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ChemoSense

EDITORIAL

ChemoSensory Science Gets Soaked in 2010

By Graham Bell
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As the rain squalls passed over the AACSS conference in December, and the Yarra Valley wine flowed freely, few realised the rain was the start of Australia's widest, deepest and most destructive floods in its modern history.

For those tragically affected, ChemoSense expresses sincere condolences on their losses and wishes all flood victims a speedy recovery and return to prosperity.

This issue begins the thirteenth year of publication of ChemoSense, and includes abstracts of the 2010 AACSS meeting at which 30 participants gave 29 presentations – truly a sign of a meeting where

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A Bayesian approach to odour recognition

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The invention and development of rapidly responding gas sensors took impetus from wartime experience of chlorine and mustard gas. At present many single compound sensors are available for a number of industrial applications (see Barnett, 1999), and combinations of chemical sensors used simultaneously have become referred to as electronic noses or e-noses. Multi-sensor arrays meet the need to identify complex mixtures of gases and vapours, such as are produced in many smelly industries which frequently cause discomfort and fear of poisoning in people exposed to them.

In the 1980s Gardiner and Bartlett showed that a simple combination of two chemical sensors operating simultaneously, could produce data sufficient to identify mixtures of headspace

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INSIDE:

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EDITORIAL CONTINUED

ChemoSensory Science Gets Soaked in 2010 continued

everyone contributes. Please mark the dates (first week in December 2011) and begin your plans to attend the landmark AACSS meeting in New Zealand.

Our main article describes the Bayesian approach to odour recognition in artificial olfaction, devised by Brynn Hibbert and based on the seminal paper of Thomas Bayes in 1763, a reference old enough to wake the sleepiest proceedings editor.

The Bayesian approach to the vexed problem of rapid recognition of odour quality allows an accurate identification of an odour to be made in a fraction of a second, thereby removing the necessity for the capture of a comparatively large data set, delaying the process over several minutes, as is required in alternative methods where an unknown sample is matched with one previously recorded. This real-time advantage opens applications for artificial olfaction in guidance and control of moving vehicles and robots.

A Bayesian approach to odour recognition continued

volatiles from various foods and beverages (see reviews, Gardiner and Bartlett, 1994, 1999). The key to this achievement lay as much in the statistical treatment of the data as in the specifications of the sensors themselves. More importantly, perhaps, was the shift in emphasis from identifying and measuring key chemical components of complex mixtures of volatile compounds, in order to effect an identification of the source of the mixture, to embracing the complexity of the continuous stream of data produced simultaneously by a number of sensors exposed to the mixture.

The complex data produced by e-noses lent themselves to several statistical methods, including analysis of variance and principal components analysis. The newly advanced field of Artificial Neural Networks (ANNs) was recognized as a potentially useful tool for classification of the sources of food odours sniffed by the e-nose arrays (Levy and Naidoo, 1999). Research for the Australian Meat and Livestock Association, using ANNs and other statistical approaches to identify odours recorded with an electronic nose (Bell, 2004; Bell and Wu, 2006), demonstrated the effectiveness of these approaches in identifying abattoir odours by source of emission and in predicting, by ANN, the likely occurrence of odours at perceived intensity levels likely to give offence to neighbours, up to 30 minutes into the future (Barnett et al, 2005).

For several years, however, the problem of real-time recognition of odours remained an elusive goal in any practical sense, while, in the same period, community concerns grew steadily about discomfort and health implications suffered from living in the vicinity of industries that smell.

These emissions may be single species of chemical compound, but, more commonly complex mixtures of many species of compound. These complex mixtures are perceived by people as specific smells, usually by a name indicating their source, e.g. "pig farm". At the heart of most complaints are two concerns: that the odour is spoiling the quiet enjoyment of their own homes, and secondly, that the odours signal something toxic.

Complaints from communities downwind of industrial air emissions form a large proportion of complaints received by environmental protection agencies (EPAs). We will describe below, several cases in which an e-nose has addressed these concerns, and the development of real-time recognition of odours achieved by an algorithm based on Bayesian probability (Bayes, 1763).

The four series of e-noses produced by E-Nose Pty Ltd (Mk 1 to 4) have up to six doped metal oxide chemical sensors and two other sensors for supporting information: temperature and humidity or wind speed and direction. Sets of sensors have been developed with reference to gas chromatography-mass spectrometry profiles of odours from the emitting industries. These were found to perform adequately over long periods, continuously. The main task then became how to obtain decisions about what the data output signified in terms of what was important to the client. Real-time identification of odours from e-noses has been an elusive goal amongst e-nose developers. A schematic for the operation of an e-nose is shown in Figure 1right.

The problem of odour identification is not particularly taxing if it can be done with the luxury of sufficient time to compare two relatively large sets of data from the

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A Bayesian approach to odour recognition

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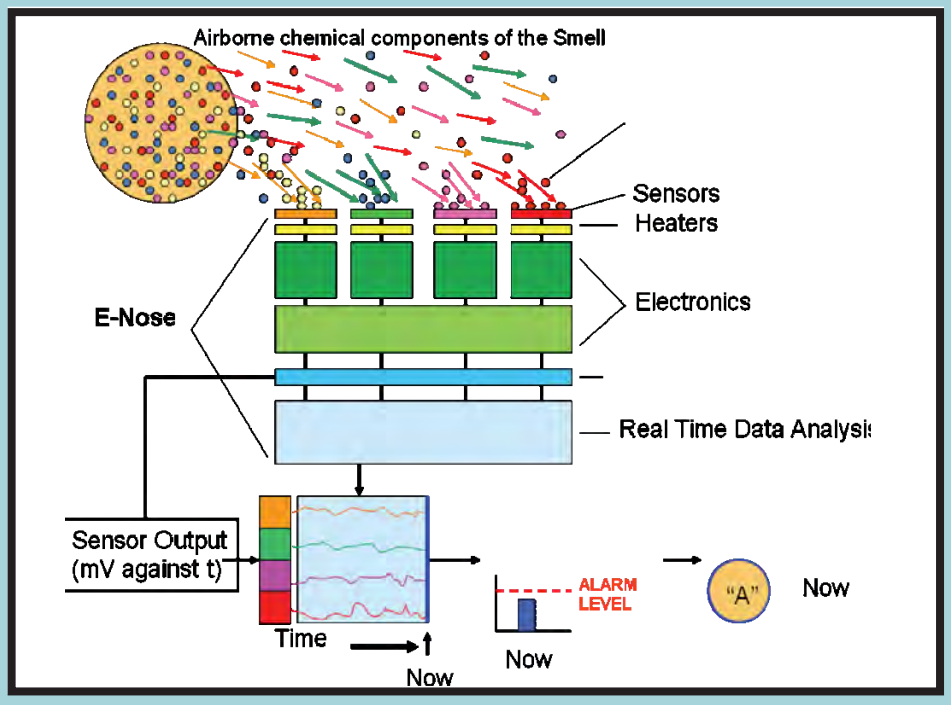

Figure 1: Schematic for the operation of an electonic nose.

odour source: the test data derived over several minutes, and a template of data recorded over at least that much time on a previous occasion. Gathering enough data for the test set imposes a time lapse on the decision process. To make the identification within a second, say, to meet practical real-time requirements, is a more vexing problem which has been solved in the manner described below.

THE BAYESIAN APPROACH TO CONTINUOUS ODOUR RECOGNITION IN REAL-TIME

Understanding the environment often rests on chemical analysis, of point samples and for a restricted range of analytes. Any inference about the nature of the environment requires both prior knowledge and a good understanding of the system. Here lies the problem: an ideal chemical analysis requires complete knowledge of the system (what chemicals, what concentration ...). For real environmental systems, we only have concerns (there has been a pollution incident, for example) and partial prior knowledge. So there is a need to take such chemical information we gain, plus what is known about the problem and synthesise an explanation for the client; who might be the courts, the EPA, the neighbours, or the company.

This paper will introduce Bayes' Theorem as an approach to expressing all knowledge about a problem. Examples will be taken from gas analysis by electronic noses.

BAYES' THEOREM

In the 18th Century, the Reverend Thomas Bayes derived possibly the most important theorem in the field of mathematical statistics. He was a non-conformist minister, and amateur mathematician and was reputed to be trying to calculate the probability of the existence of God from first principles. As we will see, the requirement of an a priori probability, before the evidence was taken into account, was too much for him to cope with and his ideas were not published until after his death in 1761. His paper *"Essay towards solving a problem in the doctrine of chances"* is a seminal work (Bayes, 1763). Here we state the form of the equation for discrete choices (Sivia and Skilling, 2006) in the context of the analysis of environmental odours and gases.

