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Chemo Sense

EDITORIAL

Reproduction in Asian Elephants: Precise Chemical Signaling has Behavioural and Biochemical Foundations

By Graham Bell

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Social behaviour and reproductive selection in mammals is determined in many species by chemical signals between individuals. In this issue, Bets Rasmussen and David Greenwood describe the complexities of elephant social behaviour and its chemosensory foundations. We are delighted to carry once again their excellent work.

This issue is also dedicated to the 40 papers presented at the AACSS 2005 Conference at Heron Island from 2-6 December 2005.

Congratulations to Australians Barry Marshall and Robin Warren on winning the 2005 Nobel Prize for Physiology and Medicine. They discovered that gastritis and peptic ulcers are caused by the bacterium *Helicobacter pylori* and not "hurry, worry and curry". We look forward to a chemosensory spin-off of their work: that the odours produced by these bacteria may be measured and used for non-invasive diagnosis and monitoring of treatment of H.pylori infections ■

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Reproductive strategies among large mammals may vary depending on the social structure, the degree of territoriality exhibited, and the sensory systems employed in conjunction with mating. Sexually dimorphic non-territorial Asian elephants, *Elephas maximus*, use a multiplexed olfactory chemical signaling system to assist in ensuring effective reproduction is attained (Rasmussen, 1999; Rasmussen et al., 2005). In examining the impact of chemical signals on reproductive strategies, the differences between male and female societal structure in Asian elephants become important.

Female Asian elephants, along with their daughters and pre-pubertal sons, stay in matriarchal family groups with close bonds among related individuals and between related family groups. A recent microsatellite study of Asian elephants [in a Southern India region overlapping that of the Rasmussen et al., (2005) study] genetically confirmed that the average

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relatedness between adult females across groups or locations was not different from zero, nor was the average relatedness between adult females and either adult males or subadults within a location different from zero, but the relatedness between adult males within a location was different from zero (Vidya & Sukumar, 2005).

In contrast to female calves, male juveniles receive preferentially longer suckling privileges and thus are larger at similar ages. As youngsters, males venture away from but then rejoin their natal groups. As these males get older, the wandering distance from the natal unit increases and the frequency of return visits decreases until independence is achieved by about 12-15 years of age. Teenage males then move solitarily or in temporary all-male groups composed of 2-4 individuals.

Male Asian elephants do not defend territories. Instead, interwoven in the loose structure of male society are developmental degrees of a condition unique to elephants, termed *musth*. Male elephants undergo a two-decade-long maturation process that involves physical, physiological, sexual and social maturation. As teenage elephants get older, gradual physiological changes related to musth begin, including transitory elevations in serum testosterone that affect behaviour. Young males begin to experience short musth episodes. These short [termed *moda*] musths are characterized by erratic behavior, displaced aggression, widely fluctuating hormone levels and the bursts of honey-like secretions from the mildly swollen facial temporal gland, a chemosecretory organ unique to elephants. Not only does this bouquet of floral-like odors, comprising numerous acetates, 3-hexen-1ol, acetophenone and 2-heptanone, smell like honey and contain compounds similar to those in honey but, fascinatingly, has some compounds identified as honeybee pheromones (Rasmussen *et al.*, 2002).

Generally when teenage males reach their early twenties sweet smelling acetates are no longer detectable and are transitionally replaced by unesterified acids. Then pleasantly odoriferous ketones make way for malodorous nonanones and undecanones, although the early phases of a discrete musth cycle are still characterized by the pleasantly odoriferous 2-butanone. Older males broadcast progressively malodorous combinations containing pongy mixtures of less volatile, more long-lasting ketones, especially during the mid-point of a particular musth episode (Rasmussen *et al.*, 2002). Significantly increasing amounts of an acrid bicyclic ketal, frontalin [1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane] are released. During musth older males dramatically increase their home range, although during nonmusth periods their home ranges are smaller than those of female family groups. These older widely roaming, secreting musth males expand and reinforce their domain of loose, subtle control over nonmusth and younger males (Rasmussen, 2003). Additionally these males older than 30 years are generally large, sexually active, socially adept and capable of sustaining long periods of musth. Accordingly, they demonstrate significantly more chemosensory responses and premating behaviours than their younger or nonmusth counterparts and moreover, they apparently are more skilled at detecting the precise ovulatory status of females (Rasmussen *et al.*, 2005).

Superimposed on this fluctuating separation of the sexes, differential and expanding home ranges, and the lack of male defense of territories is an infrequent female oestrus that is highly tuned with brief ovulatory period. However, the prevulatory period is extended and during this time females release increasing concentrations of female-to-male urinary pheromone (Z)-7-dodecenyl acetate, (Z7-12:Ac), timed to reach a maximum just before ovulation. Z7-12:Ac elicits rates of chemosensory response frequencies, including flehmens, and premating behaviours, including penile erections and mounting among males that increase proportionally to pheromone concentration (Rasmussen, 2001). Response frequencies, however, are affected also by male social contexts; in forest camps the presence of dominant older males reduced responses to Z7-12:Ac by younger males (Rasmussen *et al.*, 1997). In the wild, older musth males had both superior access to females and, because of their wide roaming behaviour, encountered greater numbers of females (Rasmussen *et al.*, 2005). While olfactory mechanisms allow adult males to locate females and to precisely assess their ovulatory status, male access to mating-ready females is partially dependent on

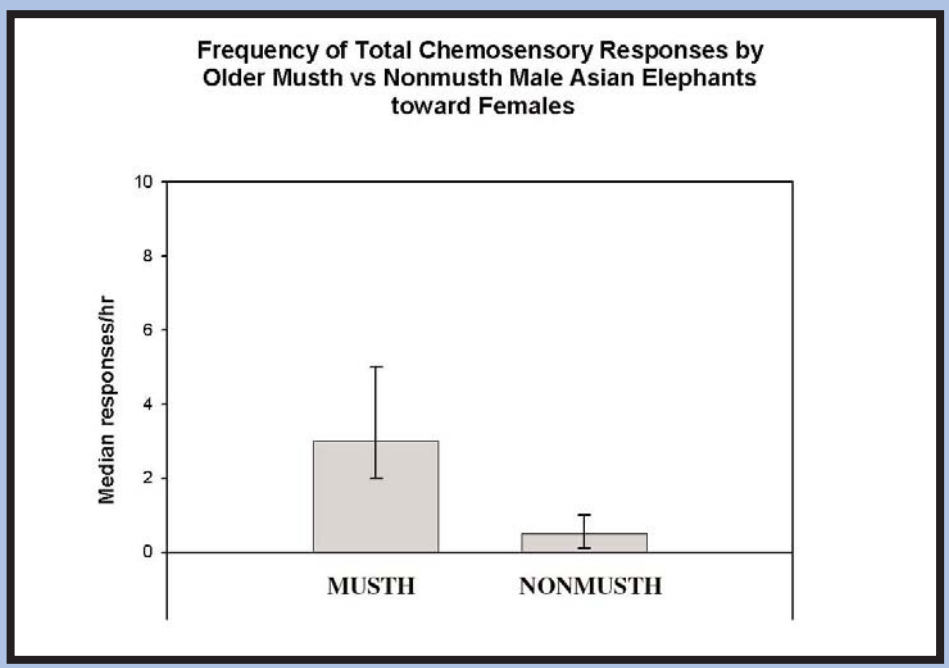

Figure 1. Median total chemosensory responses (frequency/hour) by older musth and nonmusth male Asian elephants to pre- and periovulatory females in the wild. Mann Whitney Rank Sum, T=528.000, P=<0.001.

Chemosensory responses scored included distant and close sniffs, expelled urine checks, urogenital checks and flehmens.

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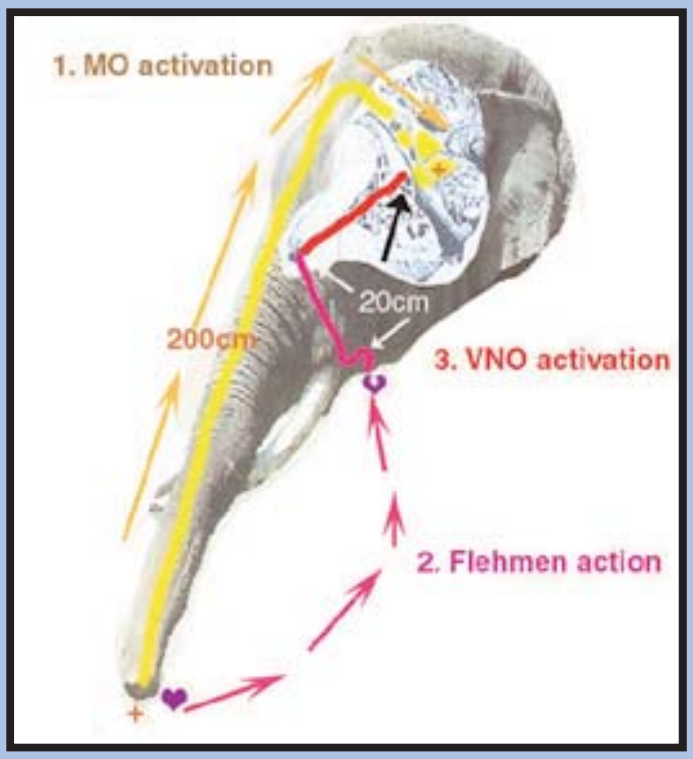

Figure 2. VNO systems in the Asian elephant. 1. MO **activation: An odorant (+) during a sniff travels up the truncal passageways at a velocity of ~66 cm/s, taking ~3 s to reach the olfactory epithelium of 37 extensive turbinates (yellow).** 2. Flehmen action: **Volatile pheromones may travel as in (1) or are deposited by flehmen action (purple arrows) to openings of the VNO ducts (purple). Time from initial sniff until flehmen is completed (i.e., trunk tip contents reach the orifice) is 4.8 ± 0.2 s.** 3. VNO activation: **As mean time between successive flehmens without additional sniffs is 11.5 ± 0.7 s, pheromonal complexes may travel through the ducts at a rate of at least 2 cm/s. Mean data from 12 elephants and 50 timed responses.**

olfactory signals not only from females-to-males, but also on signals between males. The male-emitted pheromone, frontalin, influences both male responses and female behaviour (Rasmussen & Greenwood, 2003). Female reactivity varies with hormonal state and male reactivity varies with age and musth status. The released frontalin by older males attracts reproductively ready females, but elicits apprehension from pregnant females whereas luteal phase females are indifferent. Among males, older adult males are also mostly indifferent to frontalin, whereas subadult males are highly reactive, generally exhibiting repulsion or avoidance (Ramussen & Greenwood, 2003).

