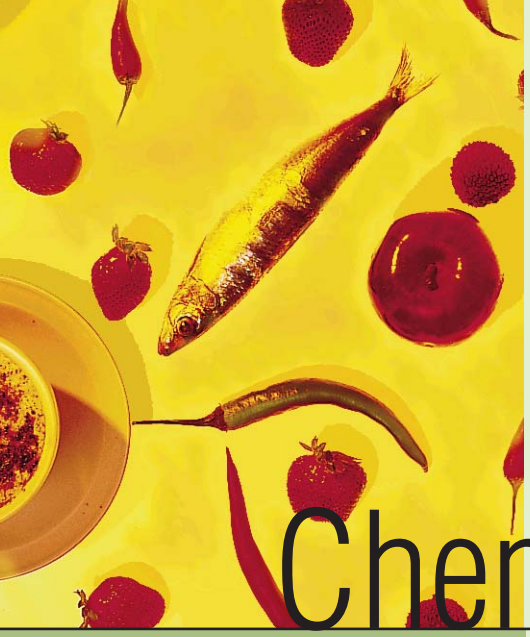

Vol. 12 No.1 December 2009

Chemo Sense

Editorial

By Graham Bell
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Learning to Taste

In the 1950s, children in Australia were given a small bottle of milk in the classroom, every day, as part of a post-war national nutrition program. The milk had often been warmed in the sun outside the classroom for too long. Nevertheless, the order was that each child must drink his/her share. The result of enforced ingestion of soured milk has left a life-long aversion to drinking milk in some of those children. Early sensory experience can be the basis for long lasting dietary habits.

On the positive side, as Sari Mustonen and Hely Tuorila show in this issue, sensory experience delivered in a systematic educational program, will decrease fear of trying new foods by young children. Sensory education may therefore provide an important

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Sensory education of school children: What can be achieved?

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More than twenty years ago, French authors Puisais and Pierre (1987) published the book *Le goût et l'enfant* that describes a program to activate school children to using their senses in food perception. The program consists of practical exercises, with ten lessons demonstrating sensory perceptions in different modalities, their interactions and their implications. This program was the basis for a combined effort in which several European institutions worked to bring a sensory education program to public schools. There remains inadequate information of the impact of the program. In this article we describe our efforts to scientifically quantify the value and effect of sensory education.

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INSIDE:

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Editorial
continued

learning experience for children lacking a sufficiently "enriched" home environment. “Learning to taste” may prevent development of unhealthy, restrictive eating habits that contribute to childhood obesity or other eating disorders.

Another successful AACSS conference took place recently at Heron Island on the Great Barrier Reef. The abstracts are reproduced herein. The next AACSS conference will be in or near Melbourne in 2010 and in New Zealand in 2011. A return to Heron or a similar island is planned for 2012. Plan to join us.

The *ChemoSense* team wishes our valued readers, authors and advertisers, a happy holiday, health and prosperity in 2010 ■

Sensory education of school children: What can be achieved?
continued

Introduction

Information concerning children’s use of their senses of taste and smell, and learning food preferences, is still rather scarce. Flavor preferences established early in life are known to track into later childhood (Mennella et al., 2005). Birch (1979) found that children under 5 years base their food preferences on two dimensions: sweetness and familiarity. Children tend to like sweeter, saltier and also, to some extent, more sour taste intensities than adults (see Popper & Kroll, 2007).

A common belief is that children are more sensitive to smell and taste stimuli than adults, but no such differences have been scientifically proven (Guinard, 2001). The misconception may be due to neophobia, the tendency to reject new or unfamiliar foods, being interpreted as a sign of sensitivity. Neophobia is a protective mechanism preventing animals and humans from eating what could be harmful to them. Pronounced neophobic behavior in children is probably due to the lack of earlier experiences on a large variety of foods (Birch, 1999), as exposures to unfamiliar foods can reduce neophobic reactions towards other novel foods, at least those similar to familiar foods (Birch et al., 1998). As children grow, their experiences of different foods accumulate, helping them to become less neophobic because fewer foods are novel to them (Cooke & Wardle, 2005). Food neophobia appears in all age groups, and its strength varies between individuals (Pliner & Salvy, 2006). Using a multi-item verbal instrument for the measurement of food neophobia (Pliner & Hobden, 1992), neophobia was shown to be a heritable trait (Knaapila et al., 2007). Although considered to be a personality trait, food neophobia may decrease during childhood or adolescence, thus being an age-dependent state (Rigal et al., 2006). Neophobia tends to bias choices towards familiar and thus, “safe” foods.

In France, Puisais and Pierre (1987) developed and described “taste lessons” (*Classes du goût*). This education program for school children has been used in several European countries, including Sweden (Hagman & Algotson, 2000). Children’s sensory awareness is increased through exercises that focus on their senses and appeal to their attention and curiosity. The program is intended to teach children about the pleasures of food. Overall, the “taste lessons” aim to educate children to become well-informed and alert consumers who pay attention to the quality and differences between foods and trust their senses in this process.

The Present Study

We conducted a 2-year follow-up study with Finnish school children, representing two age groups (in the beginning of the study 8 or 11 years old) to track the development of chemosensory awareness and food related perceptions during the intervention study (Mustonen et al., 2009; Mustonen & Tuorila, 2010). Of the 244 children recruited, 175 participated in all follow-up measurements in the laboratory and 164 questionnaires were received back from the parents. One-half of children received either one or two sets of lessons of taste education. The first lesson set was *Classes du goût* program adapted to Finnish circumstances, and the second set consisted of activating lessons on separate food categories. The second lesson set of education was run only to 2/3 of the education group, to examine whether the children would benefit from prolonged education. The education sets are described

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Sensory education of school children: What can be achieved?

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in detail by Mustonen et al. (2009). The other half of the children served as a control group, thus they performed baseline and follow-up measurements, but did not receive sensory lessons.

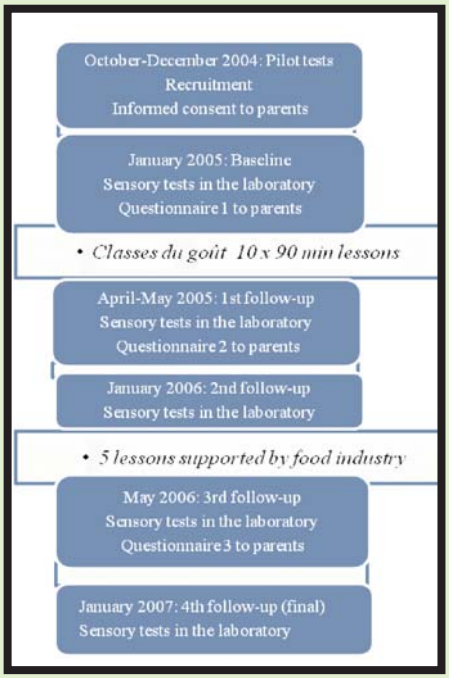

Figure 1. Sensory education: design of the intervention study

The effects of the sensory education were studied with

1) Sensory tests conducted in the laboratory (free naming of five odors, and their pleasantness and intensity ratings; taste identification of six aqueous solutions (sweet, salty, bitter, sour, umami, and water); descriptive characterization of two breads (different samples at each follow-up measurement); and aided odor naming (5 odors, list of 10 verbal labels provided);

2) Questionnaires assessing food neophobia and children's liking of and familiarity with unfamiliar and familiar foods, sent to parents three times during the follow-up period.