Suppose there are a number of different sources of environmental odour: $L_1, L_2, \ldots L_n$. The response from an electronic nose represents the evidence E. In the discrete form of Bayes' Theorem for this problem, the probability of a particular hypothesis H_i (e.g. sample comes from L_i) given response E and other information I (discussed later) is

$$\Pr(H_i \mid E, I) = \frac{\Pr(E \mid H_i, I)\Pr(H_i \mid I)}{\sum \Pr(E \mid H_j, I)\Pr(H_j \mid I)} \quad (1)$$

The likelihood, $\Pr(E \mid H_i, I)$, which reads 'the probability of finding the evidence given the truth of the hypothesis and other information', is obtained from measurements made on samples of known origin. $\Pr(H_i \mid I)$ is known as the prior probability and is the probability of the odour coming from L_i before the evidence of e-nose voltages is gathered. There are two approaches to assigning a value of the prior probability. In the absence of any knowledge of the system it might be decided that it is just as likely for the samples to come from any source; that is $\Pr(H_1 \mid I) = \Pr(H_2 \mid I) = \ldots \Pr(H_n \mid I) = 1/n$. Under this 'flat prior' equation 1 becomes

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A Bayesian approach to odour recognition

continued

$$\Pr(H_i \mid E, I) = \frac{\Pr(E \mid H_i, I)}{\sum \Pr(E \mid H_j, I)} \qquad (2)$$

and the probability of a particular hypothesis is simply the ratio of its likelihood to the sum of the likelihoods for the particular *E*. However, there is often considerable information about the samples, including intelligence from observations of the environment, statements made by parties involved, and so on. It would therefore be open to an analyst to place a probability on this prior information and incorporate it into equation 1. A good example of using general information in a Bayesian analysis is given by Coulson et al., (2001) who, when deciding how many seized tablets need to be analysed, use the information that seizures that appear physically homogeneous are more often than not chemically homogeneous (either all contain a drug or all do not contain the drug). In the example of odours, it might be that it is known that the great majority of odours emanate from a particular source, with a small fraction coming from different sources. The analysis can be weighted (using Pr(H |I)) with this prior information. The result of this weighting is that if the likelihood of two sources is about the same, the final probability will come down on the side of the more usual odour.

The method has been implemented for an electronic nose and is published in a patent (Hibbert and Bell, 2007).

EXPERIMENTAL

METHOD

An electronic nose (E-Nose Pty Ltd, Mk1, with five metal oxide sensors) was deployed in three abattoirs in New South Wales and Queensland. At the first site, there were three possible sources of odour "Lairage", "Rendering", and "Basement". The e-nose was exposed to each and the profile of the voltage resposes collected over a week in order to capture different environmental conditions (day/night, week/weekend, heavy use/ down time). To obviate the effects of concentration the variables that were analysed by the Bayesian method were four sensors' voltages divided by the fifth. The probability density function of each ratio for each odour source (which gives the likelihood) was approximated by a Normal distribution and characterised by the mean and standard deviation. It is also possible to create a fourth possibility "none of the above" with a small, flat probability density function that becomes important in regions where none of the other sources give significant ratios. This avoids the program choosing the least unlikely between two very unlikely sources ("the lesser of two evils"), when in fact the odour is a spurious one that has not been modelled.

RESULTS

The means and standard deviations of the ratios for each source are given in Figure 2. The method was tested by a set of data independently collected from 'Lairage'. The probability calculations are shown in Figure 3, indicating the correct origin of the odour. The gaps correspond to regions where the data was off scale or missing.

A set of data collected independently from the Rendering Plant was tested against the data from Lairage, Basement and "None of the Above" are shown in Figure 4. The result indicated the correct origin of the data, on each successive set of data from the sensors. The axis

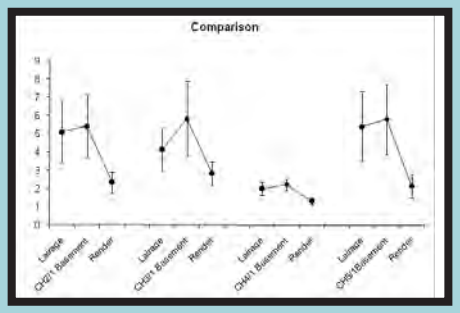

Figure 2. Mean and standard deviation for ratios of e-nose responses in three locations in an abattoir. CH*x*/1 = voltage of channel (sensor) *x* divided by voltage of sensor 1.

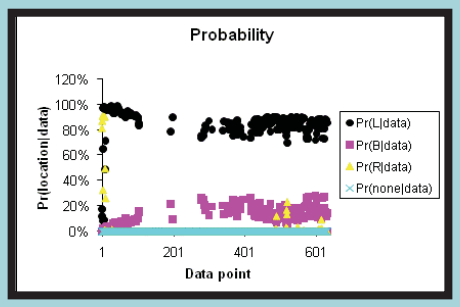

Figure 3. Continuous monitoring of probability of responses from the location 'Lairage' (=L)

labeled "Data Point" represents the incoming stream of data at one second intervals. Hence the analysis returns a correct classification of the origin of the incoming data at every time point (except with lower confidence in the case of the first time point). This process achieves an identification of the odour, in terms of its source or quality name, as rapidly as the e-nose can process new data (in this case, at a speed of one new reading on all the sensors every one second, i.e., in practical real-time). There is every reason to believe that with modern CPU speeds and a faster digitization of new data, this "practical real-time" interval can be shortened considerably.

Thus, the Bayesian Method for real-time odour identification, combined with the

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A Bayesian approach to odour recognition

continued

indication of the strength of the odour represented by the amplitude of the sensor signals, can be applied to a control system for searching and finding a source of odour, such as by robotic means (Hibbert and Bell 2007).

IDENTIFICATION OF ODOUR MEASURED FROM INCREASING DISTANCE FROM THE SOURCE USING THE BAYESIAN APPROACH

The question arises whether the data used for the recognition test (pdf or "template" of the odour source) will hold up to new test data taken from increasing distances from the source. In other words, does an odour retain its "signature" as encoded by the Bayesian method as the distance from the source increases?

At a second site, three sources of odour "S", "SW" and "Pond" were recorded near to the source and these sets formed the template against which to test an independent set taken near the Pond and one taken 500m distant downwind of it. The identification of the new pond data (within 10m source) was correct for only part of the first five minutes but was correct every second for the next 10 minutes, as shown in Figure 5.

Variation in the wind direction during the test recording can account for the sub-optimal performance of the identification of the pond odour. Identification of pond odour was successful on more than 90% of the time points when the new data was recorded 500m downwind from the pond as shown in Figure 6.

At a third site, this approach was useful in showing an industrial emitter that the odour from his site was recognizable for up to 600m from the source, at a point well within the complainants' suburb. A regression equation was then used to show how far the odour would reach before it could no longer be detected, which was approximately 1 km. This information provided the emitter with a clear mandate to take steps to minimize the fugitive odours from his site.

The following schematic (Figure 7) shows the steps required to apply the Bayesian method to odour identification and to an alarm or notification to an operator that there is an event requiring attention. The alarm criterion would most likely have an aggregate signal amplitude setting that must be met, unless circumstances required every detection, no matter how small, to be reported. In

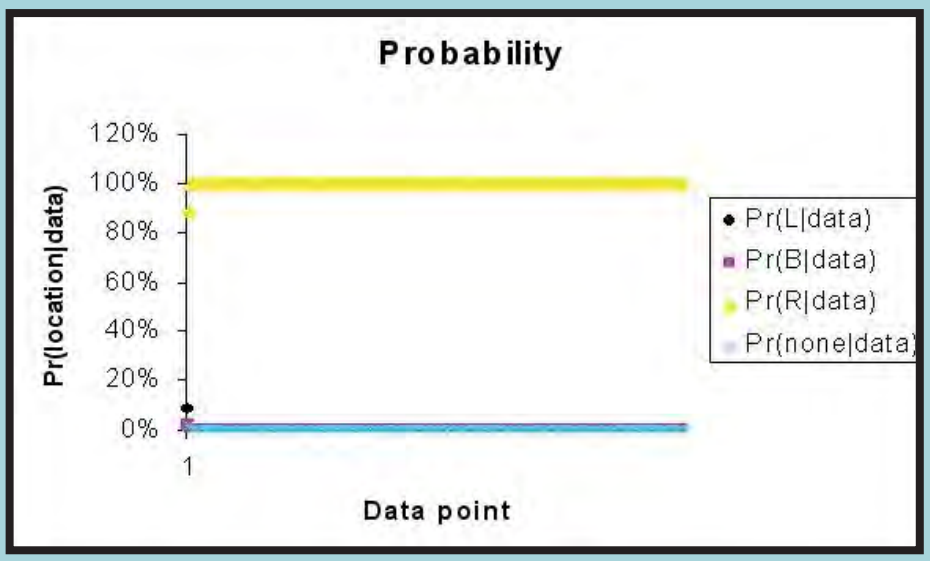

Figure 4: Continuous data from Rendering Plant R, applied to the Bayesian method, is correctly identified on all time points (approx 10 mins at 1 sec intervals).