Our captive studies demonstrated that these two intertwined pheromones facilitate concomitant male dominance interactions, female preference, and male assessment of preovulatory condition. Our field results suggest the lengthy preovulatory period effectively provides a synchrony between sexes for successful reproduction. Using chemosensory mechanisms, widely roaming, wild male elephants in musth locate periovulatory females in matriarchal-led female family units and precisely assess their ovulatory status with a significantly higher success rate than nonmusth males (Fig.1). The dual obstacles of separately living sexes and infrequent oestrus are overcome by lengthy female cycles. Thus pheromone-driven behaviors provide a firm basis for examining perireceptive events prior to neuroreception by the olfactory system.

Recently we have begun to tease apart and define the events involving pheromones and the binding proteins interacting with them while on the tortuous source-to-sink path, taking advantage of the extensive, highly mucoidal olfactory and vomeronasal systems and their interrelationships (Fig.2). We are combining studies of quantifiable responses and behaviors with biochemical and biophysical investigations of the properties of protein-ligand complexes, their sequential pathways and associated protein-ligand fluxes in accordance with Figure 2 to derive a series of timed events.

We have established that the bioactivity of Z7-12:Ac is pH dependent and protein modulated. Z7-12:Ac is bound to serum albumin (ESA, a 68 kDa alpha helical protein) and is excreted in this sequestered form in the urine of preovulatory females (Lazar, 2001; Lazar *et al.*, 2004). Male elephants sample the alkaline urine patches by mixing with acidic trunk mucus releasing volatile pheromone in a pH-mediated fashion that transitions eventually to olfactory receptors in the VNO via the flehmen response (Fig. 3). A portion of this free pheromone is "mopped-up" by copious odorant binding protein (OBP, an 18 kDa beta-barrel lipocalin) (Lazar, 2001; Lazar *et al.*, 2002; Greenwood *et al.*, 2003; Greenwood *et al.*, 2004; Greenwood *et al.*, 2005).

Binding of the Z7-12:Ac pheromone was demonstrated to both proteins by passive attachment using both a GC-based volatile odorant binding assay and on polyacrylamide gels using a radiolabeled pheromone analogue and autoradiography. We also used covalent attachment to the diazoacetate photoaffinity analogue (Z7-dodecen-1-yl diazoacetate) using both non-labelled and tritiated forms. Using archival polyacrylamide gels of several years standing we have now performed a proteomics analysis on excised Coomassie-stained protein spots of OBP and albumin samples reacted with the diazoacetate analogue, examining for covalently attached adducts using electrospray ion-trap mass spectrometry. Pheromone analogue fragments were detected attached to several tryptic peptides that map in close proximity to suspected binding site residues based on 3-D homology models of these proteins. Moreover, for albumin the adduct-peptide profile consistently mimics the stoichiometry of binding observed at varying pH values (Greenwood *et al.*, 2005).

Concluding thoughts: Asian elephants share breeding strategies common to other cognitive mammals including some primates (e.g. orangutans) and whales, while the musth factor adds a unique feature. Male roaming proclivities increase with age and are greatly expanded during musth. Reciprocally musth influences temporary social fusion-fission events as roving males join female groups while tracking preovulatory pheromone concentrations. Such behavior, often chemosensory mediated, in a time-dependent system involving pheromones, heavily influences successful elephant reproduction. From the biochemical perspective we have demonstrated that the bioactivity of Z7-12:Ac is pH dependent and protein modulated, both factors affecting its signal lifetime., The presence of ESA in a bioassay media with Z7-12:Ac preserves the bioactivity of the pheromone sample for 12 hours (Figure 4, Lazar

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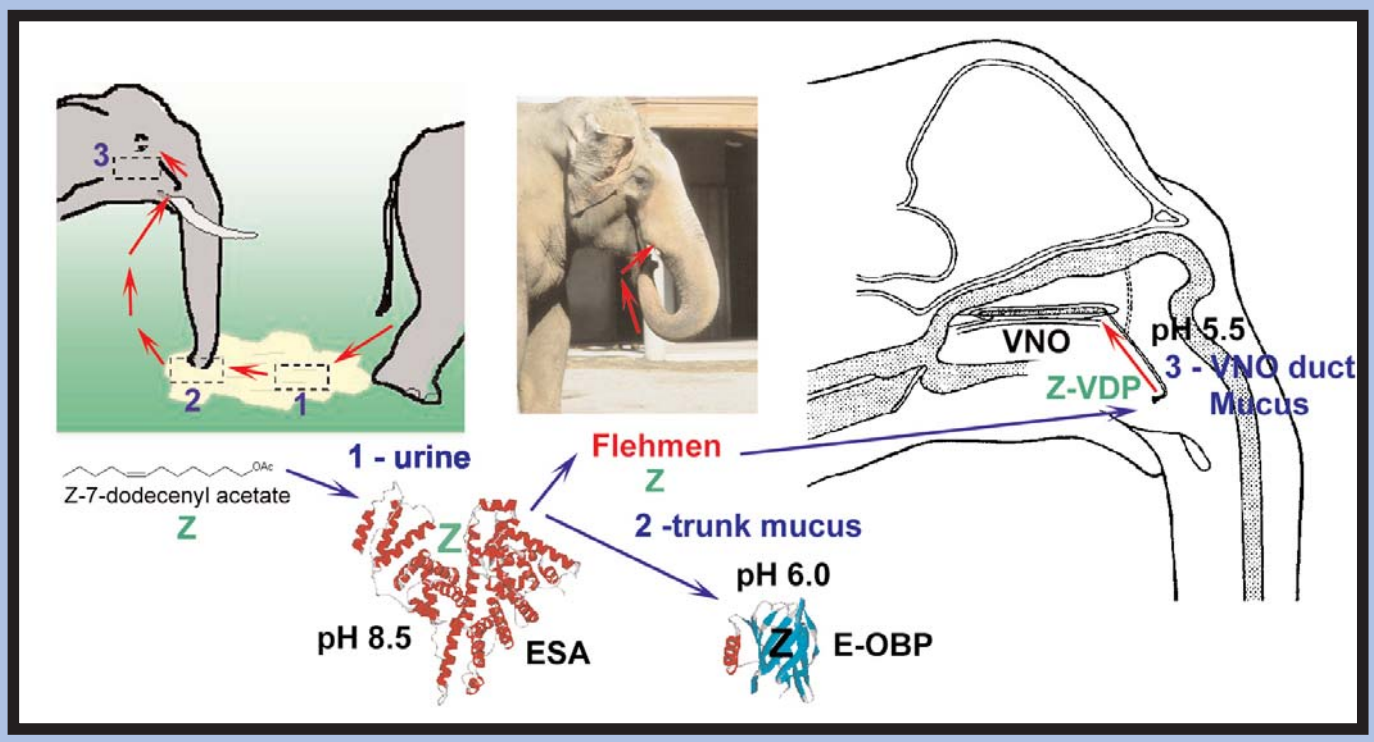

Figure 3. Perireceptive trail toward vomeronasal organ of the pheromone Z7-12:Ac (Z) showing its interactions between its protein transporters [ESA; E-OBP (sequestrant); and VDP (vomeronasal ductal protein)]. Z = Z7-12:Ac, VDP = vomeronasal ductal protein.

et al., 2004; Rasmussen et al., 2003). Recent field tests of this pheromone and musth ketones in protein based substrates have considerably extended this signal lifetime (Rasmussen & Riddle, 2004). Thus both laboratory and field studies of signal lifetimes underpin the roles of proteins. We are rigorously examining frontalin in a similar context with the hope that an overall coherent picture may emerge defining the temporally staged complex of pheromones exhibiting their endocrinological, physiological and ultimately behavioral roles in elephant reproduction with important practical implications for conservation applications.

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ABSTRACTS: oral sessions

ORAL SESSION 1: Chemical Senses and Signals in Invertebrates I

1

CHEMOSENSORY SYSTEMS IN ANIMALS: WHAT NOSES AND ANTENNAE HAVE IN COMMON

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Olfaction, or more broadly chemoreception, is the most ancient of the senses and still plays a vital role in mating and food searching of many animal species. Across the animal kingdom, chemosensory systems are remarkably similar, sharing a number of fundamental mechanisms. In both vertebrates and invertebrates, odorant molecules reach the dendrites of olfactory receptor neurons through an aqueous medium via odorant binding proteins. While olfactory receptor proteins are believed to have evolved independently in vertebrates and invertebrates, olfactory receptors all belong to the family of G-protein-coupled receptors, and the biochemical machinery of the signal transduction pathway is common to insects and vertebrates. Furthermore, olfactory neuropils in the central nervous system of invertebrates and vertebrates share close neuroanatomical and physiological characters. Since the olfactory tracts have common features across species, a better understanding of invertebrate olfaction may be relevant to olfaction in general.

