Vol. 9 No.4 October 2007

Chemo Sense

EDITORIAL

Olfaction in Flight

By Graham Bell
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The sense of smell is conserved across the Animal Kingdom, yet it is little appreciated as a well-developed sense in birds. In his mini-review, George Gomez points out that the olfactory system in birds offers a valuable model for studying the evolutionary pressures that both shape variation and conserve neural structure and function.

George's paper is based on one of a group on bird olfaction and nervous system presentations made at an IBRO Satellite Meeting, held in the idyllic location of Heron Island, Queensland, in July. The avian meeting was well attended by Australian, New Zealand, Japanese, American and European scientists.

This issue also carries the abstracts of the 9th Scientific

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Olfaction in Birds: An Untapped Resource for the Study of Olfactory Systems

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Olfaction in birds

The ability to detect chemical stimuli is a capability that is found in nearly every animal species. In terrestrial animals, the detection and identification of volatile chemicals that are released from distant stimulus sources is mediated by the olfactory system. Insect antennae, the mollusk osphradium, and vertebrate noses are different implementations of a basic design: chemoreceptor neurons whose dendrites contain molecular receptors that bind to odorant molecules, translate the odorant-receptor binding events into neural activity, and transmit this information directly to the central nervous system. The sense of smell is ubiquitous, and its functional properties are remarkably similar in many animal species.

Few, but the initiated, credit birds with a well-developed sense of smell.. This may be because birds have highly developed visual capabilities, and many species vocalize, and so most of our neural and behavioral investigations focus on avian vision and acoustics, and their role in feeding, navigation, mating, prey detection, or social interactions. Thus, olfaction in birds has been

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EDITORIAL CONTINUED

Olfaction in Flight
continued

Meeting of the Australasian Association for ChemoSensory Science (AACSS) which was held in the Barossa Valley in July. The next meeting of AACSS will be held in Brisbane from 4-6 December 2008. For more information contact: j.reinhard@uq.edu.au.

A new electronic nose group is emerging in South Africa, with the establishment of a distribution and R&D company, trading as "E-Nose Africa". South Africa is moving to reduce its air pollution, which is contributed to by its gigantic mining, minerals and energy production industries. We wish every success to Jenny and Peter Highly and electronics engineer, Bashan Naidoo, in contributing to meet these worthy goals ■

Heron Island

Olfaction in Birds: An Untapped Resource for the Study of Olfactory Systems
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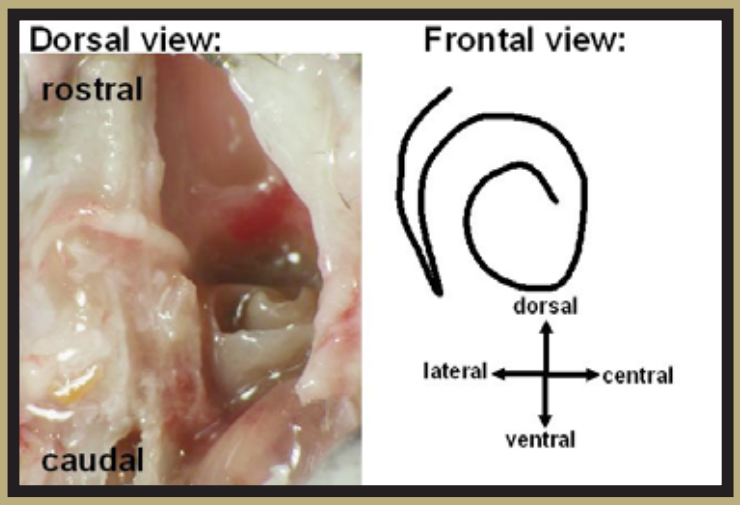

Fig. 1. Photograph of a dissected nasal cavity of a crested auklet (left) and a schematic illustration of the shape of the turbinate (right). The single spiral turbinate is attached to the lateral wall of the skull. When viewed from the front in cross-section, the turbinate coils clockwise.

regarded as an oddity. Yet every species of bird that has been systematically tested for olfactory capability has been shown to possess a well-developed sense of smell. There is strong evidence that birds use olfaction to detect important cues for their different behavioral repertoires (Roper and Jones 1997). These behaviors include: food selection, mate choice, nesting, alarm and conspecific warning, detecting distant sources of food, and navigation. The use of olfaction in different bird species is as varied as the lifestyles that they lead; they run, fly, burrow, and swim. They are predatory, herbivorous, omnivorous, and scavengers. They inhabit a broad range of habitats, from the tropics, to the temperate regions, to the polar continents and seas. They also possess a unique physiological and evolutionary position: they are endothermic (physiologically similar to mammals) but are cladistically more closely related to reptiles (Ostrom 1971). Birds may therefore provide unique insights into the influence of physiological/ecological versus evolutionary constraints on nervous system structure and function.. An understanding of the principles of odorant signaling in vertebrates may be advanced by the study of major taxonomic groups under different environmental and behavioral conditions to uncover common functional principles and themes of sensory systems.

Why study the chicken olfactory system?

To understand bird olfaction, it is convenient to do so in a model species that is readily available to all researchers all over the globe. The avian equivalent of the "laboratory rat" is the domestic chicken (*Gallus domesticus*), which is a cosmopolitan species that is commercially available and can be easily reared in the laboratory. When fertilized chicken eggs are incubated at 38 °C, they follow a precise developmental pathway that culminates in hatching after 21 days; this has made them a convenient model for the study of development. Studies have also shown that the chicken olfactory system has anatomical and functional features in common with those found in mammals (see below).

The peripheral olfactory system of birds and other vertebrates

In all vertebrates, the olfactory sensory neurons (OSNs) are found in the epithelium lining the nasal cavity. In the bird, the nasal cavity contains a single spiral turbinate that occupies the nasal cavity (Figure 1); the turbinate and apposed septum is lined with respiratory and sensory epithelium. As in most vertebrates, the chicken OSN has a distinct morphology: a generally goblet-shaped cell body, a slender dendrite with sensory cilia emanating from a knob-like tip, and an axon that traverses the deeper epithelial tissue and innervates the olfactory bulb in the forebrain (Briepohl and Fernandez 1977). This characteristic appearance is remarkably conserved across a variety of vertebrate species (Schild and Restrepo 1998). In addition to these ciliated cells, the chick olfactory epithelium also has microvillar receptor neurons morphologically similar to the ciliated cells (Matsuzaki 1995). In fish, (which also have ciliated and microvillar cells in their olfactory epithelium)

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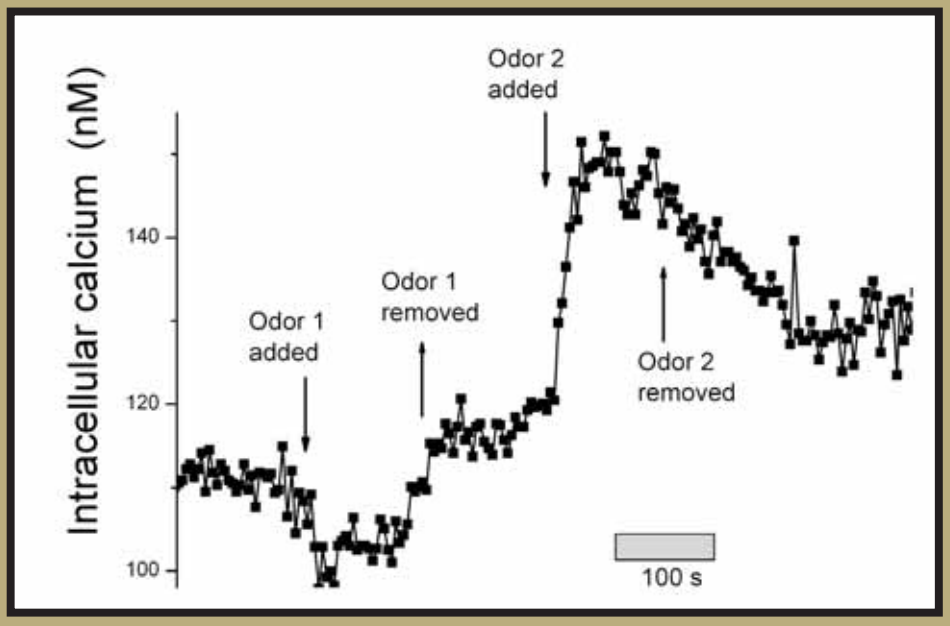

