Olfactory marker protein (OMP) is expressed in mature olfactory receptor neurons (ORNs) and may represent as much as 4% of their cellular protein. To explore the function of OMP, a null mutant was generated by gene targeting. Analysis of the electroolfactogram (EOG) of OMP-null mice showed a decrease in the initial kinetics of response to odor stimuli, a decline in the rate of return to baseline, and a decrement in the maximal amplitude achieved (Buiakova et al., PNAS, 1996). In addition, the ability of the EOG to respond to multiple stimuli was compromised (Margolis et al., ISOT,1997). The present study assessed the effect of OMP deletion on the conduction of the compound action potential along ORN axons in OMP-null mice and genotypically matched controls.

Extracellular compound action potentials were recorded from chloral hydrate anesthetized mice. Isolated square-wave stimuli were applied to the surface of the olfactory nerve layer of the rostral bulb with a twisted pair of 125 um wires. A triphasic compound action potential was recorded at each of several distances from the stimulation electrode. The latency to a large negative trough was measured, and the conduction velocity calculated. The compound action potential was distinguished from postsynaptic field potentials by its shorter latency, its ability to survive blockage of postsynaptic responses by applying cobalt or kynurenic acid to the bulbar surface, and its ability to follow twin pulse stimulation at 300 Hz. Blockade of the postsynaptic field responses by kynurenic acid indicates that, as in the rat, glutamate is the mouse olfactory nerve transmitter.

The mean conduction velocity of ORNs in control mice was 0.40 ± 0.09 m/sec (n=5), similar to the conduction velocity reported for cat and rabbit. The mean conduction velocity of OMP-null mice was 0.38 ± 0.03 m/sec (n=6); this was not significantly different from control (p=0.1). The normal conduction velocity of action potentials along the ORN axons in OMP-null mutants indicates that this mutation does not cause a general impairment of the axonal membrane's ability to generate electro-physiological responses. Thus, the reduction in the sensitivity and amplitude of the EOG in the OMP-null mutant mouse more likely reflects a defect in the receptor potential, initial transduction events, and/or initiation of the action potential in the ORNs.

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Poster 260

Concentration tuning derived from intracellular gain and spare receptor capacity distributions among olfactory sensory neurons: a theoretical study. THOMAS A. CLELAND[1], CHRISTIANE LINSTER[2], JOHN S. KAUER[1], [1]*Department of Neuroscience, Tufts University, Boston, MA 02111,* [2]*Department of Psychology, Harvard University, Cambridge, MA 02138.* tcleland@emerald.tufts.edu.

The olfactory system is capable of detecting odorants at low concentrations. Physiological dose-response curves for odorants have yielded EC_{50} values between 10^{-4} and 10^{-10} M in preparations from the sensory epithelium. However, the contemporary model for olfactory signal transduction provides that odorants bind to olfactory receptors with relatively low specificity and consequently low affinity, making the relationship between binding affinities and response thresholds difficult to reconcile.

We employ a computational model based upon pharmacological occupancy theory to propose a potential mechanism by which olfactory sensory neurons (OSNs) are able to exhibit both (1) an enhanced sensitivity to odorants, without requiring increased odor-receptor binding affinities or specificities, and (2) an increased concentration tuning range at the level of glomerular convergence. Distributions of binding-activation relationships among OSNs expressing identical odor specificities were found to both increase sensitivity to low-concentration odorants and to parsimoniously extend the concentration tuning range of a homogeneous, convergent OSN population. Both the modulation of CNG channel sensitivity to second messengers (Mueller et al., 1998, *J. Neurosci.* 18(1):164-173; Liu et al., 1994, *Science* 266:1348-1354; Chen and Yau, 1994, *Nature* 368:545-548) and variable overexpression of odorant receptor proteins (spare receptor capacity) are theoretically capable of generating the appropriate binding-activation distributions without affecting odor quality tuning. These secondary concentration-tuning mechanisms could play an important role in the olfactory system's capacity to reliably detect low odor concentrations, discriminate odor intensities, and segregate intensity information from representations of odor quality.

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Poster 261

Neural coding of complex odorant mixtures: inhibitory receptor binding events contribute to mixture suppression in olfactory receptor neurons of spiny lobsters. STUART I. CROMARTY, CHARLES D. DERBY. *Dept. of Biology, Georgia State University, Atlanta, GA 30303.* cderby@gsu.edu.

Inhibitory events, including activation of inhibitory ion channels by mixture components and inhibitory interactions between components at the membrane receptor sites (= binding interactions), can contribute to the transduction of mixtures. Previous studies suggest that these inhibitory events are important in shaping the responses of ORNs of spiny lobsters (*Panulirus argus*) to 2-component mixtures (Daniel et al., 1996). In the current study, we extended this analysis to more complex mixtures, by examining the responses of spiny lobster ORNs to mixtures of up to 7 compounds. We found that responses to mixtures of excitatory odorants were often less than the response to the most excitatory component. The effect of adding an excitatory odorant to a mixture depended on the composition of the mixture and the component, but the added excitatory component in some cases had no effect or even decreased the response intensity, thus demonstrating nonlinear contributions of the components. The predictions of competitive or noncompetitive models of neural responses were improved when they included a term for empirical measurements of binding inhibition. A competitive model that included binding inhibition was the best predictor of the measured responses to complex mixtures. These results suggest that binding inhibition is one mechanism contributing to mixture suppression in neural responses to complex mixtures, and the magnitude of the binding inhibition depends on which odorants are present in the mixture.

Supported by NIH grant DC-00312.

Poster 262

Numerical modeling of odorant uptake in the rat nasal cavity. GEOFFREY C. YANG[1], PETER W. SCHERER[1], JAMES E. SCHWOB[3], and MAXWELL M. MOZELL[2], [1]*Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104,* [2]*Department of Physiology and* [3]*Department of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210.* FAX: (215)573-2071.

A 3-D anatomically accurate numerical model of the rat nasal cavity has been developed to simulate the flow distribution in the nasal passage and the odorant uptake in the olfactory region. Based on the two physiological inspiratory flow rates of 125 and 250 cm^3/min in the real rat nose, the model allows us to determine the effects of various transport parameters (e.g. flow rates and physiochemical properties of the inhaled gases) on the odorant uptake.

Streamlines are traced by specifying neutrally buoyant particles at the external naris and computing the subsequent particle trajectories with a small time increment to help us better understand the bulk flow patterns in the rat nose. Two major stream patterns are observed that agree well with those found by Morgan *et al.*(1991) and Kimbell *et al.*(1997). One major stream of the bulk flow goes along the nasal floor and exits through the nasopharyngeal meatus. The other stream, originating from the dorsal side of the external naris plane, takes a more circuitous route to enter the convoluted ethmoid recess containing the olfactory epithelia, then bends proximally toward the external naris, and finally exits through the dorsal region of the nasopharyngeal meatus.

Several odorants commonly used in rodent olfaction studies were used in the simulation. The preliminary results of odorant uptake suggest that there is a correlation between the local mass peak mucosal surface flux and one of the circumscribed zones for specific receptors found by researchers using *in situ* hybridization. Further investigation is needed to explore the correlation between the numerical model surface flux predictions and other olfactory neuro-anatomical regions.

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Poster 263

A portable artificial nose based on olfactory principles. JOHN S. KAUER, JOEL WHITE. *Department of Neuroscience. Tufts University School of Medicine. Boston, MA 02111.* jkauer@opal.tufts.edu

Information processing of odors by biological olfactory systems depends strongly on neuronal signals distributed both in space across many cells at different levels of the pathway and in time over the stimulus pulse. A number of artificial systems have been developed which use patterned activity across various kinds of sensor arrays to recognize odors (see, for example, Persaud and Dodd, *Nature*, 1982). Such parallel, distributed events can also be exploited in artificial olfactory systems that include analysis of response time. We have previously shown that one can obtain robust odor responses from a device that uses space and time information (White et al., *Anal. Chem.*, 1996) that is based on optical sensors observed by a research grade, lab-based microscope, a CCD video camera, and positive-pressure odor delivery system. In this paper we describe an initial portable version of this device that incorporates a negative pressure odor delivery sniffer, an array of wide-dynamic range photodiodes serving as detectors, and an embedded microcomputer for controlling stimulus delivery, data acquisition and storage, and pattern recognition. After training, the device is capable of discriminating and identifying a number of odorant substances with several seconds.

Supported by grants from the NIH-NIDCD, ONR and DARPA.

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Use of an electronic nose to evaluate methods for odor remediation. SUSAN S. SCHIFFMAN[1] AND H. TROY NAGLE[2], [1]*Department of Psychiatry, Duke University Medical School. Durham, NC 27710 and* [2]*Department of Electrical and Computer Engineering, North Carolina State University, Raleigh, NC 27695-7911.* FAX: (919) 684-8449.

Livestock and other industries with odorous by-products are expanding rapidly in certain areas of the globe, and this expansion is causing environmental concerns. Reduction of odors emanating from these operations is desirable to improve relationships between producers and their neighbors. At the present time, the most frequently used methods for evaluating the efficacy of odor remediation techniques are human odor panels and gas chromatography/mass spectromety (GC/MS). An alternative method utilizes an electronic nose (E-Nose) to provide rapid, accurate, cost-effective evaluation of techniques to reduce odor production. An electronic nose (E-nose) is an instrument consisting of a gas sampling apparatus and an array of gas sensors interfaced to a personal computer. Odorous stimuli may consist of hundreds or thousands of different volatile organic compounds (VOCs). The E-nose examines a changing pattern of response across its sensor array to differentiate between different sets of odor stimuli. This differs from classical GC/MS which tries to identify the individual compounds in an odor, a formidable task. In this project, we have developed a method for odor evaluation based on an electronic nose which correlates with a human odor panel. The main findings are that electronic nose could differentiate three odor sources (unfiltered synthetic slurry, biofiltered slurry, and biofiltered blank/control samples). In addition, the electronic nose could differentiate between serial dilutions of the three odor sources. Discrimination of the three sample types was achieved on two different kinds of electronic noses (the AromaScan A32S based on polymer technology and the North Carolina State University E-Nose based on metal oxide technology). A neural network was successfully trained to mimic the performance of the human panel in a field test of swine odors. The results suggest that further refinements of electronic nose technology should prove capable of on-site, real time odor measurement at production facilities.