2

ODORANT RECEPTORS AND OLFACTORY SIGNALLING IN *DROSOPHILA*

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The fruitfly *Drosophila melanogaster* has emerged as a premier model organism for the study of the chemical senses because of the availability of a sequenced genome, powerful molecular genetic techniques, simple behavioral assays and electrophysiological tools. In *Drosophila* odor signals are detected by a large family of 62 putative G protein-coupled receptors, the odorant receptor (Or) family. The Or family members are extremely divergent in sequence from each other and from all other known proteins, including odorant receptor proteins from other organisms. The function of a number of the *Drosophila* Or genes has been recently studied, and for these genes it is known that the Or gene endows an olfactory receptor neuron with all its response properties to odors, namely what odors it responds to, and how it responds. It is thus clear that the Or genes represent essential elements in olfactory information coding in insects. The signal transduction pathway(s) activated by the *Drosophila* Or proteins are largely unknown. We are taking a number of approaches to identify genes involved in these pathways, and results from two approaches will be presented. Firstly we are using a combination of comparative genomics across *Drosophila* species and structure-function analysis in *Drosophila* S2 cells to identity regions of the Or proteins which potentially interact with downstream signalling components, as well as with ligands. Secondly, we are determining what signal transduction pathway(s) the Or genes couple to when heterologously expressed in *Drosophila* S2 cells and insect sf9 cells. We have demonstrated that myc-tagged versions of Or22a and Or33c localise correctly to the plasma membrane when expressed in S2 cells. Surprisingly, although the atypical Or protein Or83b is required for correct targeting of other Ors to the cell membrane *in vivo*, we have found that Or22a is able to reach the cell surface *in vitro* without Or83b. Now that we have established this system we are proceeding to use tagged Or proteins to examine the general membrane topology of these proteins, and to use several methods to identify the signalling pathway to which these receptors are coupling.

3

IDENTIFICATION AND CHARACTERISATION OF GENES INVOLVED IN OLFACTORY SIGNAL TRANSDUCTION IN *DROSOPHILA*.

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In the fruit fly, *Drosophila melanogaster*, odorant molecules are initially detected by a family of odorant receptors believed to be G-protein coupled receptors. Subsequently signal transduction pathways are activated in order to transmit the information to the brain. We aim to identify genes involved in the olfactory signal transduction pathways used by *Drosophila*. There are two candidate signal transduction pathways: the inositol phospholipid (IP3) signalling pathway and the cyclic nucleotide signalling (CN) pathways. We initially performed database searches to identify genes encoding components of these pathways and then performed RT-PCR to determine whether these genes are expressed in the olfactory organs. We have found the following genes expressed in olfactory organs: 9 G protein subunits, 2 guanylate cyclases, 4 adenylate cyclases, 3 phospholipase C's, 2 cyclic nucleotide-gated ion channels and 3 members of the Trp family of ion channels. Immunolocalisation experiments have further shown that both the G•76C and G•30A proteins are localised in olfactory receptor neurons (ORNs) of the adult olfactory organs. We have performed larval chemotaxis assay on strains containing mutations in the Gq•, *norpA* (a phospholipase C) and *trpl* (a calcium ion channel). The *Gq•* mutant shows reduced attraction to ethyl acetate, but normal response to other tested odours, whereas *norpA* and *trpl* exhibited normal olfactory behaviours to all tested odorants. These results suggest that the IP_3 pathway is involved in larval olfactory signal transduction and that perhaps it utilises different phospholipase C's and ion channels to those involved in phototransduction. Since the *Gq•* mutant showed normal responses to many odorants, larvae are likely to utilise other signal transduction pathways as well as the IP3 pathway.

4

FLIES AND BANANAS: HOW *DROSOPHILA* DETECTS AND RESPONDS TO ODORS

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With their olfactory system, insects are able to monitor their chemical environment. They detect and respond

to those molecules, which can lead them to food, oviposition sites or mates. Certain odors may also help them avoid toxins or other dangerous situations. *Drosophila melanogaster* feeds on fermenting fruits, which produce many odorants as well as CO_2. The entire olfactory input to the brain is made up of ca. 1300 olfactory receptor neurons (ORNs), organized into 40-50 classes. We have characterized the response spectra of many of them. Each expresses a different member of a family of olfactory receptors (OR). Electrophysiological recordings demonstrate a variety of response properties such as broad vs. narrow tuning, odor-specific temporal profiles and high vs. low sensitivity. These will determine how odors are encoded across cell classes. Several ORNs are tuned to typical fruit odors such as esters. The CO_2-sensitive ab1C neurons take up a special place, being narrowly tuned and expressing a member of the gustatory receptor gene family; Gr21a. We determined the relation between neural coding of CO_2 concentration to behavioral output. Flies avoid CO_2 concentrations above 0.1%. However, in a background of attractive odor, such as apple vinegar, behavioral responses of females are at the detection threshold of 0.02%. Our analysis of CO_2 emissions from fruit indicates that they fall within the range of detectability. Using available genetic tools for targeting specific ORNs we calibrated calcium dynamics to action potential frequency in the olfactory epithelium and in the brain. We have also determined how responses from populations of antennal ORNs relate to the electroantennogram (EAG), a popular technique for measuring an insect's detection of natural odors.

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ABSTRACTS: continued oral sessions

5

DEVELOPMENT OF AN OLFACTORY RECEPTOR ASSAY SYSTEM IN *Sf*9 CELLS

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The discovery of genes encoding olfactory receptors (Ors) within the genomes of vertebrates, and subsequently in *C. elegans* and insects, has significantly increased the breadth of possible research in olfaction. One important aspect of olfaction that can now be investigated is the question of what these receptors bind and recognise. The ability of insects to detect and respond to such a wide range of volatile compounds with only a limited number of Ors (for example, 62 Ors in *Drosophila*) poses interesting questions regarding the manner in which odorant receptors bind odorants. Despite the importance of these issues, little in the way of functional analysis of Ors has taken place. We have developed an assay system for Ors using calcium imaging in *Sf*9. We show that the interaction of the Drosophila odorant receptor 22a (Or22a) with ethyl butyrate occurs in a dose-dependent manner, and that expression of either genomic or cDNA clones of the receptor elicits a similar response. This system does not require the co-expression of a G αprotein, and hence *Drosophila* Ors are able to couple to the endogenous Sf9 signalling system, presumably through the cells own G α proteins. We have also been able to demonstrate that the response of Or22a to a range of compounds in *Sf*9 cells is similar to that obtained using electrophysiological measurements in Drosophila. We believe this system is highly applicable to the de-orphaning of Ors from a range of invertebrate species, and provides a new tool for the analysis of odorant receptor compound affinity and receptor function.

6

DROSOPHILA NORPA: ROLE IN INSECT VISION AND CHEMORECEPTION

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Drosophila norpA (no receptor potential A) gene encodes phosphatidylinositol (PI)-specific phospholipase C (PLC-ß) and yields two gene products: subtype I & II. PLC hydrolyses phosphatidylinositol 4,5-biphosphate (PIP_2) into second messengers diacylglycerol (DAG) and inositol trisphosphate ($InsP_3$), which ultimately leads to Ca^{2+} release from the intracellular stores. The best studied example of a transduction pathway involving *norpA* gene product is the *Drosophila* photoreception; flies with strong alleles of *norpA* are blind due to a dramatic decrease in the photoreceptor PLC levels. Subsequently, the *norpA*-encoded PLC has been shown to be required for Drosophila olfaction (Riesgo-Escovar *et al.*, 1995). Here, we report that norpA may also be involved in *Drosophila* gustation. Firstly, RT-PCR results indicate that taste structures, primarily labella and tarsi, contain detectable levels of subtype II *norpA* transcript; in contrast compound eyes show high levels of subtype I. Secondly, using a GAL4/UAS approach with the minimal *norpA* promoter (Doh *et al.*, 1997) fused to GAL4 to drive expression of reporter genes (*e.g.* encoding GFP) we show that norpA is expressed is a relatively large subset of gustatory neurons. Our findings suggest that gene products of a single gene may be involved in three separate sensory transduction cascades in *Drosophila*.

ORAL SESSION 2: Human Psychophysics and Behaviour

7

LOCALIZED ORAL PATHOLOGY AFFECTS LONG-TERM HEALTH RISK BY DISINHIBITING REMAINING ORAL CUES

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Several afferent nerves carry oral sensory information, and mounting evidence suggests that some of these inputs inhibit others centrally. Supporting this idea, our psychophysical data indicate that localized oral sensory loss selectively disinhibits distant oral loci: Chorda tympani (CT) anesthesia leads to elevated glossopharyngeal (IX) and trigeminal (V) sensations, while compromising retronasal olfaction. Childhood ear infections (otitis media, OM) produce similar sensory changes in adult subjects, consistent with long-term CT loss. Supertasters (ST) of 6-n-propylthiouracil (PROP) perceive the most intense oral sensations, so they may experience extreme disinhibition resulting in clinical symptoms. For example, patients with burning mouth syndrome (BMS) tend to be STs; these individuals show CT loss, report taste phantoms, and respond favorably to GABA agonist treatment, suggesting that BMS is a pain phantom induced by trigeminal disinhibition. More subtly, CT loss may alter long-term dietary health by enhancing tactile perception and fat preference via V. Recent findings show that adults with histories of severe childhood OM have body mass indices (BMI) approaching clinical obesity, and we have linked OM-related BMI gain to increased bitter (reflecting CT damage) and sweet-fat (promoting obesity) food preferences. Further oral pathology (e.g., head trauma, tonsillectomy) appears to exacerbate both the sensory and diet-related effects of OM. Finally, postnatal secondhand smoke exposure increases OM risk, and adult men raised before age 10 in homes with 2+ smokers have elevated BMI. Based on these results, we strongly believe that localized oral sensory damage contributes to chronic health risk (e.g., orofacial pain, obesity) by altering neural interactions that mediate taste and trigeminal perception.