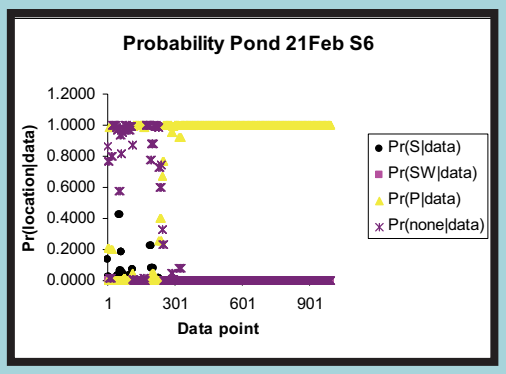

Figure 5: Identification of Pond Odour within 10m of the source.

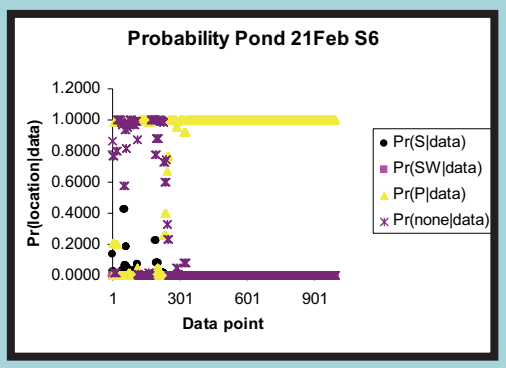

Figure 6: Identification of Pond Odour 500 m from the source. Windshifts may account for loss of 100% certainty of correct identification, shown by the dips in the pond trace in this figure.

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A Bayesian approach to odour recognition continued

the application of finding an odour source, the increase or decrease of the aggregate amplitude would inform directional guidance toward the source.

CONCLUSIONS

An electronic nose array can be used to monitor and identify sources of odours in real-time given a suitable training set. It is possible to update the library of odours with data collected in the field and verified as coming from a particular origin. The assignment can be weighted with knowledge of the likely source.

The method has been validated in the field and found to be reliable. It has been applied to testing the distance over which the identity of the odour holds up. Used in combination with the strength of the odour as indicated by the amplitude of e-nose sensor outputs, the source of an odour held in the library of odours can be located. This opens the application to potential use in various forms of robotics.

Acknowledgement

A form of this paper was presented at IUAPPA World Clean Air Congress, Vancouver, Canada, September 2010 and at NACA Clean Air Conference. Polokwane, South Africa, October 2010, and has been submitted to CASANZ 2011.

The authors wish to thank Mr Martin Kwong for editorial assistance on this paper ■

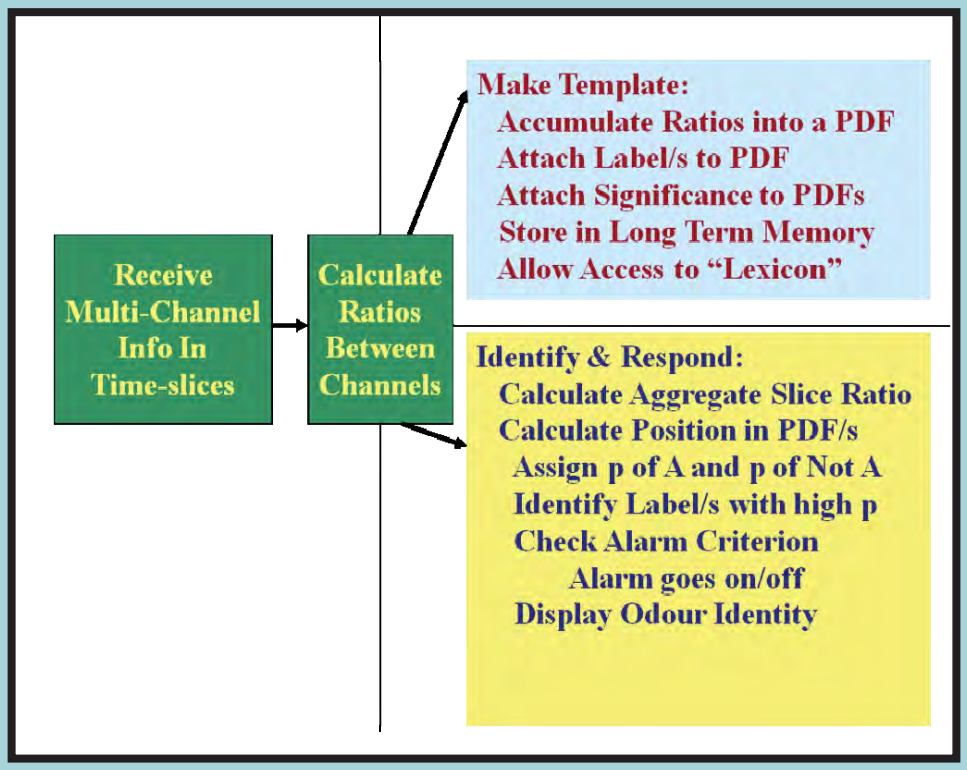

Figure 7: Schematic for steps in the Bayesian method for real-time continuous recognition of odour quality using an electronic nose.

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BOOK REVIEW *by Graham Bell*

The Great Pheromone Myth

Richard L. Doty: The Great Pheromone Myth.

Hard Cover, 278 pages. Published by The Johns Hopkins University Press, Baltimore, 2010.

Price: US$65 (excluding discounts and shipping). Order from sales@sensonics.com or www.amazon.com

This book is immediately disturbing to the point of annoyance. Don't we all know there are pheromones? Five million Google entries say there are. Are not crucial messages ("come hither") conveyed between members of a species by a few hormone-like chemicals?

Don't we all know there are specific airborne or liquid-borne chemical compounds that act instinctively on invertebrates, vertebrates, mammals and humans, to initiate the "fs": fear, flight and fornication? Don't baby humans, rabbits and kangaroos get drawn to the nipple by a sweet smell of motherhood? Isn't this the work of pheromones? Is mate selection not the work of pheromones? Is fear of a dominant male not the work of pheromones?

The answer we learn from this scholarly book is NO: a compound or simple group of compounds is neither necessary nor sufficient to produce these behaviours: there is always learning and a host of other sensory cues involved. And when some chemical compound is offered up as the key to the behaviour, the proofs invariably fail.

The senses of sight, sound, touch, taste and smell conspire to invoke behaviour, often with more cognition involved than previously admitted.

It's not just poor chemical analysis: in enough instances, few though they may be, very precise compounds have been nominated after decades of careful chemistry. Mostly, however, chemistry has not been forthcoming. The idea that a pheromone triggers a specific receptor which is genetically programmed to initiate, say, mating behaviour is sadly a myth. The myth grows stronger when applied to mammals and reaches absurd levels at the hand of some writers on human behaviour.

To back his conclusions, Richard Doty offers a formidable review of scientific literature. The reference section stretches for nearly 60 pages. Particularly impressive is the concentration applied to the main argument. Anyone who has performed a literature review for a thesis will be impressed by the scope of this review. Anyone contemplating reviewing the literature on a scientific question should take a good look at this book. Certainly, anyone interested in chemical communication must read this book.

The initial review is of definitions of pheromones. Here there has been surprisingly little agreement. Some assert they are volatile and work through the olfactory system, some that they are not, are odourless and work through the vomeronasal system (which, by the way, a weight of evidence suggests, is not functional in humans). You don't like my definition? I've got others!

Invoking a pheromone is an easy way out of explaining social behaviour which on careful observation is actually very complex. The pheromonal explanation is sometimes proclaimed without further recourse to identifying the chemical or chemicals involved. How sad to discover that scientists have clay feet. Doty shows by critical analysis of the literature, that many major claims of identifying "releaser" or "primer" pheromones are either not reproducible, inadequately account for the role of learning or novelty, or provide chemical compounds that fail to meet the claim.

One by one the icons fall. What? Surely not the holy icon of insect pheromones?

Yes: in most instances, the components that make up the insects' "pheromone mixture," have different effects on the receiver. The good news for chemosensory science is that there is clearly much more to learn about how and what insects communicate using chemical signals, and what exactly is communicated through a possible lexicon of signals able to be generated by the mixture. Some inspired research is needed.