Poster 265

Insulin supports olfactory neuron survival *in vitro* and may be produced in the nasal mucosa. JACQUELYN. K. MCENTIRE, and SARAH. K. PIXLEY, *Dept. of Cell Biol., Neurobiol, and Anat., Univ. of Cincinnati, Cincinnati, OH 45229,* mcentijk@email.uc.edu.

To aid in understanding the developmental and regenerative properties of the olfactory epithelium, we have developed partially purified cultures of the newborn rat olfactory epithelium using serum-free medium. The olfactory neurons in the cultures require insulin for their survival. Addition of insulin to the serum-free medium greatly increased the number of neurons, while not increasing the number of bromodeoxyuridine positive (BrdU+) neurons. However, there was no detectable effect on other cell type numbers. Addition of high levels of insulin like growth factor-1 (IGF-1), instead of insulin, did not support neuronal numbers as well as adding insulin. This suggests that insulin's neurotrophic effect is through the insulin receptor and not entirely due to cross reactivity with the IGF-1 receptor, as is found with other neurons.

Because insulin is produced in the brain, we used RT-PCR to detect insulin I and insulin II mRNA in RNA extracts from the nasal mucosa. Immunostaining with an anti-insulin antibody in the cultures showed that neurons were labeled. This suggests that the nasal mucosa has the potential to provide its own insulin source. Based on the immunostaining data the olfactory neuron may be a candidate cell for insulin production.

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Poster 266

Insulin-sensitivity of the olfactory system. NANCY E. RAWSON and PATRICIA M. ULRICH. *Monell Chemical Senses Center, Philadelphia PA 19104-3308.* rawson@monell.org.

Levels of insulin and insulin receptors in the olfactory bulb are higher than elsewhere in the brain in adult animals, but little is known about the role of insulin in the olfactory system. In peripheral nerves and neuronal cultures, insulin promotes neuronal growth and differentiation. It has also been suggested that insulin in the brain may have neuromodulatory effects. We have previously shown that insulin receptors are expressed at low levels in adult rat olfactory epithelium, but expression in olfactory receptor neurons is increased when plasma insulin is reduced by destruction of the pancreatic islet cells with streptozotocin.

Acute effects of insulin on olfactory receptor neurons are being studied in freshly isolated cells from adult normal and streptozotocin-diabetic rats. Isolated cells are attached to coverslips and loaded with the calcium-sensitive indicator Fura-2 for ratiometric imaging. Results indicate that insulin can potentiate odorant responsiveness of some olfactory neurons from normal, adult rats, and that insulin alone can influence calcium homeostasis in olfactory neurons isolated from diabetic rats. Studies are in progress to determine how these effects of insulin are mediated, and to further investigate the impact of streptozotocin-induced diabetes on olfactory structure and function.

Supported by NIH DC08276 and a Morley R. Kare fellowship.

Poster 267

Decreased olfactory ability in chronic rhinosinusitis is related to aspects of mucosal disease. DONALD A. LEOPOLD[1], FRANCISCO S.N. SAMPAIO[2], BIRGITTA E. MOYLAN[2], DAVID PROUD[2], ALKIS TOGIAS[2], *[1]Dept. of Otolaryngology-Head and Neck Surgery and [2]Division of Clinical Immunology, Johns Hopkins Univ., Baltimore MD 21287.* FAX: 410-955-0035.

Decreased olfactory ability is often noted to accompany chronic rhinosinusitis. Since the olfactory and respiratory epithelia are both in the nasal cavity, we have studied how this inflammatory disease affects the clinical expression of rhinosinusitis and olfactory ability.

Thirty one individuals with chronic refractory rhinosinusitis and 29 control subjects with no nasal or sinus symptoms were evaluated with systematic assessments of history and severity of upper airway disease, atopic status, and mucosal responsiveness to histamine. Olfactory ability was assessed in all using the 40 odorant Smell Identification test^{R} (Sensonics, Inc., Hadden Heights, NJ), and scored in three groups (normal, mild/moderate loss and anosmia/severe loss). Olfactory ability decreased with increasing number of surgeries ($p<0.0001$) and number of positive skin tests ($p<0.002$). Histamine responsiveness was assessed by stimulating the left nostril with histamine, and measuring the liquid produced on the anterior septum of each nostril using weighed filter paper disks. On this test, the weight of secretions only on the stimulated side decreased as the olfactory ability decreased ($p<0.03$). We also found a trend for decreased sneezing in response to histamine as the olfactory ability decreased. However, olfactory ability did not correlate with either of two questionnaires assessing the severity of nasal and sinus symptoms.

The number of surgeries and the degree of atopy can be considered rough estimates of mucosal disease severity. Symptoms can vary greatly independent of the amount or location of mucosal disease. We interpret the reduced responsiveness to histamine as an index of mucosal dysfunction. Given that this test is performed at a site distant from the olfactory epithelium, our findings indicate that increasingly refractory rhinosinusitis is associated with abnormalities of the entire nasal mucosa.

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When regeneration fails: Methyl bromide lesions and the replacement of olfactory epithelium by respiratory epithelium. JAMES E. SCHWOB, STEVEN L. YOUNGENTOB, *Departments of Anatomy and Cell Biology and of Physiology and the Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210.* schwobj@vax.cs.hscsyr.edu

Despite the olfactory epithelium's (OE) remarkable capacity to regenerate itself after either experimental or natural lesion, the failure to reconstitute damaged epithelium as olfactory is, in the extreme, a cause of olfactory dysfunction in humans. Indeed, replacement of OE by respiratory epithelium (RE) is, to a more limited extent, a consequence of "normal" life for humans. We have developed an experimental paradigm that reliably produces patchy replacement of large swathes of the OE in the anterior and ventral olfactory epithelium of the rat, namely exposure to MeBr of animals that are maintained at 75% of their free-feeding weight. Thus, we have contrasted the cellular consequences of damage between areas that always recover as olfactory and ones that recover as respiratory. We have analyzed the pattern of cellular proliferation using BrdU. In whole mount preparations of the septal mucosa, proliferation increases between 24 and 48 hours after lesion across the whole extent of the mucosa, including areas that will regenerate normal olfactory epithelium and others that will not. However, by 5 days after lesion only those areas that will regenerate as OE retain a high rate of proliferation. We have also contrasted the types of cells that are dividing between these two types of areas. In both, cells derived from Bowman's glands proliferate and migrate away from the duct. In the areas reconstituting as OE, the migrating duct/gland cells become sustentacular cells, as we have shown previously using retroviral lineage techniques. In areas that become RE, only ciliated columnar epithelial cells appear. The most striking contrast between areas that regenerate as OE and those that do not is the absence of cells that stain with monoclonal antibodies GBC-1, 2, and 3, which label globose basal cells, from those areas that become lined by RE. Thus, we hypothesize that the proximate cause for the patchy replacement of OE by RE after lesion is the total destruction of GBCs, and conversely, that transplantation of GBCs might alleviate that particular pathology . The data also suggests that the formation of sustentacular cells by cells derived from Bowman's glands depends on signals from the surrounding epithelium that are evident only when that epithelium retains its character as olfactory.

Supported by NIH grant P01 DC00220.

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Olfactory pathology in chronic rhinosinusitus. ROBERT C. KERN[1], DAVID B. CONLEY[1], KAREN J. FONG[1], G. KENNETH HAINES[2], *Departments of Otolaryngology[1] and Pathology[2], Northwestern University Medical School, Chicago, IL 60611.* FAX: 312-503-1616.

Chronic rhinosinusitis is common syndrome manifested by an inflammatory response of the mucus membranes of the nasal cavity and paranasal sinuses. This entity is likely the most common cause of clinical olfactory deficits and over 10 million people may be affected. Rhinosinusitis is often associated with the presence of allergic and non-allergic rhinitis, as well as nasal polyposis. Histopathologic changes in the respiratory mucosa of patients with chronic sinusitis reveal thickening of the lamina propria and an influx of plasma cells and lymphocytes. Pathologic changes in the olfactory mucosa have not been documented and olfactory deficits in these patients are presumed to result from respiratory mucosal edema, nasal obstruction and decreased airflow to the olfactory cleft. Clinical studies, however, have failed to show a correlation between nasal resistance and the degree of olfactory deficit. The current study examines the olfactory mucosa of patients with chronic sinusitis undergoing surgical treatment. Anosmia was evaluated with an UPSIT immediately prior to surgery. Pathologic changes in the olfactory mucosa included thickening of the basement membrane with an influx of macrophages, plasma cells and lymphocytes. Examination of the neuroepithelium revealed flattening of the cells, with an increase in the nuclear to cytoplasmic ratio. Lymphocytes were also present throughout the epithelium. The current study demonstrates changes in the olfactory mucosa that are similar to that seen in the respiratory mucosa of patients with chronic sinusitis and anosmia. This study further suggests that the mechanism of anosmia in these patients may be related to the direct effects of inflammatory mediators in the olfactory mucosa, rather than gross changes in nasal airflow.

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A molecular basis of cell death in olfactory epithelium. ALBERT I. FARBMAN, JUDITH A. BUCHHOLZ, ALEXANDRA COINES, DEBRA SPEERT. *Department of Neurobiology & Physiology, Northwestern University., Evanston, IL. 60208 USA.* afarbman@nwu.edu.

When the membrane receptor Fas binds to its ligand, Fas ligand (FasL), an apoptotic cascade is initiated in the cell bearing the Fas receptor. The same can be said about the Tumor Necrosis Factor Receptor 1 (TNFR1) and its ligand, TNF-α. In this study we have shown that the mRNAs of both sets of ligands and receptors, Fas/FasL and TNF-α/TNFR1, were present in unperturbed olfactory epithelium. Fas was shown by immunohistochemistry and by Western blots of bulbectomized animals to be in the neurons. FasL was primarily present in non-neuronal, microvillar cells of unperturbed rat olfactory epithelium. Addition of either FasL or TNF-α to organotypic cultures of fetal rat olfactory epithelium resulted in a significant increase in the number of apoptotic bodies after 6 hrs. This suggests that either or both ligands can induce cell death in olfactory epithelium.