Age group	Sweet (2% sucrose)	Salty (0.2% NaCl)	Sour (0.04% citric acid)	Bitter (0.04% caffeine)	Umami (0.3% monosodium glutamate)
8-year-old (n = 75)	78	52	47	18	21
11-year old (n = 100)	79	69	56	31	25

Table 1. Correct taste identification (% of the age group) at the baseline, aqueous solutions.

The schedule is described in Figure 1. Sensory tests were conducted a total of five times and the questionnaire was completed by the parents three times during the study period.

Sensory Influences

The sensory educated children improved their skills in identifying tastes and odors, and characterizing foods, while no change was seen in the performance of the control group. Effects of sensory education were mainly observed in the younger, but not in the older children.

At the beginning of the study, most of the children identified sweet and salty taste and half of them identified sour taste (Table 1). Umami (described to children as "meaty") and bitter tastes were identified by less than one-fourth of the children. Younger children were poorer in identification of salty, sour, and bitter taste. For sweet and umami, the identification rates were approximately similar in both age groups. Within the younger age group, boys and girls identified the tastes approximately similarly. In the older children, the boys identified the bitter taste better than girls (boys 41%, girls 19%), whereas the girls identified sweet and salty tastes slightly better than boys (approx. 10% difference). In our pilot study with a larger sample of 8-, 9-, 10- and 11-year-old children (n = 348), the results were similar in that 82, 66, 52, 27 and 23% of children identified sweet, salty, sour, bitter, and umami tastes (Oerlemans et al., 2006). The age groups performed differently only in the identification of salty taste: 8-year-olds did not reach the level of the older children.

Sensory education improved the identification of the most difficult taste, umami, in the younger age group, whose identification rate at the baseline was 8.3% and in the final measurement 30.5% (Figure 2). Umami was a new term for almost all of the children and the curiosity towards this new taste may have facilitated the identification. Overall, taste identification of the younger children improved after the first education period and remained more or less at the achieved level throughout the study period. In the older group, no clear improvement was observed.

Education effect was seen also in the sensory descriptive skills, as the number of descriptive words for breads increased in the younger children of the education group during the study period, while for the control group the number of words was approximately the same for the whole study period. In the older children, both the education group and the control group, the number of descriptive words surprisingly decreased from baseline to the final follow-up measurement. Overall, the number of descriptive words was higher in the older than in the younger children. In the sensory characterization of breads, older children used a greater number of words than did the younger. Similarly, verbalization of sensory experiences has been shown to be

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Sensory education of school children: What can be achieved?

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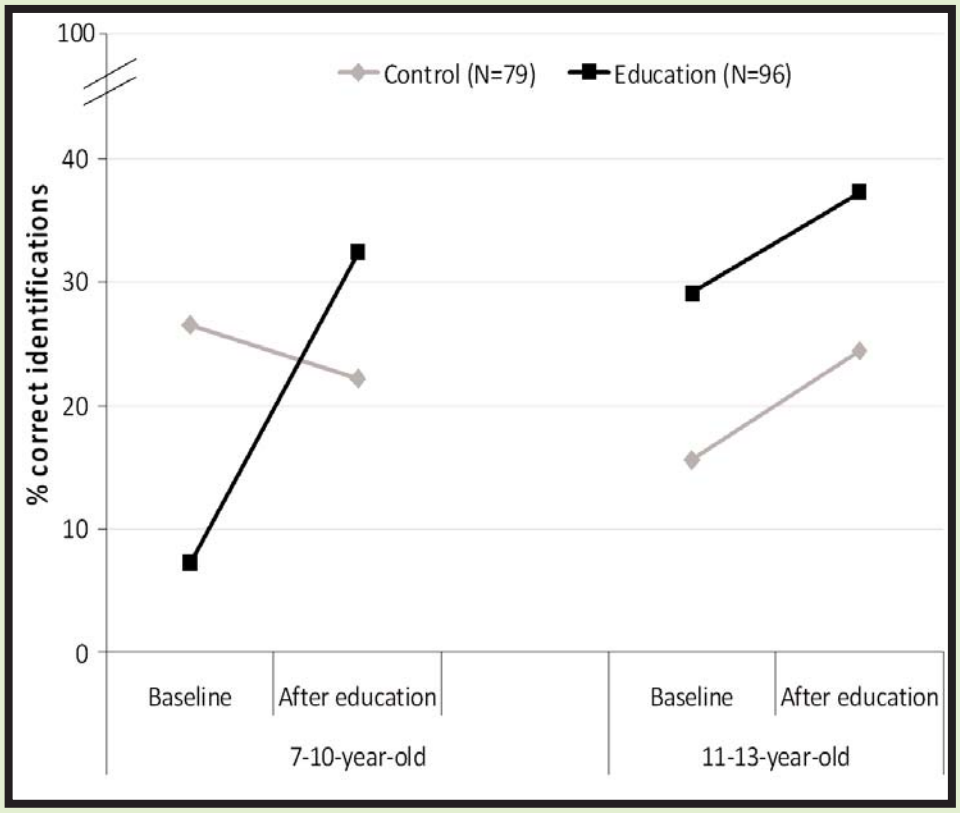

Figure 2. The identification of umami was significantly improved in the younger age group after the sensory education, while no difference was seen in the control group. In the older age group, improvement was seen in both control and education groups (Mustonen et al., 2009).

supported by taste lessons in 12-year-old Swedish children (Jonsson et al., 2005). In contrast, Sune et al. (2002) found that despite attending the taste lessons (*Classes du goût* program), French children described chocolate in their own way that was different from that of adults.

Although the improvements in the chemosensory awareness and descriptive skills were small and not always consistent over the study period, their general direction was encouraging. Based on the findings, sensory education has potential to activate children's chemosensory awareness and improve their attention towards foods and their skills to describe foods.

Responses to Unfamiliar Foods

Clear education effects were seen in children's increased preparedness to taste foods they had not tasted before. The unprejudiced disposition to new food experiences was seen also in the decreasing food neophobia score. Figure 3 shows that after the sensory education sessions, both education groups had tasted a larger number of the queried ten unfamiliar foods than at the baseline. In the control group, the number of tasted foods remained at the baseline level throughout the study period. The effect was significant for the younger, but not for the older age group, despite the similar trend (Figure 3).

The sensory education program aimed to encourage variety in eating, by exposing children to diverse foods during the lessons. This aim was accomplished, as shown at the end of the study, when children in the education groups had tasted a larger number of unfamiliar foods than at the baseline, while no change was seen in the control group. The greatest increase was in the younger children of the group receiving two lesson sets of sensory education.

Sensory education decreased food neophobia scores. The effect was strongest in the group receiving both lesson sets of education. In the younger group of children receiving only one education session, food neophobia scores decreased and remained at the same level until the end of the study. In the older children, food neophobia tended to decrease after the first education lesson set, but in the final measurement nine months after the education their food neophobia tended to rise again. Similar to the French study (Reverdy et al., 2008), the effects of sensory education on the decrease in food neophobia seemed to be only temporary in the older children. By contrast, the younger children maintained the increase in the number of tasted foods and the decrease in food neophobia scores over the one-year period even when they did not receive further lessons, suggesting a more stable effect.