When you read this book you are very likely to experience a shift from frustration to enlightenment. Far from thwarting your path to knowledge, Doty will remove some much-neglected clutter and smooth the way to new understanding. This has to be good for the whole field. This review offers a very inadequate sniff to stimulate your hunger: please buy and read the book to benefit from its messages. If you are new to the chemical sciences, this is a good start. If you are steeped in the field, you will enjoy reading about work you know well (and may have contributed to) in a new light. If you are intending to work in research in chemosensory science, this book is a significant investment in your future.

AACSS 2010: Yarra Valley Abstracts

Australasian Association for ChemoSensory Science Inc. (AACSS)
Proceedings of the 12th Scientific Meeting

Balgownie Estate Vineyard Resort and Spa,
Yarra Valley, Victoria, Australia
2-4 December 2010

Conference Organiser: Coral Warr
Organising Committee: Alisha Anderson, Richard Newcomb, Marien de Bruyne

ABSTRACTS – ORAL SESSIONS

PLENARY LECTURE

ODOR CODING IN BEES AND FLIES – LEARNING IN MULTIPLE NETWORKS

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In our lab, we study odor coding and olfactory memory in bees and flies, using a combination of physiological, immunocytochemical and behavioral approaches.

Bees collect nectar and pollen, and learn to associate color and odor with these rewards. This appears as an adaptive behavior that ensures efficient foraging by the hive. But how are these memories stored in the nervous system? We investigated the effect of associative learning on early sensory processing, by combining classical conditioning with in vivo calcium imaging of secondary olfactory neurons, the projection neurons in the honeybee antennal lobe. We found associative changes of odor representations 2 to 7 hours after appetitive odor conditioning. These changes affected both the global projection neuron response strength and the spatial pattern of activated neurons. Our data suggest that odor learning affects the intra-glomerular network at the level of olfactory receptor neuron-to-projection neuron synapses and inhibitory local neuron-to-receptor neuron synapses. The observed changes are consistent with the idea that odor learning optimizes odor representations and facilitates the detection and discrimination of learned odors.

Which are the networks that shape odor information? Multiple populations of local neurons are present, with different properties in physiological odor responses and in biochemical complements. One approach to understand this diversity is to look at the expression of neuropeptides in the brain. Indeed, neuropeptides may be the most ancient chemical messengers between neurons. As all insects, bees have are a large number of different peptides and peptide receptors, most of which have been characterized only poorly, if at all. Neuropeptides are also a powerful tool for neuroanatomical studies, because they can be used to characterize small populations of neurons based on their neuropeptide expression patterns.

Another approach to study multiple networks is to make use of Drosophila melanogaster. Here, the use of GAL4/UAS allows to selectively lable genetically defined populations of neurons with a calcium reporter, and then characterize their odor responses. Moving through the layers of the antennal lobe, we characterized projection neuron responses, populations of local neurons, and receptor neurons. Together with data from literature, we also generated an openly accessible database for receptor neuron odor responses, DoOR (Database of Odor receptor Responses, http://neuro.uni-konstanz.de/DoOR).

Session 1: Development of the mammalian olfactory system

PLENARY LECTURE II

AXON GUIDANCE IN THE MAMMALIAN OLFACTORY SYSTEM

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Olfactory sensory neuron (OSN) axons follow stereotypic spatio-temporal paths in the establishment of the olfactory pathway. The topography of olfactory projections from epithelium to olfactory bulb (OB) is an essential determinant of odor coding. The mechanisms subserving the sorting and targeting of axons are complex. In mammals odor receptors (ORs) have dual roles in OSNs, detecting odors in the olfactory epithelium (OE) and playing an instructive role in the axonal convergence of OSNs into the OB. While ORs are required for targeting, they are insufficient for correct targeting. Consistent with this notion, a plethora of guidance molecules have been implicated in OSN axon targeting in the developing olfactory pathway. A key question, however, is how are these different sets of cues coordinated to establish olfactory topography?

We have identified members of the tetraspanin family in OSNs. Tetraspanins are molecular organizers which act to form multimolecular membrane complexes called tetraspanin-enriched microdomains (TEMs), a specific type of signaling platform, distinct but similar to lipid rafts. TEMs are known to regulate cell morphology, motility, invasion, fusion and signaling, in the reproductive and immune systems, as well as in viral infection and metastasizing cells. Tetraspanins specifically form interactions with cell adhesion molecules (such as integrins and Ig-containing CAMs), ectoenzymes (such as MMPs and ADAMs), transmembrane receptors (such as GPCRs) and intracellular signaling molecules (such as G-proteins and Rho GTPases). We have hypothesized that TEMs provide an ideal platform to coordinate multiple guidance cues in growing OSNs, thereby regulating OSN sorting and synatptogenesis.

To test our hypothesis we looked for components of TEMs, and for evidence of interactions between them. Tetraspanins CD9 and CD81, Ig superfamily member EWI2/IgSF8 and membrane-type MMPs are all present in developing OSNs. Moreover, EWI-2/IgSF8 and CD9 interact in biochemical studies, and are present in discrete puncta in cultured cells consistent with their being in membrane microdomains. We also found EWI-2/IgSF8 was selectively expressed in OSN terminals during synaptogenesis but downregulated in adults. Expression in axon terminals could be upregulated if the OE was lesioned and OSNs were forced to regenerate. This suggests that TEMs may also facilitate adhesion in synaptogenesis. We propose that TEMs provide a structural scaffold which facilitates protein-protein interactions between multiple guidance cues within axons and growth cones, allowing them to signal through a common signaling pathway.

OLFACTORY ENSHEATHING CELLS PROLIFERATE FROM STEM CELLS AFTER INJURY

Fatemeh Chehrehasa, Jenny Ekberg, Katie Lineburg, Daniel Amaya, Alan Mackay-Sim and **James St John**

National Centre for Adult Stem Cell Research, Griffith University, Queensland, Australia

Olfactory ensheathing cells (OECs) support the regeneration of olfactory sensory neurons throughout life. However, it remains unclear how OECs respond to a

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AACSS 2010: Abstracts continued

major injury and whether stem cells give rise to new OECs in those conditions. We examined the proliferation and migration of OECs by surgically removing an olfactory bulb from neonatal mice. The outer layer of the olfactory bulb, the nerve fibre layer, is rich with OECs and thus bulbectomy removed the OECs of the olfactory bulb. The peripheral region of the olfactory nerve within the nasal cavity and the olfactory epithelium where the stem cells are located remained untouched. Proliferating cells were labelled by the thymidine analogue, ethynyl deoxyuridine (EdU). In the unilateral bulbectomy model, there was a large stimulation of OEC proliferation throughout the olfactory nerve up to 14 days after bulbectomy. Tracking cells that had proliferated revealed that stem cells lining the basal layer of the olfactory epithelium also gave rise to OECs that subsequently migrated along the length of the olfactory nerve. These results demonstrate that OECs actively respond to widespread degeneration of olfactory axons and that both local proliferation of OECs as well as stem cells give rise to new OECs that migrate along the olfactory nerve to the regions of need.

REMOVING THE DEAD: OLFACTORY ENSHEATHING CELLS PHAGOCYTOSE AXONAL DEBRIS

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Olfactory ensheathing cells (OECs) are the glial cells of the olfactory system. Their primary role is thought to be to provide support and guidance for primary olfactory axons. However, OECs are known to phagocytose bacteria and express immune markers and thus they may help to maintain a healthy environment. Interestingly, following widespread death of primary olfactory axons, there is minimal mobilisation of macrophages but yet the axonal debris is rapidly cleared. We have therefore investigated whether OECs are the cells that are primarily responsible for removal of axonal debris. We cultured red fluorescent OECs from S100beta-DsRed mice and green fluorescent primary olfactory neurons from OMP-ZsGreen mice. In explant cultures of DsRed-OECs and ZsGreen-neurons, OECs clearly contained green fluorescent axonal debris. When cellular debris from green fluorescent neurons was added to cultured OECs, the OECs extended pseudopodia and rapidly phagocytosed the axonal debris. We examined sections through the olfactory system in healthy animals throughout development (E15 to adult) and found that with increasing age OECs contained increasing levels of axonal debris. Following degeneration of olfactory neurons by injection of methimazole, OECs had significantly more axonal debris (30-50% more, n=5, $p<0.005$) compared to controls. In comparison, macrophages within the olfactory system did not display increased levels of axonal debris, indicating that OECs, rather than macrophages, are the cells that are primarily responsible for removal of axonal debris. These results clearly demonstrate that OECs actively phagocytose cellular debris and thus is another mechanism by which they maintain the health of the olfactory system.