Fig. 2. Sample data trace from calcium imaging studies show odorant responses from an acutely-isolated chick OSN. The Y-axis shows computed [Ca2+]i. The scale bar indicates a time period of 100 s. Odorant-elicited calcium changes typically occur over several seconds; this time frame is normal for these studies. Bird OSNs show characteristics that are typical of vertebrate OSNs, but also have unique features. For example, this cell responded to one odorant mix (Odor 1) with a decrease in [Ca2+]i and to another odorant mix (Odor 2) with an increase in [Ca2+]i For most other species, it is unusual for single OSNs to respond to different odorants with [Ca2+]i changes of differing direction.

it has been shown that these different cell types have distinct functions, and innervate different regions of the brain (Mullet and Marc 1984). It is not certain whether this is the case in chicks as well.

In the olfactory system, the physiology of single OSNs has been studied at multiple levels. When odorant stimulus molecules enter the nasal cavity, they come in contact with the OSNs and bind to olfactory receptor proteins (OR, which are 7-transmembrane domain G-protein coupled receptors) embedded on the OSN dendritic membranes. The specificity of the cells' response is thought to be dependent on the type of OR expressed by the cell. Different vertebrate species have varying numbers and types of ORs: the mouse expresses about 1000 different types; the human, about 350 (for review, see Schild and Restrepo 1998). The chick has thus far been shown to express a limited complement of about 15 different ORs, although it is estimated that they have genes that could encode about 100 functional OR variants (Leibovici et al 1996).

The binding of odorant molecules to the ORs initiates the odorant signal transduction cascade within the OSN that culminates in the generation of electrical activity that is transmitted down the OSN axon. The electrical activity generated by the OSNs has traditionally been assessed using extracellular nerve recording techniques. Odor stimulation elicits large-scale currents across the surface of the olfactory epithelium that can be measured using an electro-olfactogram (EOG). EOG recordings have been measured in different vertebrates (fish, rodents, and humans) and can be used to quantify features of the olfactory system, such as the types of molecules that are detectable (spectral sensitivity), threshold, and spatial distribution of olfactory receptor expression across the entire epithelium (Scott and Scott-Johnson 2002). In chicks, this recording technique has been used to measure the emergence of odor sensitivity of OSNs during embryonic development (Lalloue et al 2003). Electrical recordings from OSNs have also been measured using extracellular electrodes attached to the olfactory nerve (which carries action potentials from the axons of the OSNs to the olfactory bulb). Such studies were among the earliest investigations on bird OSN physiology; recordings from pigeons show that the electrophysiological properties of these bird OSNs appear to be similar to those seen in other vertebrate species (Tucker 1965).

In addition to generating electrical activity, OSNs respond to odor stimulation with changes in intracellular calcium (see Fig. 2). The activation of the odor signal transduction cascades results in an influx of calcium and opening of voltage-gated Ca^{2+} channels (see Schild and Restrepo 1998). While odorant-elicited electrical signals typically last for less that a second, the resulting change in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) lasts for several seconds to minutes. The increase in $[Ca^{2+}]_i$ influences a number of cellular events such as signal termination or adaptation since many molecules involved in odorant signaling are sensitive to $[Ca^{2+}]_i$. Thus, $[Ca^{2+}]_i$ changes are commonly used and widely accepted as a means for studying OSNs cell physiology and odor signaling mechanisms.

In birds, studies in our laboratory (Jung et al 2005, see fig. 2) have shown that newborn chick OSNs also respond to odorants with changes in $[Ca^{2+}]_i$. For these studies, OSNs were isolated from the newborn chick olfactory epithelium, loaded with calcium-sensitive fluorescent dyes, and tested with odorant mixtures. Changes in $[Ca^{2+}]_i$ were measured under computer-controlled calcium imaging systems. To stimulate the OSNs, an odor stimulus mixture consisted of odorants that were previously shown to be detectable by birds (amyl acetate, cineole, ethyl vanillin, eugenol, geraniol, limonene, octanal, and octanol, each at 100 μM). Results from the study showed that chick OSNs respond to odorants (mixtures or single compounds) with changes in $[Ca^{2+}]_i$ in a manner similar to those seen in studies on other vertebrate species. Among the common features were the following: 1) odor induced $[Ca^{2+}]_i$ changes were either increases or decreases; 2) increases in $[Ca^{2+}]_i$ are mediated primarily by a flow of calcium ions through channels on the cell membrane; 3) single OSNs are sensitive to only a subset of potential odorant stimuli, and this sensitivity varies from cell to cell; 4) cellular elements that constitute the signal transduction cascade appear to be common

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continued

among several species. Many features of odor and/or calcium signaling appear to be conserved among the species studied, suggesting that these mechanisms are remarkably effective for coding olfactory information across different the habitats and lifestyles of the representative species (Hildebrand and Shepherd 1997). It is also possible that the olfactory system is not under stringent evolutionary selective pressure, allowing it to adapt as-is to other behavioral and ecological constraints imposed by the organism as a whole.

However, as is the case with other vertebrate species, the chick olfactory system has some unique features. One interesting feature is a complex response pattern, wherein individual cells that would occasionally respond to one odorant with an increase in $[Ca^{2+}]_i$ and to another odorant with a decrease in $[Ca^{2+}]_i$. This pattern of response has not been observed in the other species tested (rodents, cats, humans, amphibians, or fish). The prevalent hypothesis of olfactory coding states that single cells express one type of OR, and the pattern of activity across the different cells that express the different ORs serves as the basis for encoding the odorant stimulus (Buck 2004). Since individual chick OSNs also appear to express a single type of OR (Leibovici et al 2003), the ability of an OSN to respond with either increase or decreases suggests that single olfactory receptors are linked to multiple signaling pathways that are differentially activated in an odorant-specific fashion. These single cells may therefore act as complex stimulus processors (Ache et al 1998), perhaps as an adaptation to compensate for the relatively fewer number of ORs expressed in chicks. This property of bird OSNs would allow the entire system to detect and encode several different types of odorants with a limited number of neurons dedicated to odor processing.

The olfactory bulb

The axons emanating from the OSNs converge into multiple olfactory nerves that traverse the base of the cranium (the cribriform plate) and innervate clusters of neurons (glomeruli) in the olfactory bulb. The vertebrate olfactory bulb is organized in concentric layers, starting with the outermost olfactory nerve layer, followed by the glomerular cell layer, and subsequent cell populations dedicated to the initial stages of signal processing (for review, see Scott and Harrison 1987). The anatomical organization of the olfactory bulb is another feature that is remarkably conserved across a variety of vertebrate species (Hildebrand and Shepherd 1997). In addition, glomeruli are found in the invertebrate central nervous system in regions that mediate olfactory detection and discrimination, suggesting that this arrangement of neurons appears to be ideally suited for decoding and processing of odor information.