Conclusions

The measureable effects were more pronounced in food neophobia related issues than in chemosensory indicators. The relatively minor effects seen in the chemosensory indicators may be due to their insensitivity, but equally well to the quality and extent of sensory intervention. After all, the maximum number of lessons

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Sensory education of school children: What can be achieved?

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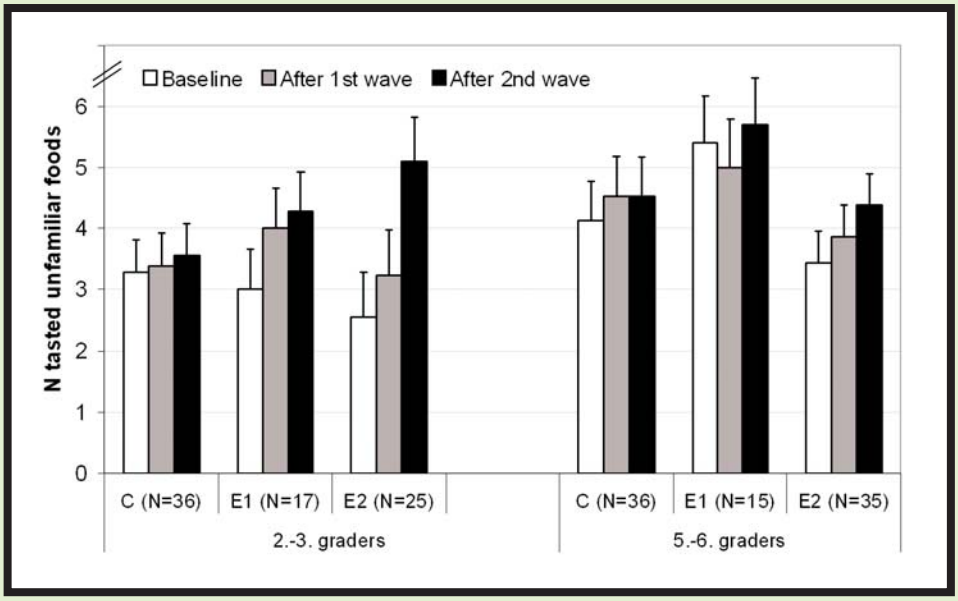

Figure 3: Mean number (+SEM) of tasted foods among the10 most unfamiliar foods (at baseline, after 1st education set and in the end) of the control group C (N=72, no sensory education), in the experimental group E1 (N=32, one set of sensory education), and in the experimental group E2 (N=60, two sets of sensory education). Number of tasted foods was increased in the younger age group receiving two sets of sensory education (Mustonen & Tuorila, 2010)

was fifteen and they were conducted over the time span of two years. Given the many activities and other development during the intervening interval, it may be unrealistic to expect very strong effects.

The effect of sensory education was stronger in the younger children, suggesting a suitable age to start the sensory education program could be eight years (or even earlier). Similar results were achieved in France (Reverdy et al., 2008). At younger age, flavor preferences and eating habits are still forming (Nicklaus et al., 2004). This has also been shown in rats: in a study by Capretta et al (1975) an exposure-to-novel-foods manipulation had an effect on younger but not on older rats (although the older group were adult rats). Thus, the sensory and cognitive information given in the sensory lessons can effectively intervene in the development of food preferences, if given to young individuals.

Our research on the impact of sensory education program paralleled the corresponding French study (Reverdy et al. 2008), and the outcome was substantially similar. A recent comparison of the odor worlds of French and Finnish children suggests that the children in these two European countries are by and large at the same level with respect to their chemosensory attention, interest and olfactory performance (Ferdenzi et al., 2008). We therefore assume that results can be generalized to Western countries.

The Potential Value of a Sensory Education Program

Sensory education appealed to children as well as to their parents and teachers. It provided a positive and enjoyable way of highlighting food-related sensory issues in the school, as seen in focus group interviews of children and parents (Salo, 2007), and teachers (Huotilainen, 2005). At its best, sensory education encourages children to try different foods and pay attention to food quality, which may lead to more healthy and balanced eating habits. Positive attitudes towards food together with preparedness to taste unfamiliar foods should serve as an incentive to set up sensory education lessons as part of school program of young children. Due to these encouraging findings, further applications of sensory lessons for school children, and also for preschool children, have indeed been started in Finland.

Acknowledgements

This work was supported by grants from the Ministry of Agriculture and Forestry, Finland, and the Finnish Innovation Fund (Sitra). We are indebted to the children who participated in the study as subjects, and to their parents and teachers for making the study possible.

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Sensory education of school children: What can be achieved?

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AACSS 2009: Abstracts

Abstracts of the Australasian Association for ChemoSensory Sciences
11th Annual Meeting
2-6 December 2009
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ABSTRACTS – Day One, Session 1

CONCENTRATION INTERACTIONS CHANGE THE NO PRODUCTION PATTERNS IN THE ANTENNAL LOBE OF MANDUCA SEXTA

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Nitric oxide synthase, the enzyme that produces nitric oxide in response to a calcium signal, is present in the olfactory systems of almost every animal examined to date. We are using the moth, Manduca sexta, as a model system in which to investigate the function of nitric oxide in the processing of olfactory information. Using electrophysiological and optical recording methods we have found that NO is present at low levels at basal states and is actively produced in a glomerular pattern in response to odor stimulation. The odor-induced nitric oxide is apparently confined within glomerular borders and it pattern of production is dependent on the concentration and context of the odor stimulus. Using the optical recording with the NO sensitive dye DAF-FM DA, and the calcium sensitive dye calcium green, we examined the responses to individual odorants at different concentrations and mixtures. We found that the patterns of glomerular activity was not strictly additive with increasing concentration and, more interestingly, that the patterns of activity of calcium and NO were not always the same. These results suggest that local interneuron interaction may play an important role in shaping the NO production pattern within the antennal lobe.

THE GENETIC BASIS OF OLFACTION IN THE LIGHT BROWN APPLE MOTH, EPIPHYAS POSTVITTANA

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The light brown apple moth (Epiphyas postvittana) is a significant horticultural pest in Australia and New Zealand, but also found in Hawaii, the UK and more recently California. We have been developing gene databases and functional genomic tools for the moth, with a long-term goal of developing new sustainable control technologies based on odours and pheromones. From these databases we have found and characterized a number of possible targets on which to base the design of new odour-based control measures, including odorant binding proteins, odorant hydrolases and odorant receptors. Among the odorant receptors we have found two that recognize odours produced by plants, including the important plant signaling compound, methyl salicylate.

