Vol. 11 No.1 December 2008

Chemo Sense

Editorial

By Graham Bell
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Finding Meaning in ChemoSensory Experience

Many gifts purchased in the coming holidays will be of better than average quality as people seek "premium value" in what they give and receive. Some people can afford and perhaps cannot resist the very best, as they perceive it to be. They are "High End" buyers and when they choose branded products they are responding to "High End" brand design. This issue leads with the thoughts of three executives in the business of creating global brand images for goods and services that will fetch the highest prices in their categories: so called High End products. They argue that sensory experience designed into the "personality" of the brand creates

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A Brief 'Taste' of the High End Sensory Experience

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Brands and their 'Sensory Experience'

Adapting sensory preferences by countries to each product or service is the ordinary approach to creating a successful, differentiated product. Yet, when we think about 'brand', we have to travel deeper. Pursuing 'personality' is an eternal theme for a brand. The so-called 'brand personality' has to be coherent across countries. Through experience with the five senses, strong brands build a bond to consumers with a defined, sensorial 'personality'. If you want to get a sense of this bond, just think of your feelings when you cuddle up someone you love. You feel comfortable with his/her not only appearance but also feeling of skin, smell of body, and lovely voice.

Applying our knowledge on the practical, applied end, we have come to recognize that 'building' a clear 'sensory' personality is essential requirement of High End, especially for some brands such as *Whole Foods*, *Apple*, and *Bang & Olufsen*. The

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its "premium value". They challenge sensory scientists to help to determine what to include or exclude in "experience design", by creating and applying an understanding of chemosensory perception in the context of different cultures.

Sensory experience and meaning come together with a bang in the review of the "Proustian" phenomenon of "autobiographical memory" by Burt Slotnick. His analysis of Proust's novel *"Remembrance of Things Past"* shows that Proust's sensory stimulus for biographical memory is taste, not odour, as assumed in many empirical studies.

Abstracts from the Tenth Annual Scientific Meeting of AACSS are published herein.

The meeting confirmed its commitment to hold the 2009 meeting at the Great Barrier Reef in the first week of December 2009. The next issue of *ChemoSense* (March 2009) will have details ■

A Brief 'Taste' of the High End Sensory Experience
continued

questions are 'what' is the sensory signature, and 'how' does the company create that signature?

During the past decade, Hakuhodo has focused on methods to create the brand personality through a unique sensory 'signature'. This we call 'Five Senses Branding'. From the perspective of applied research in the senses, rather than the more basic research to be found in journals, we can summarize the approach as follows (always keeping in mind that we focus on brands and experiences as the key topic, with the sensory perceptions as components to establish the brand):

1. Our first task penetrates into the brand's underlying character and finds out brand's raison d'etre within people's mind. ' What is happening? How do people describe the brand? Why do they use the product?
2. Our second task isolates the sensory experience. How do people describe what they sense? What do they 'feel' that they perceive, at a deeper level, where meaningfulness enters? How do the people interact with the product? What specific senses are called into play and what is the language of description?
3. Our third task creates a sensory brief, consistent with the 'brand personality'. What are the sensory characteristics that should be experienced? How do people differ from each other? Are there cultural and country differences which dictate certain sensory profiles, and not others?

The above approach seems particularly relevant in the area of creating *premium value.*

High End and the Human Senses

The High End, or affordable luxury, is that world of products and experiences that cost a bit more, that are desired because they move us beyond the everyday, but that always lie within the reach of many people, even at the somewhat higher cost. The senses will play an ever more important role in the future especially in the High End of products and services, since the High End incorporates more refined sensory experiences, including visual, auditory, and tactile presentations.

With this in mind, let's look at the nature of such High End sensory experiences. We will see taste and smell popping up in a variety of experiences, and cultures.

A key finding from several Hakuhodo, Philips Design & Moskowitz Jacobs Inc., studies is that the aforementioned High End will be marked by breakthroughs in 'experience-design'. Just consider three commercial examples: *Whole Foods* for tastes and smells, *Anthropologie* for visual design, and *Philips Aurea* for immersive sound, vision and light. We talk here of people-driven experiences, of products and situations designed to delight our senses, whether the delight is subtle such as *Anthropologie's* fit with the local environment, or insistent and demonstrable, such as the better, richer tastes of foods to be discovered in *Whole*

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Preliminary program

8.00 Light breakfast for delegates and exhibitors

8.30 **EcoForum keynote address** Why environmental behaviour changes away from home Prof. Joe Arvai *Michigan State University USA* e9167

Odour analysis

9.30 **Stream keynote address** Human olfaction Prof. EP Köster *Utrecht University* The Netherlands e9144

10.00 Can statistics help environmental analysis? Brynn Hibbert *University of NSW* e9142

10.30 – 11.15 Morning refreshments

Human health and vapour intrusion

11.15 Health screening levels for petroleum hydrocarbons being developed by CRC CARE—project update Eric Friebel *GHD* e9028

11.25 Risk management at residential and commercial sites impacted by methane and other landfill gases Chris Jewell *C M Jewell & Associates* e9054

11.35 Assessment of human health risk from vapour intrusion into residential dwellings Adrian Heggie *Parsons Brinckerhoff* e9036

11.45 Modelling and managing vapour intrusion—balancing risk and commercial considerations Chris Jewell *C M Jewell & Associates* e9056

11.55 Review of data for assessing vapour intrusion on 47 petroleum sites in Australia Christina Robinson *URS Australia* e9097

12.05 **EcoForum discussion** e9184

12.45 – 2.15 Lunch

Odour measurement

2.15 Selection of effective sampling methods to refine health risk assessments Judith Barnes *URS Australia* e9083

2.25 Biofilter design and operation in 2008—a technology review Terry Schulz *The Odour Unit* e9131

2.35 Improving odour management using olfactory GC-MS Richard Stuetz *University of NSW* e9133

2.45 Development of e-noses for real-time recognition of complex odours regarded as nuisances by local communities Graham Bell *E-Nose* e9118

2.55 Measurement of chemical emissions from building products Robert Schiller & Subbalakshmi Yerramilli *Cetec* e9062

3.05 BIOVAPOR—a spreadsheet model for evaluation of vapour intrusion with oxygen-limited biodegradation Curtis Stanley *Shell Global Solutions* e9119

3.15 **EcoForum discussion** e9183

3.45 – 4.30 Afternoon refreshments

Air quality—can we do better?

4.30 Indoor air quality, odours and ventilation energy use Paul Spry *Spry Consulting* e9143

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A Brief 'Taste' of the High End Sensory Experience

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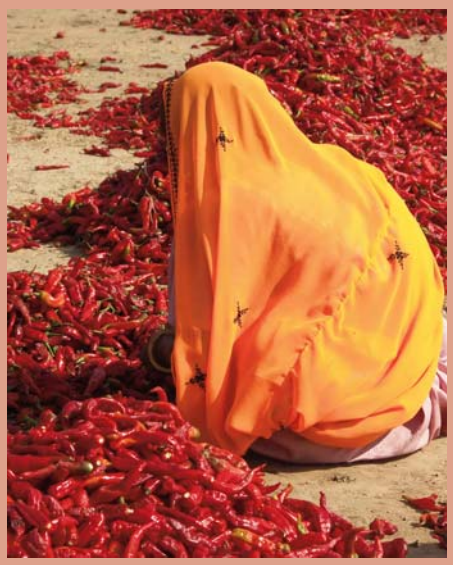

Foods stores.

How do people around the world differ in their reactions to sensory inputs? Of course, for the most part all people have similar performing sensory organs. Clearly we don't ever assume that certain cultures are tone deaf, others indifferent to specific flavors, etc. Yet we find culture-to-culture deep variation in aesthetics and design, some of which may be traced to one's emphasis on the different senses. These variations will affect the High End. Cultures that value taste and smell will evolve to High End experiences that pull in foods and perfumes. Cultures that value appearance will evolve to High End experiences that pull in visual design, and so forth.

Experience Design with the Five Senses: Differences by Cities and Countries

Let's look at some results from studies done by Hakuhodo, their ***Global Habit Survey***, focusing on the interaction between the senses and the city – a sort of sociological psychophysics, if you will. From these patterns, we will be able to guess development directions, based on what consumers in the different countries respond to.

Part of the Hakuhodo database focuses on the relative importance of the five senses in the various countries and cities of the world, as well as their practical impact on retail design and point of sale. The information describes and then synthesizes the fundamentals of cultural differences. There is, of course, a practical end to the study – namely, the opportunities for businesses around the world to create the appropriate experience design. And, of course, in so doing, teach us something about the chemical senses, as one group of sensory inputs.

Let's look at each sense, one by one:

Sight incorporates fundamental design qualities such as aesthetic appearance and colors. The most visually oriented urban cultures are to be found in Delhi and Mumbai. The least visual urban hubs appear to be, in decreasing order, Tokyo and the Kansai area in Japan.

Hearing, the sense associated with sounds and music, is much more important in London and New York, but far less relevant for the Chinese and Indian major cities, as well as Taiwan. Tokyo is perhaps the lowest ranking when it comes to the importance of sound.

Touch comprises the key High End dimensions of texture and feel. The sense of touch is most relevant for Asian cities in Taiwan, Hong Kong and Thailand. This seems yet another important regional implication, especially for product designers.

Smell shows its strongest appeal in New York and London leading the pack at a great distance, with the Kansai area of Japan quantitatively positioned as third. "Smell" is highly regarded as a sensory input for enchantment and experiential design, especially in cities such as Milan, or by the Chinese wealthy classes in major cities.

Taste (and flavor), our final sense, strongly resonates with Japanese in Tokyo, with Koreans and Philippines (especially Manila. Turin, virtual capital of the Slow Food movement focusing on traditional methods and artisanal production, was not studied by Hakuhodo, but you might expect Turin, Italy, to lead here.