As outlined earlier, OSNs have OR proteins which selectively bind to odor molecules. Research has shown that OSNs each express one type of receptor (out of several hundred to a thousand, depending on the species), and a single type of odor molecule can stimulate several different kinds of receptors. Since, axons from OSNs that express the same receptor converge onto the same glomeruli in a precise manner, it is thought that neural signals from the OSNs form a pattern of activation across the glomeruli in the bulb that is indicative of the differential odorant-OR binding activity across all the OSNs. This pattern could be the mechanism of how the olfactory system encodes odorant stimulus identity, intensity, and duration (for review see Buck 2004).

To study coding of odor stimuli in the brain, it is possible to measure the activity of glomerular neurons using a number of different techniques. Electrophysiological recordings are typically conducted on neurons that constitute the olfactory bulb circuitry. Such recordings conducted on the bird have shown that their olfactory bulbs function in a manner similar to that seen in other vertebrates (McKeegan 2002). In addition, cytochemical techniques been used as a tool for creating a "map" of glomerular activation by different odorants. In one such approach, glomerular activity leads to the transcription of the immediate early gene cFos within 30 minutes [43,110], and post-stimulation cFos gene expression can be used as a universal marker for this neuronal activity. Activity of neurons in response to different odorant mixtures elicits differential cFos expression pattern across the entire olfactory bulb; this has been shown to be true in chick olfactory bulbs as well.

The avian olfactory bulb exhibits a high degree of structural similarity to those seen in other vertebrates (Bang 1971). The outermost olfactory nerve layer, and the adjacent glomerular layer is initially activated by odor stimulation, which increases electrical activity of neurons that is correlated with the stimulus quality, intensity, and temporal pattern (McKeegan 2002). Although it has not been systematically tested, one could assume that the avian olfactory bulb encodes odorants using an activation pattern across glomeruli, as in other vertebrates. The presence of glomeruli in the olfactory system appears to be a highly conserved neural feature that is, presumably, ideally suited to processing odor information.

While the overall organization of the olfactory bulb of birds is similar to other vertebrates; other regions of the avian brain (such as the olfactory tubercle) that receive the outputs from the olfactory bulb appear to be radically different from those of other vertebrates (Reiner et al 2004). There are developmental, biomechanical, and evolutionary constraints that influence the structure of the bird brain. The enlarged bird eyes (for improved visual acuity) take up much of the space in the skull, leaving less available space for brain structures. Birds that fly must have light, hollow bones and cannot have excessively large heads. Yet birds must have numerous neurons dedicated to visual processing, spatial memory, and/or bird song generation and memory. Thus the avian brain appears to be a model of efficient organization and wiring to maximize function with a limited number of neurons. The differences in the stereotypic organization of the higher brain centers of the mammalian and avian brain prompted a reclassification of avian brain nomenclature (Reiner et al 2004) to facilitate the application of avian brain studies to mammalian models. Despite these

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continued

differences in the higher brain centers, the olfactory bulb in all these vertebrates appears to be organized in a similar fashion. This suggests that mechanisms for the initial steps of olfactory detection and coding are optimally served by the existing structural organization of the olfactory bulb. Once the olfactory bulb processes the information, the higher order functions (such as odorant identification or behavioral responses) can vary from species to species depending on their environmental and behavioral constraints. Interestingly, while glomerular organization is retained in all bird species studied, other features such as the overall size of the olfactory bulb of different species of birds are different (Bang 1971). This hints at the possible influences of the differences in olfactory anatomy (some birds have more OSNs than others) or use of odor information (some birds may use odor information more extensively than others) may result in these differences in olfactory bulb size. The olfactory bulb in these birds may therefore reflect or influence the bird's behavioral repertoire. This idea merits further investigation.

Lessons from the olfactory system

It is well-known that OSNs regenerate throughout the adult lifespan. The generation of mature OSNs from an adult stem cell population involves a series of steps that result in fully mature functional neurons (for review see Calof et al 2002). Among the maturation steps in the growth of new OSN axons that target the appropriate glomeruli in the olfactory bulb. The maturation of olfactory neurons is influenced by growth factors that are secreted by other cells of the olfactory epithelium or by the olfactory bulb. In mammals, the generation and maturation of these OSNs is known to be influenced by a series of growth factors: compounds such as TGF-α, IL-6 and -β, bFGF, BDNF, IGF-I, and LIF have been shown to affect cell differentiation and neurogenesis in the olfactory epithelium. Since the olfactory system has multiple cell types, growth factors, and maturation stages, it may serve as a useful model for the study of factors employed by the body to generate and/or regenerate neurons.

There is also strong evidence for continuous neurogenesis in the vertebrate olfactory bulb throughout the adult lifespan. In mice and rats, it is known that neuronal stem cells migrate from the subventricular zone through the rostral migratory stream and into the bulb, where they form local interneurons and granule cells. Odor exposure increases neurogenesis in this system, leading to improved odor memory (Gheusi et al 2000). Since it is known that neural reorganization occurs in the adult bird brain, it is possible to gain insight into the details and mechanisms involved in this process using the olfactory system as a model. The bird olfactory system holds a unique advantage for such studies on olfactory neurogenesis because their embryonic development is well-characterized, and conditions for embryonic development are easily controlled in the laboratory, and the entire olfactory system has all the features that are representative of vertebrate olfaction in general.

Summary

An understanding of olfaction in general necessitates the systematic study of all major vertebrate groups. Studies on olfaction in birds has largely been marginal. Yet the bird olfactory system has the physiological and anatomical characteristics similar to those found in other vertebrates (including mammals), allowing the use of the bird olfactory system to study the features of vertebrate olfaction. In addition, the variety of ecological niches, behavioral repertoires, and anatomical differences within members of this vertebrate class provide an intriguing system for investigating the influences of these constraints on the structure and function of nervous systems. ■