8

OLFACTORY SENSITIVITY THROUGH THE COURSE OF PSYCHOSIS: RELATIONSHIPS TO OLFACTORY IDENTIFICATION, SYMPTOMATOLOGY AND THE SCHIZOPHRENIA ODOUR

Brewer, W.J.1, Wood, S.J.[2,4], Pantelis, C.[2], Berger, G.E.[1], Copolov, D.L.[3] & McGorry, P.D.[1]

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There is some evidence for an unusual body odour in schizophrenia that has been linked to a hexenoic acid derivative (*trans*-3-methyl-2-hexenoic acid; MHA). Poor body odour has been linked to increased negative symptoms and reduced olfactory identification ability. However, the relationship between these findings and MHA, including olfactory sensitivity for MHA, has not been examined. Olfactory sensitivity thresholds were assessed for MHA and n-butyl alcohol (NBA), which is commonly used in such paradigms, in normal controls (CTL; n=24), patients with chronic schizophrenia (CHR; n=32) and a first-episode psychosis cohort (FE; n=31). In addition, forced-choice detection of the pheromonal steroids 5-∝-androst-16-en-3-one, androsterone-sulphate and estrone-3-sulphate was performed along with a measure of olfactory identification. CHR patients had significantly reduced sensitivity to MHA, but not NBA, compared to FE and CTL subjects. While sensitivity to pheromones was not different between the groups, CHR patients who could not detect them also showed poorer sensitivity to MHA. Further, the CHR group showed a significant association between reduced MHA sensitivity and greater levels of disorganised and negative symptoms. No relationships between identification and sensitivity for any substance were found. Our findings are the first to report reduced sensitivity for MHA in chronic schizophrenia patients, in the absence of similar impairment for more traditionally used substances. This may be linked to olfactory habituation effects, abnormal chemical processing or a genetic predisposition.

9

A ROLE OF EXPECTATION IN PERCEPTION OF FLAVOR AND/OR ODOR

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Some studies reported that vision affects gustation, olfaction and/or flavor perception. Although vision is the most important one of extroceptive sensations for humans, flavor, which consists of gustation, olfaction and other "mouth senses", is one of introceptive sensations. Thus, it is suggested that there are no interaction between vision and flavor in physicochemical, peripheral or sensory levels. In this presentation, three experiments investigating the effect of vision on perception of flavor will be reported. In the first experiment, perceived intensity of flavor of beverages was compared between when the beverages were presented with their pictures and without them. In the second experiment, perceived intensity of flavor of beverages was compared between when the beverages were presented with their own pictures and with pictures of other beverages. In the third experiment, perceived intensity of flavor of juices was compared between when the juices were

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presented with their own colors and with colors of other juices. In all these experiments, preferences for the stimuli were also asked. As a result, it was found that these visual stimuli enhanced flavor intensities and preferences when they are congruent with the flavor. These results were compatible with those of preceding studies, which investigated the interactions of olfaction with gustation and/or vision and suggested that these interactions occur in cognitive level. A role of expectation of the flavor, which is activated by vision, will be discussed in these experiments.

Acknowledgement: A part of this study is granted by Grant-in-Aid for Scientific Research (Japan Society for the Promotion of Science to N.S.).

10

COMPARING THE DIETS OF 6 -24 MONTH OLD CHILDREN IN TERMS OF FOODS BASIC TASTES

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It is believed that dietary habits established during infancy and early childhood can contribute significantly to dietary habits in later life. However, the dietary habits of many children provide poor nutrition. The taste of food is very important in food choice as it helps signal nutritional value. Dietary records for 193 children aged 6-24 months, collected from a random sample of South Island New Zealand Children during 1998, were analysed. A trained sensory panel (10 adult assessors) quantified the intensity of sweet, sour, salt, bitter and overall flavour intensity in 263 food items frequently consumed by the children using scales that were anchored using multiple references. New diet record software that records both the sensory and nutritional properties of foods consumed was developed. In terms of taste, foods can best be described as sweet or salty, and either of low or high overall intensity. Diets were found to have either a relatively high proportion of sweet taste, or a relatively high proportion of salty taste. Significant correlations were found between food composition, energy content and the basic tastes of foods. Significant relationships were determined between intake of sodium and available sugars, and the proportions of salty and sweet tastes in diets. The proportions of sour and bitter tastes also differed widely between children, but were less pronounced. Toddlers were found to differ widely in the foods they consumed, in the patterns of their food consumption. Differences in the tastes of diets could be traced to differences in dietary variety, and in the types of foods consumed by children, independent of the energy intake of the child, the child's BMI or gender.

11

SWEET ODOURS INCREASE PAIN TOLERANCE

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Several studies in humans have documented an impact of odours in reducing measures of pain. One hypothesis is that odour pleasantness influences pain, possibly via its impact on mood. Sweet tastes reduce pain, and we tested whether odours that are sweet-smelling through prior association with such tastes might similarly reduce pain. In the study reported here, adult subjects (Ss) underwent a pain-inducing cold-pressor test (CPT) during which they inhaled air containing a sweet-smelling odour. Ss in two control groups also underwent the CPT with other odours. These groups were to control for an effect due simply to pleasantness (Ss received a pleasant, but not sweet smelling odour), on the one hand, and a distraction effect (an unpleasant odour), on the other. In the CPT, Ss immersed their dominant forearm in water at ~ 5°C for up to 4 minutes on two occasions, 15 minutes apart: once with the odour present (CPT+), and once without (CPT-), order balanced across Ss. For each S, we then determined the impact of the different odours by comparing latencies (secs) to remove their arm in the two CPTs. Ss also rated the pain intensity immediately after immersion, again after another 30 secs, and then immediately on withdrawing their arm from the water. The group receiving the sweet odour had a significantly longer mean latency during the CPT+ than the CPT- condition, and a longer latency than both control groups for either CPT+ or CPT-. There were no group differences in pain ratings at any of the rating periods. Hence, these results most likely reflect differences in pain tolerance rather than to pain reduction per se. These results are discussed in terms of Pavlovian conditioning models.

ORAL SESSION 3: Chemical Senses and Signals in Invertebrates II

12

EXPRESSION OF INVERTEBRATE ODORANT RECEPTORS IN THE NEMATODE *CAENORHABDITIS ELEGANS*

Liao, C., Dumancic, M., Beard, C., Wanjura, W., Cowper, B., Weller, G. & Trowell, S.

Food Futures Flagship & CSIRO Entomology, Black Mountain Laboratories, Canberra, Australia

Until very recently, research on odorant receptor (OR) function has been hampered by the lack of simple experimental systems in which ORs may be functionally expressed. Recent research has demonstrated that functional expression of ORs requires co-expression of accessory proteins such as OR83b and its homologues in insects or, in mammals, RTP1 and RTP2. Because of these limitations, intact olfactory neurons have been the most effective expression systems for ORs. For example, the rat I7 receptor has been functionally expressed in intact rat olfactory epithelium (Firestein and co-workers) and in the AWA and AWB neurons of the intact nematode C. elegans (Milani and co-workers). However such approaches are experimentally complex and suffer from the limitation that olfactory responses due to the exogenous receptor are superimposed on the summed endogenous responses of all other ORs expressed in the tissue or organism. The development of the Drosophila "empty neuron" system in Carlson's lab and its subsequent exploitation by Hallem et al. (2004) represented a genuine breakthrough in odorant receptor research and allowed the assignment of odorant receptivity to approximately 30 Drosophila ORs, four times more than the total of all ORs characterised previously. Nevertheless, exploitation of this system requires Drosophila transgenesis and the ability to perform single-cell recordings from the antenna of the fly. We reasoned that the molecular and classical genetic techniques that are feasible in the nematode worm, combined with its easily-measured chemotaxis towards or away from odorant sources, would simplify the routine expression and characterisation of invertebrate odorant receptors. Progress towards this goal will be described in relation to the development of a cybernose instrument.

13

OLFACTION IN MOTHS: A REVIEW OF THE MECHANISMS UNDERLYING PERIPHERAL SIGNAL TRANSDUCTION

Newcomb, R.D.[1], Jordan, M.[1,2] & Greenwood, D.R.[1,2]

[1]Molecular Olfaction Group, HortResearch, Auckland, New Zealand; [2]School of Biological Sciences, University of Auckland, Auckland, New Zealand

Moths have an exquisite sense of smell, which they use to detect host plants and potential mates. Best characterised in moths are the long distance sex pheromones that are specific for each species, used by males to locate females. The sensitivity and specificity of sex pheromone reception in moths is legendary. Here we will describe the current models for the peripheral mechanisms in the antennae that underlie the ability of moths to detect volatiles, including sex pheromones. Specifically, we will review the evidence for the involvement of various families of odorant-binding proteins, odorant-degrading enzymes and membrane proteins. Olfactory receptors are now being identified from the genomes of moths including *Bombyx mori*, *Heliothis virescens* and *Epiphyas postvittana*. Future challenges will be to link different moth olfactory receptors with the ability to detect various plant volatiles and sex pheromones. This is being achieved through screening of recombinantly-expressed receptors in cell lines or *Xenopus* oocytes. For example, in *B. mori* receptors have been identified that bind the sex pheromone, bombykol, and its inhibitory partner, bombykal.

14

PERIPHERY OLFACTORY PROTEINS OF *EPIPHYAS POSTVITTANA.*

Stanley, D.[1], Jordan, M.[1,2], Greenwood, D.R.[1], Marshall, S.D.G.[1], Crowhurst, R.N.[1], Gleave, A.P.[1] & Newcomb R.D.[1]

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The olfactory signal transduction system of insects involves numerous steps leading to the generation of a neuronal

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Useful Chemical Senses Book

Tastes and Aromas: The Chemical Senses in Science and Industry, Edited by Graham Bell and Annesley J. Watson. 214 pages. Published by UNSW Press and Blackwell Science, 1999. ISBN: 0-86840 769 0. Hard Cover. Price: US$ 30 / AUD$ 40 (includes tax if applicable, postage and handling). Order from: g.bell@atp.com.au

A limited number of this extremely useful volume are, for a short time only, available at a 50% discount. *Tastes and Aromas* has been hailed as a great teaching aid and resource for the practicing sensory scientist. Written by leaders in their fields as fundamental information, the volume retains its value and is rich in scientific and practical quality. Beautifully packaged in hard cover, it will continue to be a durable reference for many years to come.