Summing Up

We write this article with a bit of trepidation, knowing that the study of taste and smell has expanded tremendously over the past forty years, and even during the past ten years, when *ChemoSense* has been on the scene. We realize that there are many worlds involved in taste and smell. Yet, at the same time we are compelled to emphasize that sociological differences in the service of product design is not well explored. Certainly we know that people differ from culture to culture. Yet we feel that when it comes to the High End, and to experience design, there is a world of insights, knowledge, and application waiting for the researcher. We hope we have inspire readers to move into this area of experience design, taking with you knowledge of taste and smell as key tools with which to make your own contribution ■

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Sautéed Onion Concentrate	2730	Fresh yellow onion sautéed in corn salad oil, strong aroma & avour
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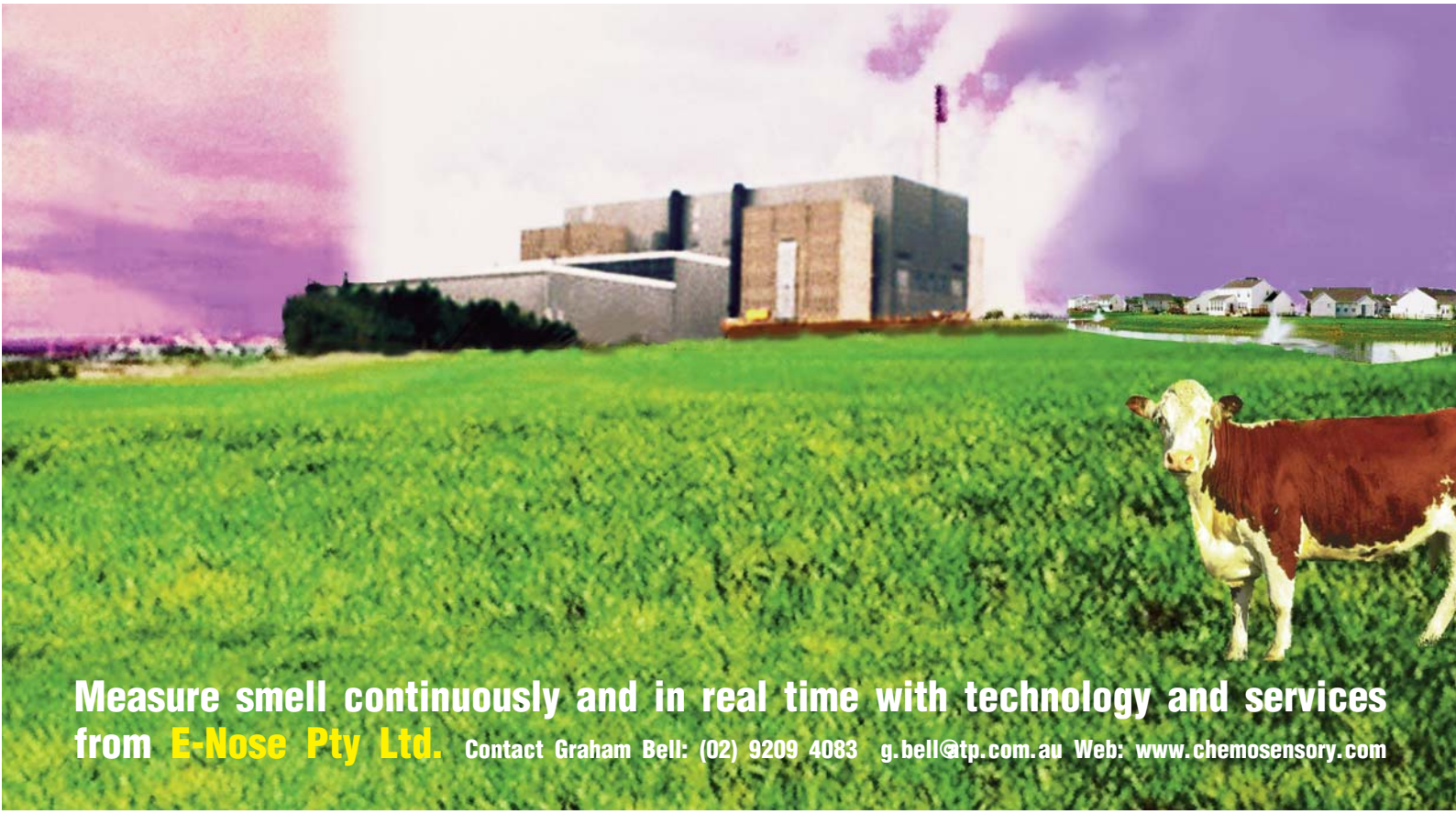

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Proust, Taste, Smell, and Memory

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An analysis of Marcel Proust's novel, 'Remembrance of Things Past', reveals that the chemical sense stimulus most often identified as evoking an autobiographical memory is taste and not, as assumed in many empirical studies of the so-called 'Proust Phenomenon', an odor.

The so called 'Proust Effect' or 'Proust Phenomena' refers to the widely held belief that an odor can produce a seemingly long forgotten (and generally autobiographical) memory. The attribution of this effect to Marcel Proust is based on an oft quoted passage from the first volume (Swann's Way) of Proust's seven volume autobiographical novel Remembrance of Things Past. In this passage, Proust, while consuming a cup of tea and a small cake (a *petite madeleine*) recalls vividly and in detail a set of childhood experiences he thought had been long forgotten.

Does this passage from Proust provide an example of the special properties of odors to produce long lost and emotionally laden memories or is it merely a beautiful and poetically described incident from the life of the author? The issue remains a subject of empirical studies (e.g. Aggleton and Waskett, 1999; Chu and Downes, 2000; 2002; Herz, 1996; Herz and Cupchik, 1992; 1995; Herz and Schooler, 2002; Willander and Larsson, 2007; 2008) and controversy (e.g., Jellinek, 2000; Willander and Larsson, 2006; Gilbert, 2008, pp 189-204). The methodologies used in empirical studies vary considerably but most appear to support the contention that odors are more likely to evoke earlier and more emotionally charged autobiographical memories than do verbal labels but it is unclear whether any of these experimental studies captures the *recherche du temps* described by Proust.

The uncertainty stems from two major problems with these Proustian studies. The first is methodological and described most clearly by Jellinek (2000) who questioned whether experimental studies can capture the essence of the Proust phenomenon. For example and as observed by Jellinek (2000), in the Proust account there occurs a surge of emotion and surprise even before awareness of the sensory stimuli which initiates the sequence of events leading to the autobiographical memory, a memory that had been 'lost' but returns rich in contextual detail. In brief, it remains unclear whether the phenomenon described by Proust can be replicated in an experimental setting in which subjects are presented with somewhat arbitrarily selected odors and judge or rate presumably lost memories and their affective content.

However, the purpose of this report is not to elaborate this issue but, rather, to describe a second and perhaps more basic problem: whether, in Proust's account, the evoked memory was, in fact, initiated by an odor. Although in both the popular and scientific literature the Proust effect is attributed to an odor, there is surprisingly little evidence for this almost universally held assumption.

In the oft cited selection of a memory being evoked after sipping tea and eating a madeleine in Swann's Way (pp 34 – 36 of the 1938 edition of the Moncrieff translation) Proust clearly attributes his memory to the taste of the tea and madeleine. Indeed, in this section there are multiple references to taste but only one to odor. Although Jellinek (2000) recognizes the possibility that stimuli other than odors (e.g., taste, temperature of the tea, texture of the madeleine) may have played a role in the Proust account, he too attributes the memory effect to smell.

If, in fact, it is uncertain that an odor was the stimulus that initiated Proust's memory in the

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Proust, Taste, Smell, and Memory continued

'madeleine passage' then (and contrary to popular usage) the passage hardly qualifies as an example of odor induced evocation of lost autobiographical memory. To examine further Proust's references to taste, smell, and memory, an on-line electronic version of Remembrance of Things Past (http://gutenberg.net.au/plusfifty-n-z.html#proust) was searched. This version is based on the C. K. Scott Moncrieff translation of the first 6 volumes (Swann's Way, Within a Budding Grove, The Guarantees Way, Cities of the Plain, The Captive, The Sweet Cheat Gone) and on the Stephen Hudson translation of the final volume, Time Regained.

The text was searched for the singular and plural of the words taste and memory, for those words specifically related to perceiving an odor (*odour, scent, fragrance, perfume, smell*), and for the word *flavour*. For this analysis, the use of the word taste as a non-sensory descriptor or adjective (e.g. "... she thought it in not good taste that M. Legrandin ... should deliver himself of such violent attacks ...", "You must conquer your vile taste for A. de Musset, Esquire", "But when his visit synchronized exactly with Eulalie's it became frankly distasteful to my aunt.", "And then, she brings the same good taste to the choice of her costumes ...", etc.) was ignored.

Next, each passage in which the selected word occurs was examined to determine if it was associated with the word memory or was used to indicate the evocation of a memory. The results are summarized in Table 1. As shown, the word taste as a sensory stimulus was used a total of 63 times (most often in Swann's Way) while there were 226 uses of words relating to odor perception (smell, odour, scent, perfume, fragrance). However, across all chapters, 33 of the 63 (52%) instances of the word taste were specifically related to evocation of a memory, while only 17 of the 210 (8%) odor-related words were so used. The word flavour is used a total of 13 times but only once in conjunction with an evoked memory.

The word memory occurs frequently in all chapters but is most often associated with a chemical sense word in Swann's Way and in Time Regained. The oft quoted passage in Swann's way contains the longest continuous text relating taste and smell to an extended memory. In Time Regained, the references to this association are briefer and concern separate memories. The relevant passage in Swann's Way bears close scrutiny as it is the one most often quoted as evidence for the Proust Phenomenon.