Session 2: Chemosensory receptor molecular mechanisms

INSECT ODORANT RECEPTOR STRUCTURE AND FUNCTION

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In animals olfaction is mediated by the interaction of odorant receptors (ORs) with small volatile molecules. Mammalian and nematode ORs are GPCRs that activate classical G protein signaling cascades, leading to the eventual opening of ion channels. Insect ORs also contain seven transmembrane helices, however they adopt the opposite orientation in the membrane to GPCRs, suggesting they belong to a novel class of proteins. Recent studies in various surrogate cell systems have shown that insect ORs can act as ion channels, with evidence in HEK293 cells that they may also be able to signal via G protein pathways. One highly conserved insect OR, Or83b, is essential for these activities, forming a complex with ligand-binding ORs.

Many questions remain concerning the structure and function of insect ORs including: What is the minimum number of receptor units required to form a functional channel? What is the stoichiometry of the Or83b/ligand-binding OR complex? Where do ligands bind in the ligand-binding ORs? How are binding events transduced within the complex? What components of the complex and additional factors are required for ionotropic and metabotropic signalling?

Approaches that will be employed to test many of these questions will be discussed, including surrogate cell and cell-free expression, co-purification studies, antibody pull down assays, ligand binding assays and studies in artificial membranes and proteoliposomes. These studies will compliment other approaches being taken including patch-clamp, FRET and X-ray crystallography to address these structure/function questions and eventually enable the use of insect ORs in odorant biosensing devices.

THE UMAMI TASTE IN PIG IS TUNED BROADER THAN IN HUMAN BUT NARROWER THAN IN LABORATORY RODENTS

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Some L-amino acids and nucleotides evoke umami taste through a trans-membrane heterodimeric receptor (the T1R1/T1R3). Laboratory rodents are commonly used in chemosensing studies as a model for humans yet their umami taste seems broadly tuned. The porcine umami taste receptor genes *pTas1r1/pTas1r3* have only recently become available and their sequences are 81 and 82% homologous compared to the human genes respectively. Among the known mammalian species, the mouse and rat have the lowest homologies with humans. Similar to the human, the T1R1 outer membrane N-terminal Venus flytrap (VFT) domain of the pig has 571 aa residues that consists of 2 globular subdomains (upper and lower lobes) containing the ligand-binding site. Both human and pig Tas1R1 VFT domain contain 10 different amino acid residues that are involved in ligand binding: H71, T149, S172, D192, Y220, E301 and R307 contribute to L-glutamate recognition while H71, R277, S306, H308 and to a lesser extent to S172 and Y220 are important for binding nucleotides. In turn, the arginine and histidine (both charged polar) residues at key positions 307 and 308 appear changed to threonine and tyrosine (both neutral polar) residues in mouse and rat sequences. Taken together, these data indicate that the pig Tas1R1 likely utilizes a similar mechanism for binding L-glutamate and nucleotides as human Tas1R1. The smaller gene homologies and the amino acid residue differences found in rodents (mice and rats), particularly at the 307 position, may explain differences in "in vivo" umami sensing compared to humans.

GREATLY ENHANCED DETECTION OF A VOLATILE LIGAND AT FEMTOMOLAR LEVELS USING BIOLUMINESCENCE RESONANCE ENERGY TRANSFER (BRET)

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Our goal is to develop a general transduction system for G-protein coupled receptors (GPCRs). GPCRs are present in most eukaryote cells and transduce diverse extracellular signals. Humans express over 750 GPCRs, which respond to a diverse range of ligands, including small organic molecules, peptides and proteins. GPCRs therefore comprise not only the largest class of integral membrane receptors but also the largest class of targets for therapeutic drugs. In vertebrates and many invertebrates GPCRs also mediate the sense of smell and some elements of taste perception. In all cases studied, binding of ligand to a GPCR leads to a sub-nanometer intramolecular rearrangement.

Here we report the creation of a chimaeric BRET-based biosensor by insertion of sequences encoding a bioluminescent donor and a fluorescent acceptor protein into the primary sequence of a GPCR. The chosen receptor, the *Caenorhabditis elegans* ODR-10 chemoreceptor, responds in vivo to the odorant 2,3-butanedione (diacetyl). The BRET^2-ODR-10 biosensor was expressed in membranes of *S. cerevisiae*. Assays conducted on isolated membranes indicated an EC_{50} in the femtomolar range for diacetyl. The response was ligand-specific and was abolished by a single point

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mutation in the receptor sequence. Novel BRET-GPCR biosensors of this type have potential application as detectors in many fields including explosive detection, quality control of food and beverage production, clinical diagnosis and drug discovery.

MOLECULAR MECHANISMS UNDERLYING OLFACTORY PLASTICITY

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Honeybees are an ideal model system to investigate brain plasticity, because they are relatively long-lived animals that encounter changing scent environments throughout their adult life. Here, we present first results on the molecular mechanisms underlying experience-dependent plasticity of the honeybee brain, both in the olfactory periphery and in higher brain centres. We found that the expression of six olfactory receptors (ORs) in the antennae that were shown to bind floral odorants varies significantly with transition from hive nurse bee to outdoor foraging bees, and with exposure to different flowering plants in the four seasons. When we conditioned bees selectively to specific floral odorants using the PER assay, we found that the respective ORs in the antennae were down regulated. OR down-regulation only occurred in context of scent learning; mere exposure to the same scents without learning induced no changes in OR expression, suggesting that the reward pathway from the learning centres plays a role in the down-regulation. Microarray and qRT-PCR analyses of the higher brain centres of the scent-conditioned bees revealed that a number of coding genes involved in metabolic processes, cellular development and biogenesis were also down regulated after scent learning. Our research demonstrates that the olfactory system is highly plastic, constantly adapting via differential gene expression to scent experiences. We propose that this plasticity enables the olfactory system to be optimally tuned to process familiar odorants as well as detect novel ones.

GENETIC ANALYSIS OF CILIUM FUNCTION IN THE CHEMOSENSORY NEURONES OF *CAENORHABDITIS ELEGANS*

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The sensory neurones of nematodes (including chemosensory neurones) are composed of the same basic elements, including a non-motile cilium, as the chemosensory neurones of other metazoans. These neurones are organised into simple sensory organs, the most prominent of which is a bilaterally symmetrical pair of neuronal bundles located in the head of the worm. These bundles are called the amphids. The sensory dendrites of some amphid neurones are exposed directly to the external environment via pores in the nematode cuticle, and can be visualised simply in live worms using lipophilic fluorescent dyes such as DiO and DiI. Variations in dye filling thus form one phenotype which may be conferred by mutations affecting dendrite structure. Furthermore, because the amphids mediate chemo- and thermosensory reception and sensitivities to some drugs, there is a range of behavioural and drug sensitivity phenotypes that are affected by amphid defects. We have used amphid mutants to investigate (a) the genetic components required to assemble a functional non-motile sensory cilium and (b) the integration of chemosensory and other inputs that underpin the coordination of the regulation reproduction by environmental signals. Our data suggest that not only do nematodes adjust their short-term reproductive behaviour in response to environmental input via the amphids, but that longer term reproductive behaviour is also modulated in response to the same signals.

Session 3: Detecting and identifying natural scents

CODING PROPERTIES OF DROSOPHILA OLFACTORY RECEPTORS FOR SENSOR APPLICATIONS

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Receptors in insect olfactory neurons hold great promise as biosensors for volatile organic chemicals (VOC) in a range of applications. We assess the suitability of *Drosophila* receptors for use in law enforcement and security and quality assessment in the wine industry. In the process, we identify new ligands and study the coding properties of this numerically simple olfactory system.

Using a small set of diagnostic odorants to identify neuronal identity we screen a large number of neuron classes on the fly's antennae and maxillary palps. We identify several receptors for volatile indicators of drugs, nerve gasses and explosives. One of these, Or43b, responds strongly to a component of explosives. We analyse how Or43b response specificity and dynamics relate to chemical structure and show that odour-specific information is partially retained in odour mixtures. Detection is tested against realistic backgrounds, and we present solutions to potential interference by odorants from fermenting fruits.

Another putative application is the detection of aroma compounds in wine. We first show that several of the fly's receptor neurons respond to Cabernet-Sauvignon wine and then screen for responses to specific aroma compounds. We find receptors for general aroma compounds as well as some taints and off-flavours and compare their sensitivity to human detection thresholds. We conclude that *Drosophila* olfactory receptors have great potential for inclusion in a biosensor that can deliver either targeted measures of specific volatiles or fingerprints of complex wine aromas.