Chapters include mini-reviews by (first authors) Stoddart; Bartoshuk; Youngentob; Prescott; Lyon; Weller; Bell; Saito; Lambeth; Noble; Morgan; Best; Barry; Sullivan; Key; Mackay-Sim; Atema; Hibbert; Barnett; and Levy.

Content covers the chemical senses in human culture; fundamentals of smell; taste; pungency; oral touch and pain; applied sensory evaluation; cross-cultural studies; perfumery and flavour chemistry; wine preference; psychophysics; sensory mapping; physiology of odour encoding; anatomy, growth and aging; emerging chemosensory technologies; sensors; marine chemical signals; electronic noses and chemosensory machines.

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NEWS

Vale: Ernest Polak (1921 - 2005)

Ernest H. Polak, supporter of chemosensory research and friend (and contributor) to ChemoSense, died on 12 September 2005. Ernest became enthused by the chemical senses in his family's fragrances business, PWF Inc., and later maintained collaborations with scientists around the world. Born in 1921 in Amersfoort, Holland, he moved to New York City in 1939 to work at PFW Inc. At this time, he also attended Columbia University and Brooklyn Polytech and later earned a B.S. degree in Chemistry from NYU. Ernest earned his M.S. degree in Chemistry from Iowa State University in 1944 and then moved back to the east coast to work as a researcher at Hoffman LaRoche in NJ. He then returned to PFW Inc. and served as the Vice President for Research and Development from 1948 until 1973. In 1975, he moved to France after the merger of PFW and Hercules.

In addition to his work in industry, Ernest had many collaborations with academic scientists. He worked with the Sensory Neurobiology Laboratory led by Patrick McLeod near Paris from 1975 to 1985 and with Jean Leveteau at the University of Paris VI from 1985 to 1997. From 1975 until 2004, Ernest sponsored and collaborated on projects with scientists focused on olfactory quality coding in France, Great Britain, Italy, and the United States. Earlier in 2005, he and his wife Ghislaine established a major endowment for AChemS and ECRO to support young scientists, dedicated as follows: "The Elsje Werner-Polak Memorial Fund in memory of our niece, gassed by the Nazis in 1944 at age 7. Donors: Ernest and Ghislaine Polak."

Ernest had a great nose and a sharp mind. He made many significant discoveries and produced thousands of important compounds. Ernest will be greatly missed by his colleagues in the chemical senses, his loving wife Ghislaine, his two sons, Clifford Polak of Paris and Elliot Polak of London, his five grandchildren, and his friends and colleagues around the world

(Acknowledgement: AChemS website) ■

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oral sessions

signal. The initial step in this system involves the interaction of soluble proteins with odorants upon their entry to the sensillar lymph. The peripheral olfactory proteins can be loosely divided into two types: odorant-binding proteins (OBPs) and odorant-degrading enzymes (ODEs). We have created a library of expressed sequence tags (ESTs) from the antennae of the Light Brown Apple Moth (*Epiphyas postvittana*), a pest of the horticultural industries of New Zealand and Australia. The library contains an abundance of potential OBPs, including pheromone-binding proteins (PBPs), general odorant-binding proteins (GOBPs), antennal binding protein Xs (ABPXs), chemosensory proteins (CSPs) and juvenile hormone binding-protein-like proteins (JHBPLPs), as well as numerous ODEs, including carboxylesterases and cytochrome P450s. Targeted OBP genes have been expressed recombinantly in *Escherichia coli* and purified for further structural and functional analysis. Three members of the PBP family have been identified from antennae. All three PBPs are expressed almost exclusively in antennal tissue, with PBP1 and PBP3 showing higher expression in male antennae than female, while the opposite is true for PBP2. PBP1 binds the major pheromone component, E11-14:OAc, as shown in binding assays. Competitive binding assays were used to screen potential ligands for a previously uncharacterised protein, JHBPL1. PBP1 and JHBPL1, together with other potential OBPs, are being crystallised to determine their structure. This will provide clues as to the ligand-binding specificity of the proteins and their role in moth olfaction.

15

DIVERSITY AND CONSERVATION OF LEPIDOPTERAN OLFACTORY RECEPTORS

Anderson, A.R.[1,2], Jordan, M.[3,4], Trowell, S.[1] & Newcomb, R.[3]

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The ability of organisms to detect and discriminate between many odours is pivotal to their survival and is primarily due to the olfactory system. In vertebrates, *C.elegans* and *Drosophila melanogaster*, odorant receptors (OR's) provide the molecular basis for odour-coding and belong to the large super family of G-Protein Coupled Receptors. In insects, ORs are extremely diverse across orders and species with the exception of the *Or83b* homologues. The *OR83b* receptor exhibits a high level of sequence conservation across four orders and appears to be required for localizing other OR proteins to the dendrites of olfactory neurons. The genomes of Drosophila, *Anopheles gambiae* and *Apis mellifera* and *Bombyx mori*, the silkmoth, are now available for sequence mining. We are mining the genome of Bombyx for homologues of the known ORs of Drosophila and *Heliothis virescens* and also for homologues of novel ORs isolated experimentally from other lepidopteran species (Jordan & Newcomb, unpublished). We are particularly interested in the extent to which specific OR sequences are conserved within an Order or other taxon and the functional significance of any such sequence conservation. We are using degenerate RT-PCR to probe for conservation of OR sequences in six lepidopteran species, besides Bombyx, representing another five lepidopteran families. RNA *in situ* hybridisation and functional expression of lepidopteran ORs in insect cell lines will help us to elucidate the functions of the ORs we have identified.

ORAL SESSION 4:
Taste & Olfactory Mechanisms

16

TRANSDUCTION AND CALCIUM SIGNALING IN TASTE BUDS

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Taste buds are sensory endorgans that transduce taste stimuli into calcium signals, resulting in transmitter release and activation of gustatory afferents. Bitter, sweet, and umami stimuli bind G protein-coupled receptors that activate the PLC signaling cascade, causing release of Ca from intracellular stores, capacitative influx of Ca, and activation of a monovalent-selective cation channel, TrpM5. Genetic ablation of TrpM5 abolishes taste responses to bitter, sweet, and umami stimuli, although its precise role in transduction is obscure. Previous studies from my lab indicated that the bitter receptor-expressing Type II taste cells lack both voltage-gated calcium channels and presynaptic specializations with afferent nerve fibers, although gustatory nerve fibers form close associations with these taste cells. In contrast, Type III taste cells have large voltage-gated calcium currents and form prominent synapses with gustatory nerve fibers. Using transgenic mice expressing GFP from the TrpM5 promoter, we have now extended these studies to include all Type II taste cells, as assessed by GFP fluorescence. The presence of voltage-gated calcium currents was assessed independently by calcium imaging with fura-2 and whole-cell patch clamp recording. To determine if TrpM5-expressing taste cells have presynaptic specializations, we used immunocytochemistry with an antibody to SNAP-25, which has been shown to be present in taste cells with conventional synapses. Taken together, the results suggest that TrpM5-GFP expressing taste cells lack both voltage-gated calcium channels and presynaptic specializations with afferent nerve fibers. Thus, how the TrpM5-mediated depolarization is conveyed to afferent fibers is unclear, but may involve novel synaptic mechanisms or communication with Type III cells by gap junctions.

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A TIMELY PROBLEM: THE NEUROTRANSMITTER LINKING TASTE BUDS TO NERVES

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The receptor cells of taste buds must transmit information about tastants to the gustatory nerves. Various candidate classical neurotransmitters have been proposed to serve this function including serotonin acting via 5HT3 receptors. Our investigation of 5HT3a knockouts show no gustatory dysfunction in 2-bottle preference tests. In contrast, mice lacking the ionotropic purinergic receptors, P2X2 & P2X3, expressed in taste nerves, are largely devoid of gustatory preferences. Further, electrophysiological recordings from the lingual gustatory nerves shows no responses to classical tastants although robust neural responses to touch, temperature and menthol persist. Finally, luminometer studies demonstrate tastant-evoked release of ATP from foliate and vallate taste buds. Taken together, these findings indicate that ATP and not serotonin is the key transmitter by which taste buds communicate with gustatory nerves.

18

MSG AND SUCROSE TASTE IN T1R3 KNOCKOUT MICE.

Delay, E.R.[1,2], Hernandez, N.[2], Bromley, K.A.[2] & Margolskee, R.F [3]

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Sweet, umami, and L-amino acid taste stimuli are believed to be detected by T1R3 receptors combined with other members of the T1R family. In molecular expression studies (Nelson et al., 2002), T1R1+T1R3 heterodimers, a broadly-tuned L-amino acid receptor, respond to monosodium glutamate (MSG) but not to sweet stimuli, whereas T1R2+T1R3 heterodimers responded to a wide variety of sweet stimuli but not to umami or most other L-amino acids. However, studies with mice lacking T1R3 have reported contradictory findings regarding the function of this receptor in taste transduction. One study reported that T1R3 knockout (KO) completely eliminated umami taste (Zhao et al., 2003), whereas another study with an independently developed T1R3 KO mouse reported only a reduction in umami taste (Damak et al., 2003). Using behavioral methods to assessed behavioral sensitivity of T1R3 KO mice obtained from Damak et al., we found that T1R3 KO mice can detect MSG and sucrose at threshold concentrations similar to those detected by wild type mice. In addition, using a shock-avoidance/water-reinforcement discrimination paradigm, we found that T1R3 KO mice can discriminate between the tastes of sucrose and MSG but only with difficulty. These findings suggest that other receptor mechanisms may be involved in detection of these substances.