HISTAMINERGIC LOCAL INTERNEURONS IN ANTENNAL LOBES OF THE HYMENOPTERA

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The information input to a neural network often undergoes some form of transformation mediated by local interneurons. Thus, the diversity of local interneurons can determine the extent to which information about the environment is refined in the brain. We describe in detail the morphological features of a population of histaminergic local interneurons (HA-ir LNs) in the antennal lobes of bumble bees and the phylogenetic distribution of this population of interneurons within the Hymenoptera. As a population, HA-ir LNs densely innervate the glomerular core while sparsely arborizing in the outer glomerular rind, overlapping with the terminals of olfactory receptor neurons. Individual fills of HA-ir LNs revealed heavy arborization of the outer ring of a single principal glomerulus and sparse arborization in the core of other glomeruli. In contrast, projection neurons, and GABA-immunoreactive LNs project throughout the glomerular volume. HA-ir LNs were present in all but the most basal hymenopteran examined, although there were significant morphological differences between the stinging Hymenoptera and the parasitoids. The ALs of other insect taxa examined lacked HA-ir LNs, suggesting that this population of LNs arose within the Hymenoptera and underwent extensive morphological modification.

ABSTRACTS - Day One, Session 2

THE ANTERIOR OLFACTORY NUCLEUS: WHERE IS IT AND WHAT DOES IT DO?

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The anterior olfactory nucleus (AON) resides just behind the olfactory bulb in the olfactory peduncle. The AON occupies a crucial position within the olfactory circuit as it is able to influence function in nearly every major synaptic processing stage of both the ipsilateral and contralateral pathway. Nevertheless, very little is known about the region internal organization and circuitry. The AON is comprised of two different subregions a) a thin strand of cells encircling the rostral end of the peduncle known as pars externa, and b) the large pars principalis, seen in coronal sections as a thick ring of cells encircling the deep subependymal layer. The present work examines the premise that pars principalis contains functional subunits. Anatomical evidence for the idea includes: a) large cells are densest in lateral and dorso-lateral regions while small cells are more numerous in medial and ventral areas b) cells immunopositive for calbindin are denser in the deep portion of the region though homogenously dispersed , while parvalbumin- positive cells are superficially located and sparse in ventral and medial regions c) CHaT+ and GAD+ fibers are denser in lateral versus medial regions, d) there are distinct patterns of interhemispheric innervation; contralateral fibers are most heavily targeted to dorsal and lateral AON subregions, while the medial and ventral areas receive relatively light projections. Golgi studies, however, suggest few regional variations in pyramidal cell morphology. The results indicate that complex processing occurs in the AON that demands further study. Supported by NIH DC00338

AGEING OF OLFACTORY EPITHELIA: MACROPHAGES THE MAJOR CULPRIT?

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Mucosal epithelia act as physical barriers and, together with phagocytic cells of the innate immune system, they form the first line of defence against microbial entry. In our research, we are particularly interested in how immune homeostasis is maintained at the level of the nasal olfactory epithelium. Because the nasal cavity provides a direct access route to the brain (via the olfactory nerve), it is self-evident that there is a crucial role for the innate immune system to protect the olfactory epithelium and brain against constant microbial infection. It is well established that the epithelial barrier itself becomes less efficient with age due to reduced regenerative capacity and general thinning. These ageing phenomena are paralleled by a decline in olfactory function and increased risk of opportunistic infections. The ageing processes that contribute to epithelial breakdown are not well understood. Our lab has recently reported that olfactory sensory neurons can directly communicate with the

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innate immune system via expression of a chemokine called CX3CL1 / fractalkine on their surface. The sole receptor for this molecule, i.e. CX3CR1, is present on intraepithelial macrophages. We will also report on the use of bone marrow chimeric mice to demonstrate that these intraepithelial macrophages are actively replenished from the circulation and that deficiency in the chemokine receptor CX3CR1 interferes with this process. Finally, we will present data from aged, 20-month old animals showing that CX3CR1-deficient mice lacked the typical signs of epithelial ageing. This unexpected finding appears to suggest that altered immune function and/or macrophages themselves contribute to break-down of the epithelial barrier with age since their reduced presence in CX3CR1-deficient mice appears to provide protection against epithelial ageing.

OLFACTORY ENSHEATHING CELLS PROMOTE AXONAL REGENERATION.

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Olfactory ensheathing cells (OECs) support the regeneration of olfactory sensory neurons throughout life and, unlike other glial cells, can cross the boundary between the peripheral nervous system and the central nervous system. Due to these characteristics, OECs are promising candidates for transplant-mediated nerve repair and have been shown to have a remarkable ability to promote neural regeneration after injury. While it has been proposed that increasing OEC migration will lead to improved axon regeneration, this has not yet been tested. We have examined this in the olfactory system during regeneration of olfactory axons. We therefore developed a regeneration model to examine the effect of OEC migration on olfactory axon regrowth. In this model, the OECs were able to proliferate and migrate, while olfactory axons were initially delayed from regenerating. Later, the olfactory axons were then able to regrow into the cavity that was now filled with OECs. Despite there being significantly fewer neurons (33% fewer) in this animal model, the axons regrew over a significantly larger area (45% more) in comparison to control animal model. We replicated the permissive glial environment by transplantation of OECs and found that although OEC transplantation also enhanced axonal regrowth, the glial environment was not as uniform and did not promote axon regrowth to the same extent. These results demonstrate that olfactory axon regrowth is dependent on the extent of OEC environment and that the creation of a permissive glial environment by OECs prior to axonal growth significantly enhances olfactory axon regrowth.

ABSTRACTS – Session 3, POSTERS

A PERIPHERALLY INTEGRATED ELECTRONIC NOSE

Wenzhi (Winston) Wu and Graham A. Bell

E-Nose Pty Ltd

When deployed remotely, e-noses need to perform their functions either at the device itself or at a remote data-processing station. Our development decision was whether to retain utmost simplicity at the device and do all the necessary processing remote from it, or to design the processing features into the device itself and transmit pre-processed information to a remote receiver for action, and/or simply activate other peripherals or machinery. We have found that needs and conditions vary greatly across the wide range of applications a remote e-nose can perform and that simplification, as far as many industrial users are concerned, actually lies in making the hardware more complex and flexible at the periphery and thereby making the remote data processing more simple, and less dependent on human expertise. We are now putting this to the test by designing an E-Nose to include a suite on onboard functions. These are embodied in removable electronic modules to reduce redundancy and facilitate maintenance and upgrades. Modules will include analog-to-digital conversion; high level odour recognition functions on a chip; data logging; wireless data transmission and user-defined outputs for activating other peripherals such as cameras, valves and responsive machinery. Consistent with the Mk 3 E-Nose will be the inclusion of interchangeable tailored chemical sensor arrays. We report here on our progress to date in reaching these objectives. Acknowledgement: This work is supported by a New South Wales Government Proof of Concept Grant to E-Nose Pty Ltd (2008-2010).

INTRAGASTRIC ADMINISTRATION OF ALLYL ISOTHIOCYANATE INCREASES CARBOHYDRATE OXIDATION VIA TRPV1 BUT NOT TRPA1 IN MICE

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The transient receptor potential (TRP) channel family is composed of a wide variety of cation-permeable channels and shows a great diversity of activation mechanisms. TRPV1 and TRPA1 are cation channels belonging to the TRP channel family that are activated by high (>43ºC) and low (<18ºC) nociceptive temperatures, respectively. Interestingly, they are also activated by spicy or pungent compounds in foods like capsaicin (TRPV1) or allyl isothiocyanate (AITC, TRPA1). Recent studies have indicated that activation of TRPV1 or TRPA1 is involved not only in nociception and thermosensation but also in thermoregulation and energy metabolism. Therefore, we investigated the effect of intragastric administration of TRP channel agonists on changes in energy substrate utilization of mice. Intragastric administration of AITC (a typical TRPA1 agonist) markedly increased carbohydrate oxidation but did not affect oxygen consumption. To examine whether TRP channels mediate this increase in carbohydrate oxidation, we used TRPA1 and TRPV1 knockout (KO) mice. Intragastric administration of AITC increased carbohydrate oxidation in TRPA1 KO mice but not in TRPV1 KO mice. Furthermore, AITC dose-dependently increased [Ca2+]i in cells expressing TRPV1. These findings suggest that AITC might activate TRPV1 and that AITC increased carbohydrate oxidation via TRPV1.