The instances in which the words taste or smell occurs in that passage are given below (italics added to identify chemical sense words):

1. "I was conscious that it was connected with the *taste* of tea and cake, but that it infinitely transcended those savours ..."
2. "I place in position before my mind's eye the still recent *taste* of that first Mouthful ..."
3. "Undoubtedly what is thus palpitating in the depths of my being must be the image, the visual memory which, being linked to that taste ..."
4. "... its inseparable paramour, the *taste* of cake soaked in tea ..."
5. "And suddenly the memory returns. The *taste* was that of the little crumb of madeleine which on Sunday mornings at Combray ..."
6. "The sight of the little madeleine had recalled nothing to my mind before I *tasted* it; perhaps because I had so often seen such things in the interval, without *tasting* them."
7. "But when from a long-distant past nothing subsists, after the people are dead, after the things are broken and scattered, still, alone, more fragile, but with more vitality, more unsubstantial, more persistent, more faithful, the *smell* and *taste* of things remain poised a long time ..."
8. "And once I had recognized the *taste* of the crumb of madeleine soaked in her decoction of lime-flowers which my aunt used to give me ..."

All but one of the chemical senses terms used refer to *taste* and the word 'smell' in this section is used only

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Proust, Taste, Smell, and Memory continued

once (in the seventh quote above) and, in this context, as an observation and not to identify the stimulus that provoked the lost memory.

Passages in Time Regained relating chemical senses to memory also offer little support to the contention that smell underlies the Proust Phenomenon. The most vivid references to chemical senses associated with memory mention only taste. For example:

9. "... as was the case when I *tasted* the madeleine. The felicity which I now experienced was undoubtedly the same as that I felt when I ate the madeleine, the cause of which I had then postponed seeking. There was a purely material difference in the images evoked. A deep azure intoxicated my eyes, a feeling of freshness, of dazzling light enveloped me and in my desire to capture the sensation, just as I had not dared to move when I tasted the madeleine because of trying to conjure back that of which it reminded me, I stood, doubtless an object of ridicule to the link-men, repeating the movement of a moment since, one foot upon the higher flagstone, the other on the lower one."

10. "...there had been the irradiation of a small zone within and around myself, a sensation *(taste* of the dipped madeleine, metallic sound, feeling of the uneven steps) common to the place where I then was and also to the other place (my Aunt Léonie's room, the railway carriage, the Baptistry of St. Mark's). And, at the very moment when I was thus reasoning, the strident sound of a water-pipe, exactly like those long screeches which one heard on board excursion steamers at Balbec, made me experience (as had happened to me once in a large restaurant in Paris at the sight of a luxurious dining-room half empty, summerlike and hot) something more than a mere sensation like one I had ..."

In the few instances in which an odor related word was used to describe a memory in this volume, the first or primary chemical senses stimulus mentioned was taste. Thus:

11. "The *taste* of our morning coffee brings us that vague hope of a fine day which formerly so often smiled at us in the unsettled dawn from a fluted bowl of porcelain which seemed like hardened milk. An hour is not merely an hour, it is a vase filled with *perfumes,* with sounds, with projects, with climates. What we call reality is a relation between those sensations and those memories which simultaneously encircle us ..."

12. "... a sensation of the same order as the *taste* of the madeleine and the warbling of the thrush. Finally, in the case of Baudelaire, such reminiscences are still–more numerous, evidently less fortuitous and consequently, in my opinion, decisive. It is the poet himself who with greater variety and leisure seeks consciously in the *odour* of a woman, of her hair and of her breast, those inspiring analogies which evoke for him "_l'azur du ciel immense et rond_" and "_un port rempli de flammes et de mâts_". I was seeking to recall those of Baudelaire's verses which are based upon the transposition of such sensations, so that I might place myself in so noble a company and thus obtain confirmation that the work I no longer had any hesitation in undertaking, merited the effort I intended to consecrate to it, when, reaching the foot of the staircase leading from the library, I found myself all of a sudden in the great salon and in the midst of a fête ..."

While it can be argued that the sensation Proust attributed to taste was primarily a retronasal odor or a flavor, his text provides no direct evidence for this. Nor can it be argued that Proust preferred to use the word taste in referring to chemical senses stimuli: terms referring to odors in the novel far outnumber those citing taste (Table 1), even if one adds to that total for taste the few instances in which the words flavour or savour are used.

Most empirical studies provide support for folk wisdom that odors are particularly potent stimuli for evoking affectively toned and early autobiographical memories. However, it is less than clear that such studies are testing a hypothesis derived from Proust or, for that matter, whether stimulation of other chemical senses might be equally or more effective in memory recall.

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Proust, Taste, Smell, and Memory continued

Table 1. Number of times the titled word is used in each chapter and used in passages describing an evoked memory.

Volume	1	2	3	4	5	6	7	**Total**
STIMULUS								
Taste	**23**	**5**	**6**	**5**	**2**	**5**	**16**	**62**
Olfactory:								
Smell	23	15	8	6	6	1	3	**62**
Odour	5	0	2	5	4	0	4	**20**
Fragrance	11	8	7	4	2	2	1	**35**
Perfume	17	2	2	9	3	1	6	**40**
Scent	2	13	9	7	12	4	6	**53**
Total Olfactory	**58**	**38**	**28**	**31**	**27**	**8**	**20**	**210**
Memory	105	115	91	101	113	179	134	**838**
Taste x Memory[1]	10	2	4	1	1	2	13	**33**
Odour x Memory[1]	6	1	0	0	7	2	1	**17**
Flavour	2	6	4	0	0	0	2	**13**
Flavour x Memory[1]	1	0	0	0	0	0	1	**2**

[1].Frequency with which the chemical sense word is used in conjunction with the word memory or in a passage describing evocation of a memory.

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AACSS 2008: Abstracts

PLENARY LECTURE

MULTIMODAL SENSORY INPUT IN BEES – THE INTERPLAY OF OLFACTION AND VISION

Angelique Paulk

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Bees must integrate numerous sensory cues, such as olfactory or visual cues, to be able to navigate and forage for food. How do bees integrate olfactory and visual cues to enable them to perform their spectacular learning and memory behaviors? The answer lies in the central bee brain and the complex interaction of neurons along the olfactory and visual pathways. We investigated the physiological response properties of neurons in the bee brain while presenting various visual and olfactory cues and subsequently mapped the neurons to the structure of the bee brain. By correlating structure with function, we can begin to understand how two very different sensory systems could possibly be integrated to allow the bee to interact with the multimodal natural world.

Session 1: Chemoreception of Marine Animals

CHEMORECEPTION IN MARINE MOLLUSCS: HOW THE SEA SLUG SMELLS

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Marine molluscs, as is the case with most aquatic animals, rely heavily on olfactory cues for survival. In the sea slug *Aplysia*, the rhinophores and oral tentacles are the two primary chemosensory organs. Mate-attraction is mediated by a blend of water-borne protein pheromones that are detected by conspecific rhinophores. Most recently, we have shown that candidate G-protein-coupled receptors (GPCRs) are expressed in sensory epithelia microdissected from the rhinophore. Analysis of the *Aplysia* genome reveals that these are part of larger GPCR multigene families, which possess features found in other metazoan chemosensory receptor families, including clusters of tandemly repeated genes with one or two exons. Phylogenetic analyses show that the *Aplysia* GPCRs represent three distinct monophyletic subfamilies A, B and C. Representatives of each of these subfamilies are restricted to or differentially expressed in the rhinophore, oral tentacles and ovotestis, suggesting that they encode functional chemoreceptors and that these olfactory organs sense different chemicals. These candidate chemoreceptors are expressed and often restricted to rhinophores and oral tentacles, lending support to the notion that water-borne protein pheromone detection in *Aplysia* involves lineage-specific families of chemoreceptors. Secondary signaling component proteins $G\alpha_q$, $G\alpha_i$, and $G\alpha_o$ are also found in the rhinophore sensory epithelium. Our current research is focused on analyzing the precise spatial expression of the candidate chemoreceptors within the pheromone sensing tissue.

GUSTATION IN ELASMOBRANCHS: ECO-MORPHOLOGY AND TOPOGRAPHIC DISTRIBUTION OF TASTE BUDS

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Gustation (taste) is an important sense used by all vertebrates for food localisation, acceptance or rejection essentially preventing the consumption of harmful substances. Previous research into elasmobranch gustation (taste) is scarce examining only four species, all of which are demersal and feed predominantly on crustaceans. Although the gustatory system in elasmobranchs appears comparable with that of teleost fish, detailed physiological and behavioural studies are not available to further support this observation for this large group of cartilaginous fishes. The present study reveals preliminary results for taste bud morphology in *Carcharhinus melanopterus, Chiloscyllium punctatum, Hemiscyllium ocellatum, Negaprion acutidens, Orectolobus maculatus, Orectolobus ornatus, Taeniura lymma, and Trygonorrhina Species A*, using light microscopy, scanning (SEM) electron microscopy and transmission (TEM) electron microscopy. Taste bud distribution within the oral cavity is also examined and appears to vary with prey preference and the feeding mechanism adopted. In addition to morphologically characterising taste receptors, this long term study will also identify, physiologically, taste preferences and threshold concentrations of a range of stimulants using the electrogustatogram (EGG) technique. The importance of the gustatory system will also be determined by analysis of the relative size of the facial and vagal lobes in the central nervous system. Knowledge of gustation in elasmobranchs will aid in our understanding of the evolution of taste, identify how gustation is used to selectively accept or reject "food" and provide crucial information for future development of shark repellents.