HOW THE BRAIN MAKES SENSE OF COMPLEX SCENTS: KEY ODORANT LEARNING IN HONEYBEES AND HUMANS

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Natural scents in our everyday life such as food aromas or floral perfumes are complex mixtures containing hundreds of different chemicals. While processing of monomolecular odours and small artificial mixtures has been studied thoroughly in insect models, the brain mechanisms underlying processing and learning of complex natural scents are not well understood. Using an animal with a sophisticated sense of smell, the honeybee and the well-established Proboscis-Extension-Reflex paradigm, we investigated how brains process and learn information from complex floral scent mixtures. Our study showed that honeybees did not learn every single component of a scent mixture equally well, but learnt only a selection of key odorants as representatives for the mixture. The bees could not distinguish a mixture of the key odorants from the full mixture, while a mixture of the non-key-odorants elicited a significantly decreased response. The key odorant composition was unique for each tested scent mixture. Interestingly, when we tested humans with the same floral odours, we found the same effect, namely humans identified some key odorants as more representative of the whole mixture than others. That is, our brain just like the bee brain seems to filter olfactory information thus reducing a complex mixture to a code of key odorants. We propose a model of the neural mechanisms underlying the process of key odorant learning, and discuss the implications our discovery has for the food and wine industry as well as for us as consumers.

UNRAVELLING THE MECHANISMS OF ODOUR MIXTURE PERCEPTION THAT LIMIT THE NUMBER OF ODOURS PERCEIVED

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Most smells in our environment be it in the home, garden or in the workplace are complex mixtures often consisting of dozens of odorants. Although instruments can identify the odorants, in most instances they cannot indicate those a human, animal or insect will detect and use in their behavioural response. This talk will present evidence

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that two major but very different mechanisms limit the number of odorants humans can perceive in complex mixtures. The two mechanisms, namely, combinatorial coding and temporal processing together simplify the coding of complex mixtures to the extent that a mixture can be identified within a second. Indeed combinatorial coding allows the olfactory system to process single odorants and complex mixtures in a similar way. Furthermore, the combinatorial tactic used by the olfactory system provides a mechanism for the simplification of odour signatures in the brain and a rapid 'one trial' learning of the identity of foods, environmental odorants and odours of behavioural significance. The relationship between the mechanisms underlying odour suppression and combinatorial coding will also be discussed including the role of competitive mechanisms at the periphery and inhibitory mechanisms at central locations in producing neural maps of 'mixtures' that allow the brain to rapidly identify an 'odour' regardless of whether it emanates from a single odorant or from a complex mixture[4].

Acknowledgements

I wish to acknowledge Anthony Jinks, Andrew Livermore, Geoffrey Francis, Andrew Eddy and Helmut Panhuber who made substantial contributions to many of the studies that will be discussed. Much of the work was funded by the Australian Research Council.

TRAINING NOVICES TO IDENTIFY ODOUR ELEMENTS IN WINE

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Untrained humans can generally recognise an odour that they have previously perceived but are often unable to name even some of the most familiar odours in the absence of non-olfactory cues, a phenomenon known as "tip of the nose" phenomenon. However, trained humans such as wine experts, perfumiers and trained sensory panels do not display this deficit, despite not possessing superior sensory abilities. In an initial set of experiment, participants were trained to identify odour solutions AX, BX and CX based on the unique element in each mixture (Vanilla, Melon and Banana respectively). Participants who were given the veridical labels were able to do so consistently above chance, but were not able to learn to consistently apply non-veridical labels to these solutions unless they were self-generated. In subsequent experiments, wine novices were trained to apply either varietal labels (eg Shiraz), appropriate descriptor labels (eg Shiraz = pepper) or self-generated descriptions to three red wines, but were unable to do so above chance, suggesting that these labels are essentially irrelevant for wine novices in this context. The implications of these findings and future research plans will be discussed.

EXPLORATIONS INTO ODOUR-COLOUR SYNAESTHESIA – WHAT THE NOSE SEES

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Synaesthesia refers to a condition where stimulation in one modality automatically and involuntarily evokes extra sensations in the same or another modality. The most common form is where visual stimuli, such as letters or words, are perceived as inherently coloured. Synaesthetic reactions to odours and tastes are much more rare. Four odour-colour synaesthetes from Sydney were tested and retested on 18-20 odours. Synaesthetes were asked to attempt to name the odours and rate them on a number of dimensions such as liking, intensity, strength of odour and strength of synaesthetic reaction. Finally, participants were asked to draw their visual experience of the odours either on a computer or on paper. Twenty non-synaesthetes were also recruited and followed the same procedure for comparison. Initial results will be presented, including a discussion of upcoming analyses and potential future experiments.

EXPLORING COMPREHENSIVE TWO-DIMENSONAL GAS CHROMATOGRAPHY APPROACH FOR IDENTIFICATION OF POTENT ODORANTS IN WINE AND COFFEE BEVERAGE

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A hyphenated system comprising gas chromatography-olfactometry (GC-O) integrated with comprehensive two-dimensional gas chromatography-FID (GC×GC-FID) configuration was evaluated in this work for aroma analysis of volatile constituents in wine and coffee beverages. A column set consisting of a first dimension (^{1}D) DB-FFAP phase, and a short column of ^{2}D DB-5 phase was applied to achieve the desired GC×GC separation of the volatile extract isolated using solid phase extraction (SPE). Whilst GC results in many overlapping peaks, GC×GC allowed unravelling of those co-eluting clusters of compounds which coincided with the odoriferous fractions. The character-impact odorants were identified through data correlation with the results obtained using other GC×GC systems, both coupled with Time-of-Flight Mass Spectrometry (TOFMS), as well as with Flame Photometric Detection (FPD) for sulfur speciation. Odorants of 2-methyl 2-butenal, 2-ethyl-5-methyl-Pyrazine, 2-Octenal, 1-Octen-3-ol, 2-Furancarboxaldehyde, furfuryl methyl sulfide and 3-Mercapto-3-methyl-1-butanol, methylbutyl formate, 2-methoxy-3-(2-methylpropyl)-pyrazine, 1-(2-Furyl)-2-propanone, 2-furanmethanol and iso-valeric acid were suspected to be particularly responsible for coffee aroma. In addition to the high odour impact of ethyl 3-hexenoate, the volatile sulfur compound 2-mercapto-ethyl acetate was discovered to contribute a fruity, brothy, meaty, sulfur odour to some Australian wines.

Session 4: Human olfaction and taste

PLENARY LECTURE III

OLFACTION AND NEUROLOGICAL DISEASE

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It is now generally accepted that olfactory loss is an early sign – perhaps the earliest sign – of such neurological diseases as Alzheimer's disease and Parkinson's disease. My presentation will provide an overview of the olfactory system, how it is quantitatively evaluated, and how its dysfunction is associated with a range of neurological and psychiatric diseases. Theories as to why olfaction is involved will be discussed.

OLFACTORY MARKERS OF NEURODEVELOPMENTAL ARREST & PSYCHOPATHOLOGY RISK: MAPPING AFFECT DYSREGULATION

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Developmental delay (mismatch) is normal in adolescence, where emotional arousability triggered at puberty stimulates subsequent maturation of prefrontal cortical (PFC) systems; these systems mediate language mechanisms the enable regulation of emotion. Interruption (e.g. trauma, latent genetic vulnerability) to PFC maturation during adolescence usually involves affect dysregulation and may be associated with developmental arrest, lag, and/or degeneration of limbic-PFC systems, along with associated deterioration in psychosocial functioning. Most neurodevelopmental disorders implicate compromise of PFC regions. As olfactory identification (OI) relies upon PFC integrity, essentially where language is requiring to frame lower-order (limbic) arousal once odors are detected, and where competency parallels prefrontal maturation, we have predicted and found OI deficits across a range of neurodevelopmental disorders. Utilising the University of Pennsylvania Smell Identification Test we have found OI deficits in Autism Spectrum Disorders (n= 60), ADHD (n = 40), OCD (n=31), PTSD (n = 31), Forensic (Antisocial Personality Disorder; n= 40), Depression (n=40) and in High Risk- (n= 84) and first-episode psychosis (n=74) cohorts. Implications of these findings are discussed in terms of identifying affect dysregulation via utilisation of OI tasks as a potential predictor for psychopathology. Particular reference is made to subgroups characterised by aggression problems.