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OMP gene deletion causes an elevation in behavioral threshold sensitivity. S.L. YOUNGENTOB[1] and F.L. MARGOLIS[2], [1]*Dept. of Physiology, SUNY Health Science Center, Syracuse , NY 13210, and Dept. of Anatomy & Neurobiology, Univ. of Maryland School of Medicine, Baltimore, MD 21201.* youngens@vax.cs.hscsyr.edu.

Previously, Buikova et al. (PNAS 93:9858-9863, 1996) demonstrated that OMP-null animals show a defect in mucosal EOG activity which is also reflected in the phenotype of the olfactory bulb. As an initial step in behaviorally addressing OMP's role in odor processing we: (1) assessed the ability of OMP-null animals to acquire a simple air vs. odor discrimination for a number of odorants; and (2) assessed whether OMP-null animals differ, relative to controls, in their threshold sensitivity.

A discrete trials, go, no-go successive discrimination paradigm was used to train five OMP-null and six control mice to detect the presence of ethyl acetoacetate, carvone, propanol, propyl acetate, and citral. Using standard operant techniques, mice were trained to initiate a trial (insert nose in sniffing port) and sample the odorant presented. In the presence of the S^+ stimulus (odor) the animal was trained to leave the sniffing port and lick the reinforcement cup. On trials in which only air (S^-)was presented, the animal was required to withhold responding for the duration of the trial. Each mouse was trained to criterion (>90% over ten successive blocks of trials) on each of the five successive air vs.odor discrimination problems.

On the average, control and OMP-null animals required 2.04 and 1.68 testing sessions, respectively, to acquire criterion performance on each discrimination problem. The slight difference in favor of the null group, however, was not significant ($t= 0.83$, $P = 0.41$). The results demonstrate that despite the altered EOG in the OMP-null mice these animals can easily acquire a simple air vs. odor discrimination problem.

To further investigate whether the absence of OMP results in a compromised ability to respond to odors, we compared the threshold sensitivity of OMP-null and controls animals for the odorant propanol. Two threshold estimates were determined for each of three OMP-nulls and three controls, using a modified descending method of limits procedure. On the average, threshold sensitivity to the odorant propanol was 4.6×10^{-6} vs 9.33×10^{-9} of vapor saturation for the OMP-null and control animals, respectively. Therefore, compared to controls, null animals were approximately 2 log units *less sensitive* to the odorant propanol. These results provide additional support for the hypothesis that OMP plays a modulatory role in the odor detection/signal transduction process.

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Na, K –ATPase subunit isoform expression in olfactory mucosa under the influence of corticosteroids. KAREN J. FONG, ROBERT C. KERN, DIMITRI Z. PITOVSKI, *Department of Otolaryngology-Head and Neck Surgery, Northwestern University Medical School, Chicago, IL 60611.* dz-pitovski@nwu.edu.

Na, K –ATPase is a plasma membrane enzyme that utilizes the energy from ATP to maintain the transmembrane electrochemical gradient of sodium and potassium in all mammalian cells. High levels of this enzyme are found in both ion-transporting and neural tissues. Na, K –ATPase exists as a heterodimer consisting of two noncovalently linked polypeptide subunits termed a (α1, α2, and α3) and β (β1,β2, and β3). The physiologic significance of this isoform diversity is not yet well understood, but a tissue-specific pattern of distribution has been demonstrated in other tissues, suggesting different roles for the various isoforms. The specific isoform distribution within the olfactory mucosa has not been previously studied, but the presence of both ion-transporting and neural components would lead us to anticipate the expression of multiple Na, K –ATPase isoforms.

In the current study, the density and distribution of various Na, K –ATPase subunit isoforms in the mammalian olfactory mucosa is examined using the techniques of enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry. Furthermore, in many other tissues, the activity of Na, K –ATPase is modulated by corticosteroid hormones. In our experimental animal model, corticosteroid levels were experimentally altered by adrenalectomy and corticosteroid replacement. The effect of altered corticosteroid hormone levels on the expression of Na, K –ATPase subunit isoforms in the olfactory mucosa were then studied utilizing the techniques of ELISA and immunohistochemistry.

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Androgenic regulation of gene expression in primary and second-order chemosensory neurons? M.L. GETCHELL[1,2], R. MARCINEK[1], T.V. GETCHELL[1,2,3], [1] *Div. of Otolaryngology, Dept. of Surgery;* [2]*Sanders-Brown Center on Aging,* [3]*Dept. of Physiology, Univ. of Kentucky College of Medicine, Lexington, KY 40536,* mgetchell@aging.coa.uky.edu.

Androgen receptors (ARs) are ligand-dependent nuclear transcription factors that in some neurons mediate the androgenic regulation of expression of GAP-43, cytochrome P450 aromatase, neuronal nitric oxide synthase, and AR genes. ARs are localized in neurons of the amygdala and hypothalamus in central chemosensory pathways (Wood and Newman, 1995, Neuroendocrinology, 62:487). We now report that AR mRNA is present in the olfactory mucosa (OM) and olfactory bulb (OB) and that AR protein is localized in olfactory and vomeronasal receptor neurons (ORNs, VRNs) as well as in juxtaglomerular and mitral cells of the OB in rats of both genders. RT-PCR with RNA isolated from the OM, OB, and, as a positive control, prostate gland of 12-week old rats and primers with unique sequences located in different exons of the AR gene (Shan *et al.*, 1995, Endocrinology, 136:1686) gave an amplimer of the expected size in all tissues. Restriction enzyme analysis verified that the PCR product corresponded to the predicted sequence of rat AR cDNA. Immunohistochemistry was performed with antibodies specific for epitopes in either the hormone- or DNA-binding domain of the AR protein; neither antibody cross-reacts with other steroid hormone receptors. AR protein was localized in the nuclei of ORNs in the upper half of the olfactory epithelium (OE), with a patchy distribution of positive nuclei extending deeper into the OE, similar to that of estrogen receptors (Getchell *et al.*, 1996, Soc. Neurosci. Abs., 22:1594), and in most but not all VRN nuclei. A subpopulation of juxtaglomerular cells and numerous mitral cells exhibited immunoreactive nuclei in the OB. ARs were also immunolocalized in the nuclei of ORNs of adult humans of both genders. By regulating gene expression in olfactory and vomeronasal neurons, androgens may influence reproduction-related chemosensation, neuronal maturation and plasticity, and development.

Supported by NIH grants DC 01715 (MLG) and DC 00159 (TVG).

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Activation of olfactory dendrodendritic reciprocal synapses through NMDA receptors and its dependence on action potential propagation in the mitral cell secondary dendrites. WEI R. CHEN and GORDON M. SHEPHERD, *Section of Neurobiology, Yale University School of Medicine, 333 Cedar Street, C303 SHM, New Haven, CT 06510.* chen@spine.med.yale.edu.

The mitral cell of the mammalian olfactory bulb has several long secondary dendrites which interact with inhibitory granule cells through dendrodendritic reciprocal synapses. This unique synaptic organization has suggested an important role for the dendrodendritic synapses in the central processing of olfactory signals. We have analysed mechanisms of activation of these synapses using patch recordings under IR microscopy in rat olfactory bulb slices. Following single action potentials elicited in single mitral cells, a large and long-lasting hyperpolarization was seen in 0 Mg ACSF but not in normal ACSF. This hyperpolarization was a recurrent IPSP rather than a spike after-potential, because it was blocked by bicuculline methiodide. Increasing intracellular chloride concentration shifted the reversal potential of the IPSP in a manner predicted by the Nerst equation, indicating that the IPSP was mediated by chloride ions. Dual patch recordings from the soma and distal primary dendrite indicated that the recurrent IPSP mainly originated from granule cells acting on the secondary dendrites rather than PG cells acting on the glomerular tuft. The persistence of the IPSP in TTX solution suggested that the IPSP was not mediated by mitral cell axon collaterals terminating on granule cells, but rather by the dendrodendritic reciprocal synapses. Pharmacological analysis showed that NMDA receptors were critically required for the recurrent IPSP whereas the antagonists of non-NMDA receptors had no effect. Under normal conditions, the recurrent IPSP could not be evoked by subthreshold depolarization, but was always associated with a preceding action potential. This suggested that action potentials need actively invade the secondary dendrites to activate the dendrodendritic synapses. Simultaneous recordings from the soma and secondary dendrites confirmed that, as in the primary dendrite, action potentials actively propagate into the secondary dendrites. The findings suggest that the dendrodendritic reciprocal synapses are dynamically involved in odor information processing as well as olfactory learning and memory.

This project was supported by grants from NIDCD, and from NIMH, NASA and NIDCD (Human Brain Project).

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Dendritic Depolarization in Mitral/Tufted Cells (MTs): Voltage Sensitive Dye Recording Evidence from Single Neurons. A. R. CINELLI. Z. XIANG. *Dept. Anat. & Cell Biol., SUNY Brooklyn, NY 11203.*

An important characteristic of mitral/tufted cells (M/Ts) is the presence ofactive depolarizations in their dendritic tree. As suggested by population studies using VSD dyes and single cell imaging of Ca2+ transients, prolong depolarizations at dendritic level might be responsible of long-lasting excitability changes. These changes follow odor and electrical stimulation affecting the integrative properties of M/Ts and the spatio-temporal pattern of the activity in the olfactory bulb. In this study, voltage sensitive styryl dyes (Di-4-ANEPPS/ EQ; Di-8-ANEPPS/ EQ) were used to optically monitored from single M/Ts membrane potential changes occurring at dendritic and somatic levels after electrical stimulation. Neurons were electively stained in vivo salamanders by retrogradely labeling, or alternatively in vitro by rintracellular pressure injection. For retrogradely labeling, dyes were injected into the dorsal olfactory cortex and/or hippocampal primordium. After M/T cell bodies and dendrites were labeled (24-48 hours), animals were decapitated and optical recordings were performed in isolated olfactory bulb horizontal slices (~500 um thickness) attached to the epithelium by olfactory nerve fibers. Following antidromic or synaptic activation, fluorescence changes 0.1-0.5 % (delta F/F) could be recorded from single MTs using either a CCD video camera, an image intensified CCD system, or discrete photodiodes. Following olfactory nerve stimulation, membrane potential changes associated to FPPs and late depolarizations were observed in apical dendritic regions, while somatic regions exhibited only a brief period depolarizations following a prolong hyperpolarization. Dendritic and somatic responses also differed in their pharmacological profile. Following antidromic stimulation, membrane potential changes were restricted to dendritic segments close to somatic regions and had a shorter time-course. These data correlate with the activation of Ca2+ transients in apical dendritic regions and the relatively long lasting (100-300 ms) depolarizations observed in the vicinity of the external plexiform layer previously reported in VSD population studies. These late depolarizations may have implications for the generation of intrinsic oscillatory events in the olfactory bulb.