Supported by DC005962 and DC7617 (ERD) and DC003155 (RFM).

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GLOMERULI: GATEKEEPERS OF OLFACTION

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Olfactory sensory neurons in the nasal cavity send axons to the olfactory bulb where they terminate in specialized neuropil structures - glomeruli, which are the first site for integration of olfactory information in the brain. Glomeruli have generally been considered simple structures surrounded by a shell of inhibitory interneurons. Findings from our lab have shown that glomeruli contain juxtaglomerular neurons multiple cell types including divided into three groups: periglomerular (PG), external tufted (ET) and short axon (SA) cells. These cell types, in turn, have distinct morphological and functional subtypes and are neurochemically heterogeneous. and are linked by complex synaptic organization. The extrinsic inputs to glomeruli derive primarily from the ON and other glomeruli with additional inputs from modulatory cortical regions. Therefore, neural processing within the glomerular layer is principally the result of *intra*- and *inter*glomerular circuits in response to sensory input from the ON. Here we present an overview of our work on the structural and functional organization of these intra and interglomerular circuits. We hypothesize that the function of these circuits is to spatially and temporally shape the transfer of olfactory information from the ON to the output neurons of the bulb.

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REGULATION OF INTRACELLULAR CHLORIDE, [CL]I, IN MOUSE OLFACTORY NEURONS BY TWO CL CO-TRANSPORTERS, NKCC1 & KCC2.

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Although secondary to the non-selective cation current, the Ca-dependent Cl current is primarily responsible for the generator potential (Cl flux carrys over 85% of the odor response in rodents). Thus, intracellular choride, [Cl]i, is important in determining the ultimate response of olfactory sensory neurons (OSNs) exposed to odor. We used MEQ, a chloride sensitive fluorescent dye, to measure [Cl]i across a population of isolated OSNs. Our results indicated a wide range of [Cl]i exist in isolated OSNs (range: 20-145 mM; mean: 62 mM). However, in any individual OSN, [Cl]i was stable during the recording period (up to 4 hours). The only changes observed in [Cl]i were due to stimulation or altering bath conditions to affect Cl co-transporters. [Cl]i of OSNs responded in a dynamic fashion, changing in response to odor stimulation or increased [cAMP]i. With [Cl]i unusually high in some OSNs , there must be an active transporter or exchanger(s) to maintain this gradient. Several different transporters have been reported to carry Cl in neurons. We focused on NKCC1 and KCC2 since these Cl co-transporters have been shown to be critical in developing neurons. Immunocytochemisty with an antibody to NKCC1 on olfactory tissue sections and isolated OSNs showed labeling over the cell body, dendrite with heavy labeling in the cilia. Antibody labeling for KCC2 showed some labeling in the cell body and dendrite but only the proximal portions of the olfactory cilia were labeled. Drugs were used to selectively inhibit either NKCC1 or KCC2. Data from these experiments, imaging and perforated patch clamp, suggest both NKCC1 and KCC2 are functional in OSNs and selectively inhibition of either can alter odor responses in many OSNs.

Supported by grants NIH-P20RR16435 and NIH-DC006939.

ORAL SESSION 5:
Olfactory Development & Regeneration

21

LAMINAR TARGETING OF OLFACTORY AXONS IS ALTERED BY GENETIC MANIPULATION OF GLYCOCODES

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Primary sensory neurons in the vertebrate olfactory systems are characterised by the differential expression of distinct cell surface carbohydrates. We have genetically engineered the terminal oligosaccharide composition of primary sensory neurons to determine whether cell surface carbohydrates contribute to axon guidance. Transgenic mice (BGAT-Tg) were generated which express the blood group A (BGA) glycosyltransferase under the control of the promoter for OMP, an olfactory neuron specific marker. In immature wild-type mice, BGA was expressed by a subpopulation of vomeronasal organ neurons whose axons terminate in glomeruli in a distinct caudal zone in the accessory olfactory bulb. In BGAT-Tg mice, ectopic expression of BGA perturbed the ability of vomeronasal axons to correctly terminate in the accessory olfactory bulb, causing axons to exuberantly grow into deeper inappropriate layers. These results provide the first in vivo evidence for a specific role of cell surface carbohydrates in laminar recognition in the olfactory system.

22

NITRIC OXIDE AND OLFACTORY NEUROGENESIS

Sülz, L.[1,2,3], Astorga, G.[1], Iturriaga, R.[2], Mackay-Sim, A.[3] & Bacigalupo, J.[1]

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Transient expression of nitric oxide synthase (NOS) in developing and in regenerating olfactory epithelium led to the hypothesis that nitric oxide (NO) regulates olfactory neurogenesis. This was tested in cultures of the non-neuronal cells from adult rat olfactory epithelium, which allow investigation of factors that stimulate neuronal precursor proliferation and their differentiation into neurons. We show here that all olfactory cell types express the three isoforms of NOS except horizontal basal cells which did not express nNOS. Growth factors that regulate olfactory neurogenesis altered the proportions of cells expressing the different NOS isoforms. NOS inhibition reduced cell proliferation and stimulated neuronal differentiation in a dose-dependent manner, including the induction of an outward potassium current. NO release stimulated cell proliferation and reduced neuronal differentiation. These effects were independent of the growth factors, leading to the hypothesis that NO may be the final common mediator for the action of growth factors on the regulation of olfactory neurogenesis. This was tested in an immortalised olfactory neuronal precursor cell line, OLF442. Differentiation of these cells by serum deprivation reduced expression of NOS and reduced levels of NO. NOS inhibition reduced NO release, reduced proliferation, induced morphological differentiation, induced expression of the outward potassium current and induced expression of neuronal proteins. NO release stimulated proliferation and suppressed differentiation. These results suggest that NO switches neuronal precursors between proliferation and differentiation.

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DIFFERENTIAL EXPRESSION AND TROPHIC FACTOR ZONES OF MEMBERS OF THE GDNF FAMILY OF LIGANDS AND THEIR RECEPTORS IN THE OLFACTORY SYSTEM

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The GDNF family of trophic factors, GDNF, neurturin, persephin and artemin, support the survival and regulate differentiation of many neuronal populations, including peripheral autonomic, enteric and sensory neurones. Members of this family of ligands bind specific GDNF family receptor (GFR) proteins, which complex and signal through Ret receptor tyrosine kinase. We previously showed that GDNF was detectable in olfactory sensory neurones (OSNs) in olfactory neuroepithelium (ON) (Buckland & Cunningham, 1999). Here we investigated the distribution of GDNF, neurturin, Ret, GFR•1 and GFR•2 in the adult rat ON and bulb. Adult male rats (150-250gm) were overdosed with IP barbiturate and transcardially perfused with 4% paraformaldehyde, olfactory tissues embedded in paraffin, sectioned and examined by multiple labelling fluorescence and diaminobenzidine immunohistochemistry. A minimum of 3 animals was used for examination of each antibody's distribution. An ApoTome device (Zeiss) was used for optical

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sectioning allowing 3-D reconstruction. GDNF and Ret were co-expressed by immature and mature OSNs, whereas neurturin was in a subpopulation of OSNs zonally restricted to a region resembling one of the known odorant receptor zones. The GFRs had differential expression, with mature OSNs and their axons preferentially expressing GFR•1, and progenitors and immature neurones more avidly expressed GFR•2. In the bulb, GDNF was highly expressed by the mitral and tufted cells, and periglomerular cells, and its distribution resembled that of Ret, although the latter was more predominant on fibres. Neurturin, was present at lower levels and more restricted in expression to the nerve fibre and glomerular layers in a particular zonal region of the bulb. Both GFR•1 and GFR•2 appeared prominently in the bulb, with complementary patterns of distribution. These data are supportive of GDNF and neurturin playing different physiological roles in the olfactory system and suggest that zones of trophic factor influence may be important in olfactory function.

Supported by the Garnett Passe and Rodney Williams Memorial Foundation

ORAL SESSION 6: Chemical Ecology

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THE ELEPHANT CHEMOSENSORY SYSTEM SAGA: ANALYSIS OF PHEROMONAL LIGAND BINDING TO CARRIER PROTEINS USING MASS SPECTROMETRY

Greenwood, D.R.1, Rasmussen, L.E.L.[2], Cooney, J.[3], Jensen, D.[3], Lazar, J.[4] & Prestwich, G.D.[5]

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We have reported that the Asian elephant sex pheromone, Z7-dodecen-1-yl acetate is bound to serum albumin (a 68 kDa alpha helical protein) while in the blood and is excreted in this sequestered form in the urine of preovulatory females. Male elephants sample the alkaline urine spots by mixing the urine with acidic trunk mucus thereby releasing volatile pheromone in a pH-mediated fashion that transitions eventually to olfactory receptors in the VNO via the flehmen response. A portion of this free pheromone is "mopped-up" by copious odorant binding protein (OBP, an 18 kDa beta-barrel lipocalin). Previously, binding was demonstrated to both proteins by passive attachment by two methods, using a GC-based volatile odorant binding assay and using a radiolabeled pheromone analogue on polyacrylamide gels prior to autoradiography. We have also used covalent attachment to binding proteins with a photoaffinity analogue, Z7-dodecen-1-yl diazoacetate, using both cold and tritiated forms of this ligand. Archival (several years old) SDS polyacrylamide gels provided the sample source for a proteomics analysis of excised Coomassie-stained protein spots of OBP and albumin samples reacted with the diazoacetate analogue. Using an ion-trap mass spectrometer operating in nanoelectrospray mode, we have now examined the peptides from trypsinolysis of the digested gel bands for covalently attached adducts. Pheromone analogue fragments were detected attached to several peptides that map in close proximity to suspected binding site residues based on 3-D homology models of these proteins. This finding demonstrates the applicability of mass spectrometry to help analyze ligand-protein associations.