RECOGNITION OF DIETARY FAT IN THE ORAL CAVITY

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Several studies have indicated that rodents and humans recognize the presence of fat in foods not only by the texture of the food but also chemically in the mouth: this suggests that the chemical perception of fat is involved in the acquisition of a strong preference for fat. CD36 is known as a fatty acid transporter in the muscle or adipose tissue. Previously we have reported that CD36 is expressed in the taste bud cells of the posterior tongue. In addition to CD36, we have found that GPR120, a G-protein coupled receptor that functions as a specific unsaturated long-chain fatty acid receptor in the gastrointestinal tract, is expressed in the taste bud cells of anterior and posterior tongue. By immunohistochemical staining of GPR120, we found that GPR120 is expressed in the taste cells of the tongue, similar to the enteroendocrine cells of the gastrotintestinal tract. We next investigated the palatability of various kinds of long-chain fatty acids by assessing licking behavior. Mice showed a higher licking response for unsaturated long-chain fatty acid but not for saturated fatty acid. The palatability of fatty acids for mice is very similar to the ligand specificity for GPR120. These results raise the possibility that GPR120 expressed in the taste cells may be also involved in the chemical reception and palatability of dietary fat in the oral cavity. This study was supported by the Program for the Promotion of Basic Research Activities for Innovative Bioscience.

SENSING THE TROUBLESOME TRIBOLIUM

Kelly Bailey, Richard Glatz & Sylwek Chyb*

CSIRO division of Entomology

Tribolium castaneum is a stored-food pest of significant importance throughout the world. The presence of these and other insect pests in stored grain facilities compromise Australia's export potential since most markets with which we trade specify a zero tolerance for live insects. Current visual methods for the detection of an infestation are not sensitive or reliable, so unless the produce has recently been fumigated with phosphine, pests are assumed to be present and the

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grain is re-treated. This leads to continued selection pressure for phosphine resistance and increases the costs of pest management in the grains industry. A reliable, sensitive, quick and cost-effective method to detect pest infestations would be highly desirable and aid in development of treatment thresholds allowing targeted fumigations, lowering the risks of resistance development in pest strains. Sequencing of the T. castaneum genome has recently been completed and has revealed a large family of putative olfactory receptor genes. It is within these genes that we are searching for one that encodes a target receptor with which to construct a sensing platform capable of highly sensitive detection of the infesting beetles. To do this, we aim to express and isolate pheromone receptors, which the insects themselves use to detect low levels of volatile chemicals released by one another. Methods such as Quantitive-PCR will be employed to expose candidate gene sequences involved in pheromone communication, followed by behavioural and physiological approaches such as RNAi silencing and cell-based assays (eg. calcium imaging), which will be investigated to determine protein function. It is envisioned that research into insect pheromone receptor systems will not only demonstrate the feasibility of using them as a potential biorecognition element in a biosensor device, but would additionally aid in our overall understanding of the mechanisms involved in invertebrate olfaction.

THE GENERATION OF OLFACTORY NEURONAL SIGNALS: GENETICS AND ELECTROPHYSIOLOGY

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Using Drosophila to study insect olfaction has yielded fruitful results. However, much still remains to be discovered regarding how the response to odours is generated in olfactory receptor neurons. In an effort to identify important genes for olfactory signaling, a previously performed mutagenesis screening identified o88, a recessive mutation caused by ethyl methanesulfonate. Mutant flies show a complex electrophysiological phenotype. Neuronal signals measured by the electroantennogram (EAG), which records gross receptor potentials across a population of neurons, are significantly reduced to all tested odorants. On the other hand, receptor potentials, measured extracellularly by single sensillum recording (SSR) as sensillum potentials, are differentially affected for different sensillum and neuron types. This project aims to dissect this complex phenotype from both genetic and electrophysiological perspectives. Using deficiency kits, the mutation was mapped to a region on the second chromosome that contains 7 predicted genes. Rescue experiments for each gene, and in vivo RNAi experiments designed to knock down the expression of each of the candidate genes, are underway to identify the affected gene. Mathematical models are being developed to explain the electrophysiological data so that the role of the mutated gene in causing the o88 phenotype can be elucidated. The integration of these results will bring about a better understanding of how olfactory neuronal signals are generated.

THE CARBOHYDRATE CT1 IS EXPRESSED IN TOPOGRAPHICALLY-FIXED GLOMERULI IN THE MOUSE OLFACTORY BULB

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Cell surface carbohydrates are differentially expressed by primary olfactory neurons and have been implicated in the growth and guidance of axons. We have previously proposed that the combination of carbohydrates present on axon subpopulations can confer a unique identity that contributes to the guidance of axons and the establishment of the topographic map. However, the majority of carbohydrates identified to date are present on large subpopulations of axons making it difficult to determine the topographical positions of glomeruli that express any particular carbohydrate. We have now examined the expression pattern of the blood group carbohydrates with GalNAc-ß1,4[NeuAc•2,3]Galß1 residues that are recognised by the CT1 antibody. A small subpopulation of primary olfactory axons was identified that expressed the CT1 carbohydrate in a highly restricted pattern with CT1-positive glomeruli being located predominantly in the medio-dorsal olfactory bulb. These axons were a subpopulation that expresses the N-acetyl-D-galactosamine epitope recognised by *Dolichos biflorus* agglutinin (DBA). CT1 carbohydrate expression varied markedly with some glomeruli exhibiting very high levels, while others expressed very low but detectable levels. However, most glomeruli were completely devoid of CT1 expression. The CT1-positive glomeruli were in topographically fixed positions and several glomeruli were immediately adjacent to both M72 and P2 odorant receptor glomeruli on both the medial and lateral surfaces of the olfactory bulb. These results demonstrate that distinct subpopulations of primary olfactory axons express specific carbohydrates consistent with these molecules playing an important role in the establishment of the olfactory topographic map.

ABSRACTS - Day 2, Session 5

A CLINICAL APPROACH TO THE SYNDROMIC ASSESSMENT OF OLFACTORY DISORDERS

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In the words of Francis Collins: "virtually every disease has a genetic component except possibly trauma". Rapid expansion of genetic information has included the basis of human olfaction. Most olfactory disorders can be divided into conductive (peripheral) problems such as sinusitis, allergic rhinitis and tumors; and sensory/neural (central) causes due to toxins, head trauma and neurodegenerative conditions. However, the remaining 10% of cases are reported to be idiopathic (or undiagnosed) with 3% considered congenital. In the literature and most medical clinics, limited attention has been paid to the association of anosmia/hyposmia with other congenital anomalies and therefore many patients lack a detailed assessment of family history and investigation for associated features. Therefore, those with identifiable genetic associations have the potential to be overlooked. Many clinicians are aware of the presentation of anosmia with Kallman syndrome but less cognizant with the overall management of such patients and their families. Other conditions with olfactory abnormalities include Down syndrome, Bardot Beidel syndrome, Fragile X syndrome and neurodegenerative conditions including mitochondrial disorders such as Leigh disease. The index of suspicion should include attention to family history, atypical presentations of common symptoms, more than one significant anomaly, and a rare anomaly. Evaluation of these cases may allow specific diagnosis and further testing and management as well as the identification of affected/at risk family members. The recognition and study of individuals with these features and correlation with the genetic basis of their conditions will facilitate the translation of advances in basic science to the bedside.