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HIGH PREVALENCE OF QUALITY-SPECIFIC TASTE DISORDERS IN ABORIGINAL AND NON-ABORIGINAL CHILDREN: EVIDENCE FOR THE DISTRIBUTION 'QUALITY-BEST' FIBRES IN THE HUMAN CHORDA TYMPANI

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A recent screening study indicated a high prevalence of taste dysfunction (8.2%) in Aboriginal children almost all of whom experience otitis media (OM) early in life. The present study aimed to confirm the latter finding and to compare the prevalence of taste disorders in 432 Aboriginal (n = 166) and non-Aboriginal (n = 266) children matched for age, gender and living in the same general and educational environment. Taste function was assessed using a 25 sample taste identification test comprised of 5 concentrations each of sweet, salty, sour and bitter tastes and water.

A high level of quality-specific taste disorders was found in 20/166 (12.0%) and 21/266 (7.9%) Aboriginal and non-Aboriginal children, respectively. Of the 41 children with disorders 27 (65.9%) had a sweet disorder. Commonly, an affected child had more than one quality disorder but no child was ageusic to all four qualities. The cause of the disorders in most of the children is likely to be OM with the higher prevalence of disorders found in Aboriginal children reflecting their more frequent experiences with OM. Growing evidence of taste dysfunction causing eating disorders leading in particular to obesity, indicates the need for a wider investigation of taste disorders and the consequences and management of this type of dysfunction. Interestingly, the finding of 65.9% of sweet disorders suggests there is a preponderance of sweet-best fibres in the chorda tympani providing yet another example of species-dependent distribution of 'sweet, salt, sour and bitter-best' fibres in this nerve.

VARIATION AMONG HUMANS IN THEIR ABILITY TO DETECT FLAVOUR COMPOUNDS AND ITS IMPACT ON FOOD PREFERENCE

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The biology behind our sensory ability is complex, controlled by a multitude of interacting genetic and environmental factors. To date more is known about the environmental influences on sensory perception such as culture and branding, but the human genetics revolution has presented the opportunity to identify the genetic determinants of sensory ability, and by proxy, whether food preferences have a genetic basis. The Plant & Food Research Gastronomics programme seeks to examine the relationships among variation in the ability to detect various flavour compounds important to New Zealand's food and beverage sector, human genetic variation, and food and beverage preference and liking. The ability to detect these various flavour compounds varies markedly among individuals. Individuals at the opposite ends of the spectrum of sensory abilities have markedly different flavour experiences, as judged by liking scores and the relative importance of terms that they use to describe the aroma. Genome-wide association using SNP arrays has been used to localize regions of the genome associated with sensory ability for these compounds, with a region on chromosome six containing a family of odorant receptor genes found to be associated with cis-3-hexenol perception. Finally, associations with sensory ability, genotype and liking are beginning to be tested in model food and beverage scenarios.

Session 5: Invertebrate olfaction and taste

ODORANT DETECTION IN *CAENORHABDITIS ELEGANS*

Harleen Basrai[1,2,] Carolyn Behm[1,] Chunyan Liao[2], Julie-Anne Fritz[1], Mira Dumancic[2], Alisha Anderson[2] and Stephen Trowell[2]

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Olfaction is a process fundamental in determining the survival success of *Caenorhabditis elegans*. Olfaction informs processes such as egg laying, locating food and responding appropriately to adverse environmental conditions. Odorant molecules present in the environment bind to olfactory receptors (ORs) expressed on olfactory sensory neurons (OSNs). This initiates signal transduction and behavioural responses. In nematodes. ORs belong to the G protein coupled receptor (GPCR) superfamily. Gaining a better understanding of ORs is important for both unravelling the complexities of the *C. elegans* olfactory system and application in biosensor research.

The first aim of my honours project was to confirm whether a double reporter system can be used to identify the location of expression of putative OR genes for the identification of potential ORs. The hypothesis being that, if a reference strain of C. elegans expressing an OSN-specific promoter fused to GFP is crossed with a test strain of *C. elegans* expressing a putative OR promoter fused to mCherry, their progeny will indicate whether the putative OR is co-expressed in a specific pair of ORN/s because co-localisation of GFP and mCherry would generate yellow fluorescence.

The second aim of my honours project was to determine whether wildtype *C. elegans* can respond behaviourally to a broad range of chemicals found in the headspace of explosives. Using chemotaxis assays, I found that *C. elegans* has a sparse expression of receptors tuned to these chemicals. This is may be due to the absence of strong selection pressure for detection of such synthetically manufactured odorants.

DISSECTION OF A COMPLEX OLFACTORY PHENOTYPE IN *DROSOPHILA MELANOGASTER*

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In an effort to identify genes that affect olfactory function in *Drosophila*, we isolated *O88*, a recessive mutation located on the second chromosome. Mutant flies show a reduction in extracellularly recorded neuronal signals from antennae and maxillary palps. Electroantennograms (EAGs), derived from combined receptor potentials across a population of neurons, are significantly reduced to all tested odorants.

In insects, odorant receptor neurons (ORNs) are wrapped in accessory cells and enclosed in sensilla, effectively compartmentalizing the olfactory epithelium. In recordings from single sensilla in *O88* flies, sensillum potentials show different levels of reduction depending on the sensillum type and the odour tested. However, action potential firing rates appear to be unaffected. If sensillum potentials are summed receptor potentials, how can they be reduced without affecting neuronal firing rates?

We show that this apparent discrepancy is due to a lack of electrical insulation between sensilla observed in wild type flies. As a result, sensillum potentials reflect receptor potentials from the ORNs housed in the sensillum recorded from, combined with signals from neighbouring sensilla. Detailed analysis of the reduction profile of sensillum potentials in *O88* mutants reveals that they are mostly reduced for ligands of ORNs housed in surrounding sensilla.

We hypothesize that the mutation affects the level of inter-sensillum leakage. The underlying mechanisms could well explain the nature of EAGs, which are widely used in research on olfaction in insects. Electrophysiological and genetic experiments are being performed to test this hypothesis. In addition, progress towards identifying the gene mutated in the *O88* mutant will be presented.

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COMPARISONS OF CONTACT CHEMORECEPTION AND FOOD ACCEPTANCE BY LARVAE OF THE *POLYPHAGOUS HELICOVERPA ARMIGERA* AND THE *OLIGOPHAGOUS BOMBYX MORI*

Hui-Jie Zhang[1,2], Cécile Faucher[2], Alisha Anderson[2], Amalia Berna[2], Stephen Trowell[2], Quan-Mei Chen[1], Qing-You Xia1[*], Sylwester Chyb2[*]

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We compared gustation between fifth instar larvae of *Helicoverpa armigera*, a generalist pest, and B*ombyx mori,* a specialist beneficial. *B. mori* feeding was more sensitive to salicin (EC_{50} = 4.9 ± 2.5 mM) than to caffeine (EC_{50}≈100 mM). Feeding inhibition was reversed for H. armigera which was tolerant (EC50≈200 mM) of the highest levels of salicin found in natural sources but more sensitive to caffeine (EC50≈10 mM). A single gustatory receptor neuron (GRN) in the medial styloconic sensillum of B. mori was highly sensitive to salicin and caffeine. The styloconic sensilla of H. armigera did not respond consistently to either of the bitter compounds. Two *B. mori* GRNs, located in the lateral styloconic sensillum, respond specifically to inositol and sucrose whereas, in H. armigera, sucrose is sensed by a GRN in the lateral sensillum and inositol by a GRN in the medial sensillum. Inositol responsiveness in both species occurred at or below 0.1 mM, a naturally occurring concentration. Behavioural responses of larvae have complex determinants that may include specific host range, metabolic capacity and gustatory repertoire.

KEY WORDS:

food preference, gustation, styloconic sensillum, gustatory receptor neuron, sugar response, bitter response

SNIFFING OUT GRAIN INFESTATIONS WITH THE RED FLOUR BEETLE

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Stored product insect pests, such as the red flour beetle (*Tribolium castaneum*), produce pheromones and other volatile chemicals that can betray their presence. They also detect these odours with high sensitivity to find mates or suitable food sources. This sensitivity and specificity relies on odorant receptors (ORs) and would be ideal for detecting infestations for targeted pest management.

We are taking two approaches to identify which of the many ORs annotated in the *T. castaneum* genome can detect infestation odours.

The first focuses on sex pheromones, since these are produced early in an infestation cycle. We hypothesise sex pheromone receptors will show sex-biased expression and are therefore examining OR expression in the heads of male or female beetles. Some differences in expression levels have been observed using real-time quantitative PCR and our search for a receptor solely expressed in one sex continues.

The second approach is based on the hypothesis that both larvae and adults can detect infestation odours in order to find food or breeding sites. This hypothesis was confirmed by behavioural studies in an olfactometer with infestations of *T. castaneum* or two other grain pests: *Rhyzopertha dominica* or *Sitophilus granarius*. Behaviour toward synthetic pheromones and beetle odours is now being investigated. We will then determine which ORs are necessary for detecting infestation odours by using RNA interference to attenuate the expression of each OR in turn.