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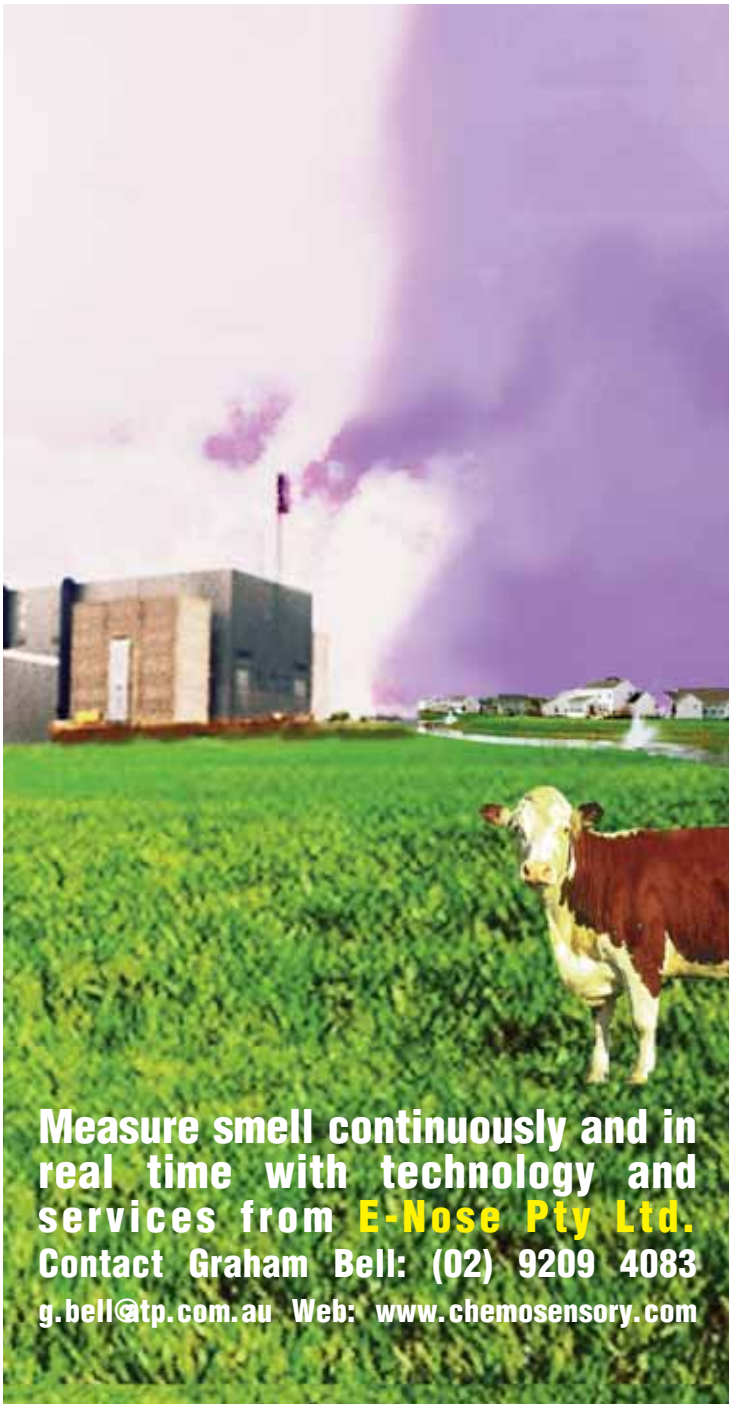

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NEWS

E-Nose Africa Launched

By Graham Bell, CEO E-Nose Pty Ltd, Sydney, Australia

In the clean crisp air of South Africa's Drakensberg, in October 2007, a new company was launched to take e-nose technology into Africa. There are few places in the world to rival the fresh mountain air around Champagne Castle, and its surrounding World Heritage sites. This was a fitting place to unveil a technology designed to measure smells.

Exhibiting at the meeting of the National Association for Clean Air (NACA), the new company, Electronic Nose Africa cc, (trading as E-Nose Africa) put on a live display of Australian technology for a highly motivated audience of conference attendees. After the launch, they visited several large petrochemical, paper and waste water companies.

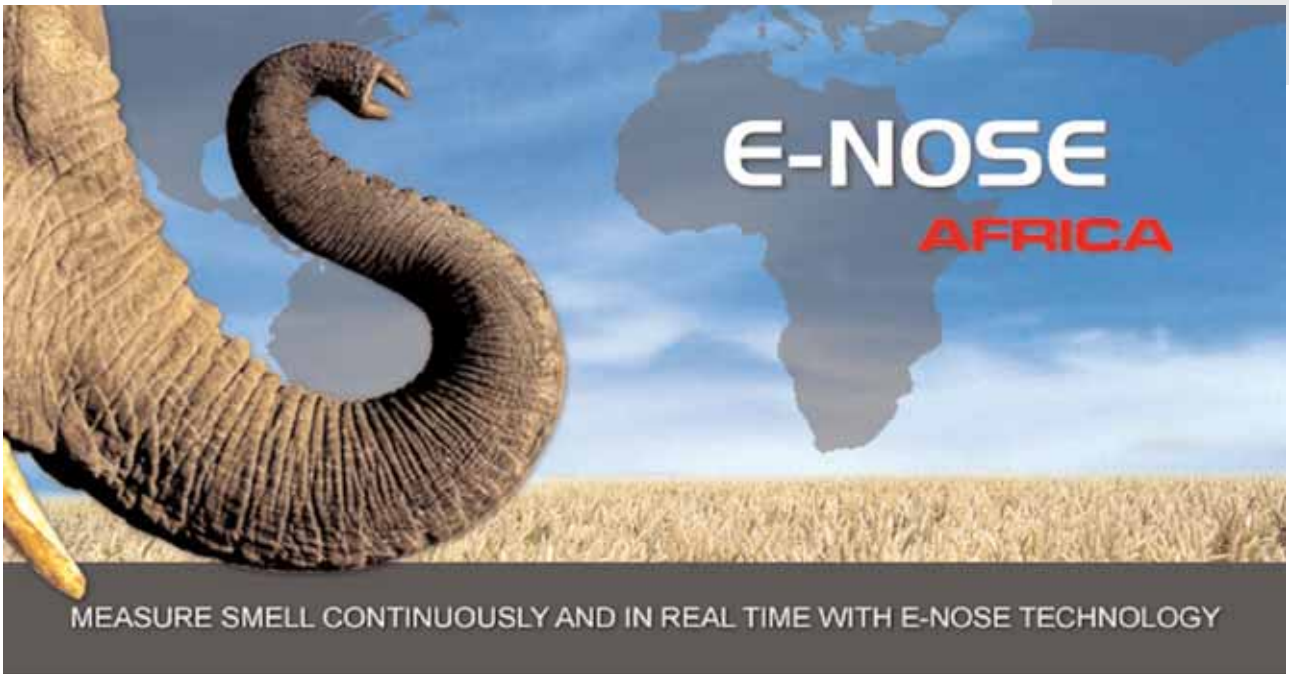
The company has exclusive rights to market the Australian E-Nose products and services in Africa. The company structure includes marketing experts Peter and Jenny Highley and electronics engineer Bashan Naidoo. It will be supported with hardware and software from Sydney. Two of the members of the Australian company are former South Africans and they have enthusiastically welcomed the reconnection to "the New" South Africa.

The technology was welcomed by South African companies and pollution control authorities, who are in the process of setting new requirements for clean outdoor air. Heavy industry located in many South African towns and cities, such as Richards Bay and on the Witwatersrand are taking stock of how they need to measure and manage airborne pollution in their operational areas. The authorities are mindful of the potential for volatile chemicals to harm the health and living quality of rural and urban South Africans, as well as workers on sites and residents close by. A delegation of South African government officials is currently visiting Australia to learn how it is tackling similar problems.

The emitting companies are aware that they need to conform to world's best practices and reduce their emissions. One emitter, for instance, currently has a "smell-reach" of sulfurous compounds from its stacks of over 300 km.

E-Nose Africa aims to empower these companies and new generations of South Africans with E-Nose technology, and to play its part in a healthy relationship between man and the environment. On the creative technical side, both companies intend to develop a productive and mutually rewarding relationship. This bodes well for future generations of chemosensory scientists and technologists on both sides of the Indian Ocean.

More information: www.e-noseafrica.co.za ■



Vol. 9 No.4 October 2007

Abstracts of Papers presented at the 9th Scientific Meeting of the Australasian Association for ChemoSensory Science Thursday 26th to Saturday 28th July 2007 Novotel Barossa Valley Resort, Rowland Flat, SA

ABSTRACTS:

Plenary Lecture

SENSORY ISSUES IN THE AUSTRALIAN WINE INDUSTRY.

Leigh Francis

The Australian Wine Research Institute, S.A.