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Mechanisms underlying dendrodendritic inhibition in slices of the rat olfactory bulb. JEFFRY S. ISAACSON and BEN W. STROWBRIDGE, *Dept. of Physiology & Biophysics, University of Washington, Seattle, WA 98195.* isaacson@u.washington.edu.

Synaptic transmission between dendrites in the olfactory bulb is thought to play a major role in the processing of olfactory information. Glutamate released from mitral cell dendrites excites the dendrites of granule cells which, in turn, mediate GABAergic dendrodendritic inhibition back onto mitral dendrites. It is unclear whether common mechanisms regulate transmitter release from both dendrites and axons, since the calcium channels and intracellular calcium dynamics that underlie dendrodendritic inhibition are unknown. To address these questions, we have explored the mechanisms governing dendrodendritic inhibition in slices of the rat olfactory bulb.

Using patch-clamp recordings from mitral and granule cells, we find that both NMDA and non-NMDA receptors are critical for the generation of dendrodendritic inhibition. Our results also indicate that high voltage-activated calcium channels of the N and P/Q class play a dominant role in dendritic transmission. We have used photometric measurements of calcium signals to test for active properties in mitral cell dendrites. These results indicate that back propagating action potentials activate calcium channels on mitral cell dendrites. To study further the relationship between mitral cell activity and the generation of the dendrodendritic response, we have made simultaneous recordings of dendritic calcium influx and dendrodendritic inhibition. The extent of reciprocal inhibition was closely related to the magnitude of calcium influx in the mitral cell dendrite. Using dual recordings from neighboring mitral cells we also studied the propagation of dendritic signals between principal cells. We show that dendrodendritic synapses can mediate lateral inhibition in a manner that does not require voltage-gated sodium channels.

Supported by a Burroughs Wellcome Fund Career Award (J.S.I.) and NIH grant NS33590 (B.W.S.)

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Initial characterization of a dopamine-activated current in the soma of olfactory projection neurons of the spiny lobster. MANFRED SCHMIDT[1,2] and BARRY W. ACHE[2,3]., *[1] Zool. Institut, Univ. Hamburg, 20146 Hamburg, Germany, [2] Whitney Lab., [3] Depts. of Zool. and Neuroscience, Univ. of Florida, St. Augustine, FL 32086, USA*

The somata of olfactory projection neurons (OPN) in the spiny lobster *Panulirus argus* are innervated by centrifugal neurons with dopamine-like immunoreactivity (Cell Tiss. Res. 278: 337-352, 1994). The terminals of the "dopaminergic" neurons are presynaptic to OPN somata and primary neurites, which in turn are coupled by gap junctions. This suggests that descending dopaminergic input moduates OPN coupling.

As a first step towards understanding the functional significance of this arrangement, we determined the effect of dopamine, if any, on the purported postsynaptic cells by whole-cell patch-clamping isolated OPN somata. "Spritizing" dopamine onto the cells evoked an inward current in ca. 90% of more than 150 cells voltage-clamped at - 60 mV. The current consisted of a very rapidly activating transient peak, typically more than 100 pA in amplitude and about 200 ms long, followed by a much smaller (<20 pA), sustained current. The amplitude of both components increased concentration-dependently between 10 µM and 0.5 mM dopamine. The current decreased in amplitude at less negative holding potentials to 0 pA at about 0 mV, but did not reverse polarity. In current clamp, dopamine rapidly depolarized the cells and elicited action potentials, indicating that the current is normally excitatory.

The dopamine receptors mediating the current have unusual pharmacological properties in that noradrenaline and acetylcholine are agonists. Phentolamine, an antagonist of vertebrate adrenergic receptors, reversibly blocked the dopamine current whereas typical vertebrate dopamine receptor antagonists like fluphenazine had little effect. Dialyzing the cells with GDP-ß-S by including the drug in the recording pipette failed to alter the amplitude of the current, suggesting that it is mediated by ionotropic receptors. The results are consistent with the idea that dopamine regulates coupling between OPNs via soma-synapses, and that its effect is mediated by a novel ionotropic receptor for dopamine.

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Poster 278

Orientation behavior in variable habitats: How do changes in substrate effect information in odor plumes and animal behavior? PAUL A. MOORE, JENNIFER GRILLS, and ROBB W. S. SCHNEIDER, *Laboratory for Sensory Ecology, Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403.* pmoore@bgnet.bgsu.edu

Many aquatic animals use a variety of sensory cues as sources of information for orientation behavior. Crustaceans primarily use chemical signals to guide orientation behavior. For chemical signals, the information contained within the signal will depend upon differences in habitat structure (such as the presence of sand, rocks or cobblestones). To use chemical signals effectively, animals must either have a foraging strategy that is general enough to function in a variety of habitats or a specific strategy for each habitat. To investigate how information within chemical signals can influence orientation behavior, we measured both the orientation behaviors of crayfish and the chemical distribution within artificial streams with different substrates. This approach allows us to correlate the behavior with the information present in the signal. Chemical signals contained faster fluctuations of concentration and were more variable with cobblestones than in other substrates. These signal changes matched our behavioral results. The substrate type did not influence the ability of the crayfish to locate the odor source, but did show that paths were different for the three substrates. By measuring the spatial and temporal distributions of a chemical signal and animal behavior under similar conditions, we can begin to get precise insight into the mechanisms by which chemical information is used by animals inhabiting different habitats.

Supported by NSF grant IBN-9614492.

Poster 279

Antennal postures of the male Sphinx Moth, *Manduca sexta*, change during upwind flight in pheromone plumes with different spatial structures. ROBB W. S.SCHNEIDER, and MARK A. WILLIS, *ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.* rwss@neurobio.arizona.edu.

In previous studies the structure of the antennae of the male Sphinx Moth, *Manduca sexta*, has been shown to act as a physical filter by altering the flow of air through and around the antennae. Airflow determines the spatial and temporal distribution of the chemical signal, thus the orientation of the antenna alters the incoming chemical information. During upwind flight in a pheromone plume male moths maintain their antennae in a specific posture. The aim of our research is to determine if this preferred angle plays a functional role in the perception of odor plumes. In this study, we have measured the antennal posture of the male moths during upwind flight in different pheromone plume structures and wind speeds. To investigate the angles at which *M. sexta* males maintain their antennae, we flew moths in a 1 m x 1m x 2.5 m wind tunnel at three different wind speeds (0.5 m/s, 1.0 m/s, and 2.0m/s). The results showed that the male moths modulate the posture of their antennae in different plume structures and in different wind speeds. On average, the moths maintained their antennae at specific values in different wind speeds. Specifically, the angle between a horizontal line parallel to the floor of the wind tunnel and the antennae decreases as a function of increasing wind speed. The angle between the antennae did not show a consistent trend with respect to changing plume structure and wind speed. Analysis of the averaged data indicates that the antennae are maintained and operated as a unit and not as individual appendages. This study adds a new perspective to the study of upwind orientation and olfactory processing of sex-pheromone.

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Poster 280

Testing a new model for olfactory imprinting in Coho salmon (*Oncorhynchus kisutch*). JASON WATTERS, DIONNE WRIGHTS, GABRIELLE NEVITT, *Neurobiology, Physiology and Behavior, University of California, Davis, CA 95616.* FAX: 530-752-5582

Salmon are known for their ability to complete long distance homing migrations using an imprinted olfactory memory to homestream odors. The mechanisms underlying the formation of this memory are not well understood. We are exploring a new model for olfactory imprinting that suggests that the formation of this memory is in part driven by a tuning of the peripheral olfactory system to homestream odors. This model suggests that during the sensitive period for imprinting ("smolting") there is a hormonally driven proliferation of olfactory receptor neurons. Neurons that are sensitive to homestream odors selectively survive while others die. This punctuated proliferation and selective survival thus contributes to an olfactory memory specific to the home stream. To begin to test this model, we have used BrDU (5 bromo-2' deoxyuridine) birthdating techniques to identify newly proliferating cells in the olfactory epithelium during smolting. Whole olfactory rosettes were embedded in paraffin and cut serially at 9 μms. Preliminary analysis indicates a doubling of clusters of proliferating olfactory receptor neurons per section coincident with smolting as compared to pre-smolt levels (4.8 ± 0.24 vs. 2.13 ± 0.13). Trials currently underway suggest a role for T_3 in this process.

Supported by grants from NIH and the Whitehall Foundation (to GAN).

Poster 281

Behavioral and biochemical effects of altering the structure of a compound that induces settlement of the barnacle *Balanus amphitrite* (Darwin). MARION MCCLARY, JR.[1], SUSAN CONOVA[2], DAN RITTSCHOF[2], [1]*Department of Biology, Georgia State University, Atlanta, GA, 30302,* [2]*Department of Zoology, Duke University Marine Laboratory, Beaufort, NC, 28516.* FAX: 404-651-2509.