With this two-pronged approach, we aim to identify ORs for application in protecting stored products, such as grain in silos or railcars, through early detection of infestation.

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Abstracts – Poster Session

THE INITIAL AXON OUTGROWTH FROM THE OLFACTORY EPITHELIUM

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The olfactory system provides an outstanding model that allows for the understanding of the mechanisms that drive neurodevelopment and axon-glia interactions. This system is unique because new neurons are constantly generated from stem cells that line the basal layer of the olfactory epithelium. The glia of the olfactory system, the olfactory ensheathing cells (OECs), are thought to be essential for the regenerative capacity of the olfactory system. However the initial outgrowth of axons and the interactions with OECs during early development are poorly understood. To visualise olfactory axons in early development we used OMP-ZsGreen transgenic mice at the ages E10.25 to E13 (n=3 at each age). The bright fluorescence of the ZsGreen enabled us to view growth cone morphology and track the trajectory of the axons as they exited the basal layer of the olfactory epithelium and projected into the central nervous system. Using the ZsGreen axons, combined with a more sensitive immunohistochemistry protocol, we have identified that olfactory sensory neurons first arise at E10.25 and their axons penetrate the telencephalon at E11.0. At E10.75 we have also identified the presence of dendrites projecting from the olfactory neurons. OECs migrate ahead of the axons and establish the pathway through which the axons extend which can be seen from as early as E11. These results demonstrate that the establishment of the olfactory nerve pathway is dependent on the migration of OECs and that olfactory axons penetrate the olfactory bulb earlier than previously thought.

OVERVIEW OF E-NOSE (MK1-4) IN ENVIRONMENTAL, SECURITY AND HEALTH APPLICATIONS

Graham Bell

E-Nose Pty Ltd

Electronic noses (e-noses) were developed by E-Nose Pty Ltd from 2003 and before that by our team of collaborators at CSIRO, UNSW and U Sydney. Our early work (1990s) funded by a CRC, was on novel host-gas materials and an optical e-nose consisting of an array of materials with chemically sensitive optical properties. From 2000-2003 we worked on a hard-wearing e-nose, with a small sensor array, for meat processing odour and developed the Mk 1 and 2 E-Noses. Five devices were placed with abattoirs in NSW, SA and Queensland. These showed that long term odour monitoring was useful to environmental management in these settings (1). Two devices were developed for the Sheep CRC, with which early detection of fleece infections by e-nose, not detectable by other methods, was demonstrated (2). An independent human health study recently showed discrimination by our E-Nose of the breath of patients with lung cancer, other respiratory diseases, healthy smokers and non-smokers (3). Early detection of lung cancer by e-nose remains a realistic goal. The Mk 3 E-Nose evolved to meet the need to measure air pollution emissions from sewage plants, waste dumps, oil tanks, and smelly industrial plants (4). Studies have been carried out in Australia, Chile, South Africa and Hong Kong using approximately 30 Mk 3 devices in various contexts. Results have assisted in identifying and understanding sources of odour that cause complaints and informing plant operators of when and how they might address odour emissions. This led to a Mk 3 E-Nose specialized for paint fumes and ink solvent detection, now called *graffit-e-nose* ® which has been successful in moderating costs of graffiti vandalism in Southern Sydney. Our latest device, Mk 4, is a platform for the previously developed sensor arrays but includes an on-board odour recognition algorithm (5), cell phone and a global positioning system amongst other electronic features. Our first 12 devices, now in production, will be put to work in security and anti-graffiti vandalism applications, while work is completed on an air monitoring version.

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AACSS 2010: Abstracts continued

AROMA CONSTITUENTS OF ULTRA-PREMIUM SHIRAZ WINE

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Shiraz grapes account for one-fifth of the all Australian vine plantings. Despite the importance of Shiraz to the Australian wine industry, little is known about the aroma compounds that are the key contributors to the perceived aroma and flavour of premium quality Shiraz wine. The objective of this study was to perform a detailed investigation of two commercial icon Australian Shiraz wines, one from a cooler and the other from a warmer grape-growing region, to characterise the constituents relating to the aroma. To enable this, automated Dynamic Headspace (DHS) sampling, coupled with Gas Chromatography-Olfactometry (GC-O) and mass spectrometric detection was employed. In addition, detailed chemical analyses were performed on the wines to quantify the aroma compounds, including alcohols; esters; acids; low molecular weight sulfur compounds; oak-derived volatiles; volatile phenols; norisoprenoids; monoterpenes and rotundone. Sensory descriptive analysis was also undertaken to profile the different characteristics of the wines.

Our findings increase the knowledge about Shiraz wine aroma and constitute progress towards a fundamental understanding of the key contributors for quality in Shiraz wine. Further work will involve reconstitution studies of the wines to test the key aroma compounds and their use as quality indicators.

MODULATING EFFECT OF ODOURS ON ATTENTIONAL BLINK IN HUMANS

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The sense of smell is our most ancient and most basic sense. It plays a crucial role in fundamental processes of everyday life, although we are not aware of how much we use it. Odours have strong effects on human emotion and episodic memory. Background odours have even been found to modulate performance on certain working memory and sustained attention tasks [1,2]. Although it is well known that vision influences olfactory perception and identification [3,4], very few studies have clearly demonstrated that odours can influence visual perception [5,6].

We investigated whether odours have an effect on a well-established cognitive phenomenon, the 'attentional blink'. When two visual targets are presented in a rapid serial visual presentation (RSVP) task, detection and identification of the second target is impaired if it is presented within 400ms of the first target. It is believed that processing of the first target prevents detection of the second target due to a bottleneck in the temporal allocation of selective attention. In our novel attentional blink paradigm we had participants monitor a rapid stream of coloured photographs at fixation, and to discriminate which of two possible objects with a characteristic smell (lemon, orange, rose, mint) appeared after an initial target. During the visual task, participants were exposed to an odorant that was either congruent, incongruent or neutral with respect to the second target object. We will present first results regarding the effect of these odours on the attentional blink.

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DOES EATING WITH SOMEONE MAKE FOODS GOOD?

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When you ate some foods with your friends and/or family members, it tasted very good. On the other day, you had a chance to eat them again by yourself, it was not so good. We experience these phenomena well in our daily lives, however there are almost no scientific studies approaching to these phenomena. In this study, ratings for taste and palatability of confectioneries were compared between when they were eaten with friends and/or family members and when eaten alone. Thirty six female university students participated in this study, and were asked to eat two kinds of chocolate and two kinds of cookies in a laboratory (alone) and in their home (with someone). Palatability ratings were higher when they were eaten with someone in their home. These results suggested that human perceptions of taste and food palatability were affected not only by foods but also by contextual and social variables.

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THE COLOR OF FLAVOURS

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White and Prescott (2007) suggested interaction of taste and smell bases on Stroop effect. In an almost same manner, flavor expected to interact with color. Here I show some data on effects of color on flavor perception.

1. The color of packages of chocolate punched in Japanese market has a type: sweet milk chocolate is in red, and bitter dark chocolate in Black. Participants reported sweeter when they taste a chocolate in red package than when they taste same chocolate in black package.
2. Participants reported strongest fishy smell to tuna when they taste tuna with a photograph of sushi in red color, compared to other colors (white, yellow and blue). It is because we know red fishes have strong taste by our personal experience.
3. When participants presented a translucent syrup including strawberry and melon flavors, the numbers of the participants reported the flavor as strawberry and as melon are same. When the syrup was colored in red, almost all participants reported the flavor as strawberry.
4. A beverage served in a yellow cup was evaluated most sour compared to other colors (pink, blue, and green). When it was served in a blue cup, it was evaluated coldest.

These results supported interaction of flavor with color bases on association between them in our daily eating experience. The memory for this cross modal association affects flavor processing as a manner of top-down effect: the cross modal Stroop effect ■

Upcoming Events

31 January – 3 February 2011	**Australian Neuroscience Society Annual Conference** Auckland, New Zealand www.ans.org.au
9-10 March 2011	**EcoForum Conference and Exhibition 2011** Australian Technology Park Sydney www.ecoforum.net.au
13-17 April 2011	**33rd AChemS** Tradewinds Resort, St Pete Beach Florida USA www.achems.org
3-5 May 2011	**ISOEN 2011** New York, USA www.olfactionsociety.org
4-8 July 2011	**20th International Clean Air and Environment Conference** Christchurch, New Zealand www.casanz.org.au
10-14 July 2011	**9th Pangborn Sensory Science Symposium** Bangkok, Thailand www.pangborn2011.com

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