IT IS LIKE THE PERFUME OF A ROSE: YOU CAN SMELL IT, AND THAT IS ALL

Peter Disler

School of Rural Health, Monash University and Bendigo Health

From the perspective of the clinician, smell is the elusive sense. Although there is evidence to suggest that an abnormal sense of smell can be used to diagnose such common conditions as Alzheimers Dementia, and it is well known that anosmia is common after frontal lobe injury, few medical practitioners are taught to assess smell comprehensively, and even fewer actually make it part of daily practice. The aim of this presentation is to generate discussion as to how the sense of smell could/should be measured in clincial practice with speed, accuracy and relevance.

MEASURING SEROTONIN RELEASE FROM THE ILEUM OF HIGH-FAT DIET RATS.

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A high fat diet is associated with subtle changes in

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gastrointestinal (GI) function. Enterochromaffin (EC) cells contain serotonin (5-HT) which is a key regulator GI of function. We characterised the uptake and release of 5-HT in a rat model of diet-induced obesity. Electrochemical methods were used to measure peak and steady state (SS) 5-HT concentrations and fluoxetine (1mM) was used to block the serotonin reuptake transporter (SERT) in control (age-matched, chow-fed; 508±16g; n=6) and high-fat diet rats (HFD; 740±26g; n=11). Levels of mRNA for tryptophan hydroxylase 1 (TPH1) and SERT were determined by quantitative PCR. Paired and unpaired data were compared with a one way ANOVA ($P<0.05$) using a Tukey-Kramer post-hoc test. In control rat, SS levels of 5-HT were 12.3±3.4µM and peak compression-evoked release was 22.3±4.2µM ($P<0.05$; n=6). In HFD rats, the levels of 5-HT were significantly increased (SS: 23.8±3.4µM; peak: 66.8±13.4µM; $P<0.05$; n=11). In control rats, fluoxetine doubled peak and SS 5-HT release (180% of control; n=3), while in HFD rats there were no significant changes. In whole ileum, there was no change in SERT or TPH1 in HFD compared to control. The numbers of EC cells/crypt-villus numbered 1.24±0.29 per crypt in control (n=4). In HFD rats, the number of EC cells had increased to 1.90±0.17 per crypt ($P=0.03$; n=4). Our data predict that a high fat diet will be associated with increased 5-HT availability. As these changes appear not to be driven by altered genetic control, further work on the physiological regulation of 5-HT availability is needed.

THE CHARACTERISTICS OF SEROTONIN RELEASE FROM HUMAN COLONIC MUCOSA ARE SIMILAR TO SEROTONIN RELEASE FROM RAT COLON

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Serotonin (5-HT) containing enterochromaffin (EC) cells of the intestine detect chemical and mechanical stimuli in the lumen and respond by releasing 5-HT on to afferent nerve terminals. Recent electrochemical studies in rodent mucosa have shown that the release of 5-HT can be quantified but whether these recordings can be made reliably from human tissue is unknown. Our aim was to characterise the release of 5-HT electrochemically from the mucosa of human colonic surgical samples and to compare this with in vitro rat colon. Fresh specimens had peak compression-evoked 5-HT release of 37±14µM while steady state (SS) levels were significantly lower (15±6µM; $P<0.05$; n=4). Nine positions on the mucosal surface were tested for compression-evoked 5-HT release; on average release was seen from 5±1 positions (n=4). SS levels were also not different when measured by gentle touch (9±4 µM) versus compression (9±4µM; $P>0.05$; n=4). These data were compared with that from rat distal colon. Our preliminary data suggests that the characteristics and level of variability in the electrochemical recordings is similar in human and rat samples. We predict that the levels of 5-HT detected electrochemically will be directly related to the numbers of EC cells in small areas of mucosa.

ABSTRACTS – Day 2, Session 6

CHARACTERISATION OF A NEMATODE ODORANT RECEPTOR THAT DETECTS CYCLOHEXANONE, AN ODORANT SIGNATURE COMPOUND FOR SOME EXPLOSIVES

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CSIRO division of Entomology and Food Futures Flagship

Mammals and insects can be trained to detect explosive vapours. Here we show that, without prior conditioning, the nematode Caenorhabditis elegans exhibits strong chemotactic responses to a range of chemicals characteristic of explosives. Responsiveness to cyclohexanone, reported to be a key odour signature chemical for C-4 explosive, was selected for further analysis using a range of genetic mutants defective in elements of the chemosensory transduction pathway. The AWC cell specification mutant ceh-36(646) failed to respond to cyclohexanone at any concentration but the AWA cell specification mutant odr-7(ky4) responded to cyclohexanone as strongly as wild-type. The putative cyclohexanone receptor was shown to signal predominantly through the odr-3, odr-1/daf-11 and TAX2/TAX4 pathway. The putative receptor has high sensitivity and preliminary results indicated that it is narrowly tuned. These results will facilitate the identification of the gene responsible for cyclohexanone receptivity. A similar approach may also assist in de-orphaning other C. elegans odorant receptors.

FIVE STUDIES RESOLVING AIR POLLUTION ISSUES WITH ELECTRONIC NOSE

Graham A. Bell*, Brian Crowley, Jessica Disler and Leigh Middleton

E-Nose Pty Ltd

Fugitive odours from industrial sites are of great concern to communities living downwind from them. Control of such emissions requires measurement, followed by abatement action and thereafter re-measurement, plus occasional or even constant on-going monitoring of the site. Currently, around the world, fugitive odour measurement relies heavily on inadequate methodology: costly sampling and lab analysis by experts, yielding few data at limited times, often after a crisis has passed. We report here a useful approach with high data yield using an electronic nose, with examples from five industrial sites. The results informed clients of: 1) whether their emissions were indeed those being complained about and not other emitters in the area; 2) how much, if any, a site was contributing to general downwind odour pollution; 3) where on the site different odour sources are located; 4) whether their fugitive odours remained identifiably theirs and at what distance from the source; and 5) at what distance from the odour source, humans would no longer be able to detect the fugitive odour. These five studies have contributed to wider acceptance by environmental protection agencies of e-nose technology in resolving air pollution issues.

CHARACTERISING THE RESPONSE OF INSECT ODORANT RECEPTORS TO VOLATILE CHEMICALS OF FORENSIC SIGNIFICANCE.