Barnacle larvae must select a habitat before they become permanently attached. Permanent attachment of barnacle larvae to a surface must occur near conspecifics to enable reproduction of the sessile adults. Barnacle larvae locate conspecifics by detecting pheromones. Pheromones are mimicked by peptides that have arginine or lysine at the carboxyl terminus preceded by several neutral amino acids. Bradykinin, a nine amino acid peptide, has the carboxyl terminal sequence of a pheromone mimic. Settlement of cyprids, less than a day old, was tested with bradykinin and bradykinin analogs. Bradykinin and bradykinin containing lysine at its amino terminus induced settlement and metamorphosis. Bradykinin that lacks arginine at its carboxyl terminus had no effect on settlement. Competitive binding studies were conducted using radioactive bradykinin. Barnacle larvae were incubated in increasing concentrations of biologically active peptides and pheromones with [^{3}H]bradykinin for 1 hour at 6°C. Larvae bind [^{3}H]bradykinin at one site in a specific and saturable manner. All peptides and pheromones tested compete with [^{3}H]bradykinin binding except bradykinin that lacks arginine at its carboxyl terminus. These data demonstrate the importance of arginine and lysine at the carboxyl terminus of settlement inducers of the barnacle *B. amphitrite*.

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Development of behavior: size-specific prey responses to predator odors. TROY A. KELLER and PAUL A. MOORE, *Laboratory for Sensory Ecology, Department of Biological Science, Bowling Green State University, Bowling Green, OH 43403*. FAX: (419)-732-2024

The information contained in environmental stimuli is context dependent and influences the behavioral decision organisms make. For organisms that undergo dramatic shifts in size and morphology through growth, the information they extract and the decisions that they make are a function of the animal's developmental stage. For example, the same chemical that indicates the presence of a predator to juveniles may signal the presence of potential prey to adults. To examine the role ontogeny plays in structuring the behavioral response of organisms to potentially important environmental stimuli (e.g., predator odor), we characterized the nocturnal orientation behavior of four size classes of crayfish (*Orconectes virilis*) exposed to predatory fish water (*Ambloplites rupestris*). The behavior of the crayfish (N=6) was filmed before and after the introduction of fish or fishless water. Crayfish responses were analyzed using a motion analysis system. Larger crayfish (>30 cm CL) oriented away from the source and turned less frequently after exposure to fish water. Fish-water did not alter the behavior of smaller crayfish (<30 cm CL). This study demonstrates that developmental stage influences crayfish response to odor cues. These ontogenetic shifts in behavior may be the result of selection for minimizing size-dependent predation. Research focusing on behavioral responses of organisms to olfactory stimuli needs to recognize the important role of ontogeny in influencing behavioral decisions.

Supported by NSF grant IBN - 9614492 (to PAM)

Poster 283

Characterization of the nature of information between chemical signal sources in the crayfish, *Procambarus clarkii*. REBECCA A. ZULANDT and PAUL A. MOORE, *Laboratory for Sensory Ecology, Department of Biology, Bowling Green State University, Bowling Green, OH 43403*, beckyzu@bgnet.bgsu.edu.

Chemical signals play an important role in communication for many crustacean communities. They typically contain information on mate location, hierarchical status, food sources and predation risk. There are several ways an organism could identify the presence of a predator. These include identifying the odor of the predator, recognizing the body fluids of a damaged or killed conspecific, or through stress odors of escaped individuals. We examined two mechanisms of recognition of predation risk by characterizing the source of chemical signals for the crayfish *Procambarus clarkii*. Previous work has shown that crayfish use two types of mechanisms to signify distress, damage-released chemicals and distress chemicals. We hypothesized that the information content of stress chemicals was different from that of damaged released chemicals. Distress chemicals were collected by isolating nephropore and gill water from distress animals through catheters while damage-released chemicals were collected by taking the water in which a crayfish chelae was crushed in. These chemicals, plus controls of no odor and non-stressed catheterized water were introduced into a flow through tank containing one male crayfish. Trial behavioral responses were analyzed using a motion analysis system to examine velocity, orientation, total distance traveled, meral spreads, and movement towards the source. Differences were found between control treatments and odor treatments in velocity, meral spread, and total distance traveled. These differences suggest that there is different information about the sender that is carried through chemical sources.

Supported by NSF grant IBN-9614492.

Poster 284

Symbiotic algae of the genus symbiodinium possess receptors for taurine, an amino acid that exhibits'host factor'activity. HENRY TRAPIDO-ROSENTHAL[1], PAOLA VALLEJO[1,2], LYNNE WHITEHEAD[3], ANGELA DOUGLAS[3], *[1]Bermuda Biological Station for Research, Ferry Reach GE-01, Bermuda, [2] Department of Zoology, University of Wisconsin, Madison, WI 53706, USA, [3]Department of Biology, University of York, York Y01 5YW, UK.* hank@bbsr.edu.

It has been suggested that free amino acids may be the'host factor' that is involved in the symbiotic association of zooxanthellae and cnidarians (Gates et al., 1995, PNAS 92:7430-7434; Bester et al., 1997, Proc. 8th Intl. Coral Reef Sym. 2:1287-1290). Recently, Wang and Douglas (1997, Plant Physiol. 114:631-636) have demonstrated that the sulfonic amino acid taurine possesses host factor activity, in that it can stimulate the export of photosynthetically generated carbohydrate from the dinoflagellate alga Symbiodinium. This latter work has led to the creation of the hypothesis that the effect of taurine could be mediated by cell-surface receptors for this amino acid. We present here results of initial tests of this hypothesis. Algal cells have an interaction with taurine that is sodium-independent and reversible; this contrasts with taurine uptake by these cells, which is sodium-dependent and irreversible. Unlabeled taurine and glycine compete effectively for the sodium-independent binding of labeled taurine, whereas the acidic amino acid glutamate does not. The basic amino acids lysine and arginine enhance this class of taurine binding. The kinetics of the sodium-independent interaction of taurine with algal cells obey Michaelis-Menten kinetics and are characterized by a K_M of 25 mM, and a B_{max} of 756 fmoles/10^6 cells. We are now designing experiments to probe the means by which the taurine signal is transduced and linked to the export of photosynthate.

Supported by NSF grant OCE 9619718.

Poster 285

Structure-activity studies of bird repellents: carbocyclic and heterocyclic compounds. LARRY CLARK[1] and EVGENY ARONOV[2], *[1]USDA, National Wildlife Research Center, 1716 Heath Parkway, Fort Collins, CO 80524, [2]Schering-Plough Research Institute, K-15 B211/2800 2015 Galloping Hill Rd., Kenilworth, NJ 07033*, nwrc.clark@worldnet.att.net

Birds can cause serious damage to agricultural commodities. In efforts to identifiy new nonlethal methods to minimize damage by birds to agricultural commodities we previously developed a qualitative model for bird repellents. The model was restricted to aromatic six-membered heterocycles that were mediated by the trigeminal nerve. Nonetheless, the qualitative model proved useful for the identification of candidate repellents. However, a quantitative model predicting level of the avoidance response was not developed. This study develops a simple multiple linear regression model that quantitatively relates structure and level of repellent activity using carbocyclic and heterocyclic compounds. Quantitative models were developed relating topographic, electrostatic, electronic and physicochemical characterizations of heterocyclic and carbocyclic molecules to relative intake at 4.7 mM. The individually characterized models were only moderately adequate in predicting repellent activity. However, a hybrid model consisting of a polarization characterization, the energy of the lowest unoccupied molecular orbital, the moment of inertia, an index of ionization potential, a negative electrostatic isopotential, a connectivity index, and a shape index proved to be an accurate predictor of repellent activity ($R^2 = 82\%$). The model forms the basis for future validation studies for the identification and optimization of trigeminally mediated primary bird repellents. These findings also raise the hope of reducing the effort needed to accurately identify candidate compounds that can be used as nonlethal, environmentally safe bird repellents.

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Canine olfactory detection signatures for explosive material. MARC WILLIAMS, MATTHEW CICORIA, MEREDITH JONES, TERESA BOUSSOM, JAN JACKSON, L. PAUL WAGGONER, & JAMES M. JOHNSTON. *Institute for Biological Detection Systems, Auburn University, AL 36849-5532.* willijm@vetmed.auburn.edu

Trained dogs are the most widely deployed technology for the detection of explosives. When a trained explosives detection dog alerts to an explosive training aid, its handler does not know exactly what the dog is responding to. Explosive materials are typically a mixture of many compounds. When a dog is trained to detect a substance, it learns to discriminate the vapor of that substance from other odors in the environment by reacting to the compound, or compounds, that best help it to earn reinforcement from the handler. With sufficient training, the compound or compounds whose detection most often results in reinforcement become what may be termed the olfactory detection signature.

This presentation describes determination of the canine olfactory detection signature for several explosive substances using operant psychophysical procedures in a laboratory setting. Dogs were trained to sample from an air stream and press one of three levers depending upon the presence of the target explosive vapor, a variety of unrelated "non-target" vapors, or clean air in the air stream. The signature testing preparation involved presenting individual or mixtures of compounds found in the target explosive vapor in probe trials. Control exerted by individual or mixtures of compounds was measured by the distribution of responding across the three levers in these probe trials. The compound or, more often, the mixture of compounds that engendered the highest percentage of responding to the target explosive lever was considered the detection signature for that explosive. The vapor constituents of the target explosives and their concentrations that were delivered by the vapor generator used in these studies were determined by thermal desorption GC/MS.

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Poster 295

Generalization between n-aliphatic aldehydes in the rat. CHRISTIANE LINSTER, MICHAEL E. HASSELMO *Dept. of Psychology, Harvard University, Cambridge, MA 02138* linster@berg.harvard.edu.

Our objective is to evaluate the relationship between mitral cell responsiveness to odorants and behavioral generalization between these odorants. Electrophysiological recordings of mitral cells in the rabbit olfactory bulb have shown that a number of mitral cells respond (with increased spike rates) to a range of aliphatic aldehydes (Mori et al., J. Neurophysiol. 67(3), 1992). These data suggest that in the olfactory bulb, the evoked patterns of activity in response to two aldehydes with similar chain length have a larger overlap than those evoked in response to more dissimilar aldehydes. We here show that this overlap in representation in the olfactory bulb correlates, in some cases, with generalization between two odorants.