OLFACTION IN WOBBEGONG SHARKS: MORPHOLOGICAL INDICATORS OF CHEMORECEPTIVE ABILITY

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Elasmobranchs are thought to possess an acute sense of smell, but the relationship between the anatomy of their olfactory organs and their sensory ecology is poorly studied. Wobbegong sharks are generally assumed to be powerful ambush predators, but habitat use, geographic distribution and behaviour vary among the different species, making it difficult to speculate on their olfactory capabilities. In this study, we measured the number of olfactory lamellae and the size of the olfactory epithelium as a means to assess relative olfactory sensitivity in four species of wobbegong shark. A comparative analysis between wobbegongs and several previously studied elasmobranchs was also done in order to compare the relative importance of olfaction in wobbegongs with other species. Significant differences between the total number of lamellae are seen among most of the species but not between the spotted wobbegong, *Orectolobus maculatus*, and the western wobbegong, *O. hutchinsi*. Sensory surface area is comparable between these two species and the banded wobbegong, *O. ornatus*, but the dwarf spotted wobbegong, *O. parvimaculatus* has significantly larger sensory surface area. When compared to other elasmobranchs, the olfactory abilities of wobbegongs are as good, or better, than other benthic elasmobranchs. Based on a comparison of diet, reproductive strategy, habitat and phylogenetic relationships between the four wobbegong species, ecological factors most likely account for the differences in olfactory ability. Some wobbegong species might rely more heavily on olfaction for prey detection, whereas intraspecific recognition and mate detection could be more important applications for olfactory capabilities in other species.

Session 2: Neurobiology of Chemoreception

STEMS CELLS FROM ADULT OLFACTORY MUCOSA: FROM NARIS TO NOSOLOGY

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Stem cells from human olfactory mucosa are multipotent, capable of generating neurons and glia but also many other cells and tissues of the body, either in vitro or after transplantation into the developing chick embryo (Murrell et al, 2005). Human olfactory stem cells are accessible through the external naris. Our focus is to explore the use of olfactory stem cells for cell transplantation therapies and as cellular models of disease. Olfactory stem cells from humans, including a patient with Parkinson's disease, proved to be therapeutic in the parkinsonian rat model, indicating the ability to generate dopaminergic neurons in vivo and in vitro (Murrell et al, 2008). We are focussing on developing cellular models of disease by investigating patient-derived, disease-specific stem cells. The National Centre for Adult Stem Cell Research now has a bank of olfactory stem cell lines from over 100 people including those with neurological conditions including Parkinson's disease, schizophrenia, motor neurons disease and mitochondrial mutation disorders. We are using a combination of gene expression profiling, proteomics, bioinformatics and functional analyses to investigate disease-specific alterations in olfactory stem cells cultures. Our initial studies have identified cellular

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signalling pathways disrupted in stem cell cultures from patients with Parkinson's disease compared to controls and these are different from those disrupted in schizophrenia. Our goal is to identify new diagnostics and new drug targets through an understanding of disease-specific alterations in key cellular pathways.

Murrell W, Wetzig A, Donnellan M, Féron F, Burne T, Meedeniya A, Kesby J, Bianco J, Perry C, Silburn P, Mackay-Sim A. (2008) Olfactory mucosa is a potential source for autologous stem cell therapy for Parkinson's disease. Stem Cells. 26:2183-92.

Murrell W, Féron F, Wetzig A, Cameron N, Splatt K, Bellette B, Bianco J, Perry C, Lee G, Mackay-Sim A. (2005) Multipotent stem cells from adult olfactory mucosa. Dev Dyn. 233:496-515.

LESSONS FROM LIVE CELL IMAGING: OLFACTORY ENSHEATHING GLIA ARE A HETEROGENEOUS POPULATION OF CELLS

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Olfactory ensheathing cells (OECs) are a specialised type of olfactory glia that show a remarkable ability to promote axonal repair after implantation in the damaged brain and spinal cord. However, transplantation of these cells results in mixed outcomes and improvements may be achieved if we better understood the mechanisms underlying migration and survival of these cells. Currently, OECs are often purified from the peripheral olfactory system or from the central olfactory system but the anatomy of the olfactory system suggests that the cells will have different functions. To determine if this is the case, we have used live cell time-lapse imaging to analyse the behaviour and migration rates of OECs taken from different regions of the olfactory system, and from different aged animals. We have found that OECs from the peripheral olfactory nerve are a homogeneous population of cells that respond uniformly to cell-cell contact with the vast majority of their interactions resulting in cell adhesion. In contrast, OECs from the olfactory bulb are a heterogeneous population of cells. OECs from the rostral olfactory bulb display an even mix of adhesion, repulsion or cross-over in response to cell-cell contact; and they maintain these responses throughout development. In comparison, OECs from the dorsal, ventral and caudal olfactory bulb also display a mix of responses but these responses change with increasing developmental age. The characterisation of the differences between subpopulations of OECs may lead to the enhancement of their therapeutic potential for neural repair.

OLFACTORY ENSHEATHING CELLS AND SCHWANN CELLS INTERACT WITH AND MODULATE SENSORY NEURONS

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Implantation of olfactory ensheathing cells (OECs) or Schwann cells (SCs) into damaged CNS tracts have led to axonal regeneration; however, the results are not optimal. In the damaged spinal cord, OECs are more efficient than SCs in stimulating axonal regrowth, however, the favorable glial type for transplantation may vary depending on the localisation of the injury. Therefore it is crucial to determine the functional differences between OECs and SCs in terms of modulation of different types of neurons. In particular, it remains unclear how these glial cells modulate peripheral neurons such as dorsal root ganglion (DRG) neurons. It is believed that direct interaction between glial cells and axons is crucial for regeneration, but the cellular mechanisms behind these interactions are unknown. We have previously shown that OECs display highly motile peripheral membrane protrusions termed lamellipodial waves. These waves constitute the main site for cell-cell contact between OECs, suggesting that they may also mediate contact between OECs and neurons. In the current study, we have for the first time demonstrated the presence of highly dynamic lamellipodial waves in SCs. Both OECs and SCs interacted with DRG axons via the lamellipodial waves. As a result of these interactions, the glial cells preferentially migrated along the axons rather than the surrounding substrate. Finally, we showed that both SCs and OECs promote survival and neurite outgrowth in DRG neurons and that the two glial types were equally efficient, suggesting that transplantation of both SCs and OECs may promote regeneration of damaged sensory neurons.

mRNA TAGGING OF THE *C. ELEGANS* OLFACTORY NEURONS TO DETERMINE GENE EXPRESSION

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C. elegans detects odours via three pairs of bilaterally symmetrical neurons AWA, AWB and AWC. In an effort to identify genes expressed in these cells, we are using mRNA tagging to enrich for odorant neuron transcripts for microarray analysis. This technique has been used to study other tissues in *C. elegans* (Roy *et al* Nature 418, 2002; Kunitomo et al Genome Biology 6, 2005; Von Stetina *et al* Genes and Development 21, 2007). Using this approach, transgenic worms are created that express recombinant polyA-binding protein (PAB-1), which is labelled with three N-terminal FLAG epitopes. The 3XFLAG::PAB-1 protein is chemically cross-linked to mRNA and these complexes are purified using anti-FLAG antibodies. The mRNA is then eluted and analysed using microarrays.

We have generated transgenic lines *C. elegans* that express 3XFLAG::PAB-1 in AWB, using the *str-1* promoter, and in both AWB and AWC, using the *odr-1* promoter. Correct expression of recombinant protein has been verified by immuno-staining using anti-FLAG antibodies. We present the results of this work to date.

ON THE OLFACTORY SENSE OF FOWL AND PIGEON

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It is believed that birds have a sharp sense of smell by the behavioral observations as well as mammals. In the meantime, the research object is few such as vultures, pigeons, crows, chickens and kiwis. Anatomical studies indicated that the birds have a well developed olfactory organ and have estimableness similar the mammalian olfactory organ. Those results studied with the sparrow, chicken, pigeon, kiwi, crow, swallow, eagle, hawk, vulture, owl and sea-gull. However , the functional significance of avian olfaction is less clear. Considerably fewer studies have been conducted in physiology. The purpose of this study is to examine the characteristics of the olfactory function of the fowl and pigeon with electroencephalographical (EEG) recording and respiration rate recording methods. Thus, for example, fowls decreased their respiration rate to some odor stimulations such as eugenol, butylic acd, phenyl isocyanate and quinoline. While, pigeons decreased their respiration rate to benzaldehyde, limonene and methyl acetate odors. Ammonia, cyclohexanone, cyclopentanone and geraniol were decreased the respiration rate of both birds. FFT analysis of EEG response between fowl and pigeon appears to characterize the response to an odor. Odor of the anthoranic acid is a well known hedonic substance to the bird. However, it did not evoke clear behavioral and EEG responses on the birds. From these results, it is speculated that the fowl and pigeon have different characteristic responses to odor stimulations.

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LEARNED PREFERENCES FOR ODOURS DETERMINED BY INDIVIDUAL VARIATIONS IN TASTE INTENSITY AND HEDONICS

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Repeated co-exposure to novel food odours with sweet or bitter tastes can lead to changes in both liking and the sensory characteristics of these odours. However, there are individual variations in perception of taste intensities such as those indexed by the genetically-determined responses to the taste of 6-n-propylthiouracil (PROP). Thus, PROP tasters find saccharin both more bitter and more sweet than do PROP non-tasters. We therefore predicted that acquired liking for odours conditioned by association with saccharin would vary with PROP taster status. Since odor perceptual characteristics such as smelled sweetness and bitterness arise via association with tastes, we similarly predicted that PROP taster status would influence such learned effects. Also, because there are consistent individual variations in hedonic responses to sweet tastes, we examined the impact of the sweet liker/disliker dichotomy. Eighty-seven volunteers evaluated two novel odours before and after co-experience of one odour with 0.0004M/l saccharin and the second with water. PROP taster status was defined using rated intensity of 3.2mM PROP, and sweet-liker status from liking and intensity ratings for 0.21 and 0.83 M/L sucrose and 0.0004M/l and 0.0010M/l saccharin. Liking for the saccharin-paired odour increased in sweet likers but decreased in sweet-dislikers. Overall liking change also varied with PROP taster status, with both odours rated less pleasant post-training in the taster but not non-taster groups. The saccharin-paired odour was rated as sweeter smelling post-training, regardless of PROP taster or sweet-liker status. PROP super-tasters rated the saccharin-paired odour as more bitter smelling post-training, in-line with enhanced bitterness of saccharin alone in this group. These data confirm that the development of liking for odours (and hence flavours), as well of the sensory characteristics of the odours, are at least partly determined individual differences in sensitivity and hedonic responses to bitter and sweet stimuli. Thus, at least some of the PROP group differences in food preferences are probably due to differential learning processes involving food flavours, and not simply to a response to the tastants in the foods.