Sensory methods have been an integral part of applied research activities at the AWRI for many years, in projects designed to assist the wine industry to continue to improve wine quality. Detailed sensory and chemical investigations through the winemaking production process, from grape-growing practices, fermentation variables, wine off-flavours and taints - including flavour derived from *Brettanomyces* yeast - to closure and shelf life studies, have advanced the ability of the industry to tailor wines to specific requirements. Studies have included fundamental elucidation of new flavour compounds and their sensory significance, to experiments designed to assess the effect of treatments on wine composition and sensory attributes. Apart from the use of trained sensory panels and of winemaker/expert panels, consumer acceptance data has been gathered in recent projects which has allowed greater insights into wine quality.

Oral Papers

COMPARING THE LEVELS OF SECONDARY METABOLITES IN GRAPES TO WINE VOLATILE COMPOSITION.

Cox, A., Tomas, A., Nicholson, E., Loveys, B., and Boss, P.K.

Food Futures Flagship and Plant Industry, CSIRO, Adelaide, SA.

Wine aroma arises from a complex mixture of volatile compounds originating from grapes and wine-making processes. Wine flavours vary according to the grape variety as well as between fruit of the same variety sourced from different geographic regions or experiencing different management techniques or environmental conditions. This indicates that grape composition must play a significant role in the final wine aroma. However, the influence of the environment and vineyard management on the development of grape-derived flavour and aroma in wine is not well understood. In order to address this, Cabernet Sauvignon grapes from various South Australian and Victorian sites, distinguished by climate and regional classification have been sampled at harvest and used for small scale wine-making. The chemical composition of the grapes and the corresponding wines has been analysed to look for links between the source grapes and the final wine product. The results are discussed in the context of how comparisons of such data sets will in the longer term help us understand the impact grape composition has on wine flavour.

COMPARISON OF THE SENSORY CHARACTER OF SOUTH AUSTRALIAN CABERNET SAUVIGNON BERRIES AND WINES.

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Differences in Cabernet Sauvignon flavour arise from a wide range of complex reactions that occur during the oenological transition from grape to wine. The quality and *potential* of wine berries are routinely assessed in the vineyard throughout the growing season in an effort to gauge the wine sensory characteristics that may be produced in the finished wines. A better understanding of how the sensory characteristics of the wine berries relate to the sensory properties of wines made from these berries can highlight indicators of quality for wine producers to look for in their berry assessments.

Cabernet berries were sourced from seven vineyard sites across South Australia and small scale wine making enabled comparison of the sensory properties of wines produced from the same berry lots. The use of paired sites within two of the vineyards sampled made it possible to correlate the sensory properties of grapes with different quality designations and value. The sensory properties of Cabernet berries and wines were compared by ten trained sensory panellists using descriptive analysis across three consecutive vintages (2004-06).

The sensory results showed where subtle differences existed between the sensory character of samples (berries and wines) from each vineyard site. There was a large vintage effect such that sensory differences in the Cabernet berries and wines were different across the three years. The talk will focus on the application of objective sensory assessments in better understanding the link between Cabernet berry and wine flavour.

FAST AROMA ANALYSIS OF CABERNET SAUVIGNON AND RIESLING GRAPES USING AN ELECTRONIC NOSE.

Amalia Z. Berna, and Stephen Trowell.

Food Futures Flagship and Division of Entomology, CSIRO, Canberra, ACT.

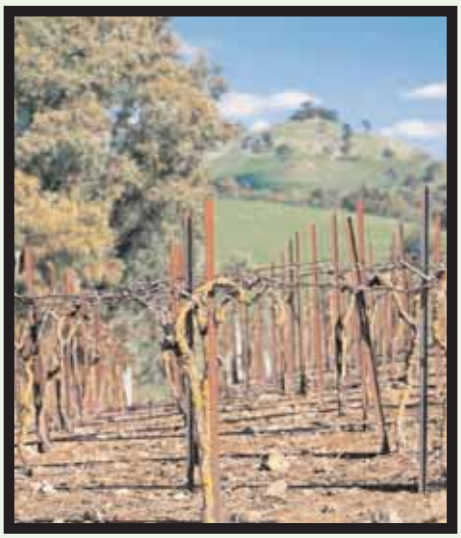
Winemakers use a range of objective measurements to assess grape juice, musts and wines at various stages of preparation and maturation. The most widely applied and established methods include soluble solids content, pH and titratable acidity. These are rapid techniques and can be automated. On the other hand, to quantify volatile compounds in wine and grapes requires the use of sensory and/or chromatographic techniques. These methods involve significant time for sample preparation and analysis, and are difficult to implement as routine quality control measures. Electronic nose (e-nose) technology offers a fast alternative for sensing volatiles and could be used to detect, for example, differences in the headspace composition of grapes grown in different regions. In this study, a metal oxide based e-nose was used to investigate the aroma of grapes harvested from five South Australian valleys. Cabernet Sauvignon and Riesling grape juice and slurry were collected at Orlando Wines. Samples were analysed for free volatiles by means of an Alpha MOS (FOX 3000) e-nose using both static headspace sampling (SHS) and solid phase microextraction (SPME). GC-MS analysis was also performed. Linear discriminant analysis of the e-nose data showed that in general SHS was superior to SPME for discriminating among different regions. Using SHS, the misclassification of juice samples for both Cabernet Sauvignon and Riesling grapes was 13%, while for slurry samples it was 15%. With appropriate choice of samples and methodology volatile measurement by e-nose may be a useful practical tool for rapid high-throughput analysis of grapes.

CAN AN OLFACTORY MACHINE PROVIDE INSIGHTS INTO THE PHYSIOLOGY OF LIVING SYSTEMS?

Graham A. Bell.

School of Medical Sciences, UNSW, Sydney, Australia, and E-Nose Pty Ltd, Australian Technology Park, Sydney.

The science of olfaction has provided important guidance for invention and development of artificial devices for detecting and identifying odour. In turn, success with the devices has suggested possible mechanisms for biological olfactory systems. This paper describes what aspects of physiology have influenced e-nose development and how a recent breakthrough



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might be applied to generate physiological hypotheses. This paper describes attempts to construct sensors and sensor arrays (e-noses). Small arrays of broadly tunes sensors have proved useful in addressing tasks where the odorant mixtures have been relatively limited. A real-time odour recognition algorithm has been discovered recently and patented. Its capacity to perform rapidly across a wide library of "remembered" odours suggests that an analogous mechanism might be found in the olfactory neuronal architecture and physiology of the forebrain.

Ref: Hibbert, D. B. and Bell, G.A. A method of predicting data sampled from an unknown source. Australian Patent Application Nos. 2006904660 and 2007903208.

MONITORING AND IDENTIFICATION OF INDUSTRIAL ODOURS WITH PTR-MS: DESIGNING ATMOSPHERIC SENSING AND SOURCE IDENTIFICATION OF ODOURS IN ALUMINA REFINING.

Michael Borgas, Ian Galbally.

CSIRO Marine and Atmospheric Research, Vic.

In this talk we will outline three things:

1. The nature of the proton transfer reaction mass spectroscopy (PTR-MS) sensor for odour sensing;
2. The nature of quantitative environmental sampling of intermittent plumes, including detection thresholds and sampling extremes; and
3. Generic examples of volatile organic compounds (VOCs) emitted from industry and suggestive links to odour impacts.