Supported by ISAT (to D.R.G and L.E.L.R), NSOF (to D.R.G and J.C) and NIH grant RO1-DC03320 (to L.E.L.R)

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THE FRONT [AND BACK] SIDE OF ELEPHANTS: AN UNEXPECTED FINDING

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Serendipitous results provide excitement in science and may occasionally lead to significant discoveries. We report a series of such unexpected, yet fortuitous findings during our studies of pheromones among the Asian elephant, *Elephas maximus*. In 1994 we identified an unusual compound in the temporal gland secretions of Asian elephants that apparently had no biological activity under the trial regime at the time [Perrin et al., 1994]. Several years later during a different style of bioassay, using this male-elephant emitted compound, frontalin, as a control substance, we discovered that this chemical was jam-packed with messages for both male and female elephants, dependent on the hormonal and/or physiological state of the receiver [Rasmussen & Greenwood, 2003]. We have further detected a similarity in the perireceptive pathway toward olfaction of this now recognized pheromone to that of the female-to-male preovulatory pheromone, (Z)7-dodecenyl acetate [Greenwood et al., 2005]. A further startling and unexpected finding occurred earlier this year when we established that the frontalin found in elephants had a specific feature that was clearly correlated with biological activity, specifically with the sexual and social maturation of the male elephant emitter of this signal. Our talk will detail these discoveries, focusing on the correlation of bioactivity with structure.

Acknowledgements: ISAT, Riddle's Elephant Sanctuary, Ringling Center for Elephant Conservation, Auckland Zoo, Biospherics Research Corporation

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ELECTRONIC NOSE WIRELESS SENSOR NETWORKS

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In many applications, networks of electronic noses may be used successfully to monitor an area for pollution, chemical spillage, odour contamination, etc. Networking the devices is often difficult because the computing resources available may be low, there may be restrictions on power consumption and there may be limited (or no) networking facilities in the area. Wireless sensor networks (WSNs) are a recent major area of research interest and will help overcome the above difficulties. WSNs are composed of large numbers of autonomous nodes equipped with modest computing, storage and communication resources. They have a range of devices to sense their environment and they communicate with each other by linking wirelessly to nearby nodes. WSNs present many research challenges and have recently attracted significant international research attention. This paper will explore their application with electronic noses for a range of monitoring applications.

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EXHALED BREATH ANALYSIS IN PATIENTS WITH LUNG CANCER.

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Lung cancer is usually detected at a stage that is too late for curative surgery. There is, therefore, a need for the early detection of this disease, particularly when the major risk group is clearly identified as current and ex- smokers. This study has used both targeted markers of lung disease, as well as an electronic nose (eNose) to identify differences between the breath of patients with lung cancer and control individuals. Those with newly-diagnosed lung cancer and control subjects (smokers, ex-smokers, non-smokers) underwent breath analysis (nitric oxide, carbon monoxide), collection of exhaled breath condensate (EBC) and breath electronic nose (eNose) analysis. Exhaled breath analysis showed similar levels of exhaled nitric oxide between the groups, but elevated levels of EBC oxides of nitrogen (NOx) were seen in those with lung cancer. Mean NOx concentrations in EBC were 26.3 +/- 6.4 •mol/L in lung cancer patients (n=14), 6.4 +/-0.6•mol/L in ex-smokers (n=7) and 14.8 +/-3.6 •mol/L in current smokers (n=11). EBC NOx was significantly higher in the lung cancer patients than the ex-smokers ($p<0.05$). Initial analyses of the eNose data suggest that there are qualitative

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oral sessions

differences between the subjects and the control subjects. These data support the idea that it may be feasible to use breath analysis for the detection of disease, but specificity and sensitivity of such tests will need to be assessed before clinical application can be determined.

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30

THE DISCRIMINATION OF ANDROSTENONE AND SKATOLE IN PIG FAT USING AN ELECTRONIC NOSE

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Androstenone and skatole are two major compounds considered to be responsible for causing a malodour in pork, that is commonly referred to as boar taint (Patterson, 1968; Walstra and Maarse, 1970). The efficacy of an electronic nose to assess for the presence of androstenone and skatole in porcine adipose tissue, was determined in this study. The electronic nose instrument was an *e-NOSE* ™ 4000 Sensor Array, Neotronics Scientific, (e-NOSE) fitted with 12 conducting polymer sensors in a modular array. The sensors though were known as Type: 258, 259, 260, 261, 262, 263, 264, 278, 283, 297, 298, and 301 which are similar to those described in Maul *et al.*, (2000). The sensor head was continuously maintained at 30oC, and the *e-NOSE* beaker and sample (vessel) was exposed to a temperature of 30oC throughout the temperature stabilising and acquisition period. The *e-NOSE* 4000 had an RH humidity sensor and a temperature sensor that acquired this data for each of the samples. The instrument contained the *e-NOSE* 4000 Series Software, Neotronics Scientific. The sensor response for the samples of fat from female pigs, castrated male pigs and entire male pigs with varying concentrations of androstenone and skatole, were analysed using the *e-NOSE*. The machine was calibrated and upon the purging of the extraneous gases, the sensor response to each sample was acquired at three time points. Multiple regression analyses were used to relate the sensor scores to the concentration of androstenone and skatole, and to determine how the relationship varied with relative humidity, sensor analysis, batch variation and the vessel and head temperatures. A simple model was established after assessing all the variables and this model was used as the basis for the discussion of the results.

Acknowledgements: We thank Australian Pork Limited (APL) for their support during these studies.

31

SMALL "TAILORED" E-NOSES FOR ENVIRONMENTAL AND HEALTH APPLICATIONS, MONITOR ODOURS CONTINUOUSLY AND IN REAL-TIME

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The current and accepted method of measuring environmental odour is by "dynamic olfactometry." It involves bagging air samples on site and sending them to be assessed by a trained human panel, which determines how many times a unit volume of the air has to be diluted before it can no longer be detected. These dilution factors, or "odour units" (OUs) are accepted by regulatory authorities as quantifying odour released from a source. However, sufficient data is difficult to obtain by this method, the relationship between the units and human perceived intensity of odour is unclear, and the time required to take the measurements precludes timely action to minimise odour emissions when they occur. In its place, a continuous odour monitoring technology is desirable. It should have sufficient sensitivity for odours that give offence to the community, produce consistent and reliable results and be validated in terms of its ability to discriminate odours from various industrial sources. This paper describes the development and deployment in an industrial site, over several months, of one of a new generation of electronic nose (e-nose). The array is tailored for a specific industrial purpose (e.g. abattoirs or sewage plants) and has fewer sensors than commercial e-noses. It uses metal oxide-based sensors that run at high temperature and are highly durable and reliable, even when subjected to a wide range of temperatures and humidity. Data were recorded remotely via the internet, and continuously over several months, from high and low odour areas. A predictive alarm system was developed, as well as an automatic calibration and cleaning system for the sensors. The daily records produced a useful profile of when and where problem-level odours were occurring. The data obtained provides a continuous record of the odour status of various sources of odour emission from industrial sites and allows timely action to be taken to avert costly complaints. A second application for these new e-noses is being found in diagnosis of odour "signatures" produced on the breath of humans or from the skin of animals. Research has begun on human breath of patients with and without lung cancer (see Thurston et al., this conference). A third application involves sniffing the fleece of sheep for various pathologies (The Sheep CRC E-Sheep Project).

Acknowledgement: The work was supported by Meat and Livestock Australia.

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STATE OF THE ART IN ELECTRONIC NOSE TECHNOLOGY

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Since two decades, a rapid commercial development in "electronic nose" technology, i.e. multi-chemical gas sensors devices coupled to pattern recognition techniques was observed. If 3 companies could be identified in 1992, 19 are present in the market in 2000. During the same period, the sensing technologies used grow from 2 (MOS, CP) to 7 (MOS, CP, MOSFET, QMB, SAW, MS, IMS, CCD). Since 2000, the classical lab devices became first handable then portable and now automated e-nose based wireless odor monitoring systems with Internet based acquisition data were available. But after 10 years of experimentation in R&D labs, what are the real industrial applications? Do e-noses be only used in Quality Control or production process monitoring or odorous sources localization as contradictors reported? Or do they be usefull also for environmental measurement (indoor or outdoor atmospheres, in industrial plant location or at the periphery) or security purposes (drugs or explosives detection) or medecinal diagnosis as promotors stated? The aim of this presentation is to give an objective state of the art of this novel sensing technology and its real applicability degree in industry.

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1

SMELL THAT? HOW INDIVIDUALS DIFFER IN THEIR ABILITY TO DETECT AND DESCRIBE FRUIT FLAVOUR COMPOUNDS

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Humans differ in their ability to both detect and describe odours. Differences in ability to detect compounds is due, in part, to inherent genetic variation. Of the roughly one thousand olfactory receptors in the human genome, only some 350 are active with many existing as non-functional pseudogenes. Moreover, this set of active receptors is not identical between individuals, perhaps, together with sequence variation, contributing to differences among humans in their ability to smell certain odours. We are interested in how this variation is structured in human populations and how it might account for preferences for various types of fruit flavours across populations or markets. To begin such an undertaking, we are developing a standardised test for the ability to detect individual compounds found in fruit. Human subjects were asked whether they could detect, and nominate a descriptor for, each of five compounds found in kiwifruit. The tests contained each compound at two different concentrations close to threshold of detection and all tests contained randomised duplicates and blanks. Various general trends were observed. For example, the higher concentrations generally increased the ability of subjects to correctly identify the compound. A 10-fold increase in the concentration of methyl benzoate increased the reproducibility of description by 35%. In contrast, the same increase in the concentration of linalool reduced reproducibility of description by only 9%. These data are now being used to identify individuals and populations with different abilities to detect fruit flavours for subsequent genetic testing.