We adopted an assay that was recently developed for studies of odor memory (Bunsey and Eichenbaum, Nature, 18(379), 1996). Subjects are conditioned to locate a food item that is buried in a dish of cage bedding. Subjects must discriminate the appropriate scented dish and dig to obtain the reinforcement. On each experimental day, subjects (male Sprague-Dawley rats) were conditioned to a particular odorant (8 trials). Each subject was conditioned with (3), (5) and (7)CHO. After conditioning, the responses (time spent digging in the scented dish) to (3),(4),(5),(7) and (8)CHO as well as to a control odor (n-amyl acetate) were recorded. No food reinforcement was given in the test trials.

We found that rats spend an average of 15 sec (+/- 0.07) digging in the dish containing the conditioned odor. On the average, subjects tended to avoid (0.7 sec +/- 0.01) the control odor. Furthermore, rats generalized between aliphatic aldehydes of similar chain lengths. Average digging time in response to an aldehyde differing by one or two carbons from the conditioned odor was 5.1 sec (+\-0.2). The response to less similar aldehydes was comparable to that to the control odor (1.3 sec +/- 0.25). There was a significant difference between the responses to aldehydes differing by 1-2 carbons from the conditioned odor and the responses to all other aldehydes. There was no significant difference between the response to the control odor and that to aldehydes differing by more than 2 carbons from the conditioned odor.

Interestingly, in no case could we observe generalization to (8)CHO. We will further investigate that particularity by including aldehydes of higher chain-lengths in our experiment. As a consequence, preliminary, we have excluded (8)CHO in the results presented here.

Poster 296

Generalization and discrimination of binary odorant mixtures and components in the rat. CHRISTIANE LINSTER[1], BRIAN H. SMITH[2], MICHAEL E. HASSELMO[1] [1]*Department of Psychology, 33 Kirkland St., Cambridge, MA 02138,* [2]*Department of Entomology, The Ohio State University, Columbus, OH 43210* linster@berg.harvard.edu

Our objective is to evaluate how rats learn about odor mixtures. In particular we are interested in determining how subjects generalize between pure components and binary mixtures that contain that component. Ultimately we hope to relate these analyses to a physiological model of elemental and synthetic processing in the olfactory bulb.

We adopted a new assay that was recently developed for studies of odor memory. In this assay subjects are first conditioned to locate a food item that is buried in a dish of cage bedding. Subjects must discriminate the appropriate scented dish and dig to obtain the reinforcement. When conditioned to a pure component (O1), we found in general that subjects spend more time digging in that scented dish than in a dish with a binary mixture (O1+O2) or in a dish scented with a different odorant (O3). There was more generalization to O1+O2 than there was to O3. When conditioned to the O1+O2 mixture, subjects generalized to O1 more than they did to O3. These results indicate that the mixture retains some information about the components, although several models could still support these data.

Finally, the exact pattern of generalization depended on the identity of the odors. Some odor groups produces more non-additive effects than other odor groups. This latter finding in particular indicates that sensory or first-order (Olfactory Bulb) interactions among sensory representations are important in mixture discrimination.

Poster 297

GABA- and nitric oxide-mediated modulation in the honey bee (*Apis mellifera*) brain differentially affect olfactory discrimination. BRIAN H. SMITH, KRISTI BUXTON, JAY S. HOSLER, *Department of Entomology, The Ohio State University, Columbus, OH 43210.* smith.210@osu.edu

Gamma amino butyric acid (GABA) and gaseous neurotransmitters such as nitric oxide (NO) subserve important modulatory systems in the vertebrate and in the invertebrate olfactory systems. Much of the evidence in support of that proposition comes from anatomical and physiological investigations. In insects, a large subclass of interneurons in the Antennal Lobe (AL) are GABAergic and underlie important, identified inhibitory circuits. In addition, both the sensory and first-order synaptic processing areas show prominent staining patterns for NO synthase. The broad phylogenetic distribution and biochemical properties NO could predispose it to mediate such fundamental properties of signal processing as adaptation and habituation.

Here we show that pharmacological blockade of GABA and NO modulation has different effects in an olfactory generalization/discrimination assay in the honey bee. Blockade of GABA disrupts discrimination of molecularly similar odorants (1-hexanol and 1-octanol) but leaves intact discrimination of dissimilar odorants (alcohols from geraniol). Furthermore, blockade during either conditioning or recall testing is sufficient to produce this disruption. In contrast, blockade of NO modulation eliminates all discrimination. But this disruption occurs only when the drug is active during conditioning. Therefore, these modulatory systems most likely subserve different functions in the honey bee and vertebrate olfactory bulbs.

This research was supported by a grant from NIMH to B.H.S.

Poster 298

Behavioral analyses of amino acid taste perception in the honey bee *(Apis mellifera)* and in the fruit fly *(Drosophila melanogaster).* BRIAN H. SMITH, YOUNG SOO KIM, *Department of Entomology, The Ohio State University, Columbus, OH 43210.* smith.210@osu.edu

Our goal is to analyze the behavioral consequences of amino acid taste sensation in insects. Amino adds are essential dietary components found in the nectars of many flowers that are visited by insects from the Lepidoptera, Diptera and Hymenoptera. Yet several studies of taste preferences have led to mixed conclusions in regard to whether insects prefer to feed on nectars that contain the amino acids. The lack of a clear consensus on this issue could reflect different pre- and post-ingestive feeding effects of amino acids.

We have evaluated amino acid-mediated feeding behavior in both honey bees and fruit flies using learning preparations that allow us to separate these effects. In honey bees, amino acids affect the perception of sucrose solutions in a dose-dependent manner. Some concentrations decrease the reinforcement power of a sucrose solution whereas other concentrations of amino add enhance it. The effects can be mimicked by direct injection of amino acid into the hemolymph. Thus these effects are most likely due to post-ingestive mechanisms.

We have also used a sensitization assay in fruit flies and in honey bees, which requires assessment of behavior within seconds after stimulation. We argue that this assay measures pre-ingestive (sensory) effects. Under this condition GABA, but not glycine, inhibits behavioral performance. The different pre- and post-ingestive effects demonstrate the need to carefully separate out these effects in future physiological and genetic analyses.

Poster 299

Discrimination of multimixtures by catfish is based on the most stimulatory component in the mixture. TINE VALENTINČIČ, PIKA MIKLAVC, AND KSENJA BABIČ. *Department Of Biology, University of Ljubljana, Vecna pot 111, 1000 Ljubljana, Slovenia.* tine.valentincic@uni-lj.si

How odorant mixtures are perceived by vertebrates was studied in catfish, organisms with well-characterized olfactory organs that are highly responsive to amino acids. To study how catfish perceive ternary and multimixtures of amino acids, brown bullhead catfish (*Ameiurus nebulosus*) were tested with single amino acids, ternary mixtures and multimixtures consisting of 13 amino acid components. The odorant mixtures were presented in two different forms. In form one, each of the components was adjusted in concentration such that all components were equipotent based on EOG recordings. In form two, the concentration of one of the components was increased from the equipotent concentration so that it was the most stimulatory component in the mixture. Brown bullhead catfish were conditioned to a ternary mixture and to a multimixture of 13 amino acids, respectively, both with L-cysteine as the most stimulatory component in the mixture. The conditioned catfish were tested with: (1) ternary and multimixtures composed of equipotent components, (2) ternary and multimixtures with L-cysteine as their most stimulatory component, (3) ternary mixtures and multimixtures with amino acids other than L-cysteine as their most stimulatory component, and (4) L-cysteine. Brown bullhead catfish were unable to discriminate the conditioned stimuli, either a ternary mixture or a multimixture with L-cysteine as the most stimulatory component, from L-cysteine and from all other mixtures containing L-cysteine as the most stimulatory component. These fish did, however, always discriminate mixtures with amino acids other than L-cysteine as their most stimulatory component and mixtures composed of amino acids at their equal stimulatory effectiveness concentrations from the conditioned stimuli. In conclusion, both simple and complex odorant mixtures in which one of the components is more stimulatory than the others are perceived by catfish as their most stimulatory component. Whether the lesser stimulatory components in the mixture can influence the perception of the mixture needs to be further evaluated.

Supported by Ministry of Science and Technology of Slovenia grant No. J-17127-0487

Poster 300

Comparison of olfaction and taste behavioral responses to free amino acids in two cyprinid fishes. ALEXANDER O. KASUMYAN, EUGENY A. MARUSOV, AMAL M.H.MORSY, EKATERINA V. NIKOLAEVA, *Department of Ichthyology, Faculty of Biology, Moscow State University, Moscow, 119899, Russia.* kasumyan@1.ichtyol.bio.msu.ru

The experiments were performed on common carp, *Cyprinus carpio,* and crucian carp, *Carassius auratus,* of one year old. Twenty one free amino acids (L-isomers) were used as olfactory and taste stimuli. Fish olfactory responses were examined by adding into aquarium water solution of free amino acids, 0.1 mM. The taste tests were performed by offering the fish agar-agar pellets containing one of substances tested, 1-100 mM.

It was found that 11 amino acids, Ala, His, Pro, Gln, Gly, Asp, Asn, Met, Thr, Arg, Glu, were very effective for stimulated food searching behavior stimulating in common carp. In oral taste tests 6 amino acids, Cys, Pro, Glu, Asp, Ala, Gln, were high palatable, 7 ones, Val, Ser, Phe, Met, Thr, Arg, Trp, were strong deterrent for common carp.

In crucian carp 12 amino acids His, Asn, Ala, Glu, Thr, Phe, Lys, Pro, Arg, Gly, Gln, Cys, using as olfactory stimuli, were produced intensive behavioral responses. Nine amino acids, Leu, Tyr, Gly, Thr, Ile, Val, Arg, Ser, Asp, stimulated pellets consumption. Four amino acids, Pro, Phe, Met, Gln, had deterrent effect.

These results indicate that free amino acids olfactory spectra in two cyprinid fishes are close. Taste spectra of these species are strong different. Within the same species olfactory and taste spectra are coincided only in part.

The present study was supported by the Russian Foundation for Basic Research, grant 95-04-11754.