The technology embodied in PTR-MS allows better quantitative estimates for common odours, both in the detection level possible and with sampling-time responses more typical of olfactory systems. The knowledge developed from the integration of this sensed information also requires the mathematical understanding of the complex make-up of VOC signals in the atmosphere and the natural variability from sources, winds and mixing near the surface of the earth.

Atmospheric chemistry and air pollution research has long grappled with these issues for climate and clean-air amenity. This recent technology accelerates and deepens the scope of scientific study of odour in the environment and touches on many practical aspects of how our current society interacts in the environment.

The application of new tools offers ways to better inform debates on odour nuisance, which are common in all Australian air-quality jurisdictions, but will also facilitate the development new perimeter monitoring tools for more efficient and timely emission management for industry.

NEUROPSYCHOLOGICAL MODELS OF ADOLESCENT TRAJECTORIES INTO PSYCHOPATHOLOGY: UTILISING FUNCTIONS OF ACUITY THROUGH TO IDENTIFICATION TO MAP ONSET AND NATURE OF PROGRESSION OF ILLNESS.

Brewer, Warrick J; Wood, Stephen J; Pantelis, Christos, & McGorry, Patrick D.

ORYGEN Research Centre, Department of Psychiatry – The University of Melbourne.

We describe the direction of neuronal maturation during childhood and adolescence as a context for a review of the limited data reflecting the "time-staggered nature" of development of lower-to-higher-order olfactory functions (acuity, discrimination, memory, identification). We demonstrate how mapping the differential maturation of various olfactory functions enhances understanding of the onset and progression of developmental disorders such as Schizophrenia: disorders in which we reported olfactory identification deficits. We discuss the hypothesis that insult to neuronal structures that are nearing maturity during childhood may have a greater impact on olfactory functions that also mature at that time (e.g. olfactory acuity and memory), as well as adversely affecting functions maturing later during adolescence. For those disorders whose onset occurs during adolescence, the impact of olfactory function may be limited to those olfactory domains that have not yet matured (e.g. olfactory identification), thereby manifesting as "developmental arrest". Further, the impact of pre- or peri-natal insults, as evident in disorders like schizophrenia for example, is considered in terms of the interaction between brain maturation and the processes relevant to the disorder. Thus, early neurodevelopmental insults affecting olfactory-related neural systems may only become manifest as olfactory deficits at a time when such function would normally be reaching maturity ("growing into deficit"). We conclude that olfactory assessment is an important tool for mapping the onset and progression of developmental disorders that also implicate olfactory related disorders.

A CLINICAL TEST FOR INTRANASAL TRIGEMINAL FUNCTION IN CHILDREN.

W Kempter[1,2], T Hummel[1], DG Laing[2].

[1]University of Dresden Medical School, Germany, [2]School of Women and Children's Health, University of NSW & Sydney Children's Hospital.

Little is known about chemosensory trigeminal function in children and there is no clinical test to screen humans for trigeminal function when investigating damage to this nerve from nasal surgery, diseases, and exposure to environmental chemicals and medications. Accordingly, this study investigates a test of trigeminal function that is based on the well- known phenomenon of nasal lateralization which only occurs with trigeminal stimuli but not olfactory stimuli.

The stimuli were eucalyptol (EUC), a trigeminal stimulus, and phenylethanol (PEA) a floral olfactory stimulant, and the 348 subjects were 3-54 yr. The stimuli were delivered to the external nares of subjects using a 2-bottle device which allowed the simultaneous presentation of non-odorous air from one bottle to one nostril, and one of the two stimuli in the other bottle to the other nostril. Each stimulus was presented 10 times, 5 to each nostril, in a random order and a subject was required to indicate the side of the nose each time a chemical was presented.

Analyses indicated that regardless of age, a chance score of ~50% correct responses was obtained by subjects when the olfactory stimulus PEA was presented, demonstrating they could not indicate the nostril to which it had been presented. In contrast, participants from 5 yr of age indicated the correct nostril when the trigeminal stimulant EUC was presented..

Thus, the lateralization phenomenon is present at 5 yr of age and can be used as a simple clinical test of chemosensory intranasal trigeminal function.

FACIAL ELECTROMYOGRAPHY: RESPONSES OF CHILDREN TO ODOUR AND TASTE STIMULI.

JE Armstrong[1,2,] Hutchinson[2], DG Laing[1,2] and AL Jinks[2,3].

[1]School of Women and Children's Health, University of NSW and Sydney Children's Hospital, [2]Centre for Plant and Food Sciences, University of Western Sydney, [3]School of Psychology, University of Western Sydney.

The study investigated the potential for facial electromyography (EMG) to be used as a clinical tool for measuring the responses of newborns to pleasant and unpleasant smell and taste stimuli. As a first step towards this goal, responses in the zygomaticus major and levator labii muscles to 4 odorants and 4 tastants were recorded from 34 children aged 6-9 yr. The results indicated that EMG activities in the two muscles discriminated between pleasant and unpleasant stimuli within each modality in a manner which indicated the children perceived the hedonic qualities of the stimuli in a manner similar to that reported for adults. Importantly, there was unanimous agreement across the children as regards the differential nature of the activities exhibited. These outcomes together with the results of earlier facial expression studies, suggest that facial EMG may provide an objective procedure that could be suitable for the clinical assessment of taste and smell function in newborns and young infants. Currently, studies are underway using facial EMG to record the responses of 6 week old infants to the same odorants and tastants

DYNAMIC LAMELLIPODIAL WAVES ON THE SHAFT OF OLFACTORY ENSHEATHING CELLS REGULATE CELL-CELL INTERACTIONS AND MIGRATION.

Louisa Windus, Christina Claxton, Chelsea Allen, Brian Key, James St John.

Brain Growth and Regeneration Lab, School of Biomedical Sciences, The University of Queensland, Brisbane.

Olfactory ensheathing cells (OECs) exhibit a unique ability to migrate from the periphery into the brain during development. Although this migratory activity underlies the regenerative properties of the olfactory system, the cellular and molecular mechanisms regulating this behaviour are largely unknown. We have generated a reporter line of transgenic mice that expresses DsRed fluorescent protein in OECs under the control of the human S100ß promoter. Time-lapse imaging of these fluorescent OECs showed that contact-mediated migration was dependent upon the activity of motile plasma membrane protrusions which were distinct from the leading edge. These protrusions, termed lamellipodial waves, advance in a wave-like motion, collapsing and expanding as they move back and forth along the shaft of the cell. Lamellipodial waves are decorated with active thin filopodia and mediate both the initial cellular contacts between OECs and the resultant behaviour of cell-cell adhesion. Without the presence of lamellipodial waves, cell-cell adhesion did not occur and thus migrational rates declined. We show that the dynamic activity of waves is modulated by both glia-derived neurotrophic factor and inhibitors of the JNK and SRC kinases. The ability to regulate waves and cell migration has implications for facilitating the regenerative properties of OECs during neural repair.

DIFFERENTIAL EXPRESSION OF TWO ISOFORMS OF THE RET RECEPTOR TYROSINE KINASE IN THE MAIN OLFACTORY BULB.

T. Kaplinovsky and A. M. Cunningham.

Developmental Neurosciences Program, Faculty of Medicine, UNSW, Sydney.