2

A "GENE TRAP" SCREEN TO IDENTIFY NOVEL OLFACTORY GENES IN *DROSOPHILA*

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In *Drosophila*, odour discrimination begins with the detection of odours by a large family of 62 highly divergent Odorant receptors (Ors) presumed to be G-protein coupled receptors. The Ors represent essential elements in olfactory information coding, however in insects the signal transduction pathway activated by the Or proteins remains unknown. Two primary candidate pathways exist, the inositol phospholipid signaling pathway and the cyclic nucleotide signaling pathway. As other novel pathways may exist, we are identifying novel *Drosophila* olfactory signaling genes based on their expression in the olfactory organs. To identify novel genes expressed in the olfactory organs we have screened a set of approximately 500 *Drosophila* gene trap lines (Lukacsovich, et al., *Genetics*, 2001, 157: 727-742). Each line contains a P element insertion within the coding sequence of a gene, this both generates a mutant in that gene and allows precise detection of the expression patterns of the trapped gene. Using the GAL4 gene in the P element inserts to drive the reporter gene UAS-GFP we have identified 16 lines with expression in olfactory organs (and, in most cases, elsewhere including gustatory organs). We are currently performing molecular genetic experiments to identify the genes that are "trapped" in these lines as well as characterizing their olfactory phenotype using behavioural and electrophysiological assays.

3

JUST NOTICEABLE DIFFERENCE FOR PERCEIVED CIGARETTE SMOKE IMPACT

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Impact is defined as the sudden, sharp but very short lived sensation (typically of about one second in duration) which is noticed when cigarette smoke makes contact with the back of the throat during inhalation. Intensity of perceived Impact is related to the level of nicotine in the smoke. The optimum level of perceived Impact varies depending on 'tar' level, tobacco blend type and product design of the cigarette. Accordingly, the level of nicotine in smoke in commercial products can range from 0.10 to 1.0 mg per cigarette. Therefore, it is important for the product developers designing new or modifying existing products to know the magnitude of change in smoke nicotine required to affect the change in perceived impact. A technique to estimate Just Noticeable Difference (JND) has been developed by assessing the Draw Resistance (DR) of unlit cigarettes through a series of paired comparisons (Prasad et. al., 2005). This technique was extended to evaluating lit cigarettes by asking smokers to take the 5th puff and compare perceived Impact of a range of cigarettes differing in their smoke nicotine levels. A series of sixteen paired comparisons were carried out by 35 subjects testing each pair four times to determine the sensory threshold for Impact across a range of cigarette samples with different nicotine levels (0.35 to 1.32 mg/cig) equivalent to 5th puff nicotine between 0.04 to 0.15 mg/puff respectively. This was achieved by blending various proportions of untreated and denicotinised versions of the same tobacco blend and designing cigarettes at two different 'tar' levels. For cigarettes with 5th puff nicotine of 0.068mg it was found that an increase of 0.0215mg nicotine was required for 50% of the population to perceive an increased Impact. In this study it was not possible to determine the decrease in nicotine required to perceive a decrease in Impact from the control (0.068mg 5th puff nicotine) as it was not possible to anchor the bottom part of the probit curve. For cigarettes with 5th puff nicotine of 0.095mg it was found that a decrease of 0.0025mg nicotine was required for 50% of the population to perceive a decrease in Impact. This value is extremely low and is almost certainly an artefact of different tar to nicotine ratios in the smoke affecting the perceived Impact. In this case it was not possible to determine the increase in nicotine required to perceive a increase in Impact from the control (0.095mg 5th puff nicotine) due to the large confidence bands at p=0.75. Over the whole range of the samples in the experiment, JND50 for the range 0.04 - 0.15mg 5th puff nicotine is between 0.028 and 0.039 mg nicotine per puff. Further work with samples at a particular 'tar' level but with a much wider range of nicotines is required to successfully apply the JND probit method and avoid the confounding effect of tar differences.

4

AN INVESTIGATION OF USING SIGNATURE-IMAGE IN ODOUR IDENTIFICATION FOR ELECTRONIC OLFACTORY SYSTEMS

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We investigate a novel approach to process E-Nose signals for odour identification. This approach is inspired by the Signature-Image signal processing technique developed by the University of Sydney for monitoring the quality in arc/spot welding. In that application, the time-sequence data from welding is analysed using wavelet transforms and then converted to a feature map, or a signature image. These images serve as the basis for statistical classification, or pattern recognition, in which the probability of an input signature belonging to each of the learnt categories is calculated. The process is carried out in less than half a second per sample on a personal computer (real-time), which makes the technology suitable for E-Nose applications. We are employing this technique for the signals generated from four TGS thick film sensors. In this E-Nose case, the unique signature images are the odour-fingerprints and hence are the foundations of the statistical odour discrimination process. We will present the initial results of adopting this

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ABSTRACTS: continued posters

Signature-Image approach for identifying some plain odours. The ultimate goal is to distinguish amongst a mixture of odours and quantitatively determine the approximate composition of the constituents.

5

SOFTWARE FOR ALARMING AN E-NOSE FOR POLLUTION MONITORING

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The project focuses on the design of appropriate software for managing information derived from chemical sensing devices that both process data and require appropriate actions to be initiated. The example being worked on is a sensory system for airborne chemicals, with decision resources that identify and quantify the incoming stream of information against response criteria, such as various levels of alarm. There will be three response options for the alarm system, viz remote paging, such as by telephone, and local audible &/or visual signals. If the machine detects the certain odour beyond the normal strength level (in e-nose output voltages or calibrated against human perceived intensity or chemical concentration measures) then the device should inform an appropriate person by one of these means, so that adjustments can be made to the odour source. The choice of response option can be set by the user, according to likely consequences of an odour reaching a certain intensity. The alarms can therefore be set at rising levels with the responses going from low level of urgency to extremely high. Generally processing speed in these circumstances is not an issue as the response can be triggered at the instant a threshold in the real-time e-nose output is reached. The criterion for triggering the alarm can also be a qualitative one, namely an identification of the quality of the odour sampled by the e-nose. Here processing time is a relevant consideration, depending on the size of the database to be interrogated in order to make a match with the incoming data, and the degree to which confidence can be placed on a decision by the system. In certain applications, such as pollution monitoring, a few seconds of processing time is unlikely to be an obstruction to efficiency, as it might be in, say, e-nose applications on a food processing line. Odour identification and response to the e-nose sensor array will be performed by a pattern recognition algorithm, or via a previously trained artificial neutral network (ANN). In all such operations, the user interface must supply appropriate information, instantly, and in a form that a human operator can understand and respond to correctly. Desired outcome is software architecture to make e-nose functions user-friendly. Device owners will then better understand what can be done with an e-nose, by applying its data to existing software packages, various algorithms, databases, classifiers, statistical and graphical packages.

6

CHALLENGING THE ACCEPTED MECHANISM OF ASTRINGENCY PERCEPTION: INTERACTION OF PLANT BASED FOOD AND BEVERAGE POLYPHENOLS WITH HUMAN ORAL EPITHELIAL CELLS

Payne, C., Humphries, A., Spriggs, C., Niemietz, C., Tyerman, S. & Bastian, S.

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Oral astringency is defined by the American Society for Testing and Materials as being a 'complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as Alums and tannic acids'. It is a characteristic that plays a primary role in the quality and mouth-feel of red wines, and in the palatability of some foods and therapeutics. Despite its economic importance, the mechanism by which the stimulus is perceived has yet to be elucidated. A long-held belief is that the primary mechanism for perception of oral astringency is de-lubrication of saliva resulting from tannin-salivary protein binding and subsequent precipitation of the complexes. The resultant decrease in lubrication of the saliva increases friction between oral surfaces, providing a stimulus that may be perceived as astringency. Research has tended to concentrate on the investigation of binding of polyphenols to salivary proteins. Increasingly, however, reports are being published which suggest that this is not the only mechanism involved in perception of astringency. It is speculated that the sensation may also involve binding of polyphenols or complexes of polyphenol-protein complexes to cells of the oral cavity. Preliminary data generated within our laboratory using a single-mix stopped flow assay demonstrates that polyphenols, specifically the monomeric flavanols catechin and epicatechin, and a mixed tannin extract of white grape seeds, bind to cells harvested from human cheek epithelium in a dose-dependent manner. Concentrations up to 1,500 mg/mL were studied, a range which encompasses concentrations of the products which are known to elicit an oral astringent sensation when ingested. Our results support the hypothesis that the perception of astringency may involve direct binding of polyphenols to oral epithelial cells. Confirmation of these results, and identification of specific binding sites, may lead to the development of a precise *in vitro* assay that could be used to determine the perceived astringency of compounds present in foods and beverages, in place of time-consuming and costly consumer trials.

Upcoming Events

26-30 April 2006	**AChemS** Sarasota, USA Abstract Deadline: 13/1/06 www.achems.org
8-11 May 2006	**Enviro 2006 Conference and Exhibition** Melbourne Exhibition & Convention Centre Melbourne Contact: Rosalind Vrettas, Ph. +61 (0)3 9741 4679 rvquitz@bigpond.com
9-12 July 2006	**39th AIFST Convention:** **"Festival of Food"** Adelaide Convention Centre Adelaide, South Australia Contact: aifst@aifst.asn.au
2-4 August 2006	**8th Sensometrics Meeting:** **Imagine the Senses** Ås, Norway. Contact: www.sensometric.org
4-8 September 2006	**ECRO** Granada, Spain www.ecro.cesg.cnrs.fr
21-25 October 2006	**Society for Neuroscience** New Orleans Info: www.sfn.org
October 2006	(watch ChemoSense for details) **AACSS** **9th Annual Meeting** Adelaide, South Australia

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