Poster 301

Behavioral responses of fingerlings of brown trout, *Salmo trutta,* to food odors and some free amino acids. EUGENY A. MARUSOV, *Department of Ichthyology, Faculty of Biology, Moscow State University, Moscow, 119899, Russia.* kasumyan@1.ichtyol.bio.msu.ru

The fingerlings of brown trout from Baltic Sea and White Sea populations living each alone in tanks with streaming water do not show significant behavioral responses to food extracts and water solutions of free amino acids (L-isomers, 0.1-1.0 mM). The fish without object sight by means of lens ablation change the stereotype of feeding behavior from throwing to searching. The water extracts of both *Chironomus spp.* larvae and *Tubifex spp.* provoked the excitement, increasing the locomotor activity, special food searching behavior with spontaneous seizing mouth actions.

The most of testing solutions of free amino acids were indifferent for fish. The weak reactions of fish from both sea basins were stimulated by solutions of cysteine, alanine and arginine; proline and aspartic acid produced poor effect in White Sea brown trout only.

The present study was supported by the Russian Foundation for Basic Research, grant 96-04-48218.

Poster 302

Comparison of MSG and L-aspartic acid using conditioned taste aversion in rats. JENNIFER R. STAPLETON[1], STEPHEN D. ROPER[2], and EUGENE R. DELAY[1], [1]*Dept. of Psychology, Regis Univ., Denver, CO 80221,* [2]*Dept. of Physiology & Biophysics, Miami Univ., Miami, FL 33101.* FAX: (303)964-5480.

It has been suggested that L-forms of alpha-amino dicarboxylic acids with an R group of four to seven carbons may taste similar to monosodium glutamate (MSG). One such amino acid, L-aspartic acid (L-ASP), appears in humans to possess taste properties similar to MSG (Maga, *CRC Crit. Rev. Food Sci. Nutr.* 18: 231-312, 1983). Based on this information, we are comparing taste properties of MSG with L-ASP in rats using conditioned taste aversion procedures. One group of water-deprived rats was conditioned to avoid MSG using LiCl and a second was conditioned to avoid L-ASP. Two days later, taste stimuli were presented in a Davis MS80 rig and lick rates emitted during 10 s presentations were counted. All solutions were treated with 50 μM amiloride. Rats conditioned to avoid MSG appeared to avoid 100 mM sucrose and L-ASP in a dose-dependent manner, but not 20 mM KCl. Rats conditioned to avoid L-ASP also appeared to avoid 100 mM sucrose and MSG in a dose-dependent manner, but not 20 mM KCl. These results indicate that rats may perceive the taste of L-ASP as similar to that of MSG.

This research is supported by NIH DC03013.

Poster 303

Dietary salt levels influence the ingestive patterns of female rats during pregnancy and lactation. DEREK J. SNYDER, ROBERT J. CONTRERAS, DONNA L. WONG, AND JAMES C. SMITH, *Program in Neuroscience, Department of Psychology, The Florida State University, Tallahassee, FL 32306-1270,* dsnyder@psy.fsu.edu.

In an investigation of the effects of perinatal salt (NaCl) experience on salt intake behavior later in life, we have previously shown that rat pups raised on a high salt diet during the perinatal period produce elevated adult salt intakes over those reared with medium or basal salt exposure. This effect is correlated with long-term alterations in physiological state. In an early effort to define more precisely the critical period for such developmental change, we examined food and water intake in female rats during pregnancy and lactation. This period corresponds with perinatal salt experience in developing offspring. Female Sprague-Dawley rats were housed individually and given ad libitum access to deionized water and diets containing 0.1, 1.0, or 3.0% NaCl (BA, MD, HI) starting at 66 days of age. 24-hour patterns of food and water intake were measured throughout the experiment. After a week of baseline measurements, a male was added to each cage for 7 days. Offspring were born ~3 weeks later and were weaned at postnatal day 21. Ingestive pattern data were collected from the mothers until 9 days after weaning. There were no distinguishable group differences in food intake, but the HI group drank considerably more water than the MD or BA groups before conception and during lactation. Only during pregnancy did varying salt concentrations not produce related differences in water intake. We believe that group differences in salt load are maximal during pregnancy and, thus, that salt load exerts a critical influence on developing offspring during this time. Food and water consumption for all three groups rose after parturition to levels greatly exceeding those seen at any other measured point; this is consistent with increased caloric need in the lactating mother. A detailed analysis of intake patterns will be presented at AChemS XX. Our data suggest that pregnancy alone may represent the perinatal critical period for the development of adult salt appetites.

Supported by NIH grant DC 02641.

Poster 304

The effect of CCK-8 on taste responses in the adult Sprague-Dawley rat. A.KURT THAW. *E.W.Bourne Behavioral Research Laboratory, Cornell University Medical College, White Plains, NY, 10605.* akthaw@aol.com.

The goal of this project was to determine if the taste system plays a significant role as a mechanism of action for the satiety effects of sulfated octapeptide cholecystokinin (**CCK-8**). If it can be shown that CCK-8 functions as a physiological mediator of postprandial satiety via a taste mechanism, this will have fundamental implications for the analysis of normal feeding behavior and for the determination of the pathophysiology and treatment of two major satiety disorders in humans, bulimia nervosa and obesity.

Ten Sprague-Dawley rats were used as subjects. Rats were trained to lick from sixteen sipper tubes during 10 days of consecutive training. Access to each tube was limited to 30 seconds and there was a 30-second delay before the next tube was presented. Once all subjects were trained, testing began. Concentrations of sucrose were presented in random order such that during a single test session rats had access to tubes containing 0.03M, 0.06M, 0.125M, 0.25M, and 0.5M **sucrose** or water. Baseline data was collected for eight days. This was followed by eight days of testing including a saline or peptide injection. Four days of additional baseline data were collected followed by testing with various concentrations of **NaCl** (0.03M, 0.06M, 0.125M, 0.25M, and 0.5M and water).

Results indicate a significant decrease in the total number of licks on all sucrose concentrations following injections of CCK-8. The decrease in intake was due to significant decreases in the mean number of bursts (defined as sustained licking with no more than a 250 msec pause between licks) for the two highest concentrations and a significant decrease in the number of licks/burst for the lower concentrations. No differences were found for any of the other dependent measures observed (latency to first lick and mean licks/second) or for the NaCl tests.

These results indicate that the satiating effect of CCK-8 are, in part, due to changes in the perceived taste of certain concentrations of sucrose, but not NaCl in the behaving rat.

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Poster 305

Non-equivalence of calcium and sodium chloride to sodium deficient Rattus norvegicus. CHARLES N. STEWART, *Department of Psychology, Franklin and Marshall College, Lancaster, PA 17604-3003.* c_stewart@acad.fandm.edu.

In a previous AChemS poster session (AChemS XV, Cianci, K., Karaefthimoglu, A. & Stewart, C.N.) it was reported that rats with a conditioned taste aversion (CTA) to sodium gluconate did not generalize that aversion to any of calcium, potassium or magnesium gluconate. This suggests that rats do not classify other salts as similar to sodium when the CTA procedure is used. It remains possible, however, that in sodium deficient rats the taste psychophysics may be altered.

In the present study male and female Long-Evans rats were fed either a sodium deficient or replete diet for 48 hours, following which the deficient animals were injected with furosemide (15mg/kg). During the following 24 hours, two-bottle preference tests were conducted with half of the animals in each group having a choice between 0.45M NaCl and de-ionized water and the remainder having a choice between 0.45M $CaCl^2$ and de-ionized water. Both male and female deficient rats showed a significant increase in NaCl intake (58.5% vs 28.7%) but deficient and control animals did not differ in their intake of $CaCl^2$ (8.0% vs 9.2%). There were no significant sex differences. Thus, the results fail to support the hypothesis that sodium deficient rats display a generalized saltiness appetite and are consistent with the results from the CTA study with the several gluconate salts. Sodium deficient rats would appear to cue in rather specifically on the sodium ion.

Poster 306

Marker-assisted selection of a high saccharin-preferring 129.B6-*Sac* congenic mouse strain. G.K. BEAUCHAMP[1,2], A.A. BACHMANOV[1], D.R. REED[2], M. INOUE[3], Y. NINOMIYA[4], M.G. TORDOFF[1], and R.A. PRICE[2], [1]*Monell Chemical Senses Center, Philadelphia, PA, 19104,* [2]*University of Pennsylvania, Philadelphia, PA, 19104,* [3]*Tokyo University of Pharmacy and Life Science, Japan,* [4]*Asahi University, Japan.* FAX: (215) 898-2084.

Relative to mice from the 129/J (129) inbred strain, the C57BL/6ByJ (B6) mice have a greater avidity for certain sweeteners and a more intense gustatory nerve response to them. Our previous studies show that these differences are largely determined by a locus on distal chromosome 4 (chr4), probably the *Sac* locus (Mammal. Genome, 1997, 8: 545). We have started development of a congenic strain, which retains the B6 *Sac* locus on the genetic background of the 129 strain. Based on microsatellite (SSLP) marker genotypes at distal chr4 characterized in 455 F_2 (B6 x 129) hybrids, we have identified three mice used as founders of the congenic strain. Each founder had one copy of distal chr4 originating from the 129 strain; the other copy was recombinant, with a short distal segment from the B6 strain and the more proximal part from the 129 strain. These three founders were backcrossed to the 129 strain. In all their offspring, SSLP markers on distal chr4 were genotyped, and 5 and 20 mM saccharin preferences in 2-bottle tests were measured. Additionally, in some mice chorda tympani responses to sweeteners were electrophysiologically recorded. In two of the three replicate crosses, offspring separated in two groups. One group included mice that inherited one copy of distal chr4 from the B6 strain; they had higher saccharin preferences and chorda tympani responses to sweeteners. The other group included mice that inherited both copies of distal chr4 from the 129 strain; they had lower saccharin preferences and chorda tympani responses to sweeteners. The ratio was approximately 1:1, consistent with monogenic predictions. The remaining genetic background from the B6 strain will be eliminated in the next few backcross generations using SSLP marker-based selection. This will lead to production of a high sweetener-responsive 129.B6-*Sac* strain congenic with the low sweetener-responsive 129 strain.

Supported by NIH grants DC00882, DK44073 and DK48095.