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The detection and identification of volatile chemicals (VCs) is essential to the successful undertaking of numerous forensic analyses. Biological olfactory systems possess the extraordinary ability to not only detect many thousands of distinct VCs (odours) but also to discriminate between them. While the fundamental aim for many developers of portable instruments is to replicate this remarkable ability, they are yet to possess the detection and discriminatory powers of biological sensors. The aim of this research is to determine the feasibility of employing insect odorant receptors (ORs) for the detection of VCs for forensic purposes. The response patterns of *Drosophila melanogaster* ORs to forensically significant compounds representing several areas of forensic analysis were characterised via expression in *Spodoptera frugiperda* (Sf9) cells followed by calcium imaging assays. It was found that Or7a and Or85f respond to benzaldehyde - an illicit drug compound - in a dose-dependent manner but with differing sensitivities. The interaction between dimethyl disulfide (a compound released during decomposition processes) and Or59b was also found to be dose-dependant and highly sensitive. The response profile of Or43a to cyclohexanone - an explosive precursor - was found to differ from those obtained from *in vivo* studies and research completed with Or94b revealed that several compounds containing benzene rings (which are common among compounds indicative of ignitable liquids and human decomposition) activated the receptor. This research provides an initial indication that insect ORs can be employed for the discriminative detection of forensically significant VCs.

ABSTRACTS – Day 3, Session 7

FUNCTIONAL STUDY OF GUSTATORY RECEPTORS IN DROSOPHILA

Cecile Faucher* and Sylwester Chyb

CSIRO Entomology

The fly Drosophila melanogaster detects taste compounds by contacting a substrate with sensory hairs located on gustatory organs, such as proboscis, legs, wings, and ovipositor. Each sensory hair houses two to four gustatory receptor neurons that send projections to the brain. Recently, a family of 60 putative gustatory receptor (Gr) genes has been identified, consisting of

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68 predicted seven-transmembrane-domain proteins. They are thought to be involved in the transduction of the chemical information into a neuronal signal. However, for the majority of these receptors, their ligand specificity and role in taste discrimination remains to be elucidated. Here, we describe the functional characterisation of two gustatory receptors heterologously expressed in an insect cell line using calcium imaging. Cell expression vectors containing one of these two gustatory receptors have been generated and transiently expressed in the Sf9 cell line either individually or in combination. Their sensitivity to tastants has been assessed by monitoring the rise of cytosolic calcium using fluo-4 as a calcium indicator. For homogeneity of the data, values were related to the maximal fluorescence emitted by the cells by addition of ionomycin, a calcium ionophore that affects the permeability of the plasma membrane and thus leads to maximal entry of external calcium into the cell. This method is based on that developed by Kiely et al., 2007. The results of this study will be discussed in light of the present knowledge of GR specificity in vivo.

CONSERVATION AND CHANGE IN OLFACTORY RECEPTOR NEURONS ACROSS THE DROSOPHILA GENUS: IMPLICATIONS FOR RECEPTOR STRUCTURE-FUNCTION RELATIONSHIPS

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Insect olfactory receptor (Or) genes are large, rapidly evolving gene families that are of considerable interest for evolutionary studies as they determine the responses of sensory neurons which mediate critical behaviours and ecological adaptations. High levels of variation in the amino acid sequence and odour response spectra of Or paralogues has hampered the identification of functionally important residues. We have investigated the functional and molecular evolution of a subfamily of Or genes across the Drosophila genus by determining the odour responses of eight classes of olfactory receptor neurons that express these genes in *D. melanogaster* from seven other Drosophila species. Despite the fact that these species have diverged over an estimated 40 million years, we find that odour specificity of neurons is largely maintained within the subgenus Sophophora. In contrast, we observe extensive changes in *D.virilis*, subgenus Drosophila, and in two neurons across the entire genus. Some neurons show small shifts in specificity while the most dramatic changes correlate with gene duplications or loss. Our phenotypic analysis enables us for the first time to compare orthologous Ors of known functional equivalence with functionally different paralogues, and thus determine that in this Or subfamily less than 24% of amino acid residues are relevant for odour specificity. For a specific ligand response change we are using in vivo and in vitro assays of Or function to perform structure-function analysis to identify the causative amino acid changes in the Or underlying the ORN response change.

REGULATION OF BRAIN GENE EXPRESSION IN HONEYBEES DURING OLFACTORY LEARNING.

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Our honeybee research suggests that changes to sensory acuity (the ability to receive sensory input) and learning and memory of scent profiles involves changes in expression of olfactory receptors in the antennae and remodeling of molecular and cellular processes in the brain. We used a classical Pavlovian learning paradigm (also known as Proboscis Extension Reflex; PER assay) to study brain gene expression of worker bees that were conditioned to simple and complex odour mixtures. Our RNA microarray analyses identified significant changes in brain gene expression of bees conditioned to a single odorant (linalool) and a mixture of floral compounds (including linalool) compared to control bees exposed to air. We found that the majority of genes differentially expressed after conditioning to linalool and to the complex mix were down-regulated (42 and 14 genes, respectively), while significantly fewer of the differentially expressed genes were up-regulated (10 and 7, respectively) when compared to air. Furthermore, we also identified many non-coding sequences that were differentially expressed in bee brains. In contrast to the coding genes, the majority of the non-coding sequences were up-regulated in both the linalool and the complex odour mixture trained bees (80% and 69%, respectively). Functional assignment of the down-regulated coding genes showed that they are mainly involved in metabolic processes, cellular development and biogenesis. We propose there is selective shutdown of genes controlling key cellular events as a means to remodel neuronal connections (eg. neurite outgrowth and synapses) that is dependent on the complexity of sensory information experienced by the bee.

ABSTRACTS – Day 3, Session 8

DOPAMINERGIC JUXTAGLOMERULAR NEURONS: OLD CELL, NEW ROLE?

Adam Puche

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THE MOTILITY OF OLFACTORY ENSHEATHING CELLS: A HITCHHIKER'S GUIDE TO OLFACTORY AXON MIGRATION

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During development and during regeneration in the adult, olfactory ensheathing cells (OECs) are intimately associated with axons of olfactory sensory neurons along their entire trajectory. At present little is known about the physical interactions between OECs and axons. We hypothesised that the motility of OECs directs the migration of olfactory axons. To address this we used timelapse imaging of fluorescently labelled primary cultures of olfactory sensory neurons and OECs to determine the mechanisms of olfactory axon extension and OEC interactions. We reveal here that the extension of pioneer olfactory axons is dependent on the motility of the underlying OECs. This intimate association is in part initiated and mediated by lamellipodial waves along the shaft of OEC processes. Moreover, as axons remain adhered to axons at all times, perturbation of OEC movement via GDNF and inhibitors of the JNK and SRC kinases significantly altered axon motility. In addition, inhibition of NCAM significantly disrupted OEC cell-cell recognition resulting in increased OEC migration, while OEC-axon adhesion was maintained. These results demonstrate that olfactory sensory axon outgrowth is dependent on cell-cell contact with OECs. Rather than merely providing support for axon growth the glia of the olfactory system strongly regulate the migration of olfactory axons.

SCHWANN CELLS INTERACT WITH GLIAL CELLS AND NEURONS VIA HIGHLY MOTILE PERIPHERAL LAMELLIPODIAL WAVES.

Jenny Ekberg*, Susan Scott, Katie Lineburg, Alan Mackay-Sim, James St John.