Session 3: Evolutionary Genetics of Insect Olfaction

INSECT ODORANT RECEPTORS: NOT JUST ANOTHER FAMILY OF GPCRs

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Vertebrate members of the animal kingdom use G protein-coupled receptors (GPCRs) to perceive their chemical world. Upon sequencing the first insect genome, that of Drosophila, a set of genes predicted to encode seven transmembrane proteins and expressed exclusively in antennae and maxillary palps was thought to be part of a specialised family of insect GPCRs involved in olfaction. Although this family shares little sequence similarity with any GPCR, subsequent work did indeed show that they encode odorant receptors. However, little is known concerning the structure and activation mechanism used by these important proteins. We have developed methods to assess the activity of insect odorant receptors transfected into heterologous cells by detecting [Ca^{2+}] increases upon the addition of odorous ligands. These assays have enabled structure/function studies and the collection of pharmacological data on odorant receptors from flies and moths. A consensus model of the topology of the odorant receptor, Or22a, from *Drosophila melanogaster* was tested empirically using immunohistochemistry of epitope-tagged versions of the receptor under different detergent conditions. While the data support a topology model containing seven transmembrane regions, the orientation of the receptor in the plasma membrane is opposite to that of classical GPCRs, instead having an intracellular N-terminus. Furthermore, the addition of inhibitors of classical G protein signalling pathways had little impact on receptor activity, suggesting that insect odorant receptors form a novel family of seven transmembrane receptors. These data, together with patch clamp studies conducted by us and others (*Nature*, 2008, 452: 1002-6, 1007-11), reveal that insect odorant receptors primarily signal through interaction with a non-specific cation channel, made up of the co-receptor, Or83b, likely together with a ligand-binding Or, such as Or22a. Thus it seems insects have developed a completely novel mechanism to detect volatiles compared with their vertebrate counterparts.

CO-EVOLUTION OF BEE ODORANT RECEPTORS AND FLOWERING PLANTS

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The complete genome sequence of *Apis mellifera* reveals significantly fewer genes overall than in other sequenced insect genomes. However, certain subfamilies of genes are disproportionately large in this species, notably those encoding odorant receptors, certain cytochrome P450s putatively associated with hormonal and chemosensory processes, and farnesyl pyrophosphate synthases implicated in lipid metabolism. *A. mellifera* also has some subfamilies not found at all in the other genomes, in particular those encoding the major royal jelly proteins (MRJPs) that provide the cues for caste determination. We first estimated nucleotide divergences among *A. mellifera* and six other corbiculate bees representing various steps in the evolution of advanced eusociality. We found that evolutionary rates have accelerated markedly down the lineage leading to A. mellifera. We then used the rate data from the A. mellifera lineage together with the fossil record to calibrate a molecular clock for synonymous sites down this lineage. This allowed us to date the expansions of the gene families above. We found that essentially all of the expansions occurred in the last 50 Myr, well after the honey bee tribe Apini emerged, and simultaneous with the evolution of flowering plants. While certain clades of odorant receptors and in particular P450s radiated 30-50 Mya, most of the odorant receptors and the MRJPs emerged in the last 10-30 Myr, when more advanced eusociality and cavity nesting evolved. One clade of odorant receptors and most of the farnesyl pyrophosphate synthases only proliferated after *A. mellifera* separated from its nearest relatives, less than 10 Mya.

EVOLUTION OF OLFACTORY RECEPTORS FOR ALIPHATIC ESTERS IN DROSOPHILA SPECIES

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The molecular evolution of olfactory receptor (Or) genes is of considerable interest because they form large, rapidly evolving gene families and each gene is expressed only in an identifiable class of sensory neurons that drive behavioural responses crucial for survival. *Drosophila* flies feed on yeast in fermenting fruits. Aliphatic esters are products of yeast fermentation and important components of fruit aromas. We have investigated the evolution of the subfamily of eight Or genes that are largely responsible for their perception across eight species of *Drosophila*. Electrophysiological recordings were made from olfactory receptor neurons (ORNs) to determine their response properties to a panel of 16 odorants. We

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recorded from the eight classes of ORNs that express the targeted Or genes in *D.melanogaster*, and compared the responses of homologous neurons in seven other species of *Drosophila*.

Our studies reveal a surprising level of conservation in odour response spectra, at least within the subgenus *Sophophora*. Ligand binding specificity of Or proteins is maintained despite considerable variation in amino acid sequence. One neuron (ab3A) shows exceptional variability across species, correlating with a high number of duplication events for the corresponding Or gene (Or22a). We also find that response properties of homologous ORNs in some species change when Or genes are lost from the genome. Our findings suggest that changes in Or gene expression are likely to play as important a role in the evolution of the peripheral olfactory systems as shifts in ligand specificity. Finally, we perform a structure-function analysis for selected Or genes to correlate Or sequence and ORN response property changes.

INSECT PERCEPTION AND THE RECOGNITION OF FLORAL ODOURS

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The production and detection of floral scents has played a major role in the coevolution of insects and plants. Floral odours are unique blends of volatile chemicals that attract pollinators, but also act as signals to insect herbivores, which damage the plant through adult and larval feeding. Using the noctuid moth, *Helicoverpa armigera*, as a model species, we have conducted (i) laboratory based (wind tunnel) experiments using artificial blends and (ii) flight cage experiments, using genetically modified plants with differing odour profiles. Our work investigates how changes in odour blend composition influence the nectar foraging and egg laying behaviour of an important agricultural pest.

Session 4: Aromas, Flavours & Human Olfaction

TEMPORAL PROCESSING OF OLFACTORY STIMULI DURING RETRONASAL PERCEPTION

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Odorants can be perceived via the nose during an inhalation or sniff (orthonasal perception) and via the mouth and nasopharynx during mastication or drinking (retronasal perception). Previous data suggest that orthonasal perception provides a more efficient route with greater difficulty being reported when detecting [24] and identifying [7] single odorants retronasally. Whether the poorer sensitivity obtained via the retronasal route is largely due to the greater adsorption of odorants by the nasopharyngeal mucus compared to the nasal mucus thereby reducing their peak concentration and/or slowing their passage, has not been resolved. Importantly, the question of whether solubility of odorants in mucus or water predicts the outcomes for perception of stimuli presented via the retronasal route has not been resolved. Accordingly, the present study investigates this question by determining whether the solubility of an odorant in mucus predicts which component of a binary odour mixture is perceived first during retronasal perception. The results indicate that solubility in mucus rather than solubility in water is a better predictor of which odour will be perceived first and identified more readily during the retronasal perception of a binary mixture. In addition, lower intensity levels of single odorants occurred via the retronasal route suggesting that adsorption was greater via this route. Whether this was due to nasopharyngeal mucus having a greater adsorptive area or different composition compared to the orthonasal pathway is not known.

THE SENSORY AND CHEMICAL PROPERTIES OF COCOA PRODUCTS

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Understanding the biochemical origin and sensory importance of key aroma compounds in cocoa, can allow manipulation during the production process to enhance or control the formation of quality-determining compounds, ensuring consumer acceptance of the final product. The present study focused on analysing the sensory and chemical properties of six cocoa samples, of different geographic origin. The objective of this study was to combine a series of scientific evaluations of chemical and organoleptic parameters, to identify key volatiles that are responsible for the distinct aromas of good quality chocolate and typical off-flavours.

The key findings from this research were that chocolates made from Asian cocoa liquors exhibited more undesirable flavours than the West African and South American chocolates, which were perceived as having desirable sensory attributes. The volatiles, 3-methylbutanoic acid, 2 methylbutanoic acid, benzeneacetaldehyde, ethyl octanoate and 2 nonanone were responsible for intense aromas in the majority of samples, implying their possible contribution to cocoa aroma. The sensory and chemical data indicates that the sensory differences between the cocoa samples could be explained by the variation in the volatile composition.

The results obtained from this flavour research provide significant information regarding the key aroma volatiles and the unique flavours of cocoa. In particular, it may aid in the future development of a targeted analysis to objectively measure the volatiles in cocoa beans that are responsible for flavour quality. To date, this is the first study that investigates the sensory importance of each volatile found in cocoa.

GASTRONOMICS: PERSONALISING FOODS FOR FLAVOUR

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Not everyone is gifted with the same sense of smell. The premise of Gastronomics is that a person's genetic makeup with respect to odour perception can be a predictor of personal food preferences and food choices. Such a link could have a transformational effect on Food and Beverage industries across the globe, enabling innovation of products optimised for taste and flavour. The notion that such innovation needs to deliver products that people like is not new. What is new is drawing on knowledge of human genetics to understand why people prefer different foods and how products that will meet the individual preferences of consumers can be developed. Initially we have chosen the genetics of olfaction as much of what we find pleasurable about eating is determined by flavour and humans are highly variable in their compliment of functional olfactory receptor genes that underlies the ability to smell. In our initial experiments to test the premise of gastronomics we have already found high levels of variation in the ability of panellists to detect certain important flavour compounds but not others. These differences will allow us to determine the genetic basis for the ability to smell these compounds and whether sensory acuity for these compounds impacts on food preference.

TASTE AND SMELL PERCEPTION AND WINE EXPERTISE

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Untrained humans can generally recognise an odour that they have perceived before but are often unable to put a label to even some of the most familiar odours in the absence of non-olfactory cues, a phenomenon known as "tip of the nose" phenomenon (Lawless & Engen, 1977). However, trained humans such as wine experts, perfumiers and trained sensory panels do not display this deficit, despite not possessing superior sensory abilities (Parr et al, 2002). These experiments aim to study various aspects of perceptual learning in smell and taste perception in order to train novices, with a particular emphasis on wine expertise. In an initial experiment, first year participants were given an

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odour-label matching task, where they had to match labels to three 2-odour mixtures that were difficult to discriminate due to a common lemon odour. Participants were given two blocks of 18 training trials before a final 18-trial test 24h later, with feedback given during training but not test. One group *(Given)* was given a list of (correct) labels to choose from, while participants in a second group (Make-your-own or MYO) generated their own labels for the samples. The Given group performed well above chance from the beginning, while the MYO group performed at chance during the first training session, but differences between the groups disappeared by test. These results indicate that appropriate labels facilitate identification of odours more than self-generated labels. In current experiments we are examining which conditions best facilitate this label effect.