Members of the GDNF family of trophic factors signal via the Ret receptor tyrosine kinase. We previously reported expression of Ret in the olfactory neuroepithelium and main olfactory bulb. However, *Ret* is alternatively spliced to yield two main isoforms, each appearing to play distinct roles in development: the

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Abstracts of Papers presented at the 9th Scientific Meeting of the Australasian Association for ChemoSensory Science

continued

Ret9 null mouse being non-viable due to renal dysgenesis, whereas the Ret51 null developed apparently normally. This study aimed to determine which isoforms were expressed in the olfactory bulb. Adult rats were perfused and olfactory bulbs examined by immunohistochemistry. Antibodies used included a rabbit pAb to Ret9 and a goat pAb specific for Ret51 (*Santa Cruz Biotech.*). This showed the two isoforms expressed in a complementary pattern with Ret9 present on many cell types, including a significant proportion of periglomerular (PG) cells, and Ret51 prominent within glomeruli and the olfactory nerve layer (ONL). Both isoforms were expressed by cells consistent with olfactory ensheathing cells (OECs) in the ONL and by approximately 50% of TH-positive PG cells. Double-labelling for Ret9/Ret51 showed co-expression in a small population of cells in the juxtaglomerular region, although single isoform-positive PG cells were more common. Finding both isoforms in OECs is likely to be important, as their migration *in vitro* recently has been shown to be GDNF-responsive. Similarly, Ret51 is the isoform selectively expressed by olfactory axons as they course towards glomeruli. Our study supports these two independent signalling complexes playing different roles in mediating the trophic effects of the GDNF family of neurotrophins in the olfactory system.

PREVALENCE OF OLFACTORY DISORDERS IN ABORIGINAL CHILDREN.

DG Laing and JE Armstrong.

School of Women and Children's Health, Faculty of Medicine, University of NSW and Sydney Children's Hospital.

Chronic nasal infections including sinusitis and rhinitis can impair smell permanently or temporarily. Despite this no one has determined whether the extremely high occurrences of chronic nasal infections that accompany chronic otitis media (COM) in young Aboriginal children result in smell disorders. Accordingly, this study determines the incidence of olfactory loss in 9-12 year old Aboriginal children and is the first to assess smell function in Aboriginals. A high incidence would indicate that early childhood impairment may result in a lifespan effect.

261 Aboriginal children (9-12 years old) from 16 communities in rural NSW participated. Olfactory function was assessed using a 16-odour identification test where a child chose from sets of 3 photographs which one best described the odour.

The data indicate that 257/261 children had normal olfactory function ie equalled or exceeded the criterion for normality of 13/16 identifications. 2 children were anosmic ie achieved chance scores, whilst 2 were classified as being hyposmic. These levels of correct responses parallel those from non-Aboriginal children.

The results indicate that the chronic nasal infections most Aboriginal children endure during early childhood do not result in long-term smell impairment. The occurrence of only 2 children with anosmia (0.76%) compares with a level of 5.8% in a recent study of 1387 adults (Bramerson et al, 2004), and suggests that smell disorders are less prevalent in children than in adults.

OTITIS MEDIA AND TASTE DISORDERS IN ABORIGINAL CHILDREN.

DG Laing & JE Armstrong.

School of Women and Children's Health, University of NSW and Sydney Children's Hospital.

The study aimed to determine whether Aboriginal children, who as a group experience high frequencies of otitis media (OM), have taste disorders. This was investigated because the chorda tympani, a major taste nerve, passes through the middle ear and could be damaged by OM pathogens. Loss of taste (sweet, sour, salty, bitter) alters how foods are perceived, food acceptance and diet, and can result in obesity or anorexia.

292 Aboriginal children (9-12 years old) from 16 communities in rural NSW participated. The taste test required them to sample 5 concentrations (weak to very strong) of sweet, sour, salty and bitter solutions and water, and to identify the taste from sets of 3 photographs. Loss of taste was defined as a score of 2/5 or less correct for a tastant. The loss was confirmed in a repeat test.

The data indicated that 24/292 (8.2%) of the children had taste loss and that the types of taste loss differed between individuals. 19.1% had hearing loss.

Taste loss prevalence (8.2%), therefore, was double that recognised by WHO (4%) for a disorder to be classified as being at an epidemic level.

The next phase of the research aims to determine the consequences of taste loss on growth and development, diet and nutrition, with the aim of developing strategies to manage the disorders.

IDENTIFICATION OF OLFACTORY SIGNALLING GENES IN *DROSOPHILA MELANOGASTER*.

Takahiro Honda, Narelle E. Tunstall, Morgan Beale, Marien de Bruyne and Coral G. Warr.

School of Biological Sciences, Monash University.

The fly *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* odour signals are detected by a large family of 62 seven-transmembrane receptor proteins, the odorant receptor (Or) family. 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 olfactory signalling in *Drosophila*, and results from two approaches will be presented. Firstly we have screened a large number of EMS-generated homozygous viable mutant strains for electro-antennogram defects, and identified two mutants which have greatly reduced responses to all tested odours. Progress towards mapping and cloning the genes involved will be discussed. Secondly we have used an approach to identify novel *Drosophila* genes expressed in the olfactory organs. We screened 500 'gene trap' lines (Lukacsovich, et al. 2001) that contain a P element insertion within the coding sequence of a gene, thus generating a mutant in that gene whilst still allowing detection of the expression patterns of the trapped gene. We have identified 16 lines with expression in olfactory organs, many of which are in olfactory receptor neurons, and are performing molecular genetic experiments to identify the genes that are "trapped" in these lines as well as characterizing their olfactory phenotype.

Ref: Lukacsovich, T., et al. (2001). Dual-Tagging Gene Trap of Novel Genes in *Drosophila melanogaster*. *Genetics* 157: 727-742.

DROSOPHILA ODORANT RECEPTORS DO NOT SIGNAL THROUGH CLASSICAL GPCR SIGNAL TRANSDUCTION PATHWAYS.

M. J. Beale[1], R. S. Smart[1], E. Vargas[2], R. D. Newcomb[3], C. Chen[2], and C. G. Warr[1].

School of Biological Sciences, Monash University, Clayton, Victoria, Australia, Prince Henrys Institute of, Clayton, Victoria, Australia, HortResearch, 120 Mt Albert Road, Auckland, New Zealand.

Drosophila odorant receptors (Ors) are predicted to be seven transmembrane proteins, and initially were thought to be G protein-coupled receptors (GPCRs). However, unlike mammalian and *C. elegans* Ors, which have been shown to signal via G proteins, there is no clear evidence for such signalling in Drosophila. In addition, *Drosophila* Ors may have a different membrane topology compared to GPCRs studied to date. As a consequence the mechanisms of *Drosophila* Or signal transduction remain unresolved.

In this study *Drosophila* Or43b and/or Or83b were expressed in Sf9 and HEK293 cells, and responses to ligands were measured via intracellular free Ca^{2+} $[Ca^{2+}]i$ imaging. In both cell types reproducible responses to several known ligands of Or43b were obtained. Inhibitors were then added to test whether components of common G protein-coupled signal transduction pathways are involved in Or signalling.

The response of Or43b to one of its ligands, ethyl butyrate, was unaffected by inhibitors of G-proteins (GDP-beta S), both cAMP and cGMP-specific phosphodiesterases (IBMX), guanylate cyclases (ODQ), adenylate cyclases (SQ22536), and all isoforms of phospholipase C (U73122). These results suggest that *Drosophila* Or-induced $[Ca^{2+}]i$ responses are independent of several classical GPCR signalling pathways.

INSECT ODORANT RECEPTORS: INSIGHTS INTO THEIR STRUCTURE, FUNCTION AND EVOLUTION.