National Centre for Adult Stem Cell Research, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, QLD

Peripheral lamellipodia in Schwann cells (SCs) are crucial for myelination of axons, however, the mechanisms by which these lamellipodia promote myelination remain unknown. Using time lapse imaging of fluorescently labeled cells, we have studied the dynamics of peripheral lamellipodia in SCs. We found that peripheral lamellipodia in SCs closely resembled the highly motile lamellipodial waves in olfactory ensheathing cells (OECs) that our laboratory was the first to characterize. The SC waves were similar to those in OECs but traveled ~2-fold faster along the cell. As previously shown for OECs, lamellipodial waves in SCs promoted cell migration. We also found that the waves were involved in most cell-cell interactions between individual SCs and between SCs and neurons. 73% of all SC-SC interactions involved the leading edge (LE) of one SC and a peripheral lamellipodial wave of another SC. Approximately half of the SC-SC interactions resulted in repulsion, while 35% of interactions led to cell-cell adhesion and the remaining ones resulted in no alteration in cell behaviour. Initial contact between SCs and cultured

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DRG axons was mainly mediated by the leading edge of the SC (77%) while the remaining interactions were mediated by peripheral lamellipodial waves. Furthermore, axon-SC contact appeared to trigger the generation of lamellipodial waves in the region of the SC that was in direct contact with the axons. In summary, we have demonstrated that peripheral lamellipodia in SCs are highly motile and closely resemble the lamellipodial waves that occur in OECs. These waves promote migration of SCs and mediate SC-SC and SC-axon contacts. While the leading edge of SCs appears to initiate most SC-axon interactions, it is possible that peripheral lamellipodial waves maintain such interactions over time. Thus, it can be speculated that peripheral lamellipodia in SCs prolong the interaction between SCs and axons, enabling myelination.

ABSTRACTS – Day 4, Session 9

REGULATION OF TISSUE HOMEOSTASIS WITHIN THE OLFACTORY NEUROEPITHELIUM

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Degenerative diseases become increasingly prevalent with an aging population. In order to develop treatments, it is imperative to understand the molecular pathways that control tissue homeostasis. Olfactory sensory neuron neurogenesis and programmed cell death are ongoing during development as well as throughout life. This is a unique feature which sets olfactory sensory neurons apart from most other neurons in the nervous system and is enabled by a population of stem cells that resides within the basal compartment of the olfactory neuroepithelium. The molecular mechanisms that control tissue homeostasis within the olfactory neuroepithelium are largely unknown. The Hippo pathway regulates organ size through controlling proliferation of stem cell populations. This pathway ultimately phosphorylates and thereby inactivates the transcriptional activator Yes-associated protein 1 (YAP1). YAP1 interacts with and modulates the activity of the TEAD transcription factors, which in turn control cell proliferation. YAP1 can also act as a pro-apoptotic protein. This growth-opposing activity is reported to be mediated through binding of YAP1 to pro-apoptotic protein P73 in a transcriptional complex. We found that Yap1 and components of its upstream regulatory pathways are expressed in the olfactory epithelium. Interestingly, Yap1 is expressed throughout the olfactory epithelium. In addition, we found that YAP1 protein is present and phosphorylated in the olfactory epithelium. These data suggest that YAP1 may control pathways that regulate both neurogenesis in basal regions of the olfactory epithelium and progammed cell death of mature olfactory sensory neurons. Our data are consistent with a central role for YAP1 in tissue homeostasis within the olfactory epithelium.

EXUBERANT AXON GROWTH AND ERROR CORRECTION IN THE DEVELOPING MOUSE OLFACTORY BULB.

Caroline Chan[1,2], Jenny Ekberg[1], Fatemeh Chehrehasa[1], Susan Scott[1]*, Louisa Windus[1,2], Christina Claxton[2], Alan Mackay-Sim[1], Brian Key[2], James St John[1]

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During development of the primary olfactory system, sensory axons course from the nasal cavity to the glomerular layer of the olfactory bulb. In the process axons can branch inappropriately into several glomeruli and sometimes over-shoot the glomerular layer, entering the deeper external plexiform layer. Establishing the extent of this over-shooting has important implications for our understanding of molecular cues responsible for glomerular targeting and axon branching. We have determined the extent of the transient over-shooting of primary olfactory axons by using an enhanced OMP immunohistochemistry protocol as well as the OMP-ZsGreen transgenic mouse line that expresses a fluorescent protein in primary olfactory neurons. Numerous primary olfactory axons aberrantly projected radially into the external plexiform layer where they form a loose tangential plexus of fibres. These axons were largely unbranched and appeared to project preferentially along the ventro-dorsal and rostro-caudal axes for over 1000 µm. The numbers of long projecting axons within the external plexiform layer decreased with age but continued to be detected until P17. We tested whether the external plexiform layer contained axon guidance cues that attracted or repelled axons at the different developmental ages by microdissecting the external plexiform and preparing a protein extract. When assayed using olfactory epithelium explants, primary olfactory axons became increasingly inhibited by extract prepared from older animals. These results demonstrate that the external plexiform layer is initially conducive to primary olfactory axon growth, but that chemorepulsive molecules become upregulated during development and inhibit axon growth within the external plexiform layer.

A NEW LINE OF TRANSGENIC MICE: OMP-ZSGREEN PROVIDES UNRIVALLED VISUALISATION OF PRIMARY OLFACTORY SENSORY NEURONS

Jenny Ekberg[1], Jessica Cornock[1]*, Susan Scott[1], Katie Lineburg[1], Louisa Windus[1,2], Christina Claxton[2], Brian Key[2], Alan Mackay-Sim[1], James St John[1]

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We report here the generation of a new line of transgenic mice, OMP-ZsGreen mice, in which primary olfactory sensory neurons express an extremely bright and stable green fluorescent protein. In these mice, the olfactory marker protein (OMP) promoter drives expression of the coral protein ZsGreen, and hence primary olfactory sensory neurons are labelled with the bright green fluorescent protein from embryonic day 11 onwards. The only cells within the olfactory system that express the ZsGreen protein are the primary sensory neurons. Immunostaining with OMP antibodies revealed that the vast majority of primary sensory neurons also expressed ZsGreen protein. However, it was clearly apparent that the ZsGreen protein provided superior visualization and resolution of the neurons and their axons in comparison to the antibody staining. The ZsGreen protein is expressed in all parts of the neurons including the dendrite, dendritic knobs, axon and cell body. The expression is so strong that individual axons within fascicles can be easily visualised and in vitro live cell imaging of axons has demonstrated that it is highly suitable for continuous long term imaging experiments. The use of this line of mice will provide unprecedented imaging capabilities, particularly for live cell imaging, and will enhance studies on the development and regeneration of the olfactory system ■

Upcoming Events

31 January – 3rd February 2010	**Australian Neuroscience Society** and **Australian Physiological Society** Annual Scientific Meeting Sydney Convention Centre, Sydney www.ans.org.au/ans-annual-conference/
23-24 February 2010	**EcoForum Conference and Exhibition** Australian Technology Park, Sydney www.ecoforum.net.au/2010/
10-12 March 2010	**Climate Change,** Copenhagen, Denmark. http://climatecongress.ku.dk
21-25 April 2010	**AChemS 32nd Annual Meeting** Tradewinds Resort, St Petersburg, Fl., USA www.achems.org
21-23 June 2010	**Air Pollution 2010** Kos, Greece http://www.wessex.ac.uk

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