CO-INOCULATED FERMENTATIONS: THE EFFECT ON THE AROMATIC PROFILE, CONSUMER PREFERENCE AND QUALITY OF SAUFIGNON BLANC WINES

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Alcoholic fermentation using specific *Saccharomyces cerevisiae* wine yeast strains is an effective means of enhancing wine aroma, through modulation and production of hundreds of volatile aroma compounds. This study investigated the effect of co-inoculating multiple yeast strains on the chemical composition and sensory profile of Sauvignon Blanc wines, and to determine whether regular wine-drinking consumers have a preference among the yeast inoculums tested. Small-scale replicated winemaking was conducted using four co-inoculations, one containing two yeast strains and three containing the same three yeast strains in differing proportions. Single-strain fermentations of the co-inoculations' single yeast components were also conducted for comparison. All wines were chemically analysed for volatile aroma compounds, including thiols and esters, and underwent a descriptive sensory analysis. A subset of four wines was subjected to consumer acceptance testing by 120 consumers. The results showed that all wines differed in their chemical and sensory properties, particularly between the three yeast strain co-inoculations, indicating that the proportions of single yeast components in the co-inoculations was very important to the final wine aroma. The results of the consumer acceptance testing indicated that there was a significant difference in consumer liking for the wines produced using different yeast inoculums. Four clusters of consumers were identified as rating the yeast inoculums differently and were investigated. This work demonstrates that co-inoculations can be used to modify the aroma profile of Sauvignon Blanc to maximise consumer acceptance, and will assist the wine industry in tailoring wine to market specifications for increased competitiveness.

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Session 5: Biosensors & Industrial Applications

BODY ODOUR FILTERS: OPTIMISING AIR FILTARTION AND VENTILATION IN BUILDINGS BY OLFACTORY MEASUREMENT

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Air quality in buildings is important for human health and safety. Air conditioning systems presently filter air (remove particulates), but do not remove odours. Odours are merely reduced by dilution, i.e. by addition of fresh air, usually as specified in an Australian Standard. Fresh air inflow strongly affects energy consumption of buildings, the size and cost of heating and cooling plants and, consequently, the cost of running these buildings.

The Australian Ventilation Standard (AS 1668.2), which specifies fresh airflow requirements determined by health and olfactory considerations, leads the world. It has, since 1991, allowed reduction of ventilation rate, when odour in occupied spaces is reduced by tested technical means. Only recently has a suitable gas phase air cleaner (GPAC) been designed and tested.

This paper describes a robust means of testing the olfactory attenuation of gas phase air cleaning (GPAC) devices. The test produces a percentage value for the efficiency of apparatus that removes body odours and other odours from buildings. Use of tested apparatus optimises fresh airflow into buildings, without compromising health or olfactory comfort. This minimises energy expenditure, particularly in large buildings.

We have developed a robust, large scale, GPAC test rig at the Australian National University. It uses human subjects to generate odours, and human noses to assess the effectiveness of a GPAC device. The methodology may be used to test many kinds of GPAC devices and odour instrumentation approaches. It offers significant economic benefit on Australian and worldwide scales.

DEVELOPMENT OF AN ALARMED E-NOSE FOR RECOGNIZING SPRAY-PAINT AND FOR THE APPREHENSION AND DETERRENCE OF GRAFFITI VANDALS

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Whether it is art or vandalism, spray-painted graffiti is costing Australian city councils, companies, property owners and taxpayers in excess of $50 Million per annum. Cities in the USA, UK and Europe, collectively, bear massively greater costs from spray-painted graffiti. This is money that is being diverted from people's living expenses, from businesses' "bottom lines" and from communities' projects such as libraries, child minding centres, services for the poor and elderly, etc. We were asked by community members and a railway company to see if we could turn our e-noses into a means of apprehending and deterring graffiti vandals. Using a sensor array previously developed by our group for detecting wet ink on plastic packaging, we tested various spray paints of the kind used by graffiti vandals to "tag" walls, windows, and the outer surfaces of trains and buses. The array could discriminate different paints, propellants and solvents. It could detect a 4 sec burst of spray emulating the painting of a train carriage, downwind, in a 5km/h breeze at 45 m distance. It can be safely concluded that the range will increase considerably with longer bursts of paint, such as happens when three vandals, each with spray-cans, typically attack a train carriage and cover one side of it, in around 17 minutes: a release of nearly 200 times more vapour into the air than was released in the experiment. The device can therefore be located some distance from the likely attack point and still be effective. Several devices may be needed to cover wide areas and provide for varying wind directions. The e-nose, now called *graffit-e-nose* ™, also discriminated between spray-paint and cleaning solvents in the experiment. A patented Bayesian algorithm (Hibbert and Bell, 2007) will be used, as application demands, to make real-time recognition of spray-paint and distinguish it from graffiti cleaning solvents that might be in use in the same area, as happens at night in large vehicle parks. The alarmed e-nose consists of the device itself, discretely located in a customized secure box, also containing a wireless data transmitter. Data is automatically received and interpreted at an unmanned remote and secure location, whereupon recognition of a "graffiti event in progress" triggers one or several SMS messages which are sent to mobile phones of people or agents responsible for the assets under attack.

Reference: Hibbert, D.B. and Bell, G.A (2007). A method of predicting the source of data sampled from and unknown source. PCT/AU2007/001214.

CHEMICAL BASED-SENSORS FOR GRAPE AND WINE ODOUR DETECTION

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The application of fast techniques for aroma and flavour sensing in food industry has been widely reported and well received. Examples include the classification of different tomato cultivars, types of cheese and grades of meat. However sensor arrays (electronic noses) have not been broadly evaluated for classification of wine

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and grapes. This is despite the significant value of these products in wine-making countries and the well-known need to have rapid and cost effective tools for quality control purposes and to detect contaminants or off-flavours in wines. For many years, a key obstacle preventing the use of electronic noses (Enose) in wines has been saturation of the sensors due to the high ethanol content. Here we present a simple technique to reduce ethanol in wine prior to Enose analysis. This combines a recently introduced filter-drying step followed by solid phase micro-extraction. Using this procedure we were able to apply Enose to a number of problems in wine as well as winegrape analysis. Examples of our findings include determining the limits of detection of metal oxide based-sensors for two "brett" taints in wines, namely: 44 µg L^{-1} for 4-ethylphenol and 91 µg L^{-1} for 4-ethylguaiacol. These values are significantly lower than the reported human sensory thresholds. We also found that, following training, the Enose can correctly classify Sauvignon Blanc wines from different regions with 90% accuracy. Moreover, we show that the instrument can discriminate among winegrapes of different qualities with the same level of confidence as Gas Chromatography-Mass Spectroscopy.

CHARACTERISTICS OF A SET OF METAL OXIDE SENSORS OF VOLATILE ORGANIC COMPOUNDS COMPARED WITH INSECT OLFACTORY RECEPTORS

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Quality Biosensors theme – Food Futures Flagship, CSIRO, Canberra, Australia

Animal noses and antennae are deployed in situations where odors must be detected or discriminated rapidly. Noses and antennae are also capable of performing discriminations among complex odorant mixtures with great sensitivity and speed. Electronic noses, E-noses, are instruments designed to reproduce the pertinent features of animal noses using arrays of chemical sensors. However, E-noses have not been very widely adopted, in large part because they perform poorly in many real-world discrimination tasks. Replicating the performance of a biological system deepens our understanding of the physiological mechanisms involved and may also lead to technological improvements in instruments, such as E-noses, designed to perform similar tasks. Following recent detailed characterisation of the molecular receptive range of a subset of Drosophila olfactory receptors (ORs) (Hallem et al, 2004) it is now possible to compare the responses of a set of technical sensors with those derived by evolution. Here we compare the sensitivity, tuning and independence of 12 metal oxide (MOx) sensors with those of 24 Drosophila ORs using the same panel of 110 test odorants. We find little difference in levels of sensitivity between the MOx sensors and ORs with the Drosophila ORs being more broadly tuned. Despite this apparent lack of selectivity the Drosophila ORs are highly independent of each other, ie each Drosophila OR is non-selective in its own unique way. We propose that an array of independent sensors, each of which display odorant promiscuity is important for the discriminating power of the insect olfactory system. The array and component MOx sensors we examined lack these features and accordingly have relatively poor ability to discriminate odorants.

UTILISATION OF OLFACTORY RECEPTORS FOR BIOSENSING APPLICATIONS

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Recent advances in molecular biology, surface chemistry, protein purification, signal transduction/amplification, lipid chemistry and nanofabrication technologies have converged in a relatively new field of science, that of molecular biosensing. A molecular biosensor is defined as a biological detector linked to a physical transduction/amplification system that reports (and hopefully quantifies) a specific interaction with the biological detector. Because of their diversity, specificity and metabolic importance, G-protein coupled receptors (GPCRs) are increasingly being investigated as front-end biological detectors for biosensing applications. A functional class of GPCRs, the olfactory receptors, hold particular interest for biosensing of volatile compounds. Here, I summarise the approaches to olfactory biosensing, and introduce a new project that aims to use data from the recently sequenced genome of *Tribolium castaneum* to isolate and characterize its pheromone receptors in order to produce a *Tribolium* biosensor for use in the agrifood industries. It is expected that the development of an effective biosensor utilising the inherent sensitivity of *Tribolium* olfactory GPCRs would allow detection of *Tribolium*-specific compounds well below the limits of current technologies. This would provide the Australian grain industry with the ability to monitor beetle populations, establish treatment thresholds, reduce phosphine fumigation of grain stores and increase confidence in "live-insect free" status of exports.