Richard Newcomb, Aidan Kiely, Colm Carraher, Andy Law, Coral Warr, Narelle Tunstall, Andrew Kralicek.

HortResearch, Auckland, New Zealand.

Olfaction is a critical sense for insects, using their chemosensory abilities to locate mates, enemies, oviposition sites and food sources. Central to their olfactory abilities are a set of odorant receptors that are being uncovered by whole genome sequencing programmes in insects. Recently *in vivo* and *in vitro* expression systems have been developed to begin to characterise these receptors. Here we present insights into their structure, function and evolution that are emerging from these expression systems and contrast the features of insect odorant receptors with those of worms and mammals.

INVESTIGATING THE EXPRESSION PROFILES OF THE OLFACTORY NEURONS OF *C. ELEGANS*.

Nicholas M. Johnson, Timothy S. Sloan-Gardner, Ulrike Mathesius and Carolyn A. Behm

The School of Biochemistry & Molecular Biology, The Australian National University.

C. elegans can respond to a wide array of volatile odours using three pairs of bilaterally symmetrical sensory neurons. The AWA and AWC neurons detect attractive odours and the AWB neurons detect repellent ones. The compositions of the signal transduction pathways that are active within these cells are beginning to be resolved, principally with the use of classical genetics. However, the global gene expression profile of these cells remains unknown. Microarray analysis is the standard technology for measuring global gene expression, but in order to use this approach, mRNA must be isolated from single pairs of olfactory neurons.

Recently, cell-specific microarray analysis for *C. elegans*

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has been achieved through the development of the mRNA-tagging technique (Roy *et al* Nature 418, 2002; Kunitomo et al Genome Biology 6, 2005; Von Stetina *et al* Genes and Development 21, 2007). This approach involves using a recombinant *C. elegans* polyA binding protein (FLAG::PAB-1) to isolate the mRNA population from a tissue of interest. FLAG::PAB-1 is expressed with the use of a tissue-specific promoter and is tagged with a FLAG epitope to enable purification of the recombinant protein. Following purification, mRNA bound by FLAG::PAB-1 is eluted and analysed with microarrays.

We are using the mRNA tagging approach to investigate the gene expression profiles of AWA, AWB and AWC neurons. Our progress will be reported.

We thank the CSIRO Food Futures Flagship Cluster Program for funding this project.

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF OLFACTORY RECEPTORS IN LEPIDOPTERA.

Alisha R Anderson[1,2], Kevin Wanner[4], Doreen Begum[3], Melissa Jordan[3], Stephen Trowell1 and Richard Newcomb[3].

[1] Food Futures Flagship & Division of Entomology, CSIRO, Canberra, [2] School of Biological Sciences, Monash University, Victoria, Australia, [3] HortResearch, Auckland, New Zealand, [4] University of Illinois, Urbana-Champagne, Illionis, USA.

Odorant receptors (Or's) provide the molecular basis of odour coding and are critical for many behaviours in insects including mating, oviposition and detecting prey. The functional analysis of these receptors has been extensively studied in Drosophila showing receptors range in their odour responses along a continuum from narrowly tuned to broadly tuned. Some studies in Lepidoptera have focused on more narrowly tuned male specific pheromone receptors, however only very few lepidopteran receptors have been identified. We have identified 41 novel odorant receptors from the recently sequenced silkworm genome, *Bombyx mori* and RT-PCR has identified six receptors that show exclusive or more highly abundant expression in females compared to males. We have also identified an Or from *Epiphyas postvittana* which is not related to any other OR from other insect species but appears to be conserved across lepidopteran families. The strong conservation of this Or, compared to other lepidopteran Or's, indicates the function may be of importance to Lepidoptera species. Functional analysis of this receptor from *E. postvittana* and the *B. mori* homologue show this Or's response to monoterpenes is conserved between these species.

Posters

FOR WINE, IS THE STATIC SIGNAL ON THE NOSE?

David Clifford, Glenn Stone, Amalia Berna, Stephen Trowell, David Lovell.

Food Futures Flagship, Mathematical and Information Sciences and Entomology, CSIRO.

Time to ripeness of grapes is an important property, and reliable predictors would be desirable. The Alpha MOS electronic nose (e-nose) aims to measure volatiles objectively using an array of sensors whose electrical properties change. Potentially these changes may be used to predict time to ripeness. The standard data analysis with an e-nose uses the relative change in resistance of each of the sensors (the static signal). However, the dynamic signal recorded for each sensor over time may contain information that can be used for classifying samples. Examples of such information include the time taken to reach peak, the time to return to baseline, the shape of the peak, and the slope of the signal on either side of the peak. Development of the e-nose as an alternative to GC/MS or sensory panels in the field of enology through the use of the entire dynamic signal may prove fruitful. Exponential decay functions are combined into an empirical model for the dynamic signal. Having fitted the empirical model, the signal can be summarised in many ways. Additionally the model parameter estimates can be used directly for classifier building and subsequent prediction of new samples. Illustration is provided by predicting the time to ripen for Cabernet Sauvignon grapes based on juice and slurry samples. The performance of the methods is compared to use of static signals only.

ARE REEP FAMILY MEMBERS INVOLVED IN MEMBRANE TRAFFICKING OF OLFACTORY RECEPTORS IN *CAENORHABDITIS ELEGANS* ?

Guangmei Zhang, Chunyan Liao, Irene Horne and Stephen Trowell.

Food Futures Flagship and Entomology, CSIRO, Canberra.

Receptor expression enhancing proteins (REEPs), as their name suggests, enhance the expression of chemosensory G-protein coupled receptors. First detected in yeast, they have also been identified and isolated from many other organisms including plants, insects and mammals. In mouse, at least six REEP genes have been identified by BLAST searching of the genome. mREEP1 is strongly expressed in olfactory neurons. Coexpression of mREEP1 and murine olfactory receptors (ORs) in HEK293 cells promoted cell surface expression of ORs and enhanced their responses to odorants. We BLASTed mREEP amino acid sequences against the *C. elegans* genome and recovered five putative REEP homologues (designated CeREEP1-5). CeREEP1 has approximately 37% amino acid identity with mREEP1 and, like mREEP1, contains two predicted transmembrane domains, within which the levels of sequence conservation are much higher. CeREEP2-5 each have three predicted transmembrane domains. Although expression of CeREEP3 has not yet been detected, RT-PCR demonstrated that the other putative REEP genes are strongly expressed in *C. elegans*. A transgenic strain of nematode expressing a fusion between the CeREEP1 promoter and green fluorescent protein demonstrated that CeREEP1 gene is strongly expressed in the amphid chemosensory neurons including AWA and AWC. These results suggest that CeREEP1 may have a similar function to mREEP1 being involved in membrane transport and targeting of ORs in *C. elegans*. ■

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Vol. 9 No.4 October 2007

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.

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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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6-8 July 2008	**International Conference on Environmental Odour Monitoring and Control – NOSE2008** Rome, Italy http://www.aidic.it/nose2008/
21-25 July 2008	**International Symposium on Olfaction and Taste (ISOT) and AChemS Meeting** Hyatt Regency Hotel at the Embarcadero San Francisco, California, USA http://www.ISOT2008.org
8-10 October 2008	**The 3rd IWA Specialist Conference on Odours and VOC** Barcelona, Spain Contact: r.steutz@unsw.edu.au
4-6 December 2008	**Australasian Association for ChemoSensory Science (AACSS)** Annual Scientific Meeting Griffith University, Brisbane Contact: j.reinhard@uq.edu.au ■

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