Poster 307

Lysine-deficient rats drink significantly more lysine than controls in a two-amino-acid choice test by increasing number of ingestive bouts. STACY MARKISON [1], BARBARA L. THOMPSON [2], JAMES C. SMITH [2], AND ALAN C. SPECTOR [1], *Department of Psychology, University of Florida, Gainesville, FL 32611* [1] *and Department of Psychology, Florida State University, Tallahassee, FL 32306* [2].

Rats deficient in the essential amino acid lysine (LYS-) drink significantly more 0.2 M lysine in 23-h two-bottle (vs. water) intake tests relative to non-depleted controls (CON). Meal pattern analysis reveals that their enhanced intake of lysine occurs as a function of increased number of ingestive bouts, not bout size. Apparently, lysine deficiency promotes approach behavior to the lysine source, but does not influence amount consumed during stimulus sampling. These effects are thought to be based on a learned association between lysine taste and the beneficial effects of its ingestion. For example, a difference in cumulative lysine intake between CONs and LYS- rats does not occur immediately; it does, however develop within 30 minutes of sampling the solution. We tested if similar results would be obtained if lysine solution were pitted against another chemical cue as opposed to water. LYS- (n=8) and non-depleted CON rats (n=8) were presented with 0.2 M lysine and 0.1 M threonine for 4 days while intake and licking were measured. When faced with this potentially more difficult choice, LYS- rats on average ingested significantly more lysine and less threonine relative to CONs, but by the last day of testing, 2 LYS- subjects had not developed a lysine preference. For the meal pattern analysis we only included rats expressing a lysine preference. Consistent with our previous findings, LYS- rats increased lysine intake by initiating more lysine bouts, not by adjusting bout size. Relative to when lysine and water were available, increased cumulative licking to lysine was delayed in the LYS- rats and did not occur until at least 90 minutes after sampling the solution. In sum, the presence of a second amino acid did not eliminate the development of a lysine preference in most of the LYS- rats. Nevertheless, for those LYS- rats displaying a lysine preference, its development was delayed, but the pattern of enhanced intake is similar to that observed previously.

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Poster 308

Effects of perinatal dietary NaCl exposure and amiloride on NaCl detection threshold in Sprague-Dawley rats. LAURA C. GERAN and ALAN C. SPECTOR, *Department of Psychology, University of Florida, Gainesville, FL 32611*, geran@psych.ufl.edu.

Rats perinatally exposed to a high-sodium diet exhibit increased NaCl preference as adults compared with rats perinatally exposed to a low-sodium diet. Our experiment was designed to test whether such perinatal manipulations may influence the rat's taste sensitivity to very low NaCl concentrations as measured psychophysically. We used a two-lever operant conditioning paradigm to measure NaCl detection thresholds in adult offspring of dams that were given one of three diets from conception through weaning. Pups were maintained on the same diet as the dam until they reached 30 days of age (high salt diet: 3% NaCl (n=5), intermediate diet: 1% NaCl (n=5), or basal diet: 0.1% NaCl (n=2)), at which time they were switched to the intermediate (1% NaCl) diet. Animals were 24-h water-deprived and trained to press one lever when NaCl was the stimulus, and a second lever when water was the stimulus. Correct responses were reinforced with water and incorrect responses were punished with a timeout (30 s). Hit rates corrected for false alarm rate were used to determine performance for each rat and manipulation. Threshold was defined as the NaCl concentration that produced one-half the maximum asymptotic performance when a logistic curve was fit to the data. No obvious differences in NaCl threshold were found between dietary groups. The effects of stimulus adulteration by amiloride (100 μM), an epithelial sodium-channel blocker, on NaCl detection threshold were also assessed. Mean NaCl detection threshold across groups was found to be 0.005 M NaCl. Amiloride adulteration increased threshold to 0.036 M NaCl, an average difference of 0.85 $\log_{10}$ units ($p < 0.0001$). The level of perinatal exposure to dietary NaCl (in the normal physiological range) does not seem to appreciably affect NaCl taste detectability in adulthood. Interestingly, amiloride raises the average NaCl detection threshold to a value almost identical to that measured by the same procedure for KCl in perinatally unmanipulated rats from a prior experiment. We are currently testing more animals to increase our sample size.

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Poster 309

Perinatal dietary NaCl exposure does not influence NaCl concentration-lick functions in water-restricted adult rats as measured during brief access trials. BRIAN C. SAUER, and ALAN C. SPECTOR, *Department of Psychology, Univ. of Florida, Gainesville, Fl 32611.*

Perinatal manipulations of dietary sodium content have been shown to influence NaCl preference behavior of adult rats in fluid intake tests. We tested whether perinatal dietary NaCl exposure would influence the responsiveness of water-deprived rats to brief presentations of NaCl. In addition, to assess the possible mechanisms by which perinatal salt manipulation may produce its effects, we added amiloride, an epithelial sodium channel blocker, to the taste solutions. Seventeen Sprague-Dawley dams were presented with diets that varied in NaCl content, within a physiologically normal range, from conception through weaning. At postnatal day 30, offspring from the three dietary groups (basal: 0.1% NaCl, n=5; intermediate: 1.0% NaCl, n=6; high: 3.0% NaCl, n=6) were all placed on the intermediate diet for the remainder of the experiment. The rats were water deprived for 23.3 h then tested for 40 min in a specially designed gustometer. Licking responses to 0.03, 0.1, 0.15, 0.3, 1.0, 1.5 M NaCl and distilled water, delivered in randomized blocks of seven trials (10 s) with water rinse trials preceding each stimulus trial, were recorded. Concentration-response functions were composed of data collapsed from 5 consecutive sessions. Unadulterated NaCl solutions were tested in the first 5 sessions and then 100 μM amiloride was placed in all of the solutions for the next 5 sessions; this regimen was then repeated. The data from the last 5 sessions for each condition were analyzed. Licking significantly decreased as a function of NaCl concentration, but there were no significant differences between dietary groups. Sigmoidal logistic functions were fit to the concentration-response data for each rat. This analysis revealed that amiloride significantly shifted the descending function to the right by about 0.1 $\log_{10}$ units and significantly steepened the slope of decay. Dietary group did not significantly affect either of these curve parameters. These results suggest that the effects of perinatal dietary NaCl exposure on NaCl preference in adult offspring may not be mediated by the gustatory system.

P01-DC02641 and K04-DC00104.

Circadian variations in blood pressure and heart rate in rats raised on low, mid, and high dietary salt levels. DONNA L. WONG, JESSICA J. WILSON, ROSS HENDERSON, ROBERT J. CONTRERAS, *Program in Neuroscience, Department of Psychology, Florida State University, Tallahassee, Florida 32306-1270.* dlwong@psy.fsu.edu.

We are investigating the long-term influence of perinatal salt exposure on the blood pressure and heart rate of adult rats. Using Data Sciences' pressure transmitters and receivers in conjunction with our own data collection system, we are able to measure mean arterial pressure (MAP) and heart rate (HR) continuously for a period of several months. Subjects were offspring of female Sprague-Dawley rats exposed to one of three different dietary NaCl levels in the solid chow from conception through lactation: high salt (3%), mid salt (1%), or basal salt (0.1%). After weaning at postnatal day 21), offspring were maintained on the respective diet of the dam until postnatal day 30 when they were given Purina 5001 diet as their solid chow. A total of 21 adult male offspring were implanted with an aortic electronic sensor for transmitting blood pressure and heart rate signals by telemetry: 8 high rats, 6 mid rats, 8 basal rats. During testing, rats were given ad lib food and deionized water while on a 12:12 light/dark cycle.

Based on data collected, MAP and HR were relatively low and stable during the light phase, compared to higher and more variable levels during the dark phase. Variations in the MAP and HR during a 24-h period indicate that circadian rhythms play a part in the physiological condition of the rat. Additionally, differences in salt exposure during the perinatal period seem to have long-term affects on adult Sprague-Dawley rats in terms of their comparative blood pressures and heart rates. Finally, we have conducted some preliminary research to examine the relationship between the physiological measures of MAP and HR and ingestive behavior.

This research is supported by NIH Grant DC 02641.

LiCl induced taste aversions show that fat is the salient taste feature of corn oil and sucrose emulsions. JAMES C. SMITH, VICTORIA MALESZEWSKI, BRIAN MCCLAIN *Department of Psychology, The Florida State University, Tallahassee, FL 32306-105 1.* FAX: (850) 644-7739.

In our laboratory, it has been shown that rats gain excessive weight over a period of a few weeks when they have access to emulsions of sucrose and corn oil along with Purina Lab Chow. By measuring the patterns of ingestion, we found that they drink considerable quantities of the emulsion at a within-bout rate that indicates a major role for taste. The present studies were designed to understand more about the salient taste qualities of sucrose/fat mixtures. We had previously reported that rats generalize to the corn oil much more than to the sucrose after an aversion to the mixture was conditioned following a LiCl injection. The present studies give more evidence that the salient feature of the sucrose/fat mixture is the fat. By giving rats extensive experience with either sucrose or corn oil, we ruled out the possibility that the fat was more "novel" to the rat, making it easier to get an aversion to the corn oil. Furthermore, we showed that rats conditioned with a sucrose solution show a minor aversion to the sucrose/fat mixture in 90 minute preference tests on the first day of testing and completely extinguish the aversion in two days. In contrast, rats that ire conditioned with corn oil as the CS exhibit a profound aversion to the sucrose/fat mixture, drinking only water during the tests. This strong aversion remains for the next week. We also have shown that the order of generalization tests is of no consequence following the sucrose/lithium pairing. Although we tested a variety of concentrations of the sucrose/oil mixtures, most of the work has been done using 84 parts of 0.25M sucrose and 16 parts of corn oil blended with Tween 80. The phase separation of the sugar water/corn oil emulsions was quantified by sampling the emulsion from the bottom half of the drinking bottles at various times over 24 hours. Corrections for caloric intake can be made based on these calibration data.

Future Meetings

AChemS – XXI
April 14 -18, 1999

AChemS – XXII
April 26 - 30, 2000

AChemS – XXIII
April 25 - 29, 2001