Abstracts – Poster Session

UNDERSTANDING THE REGENERATION OF SMELL

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Neurodegenerative diseases become increasingly prevalent with an aging population. In order to develop treatments for these debilitating diseases, it is imperative to understand the molecular pathways that control neural regeneration and guidance processes. Olfactory sensory neurons (OSNs) are constantly being regenerated throughout life by an active stem cell population in the olfactory epithelium. The newly generated OSNs project axons that target precisely defined glomeruli in the olfactory bulb. In our research project we attempt to elucidate the genetic mechanisms that control the regeneration and guidance processes of OSNs. The conserved Hippo pathway is involved in stem cell turn over and maintenance in arthropods through to vertebrates. We found that all components of the Hippo pathway are expressed in the murine olfactory epithelium, which strongly suggests that it regulates olfactory epithelium regeneration. We aim at getting clear insights into the role of the Hippo pathway in OSN regeneration through detailed expression analyses and through studying the regeneration processes in transgenic mice in which the activity of the Hippo pathway is manipulated. In addition, we are analysing the role of a novel gene *Eva1* in OSN axon guidance processes. In *C. elegans, Eva1* has recently been found to be an important DV guidance molecule that interacts with members of the *Slit* and *Robo* gene families. We found that the mouse orthologue of *Eva1* is expressed in the murine olfactory system and we are currently investigating the functions of *Eva1* in DV patterning of the olfactory bulb in conjunction with members of the *Slit* and *Robo* gene families through detailed expression analyses and gain-of-function studies in mice. The outcomes of our studies will yield crucial insights in the molecular biology of neural regeneration and may give insights into the development of neurodegenerative diseases and how to cure them.

PLANTS, PESTS, AND POLLINATORS: COMBINING TECHNOLOGIES TO CRACK THE ODOUR CODE

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Plant odours, a complex bend of many individual volatile components, play a fundamental role in insect-plant communication through the attraction of pollinators and defense against herbivores. This project aims to determine the roles that major floral terpenoid compounds play in attraction, orientation and acceptance of a real plant by *Helicoverpa armigera*, a polyphagous pest of worldwide agricultural significance. Quantitative assays of promoter activity and northern analysis of transgenic *Nicotiana tabacum* plants demonstrate successful overexpression of heterologous terpene biosynthesis genes in flowers, while successful downregulation of one gene can be observed by phenotype. Preliminary wind tunnel bioassays show *H. armigera* moths prefer one naturally occurring volatile component of tobacco flowers over another.

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EXUBERANT PROJECTIONS OF OLFACTORY AXONS AND ERROR CORRECTION DURING DEVELOPMENT IN THE MOUSE OLFACTORY BULB

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Olfactory sensory neurons (OSNs) project axons within fascicles along defined pathway to the brain where they terminate in discrete glomeruli in the olfactory bulb. During development of the mouse olfactory system, olfactory sensory axons first contact their terminal zone in the glomerular layer of the olfactory bulb during late embryonic period and target to their appropriate glomeruli over the next few days. During this period, many axons can branch inappropriately into several glomeruli and overshoot the target layer and enter deeper layers of the olfactory bulb. We have undertaken a detailed examination of the over-projection of these axons using mice that express a very bright green fluorescent protein in primary olfactory neurons (OMP-ZsGreen mice). We found that numerous aberrant axonal projections occurred within the deeper layers of the bulb for the first two postnatal weeks; after that axon trajectories became refined and over-projecting axons were infrequently observed although they continued to be present even in adult animals. These results, together with *in vitro* cultures of primary olfactory neurons with olfactory bulb explants, have provided insight into the role of the molecular and cellular cues that regulate the timing of olfactory axon guidance.

NOVEL APPROACH TO STUDY CALCIUM SIGNALS IN INSECT CHEMORECEPTION

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In Drosophila, the detection of taste compounds is thought to be mediated by a family of about 60 gustatory receptors (GRs) expressed in the gustatory receptor neurons (GRNs). For the majority of these receptors, their role in taste discrimination remains to be elucidated. Here, we describe the development of insect cells expressing the genetically encoded calcium probe G-CaMP (Nakai *et al*, 2001) as a new approach to investigate GRs function. G-CaMP is a high affinity calcium indicator with a hight signal-to-noise ratio derived from GFP. We have generated an insect cell expression vector containing G-CaMP and transiently expressed it in the Sf9 cell line. G-CamP functionality was tested by monitoring levels of intracellular Ca2+ in response to pharmacological agents either affecting the permeability of the plasma membrane (ionomycin) or evoking Ca^{2+} release from internal stores (thapsigargin). Using this system, we aim to co-express specific GR with G-CaMP in Sf9 cells in order to assess the rise in cytosolic calcium in response to candidate taste receptor ligands. Compared to usual fluorometric assays using synthetic dyes *e.g.* fura-2, this new approach offers the advantage of a stable expression of the calcium probe and the omission of the time and labour-consuming steps of loading, incubation and washing of the fluorophore.

"I'LL HAVE WHAT THEY'RE HAVING" or SOCIAL TRANSMISSION OF FOOD PREFERENCE IN MICE.

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Mice learn a socially transmitted food preference from odour cues on the breath of conspecifics. We tested whether social transmission of food preferences would occur in 2-4 month old F2 C57Bl/6x129sv mice 24 hours after a 5 minute exposure to a sender mouse while the receiver mouse was anaesthetised. The receiver mice were tested in a binary choice between the cued food and an equally palatable novel flavoured food for 30 min. Mice *anaesthetised* during the social interaction with Sagatal (60 mg/kg) and allowed to recover avoided the cued food when tested 24 h later. This aversion was not due to any overt aversive effects of this dose of Sagatal as mice which ate the food and were then anaesthetised, or could smell only the food for 5 min while under anaesthesia showed no preference or aversion. Control mice (non-anaesthetised) showed a robust preference for the cued food when they smelled the breath of a receiver mouse, but no preference was evident if they ate the food or could smell the food 24 h before testing. In a second experiment we found that the sagatal-induced aversion was not a general property of anaesthesia as there were differential results from receiver mice treated with a number of anaesthetic drugs. For example, sender mice anaesthetized with Hypnorm (0.5 ml/kg, opioid agonist) showed a preference for the cued food. These results suggest that modulating the GABA and opioid systems alters socially mediated food preferences and aversions in anaesthetized mice.

A CLINICAL TEST OF SMELL FOR SCHOOL-AGE CHILDREN

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Currently there is no standard test that clinicians can use for the assessment of smell in children. Although tests have been developed for adults eg UPSIT and the German 'Sniffin Sticks' test, their cognitive requirements and duration make them unsuitable for use with children under about 10 yr. Diagnosis of smell dysfunction in children, therefore, is rare, whilst the health and social consequences are largely unknown. The present project aimed to develop a test for children aged 5 yr and above, based on smell identification. A total of 232 children aged 5 to 7 years from Sydney public schools and 56 adults aged 18 to 51 years participated. Participants had to identify 16 food and non-food odorants, sniffed from opaque squeeze bottles, from sets of 3 photographs and words. All tests were conducted at schools on an individual basis by a trained assessor. The results indicated that 5, 6 and 7 yr olds and adults, correctly identified 88.1, 88.9, 91.1 and 97.8%, respectively, of the 16 odorants. Applying the commonly used clinical criterion of 90% for normal function, the normosmic scores for the 4 age groups were 11, 11, 12 and 13, respectively, An ANOVA indicated there were no significant gender differences within age groups. Furthermore, no odorant was identified at a level which was significantly lower than the others, and all odorants were identified at about or above the 90% correct level. In summary, an olfactory test has been developed which provides a rapid (5-6mins) method for assessing smell function in school-age children.

SMELL AND TASTE FUNCTION IN CHILDREN WITH CHRONIC RENAL DISEASE

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A major problem for renal clinicians is the loss of appetite and unwillingness to eat that is exhibited by patients with chronic renal failure (CRF). Commonly, this results in malnutrition and anorexia, compromising treatment and recovery. Since impaired olfaction and/or gustation may be a cause of the unwillingness of CRF patients to eat sufficient food to maintain normal nutrition, the present study investigated smell and taste function in children with CRF. Sixty children, aged 5-16 years, participated: 20 had CRF, 20 were clinical controls, 20 were healthy controls. All were matched for age and gender. Olfactory function was assessed using a 16- odour identification test developed for children aged > 4 years. Gustatory function was measured using a test in which children identified 5 concentrations of sweet, salty, sour and bitter solutions and water, using sets of 3 photos. The results indicated there were no differences between the odour identification levels of the 3 groups ($p > .05$), however, the CRF group was

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significantly poorer at identifying the tastants than the other 2 groups ($p < 0.001$). In addition, there was a positive correlation between kidney function and total taste identification score ($r = .43$, $p < .01$) and 55% of patients had a taste disorder. Many children with CRF, therefore, have reduced taste function but their smell function is normal. Accordingly, impaired taste function may be an important factor that affects the willingness of CRF children to eat a diet that is sufficient to maintain their nutrition.

CHEMOSENSORY FUNCTION IN CHILDREN WITH CYSTIC FIBROSIS

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Cystic fibrosis (CF) can cause olfactory and gustatory disorders, which have detrimental effects on the diet/nutrition of patients and make it difficult for dieticians to develop diets that maintain growth during childhood. Accordingly, the relationships between olfactory and gustatory function and food preferences in 40 CF and 40 healthy 5-18 yr olds were studied. A three-choice 16-item odour identification test and a gustatory identification test involving 5 concentrations of sweet, sour, bitter and salty tastes were used to assess chemosensory function. Food preferences were assessed using a 94-item food preference questionnaire and a 24 hr dietary record. CF patients identified significantly fewer odorants than controls (89.8 vs 95.7% correct; $p < 0.001$) indicating that overall they had a poorer sense of smell. However, only 6 patients and 1 control were slightly hyposmic (75 % correct) indicating the loss of olfactory function was not substantial. No significant difference was found between the groups in gustatory ability (patients 92.6 vs controls 94.2% correct), nor was there a correlation between age and odor identification ability, but taste performance improved with age ($r = 0.39$, $p < 0.05$) suggesting cognition was the cause. In summary, only mild decrements in the olfactory function of CF patients were found and CF had no significant effect on gustatory function. The results suggest that the mild changes in chemosensory function in CF patients at least to the age of 18 yr are unlikely to be the cause of inappropriate food consumption.

OLFACTORY ENSHEATHING CELLS RAPIDLY PHAGOCYTOSE CELLULAR DEBRIS FOLLOWING NEURONAL DEATH

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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 also play a role in maintaining 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, indicating that OECs may be responsible. We have therefore further investigated the phagocytic ability of OECs. We cultured red fluorescent OECs from S100B-DsRed mice and green fluorescent primary olfactory neurons from OMP-ZsGreen mice. When explants containing both red OECs and green neurons were cultured, OECs that migrated out clearly contained green fluorescent debris. Further, when cellular debris from green fluorescent neurons was added to OECs, the green fluorescent debris was clearly phagocytosed by the OECs. In cultures of dorsal root ganglion neurons, considerable cellular debris is routinely present in the culture medium. However, when co-cultured with OECs the cellular debris was rapidly removed. In addition, the survival of the DRG neurons was improved in the presence of OECs and while it may be partially due to secreted growth factors and contact with the OECs, we propose that the phagocytosis of the cytotoxic debris by the OECs improves DRG neuron survival. 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.

DISCRIMINATION AMONG GASES USING AN OPTIMIZED E-NOSE ARRAY AND CHEMOMETRICS

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Gas sensors have been shown to be useful tools for rapid identification of gases[1]. To increase the selectivity of the system, and to be able to determine simultaneously multiple compounds, arrays of sensors are used[2]. In this paper we study the performance of five different metal-oxide semiconductor (MOS) sensors to discriminate among five gas species namely: ethanol, methanol, iso-propanol, acetone and toluene. Different concentrations of the five gases were injected into the sensor array over a period of 20 minutes and the signals from all the five sensors were collected and transferred to worksheets. Principal component analysis (PCA) was used as an unsupervised pattern recognition method to classify the gas species. In a systematic study linear discriminant analysis (LDA) was used to find the minimal number and optimal combination of the sensors that can be used to distinguish among all the compounds. Different pre-processing methods were compared. A general decreasing trend in the classification percentage was observed by reducing the number of sensors. However a unique combination of three of the sensors led to a complete discrimination among the gases. This work shows the synergy between sensors in arrays, and addresses approaches to choosing appropriate combinations for specific analytical tasks.

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ATTENTION FOR LISTENING TO MUSIC AFFECTS ON PERCEPTION OF SOAP ODOR

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We are using all five senses and integrating them to recognize external things in our daily lives. Although there are many studies considering the interaction and integration of olfaction with gustation, vision and somatosenses, an interaction between olfaction and audition remains unclear. To understand the interaction between audition and olfaction, the authors investigated whether the olfactory perception is affected by focusing attention to auditory stimuli or not. Twenty participants, who were female university students, were asked to continuously evaluate an intensity of soap odor presented by an olfactometer during listening to J-POP music (Music condition) or white noise (Control condition). After finishing evaluation, participants were asked to evaluate the odor intensity and the odor hedonics on visual analogue scale (VAS) and to rate auditory hedonics on 7-point scale. There were no significant differences and interactions in continuous evaluation for odor intensity among the conditions. However, there were significant effects of conditions in both evaluations on VAS. Participants evaluated the odor stronger and less pleasant during listening white noise as an auditory stimulus than those during listening to J-POP music. Thus, it is suggested that an attention for the auditory stimuli affects the olfactory information processes. This study also showed weak, but significant, correlation coefficient between the hedonic evaluation for odor and one for auditory stimulus. The latter finding seems to be an example of mood congruency effect in olfaction and audition.

EFFECTS OF AROMA AND BEVERAGES ON SUPPRESSING OF MENTAL STRESSES

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In our everyday life, we are exposed to many stressors that induce mental stresses, and many people suffer from having mental illness, such as depression, bipolar mood disorders, and so on. Although there are many studies showing that smelling an aroma is useful for easing these mental stresses, those studies did not exclude the placebo effects, and did not distinguish psychological effect of aroma from pharmacological effect of the aroma. In this study, two aromas, which have almost same ingredients but are dissimilar to each other in the effects, were presented to participants during exposure to the stressors. The effect of easing mental stresses was compared with other sensory stimuli, music and beverages. Two hundred and thirty six participants were asked to rate their mood on the state-trait anxiety inventory (STAI), and to work out puzzle (SUDOKU), and to rate their mood on STAI again. Working out SUDOKU made participants stressful. Drinking bottled coffee or water suppressed the mental stresses significantly. There are no significant differences between control group and music group, thus listening to the music during solving a SUDOKU puzzle did not suppress the mental stress from the puzzle. The aroma labeled 'for refreshment' did not have a stress suppressive effect, but the aroma 'for relax' tended to suppress the mental stress. Because there is only a little differences in their ingredients between two aromas, the psychological effect is suggested to be caused by labeling, placebo effect.

OLFACTORY AXONS AS YOU HAVE NEVER SEEN BEFORE: THE UTILIY OF THE OMP-ZSGREEN TRANSGENIC MICE

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We report here the generation of a new line of transgenic mice, OMP-ZsGreen mice, in which primary olfactory 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 neurons are labelled with the bright green fluorescent protein from embryonic day 11 onwards. The ZsGreen protein is expressed in all parts of the cell 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. We have generated two different founder lines of these mice. In the A1F(4) line of OMP-ZsGreen mice, the vast majority of primary olfactory neurons express ZsGreen, such that expression appears almost completely uniform throughout the nasal cavity and olfactory bulb. In the B1M(5) line of OMP-ZsGreen mice, the primary olfactory neurons lining the lowest ventral zone of the nasal cavity uniformly express ZsGreen, while neurons in the upper nasal cavity mosaically express ZsGreen. The B1M(5) line of mice is useful for examining the projection of axons from the ventral nasal cavity to their terminations in the caudal olfactory bulb. The use of both these lines 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.

THE DOG CAN SNIFF OUT THE EXPIRATION-ODOUR OF CANCER PATIENT

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Several researchers have reported that dogs are able to detect mole or tumours on the basis of odour. Recently, it is reported that dogs scent detection in lung and breast cancers. Since it is well known that the patient is scented unique odour, for example, diabetic patients are scented sweet body and sweet expiration odour. The ability of dogs to recognize and cross-match many kinds of variety of odours are studied by electrophysiological and behavioral experiments. Here we show that dogs can sniff out the expiration odour of cancer patients. We used sampling-gas-bags are made by Teflon with a Teflon one way tight bulb connecter which are placed in the similar blue boxes, respectively. We trained three female Labradors. Dogs were trained to detect one expiration sample from a patient with cancer placed among three control specimens. We observed the dog's behavior and the dog's EEG from behind a partition, concealed from the dog and handler. The handler usually took an air from the prostate cancer's breath air-bag with a 20 ml syringe. Then the air puffed from the syringe to the nostrils of the dog about ten seconds. The dog smelled it and stayed and waited until utter a command "look for". Then the dog searched and she was sitting beside a box among four boxes in which there was the similar odor-air-bag. She always selected a correct box.

MILK FLAVOUR ASSESSMENT

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This preliminary study investigated, firstly whether two different brands of milk were discernibly different, secondly the ability of two different ethnic groups (Japanese and Australian) to distinguish between the milks and thirdly if either smell only or taste and smell be used to tell the milks apart.

Two triangle tests (smell only then taste and smell) plus a warm-up test were conducted with Australian and Japanese panelists, recruited from staff and students at Southern Cross University. Two different brands of milk (A and B), purchased from supermarkets, were served at room temperature (22°C) in a randomised order. The milks were different in terms of gross (milk fat 3.43% and 3.91%; milk protein 2.87% and 3.23% respectively) and volatile composition.

FFor the combination of taste and smell; the majority of the both the Australian (20 of 31, α-risk of <0.1%), and Japanese (16 of the 25, α-risk of 1-0.1%) students correctly identified the odd sample. For the smell only test; 24 of the 31 Australian (α-risk of <0.1%) and 8 of the 13 Japanese (α-risk of 10-5% students correctly identified the odd sample.

For the Australian participants there is very strong evidence that they can use either smell only or taste and smell distinguish between the two different milks. Australian and Japanese participants were equally well able to distinguish between the samples. This information will be used to guide future PhD research in milk flavour.

Upcoming Events

27-30 January 2009	**ANS 2009** Australian Neuroscience Society Annual Meeting National Convention Centre Canberra, ACT Contact: www.sallyjayconferences.com.au
15-17 April 2009	**ISOEN 2009** International Symposium on Olfaction and Electronic Nose University of Brescia, Italy Contact: www.isoen.org
22-26 April 2009	**AChemS XXXI Annual Meeting** Hyatt Sarasota, Florida, USA Contact: www.achems.org
28-30 April 2009	**EcoForum Conference and Exhibition** Australian Technology Park, Sydney Contact: www.ecoforum.net.au
19-25 July 2009	**Summer School on Human Olfaction** Dresden, Germany Registration deadline: 1 May 2009 Contact: thummel@mail.zih.tu-dresden.de Also: www.tu-dresden.de
6-9 September 2009	**19th CASANZ Conference** "Air Quality and Economic Development" Perth Convention Centre Perth, Western Australia Contact: www.iceaustralia.com/casanz2009
December 2009	**Australasian Association for ChemoSensory Science (AACSS)** Annual Scientific Meeting Heron Island, Great Barrier Reef, Australia Contact: j.stjohn@griffith.edu.au

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