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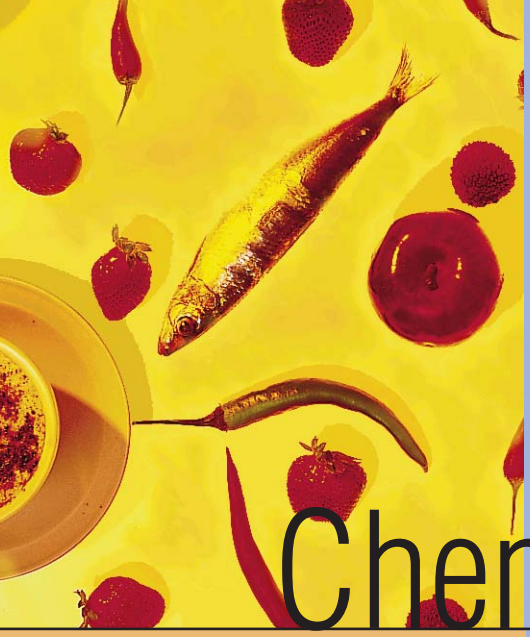

Chemo Sense

Editorial

By Graham Bell
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We know that, in mammals, there are more chemosensory systems than olfaction and gustation: the vomeronasal organ, the trigeminal/chemesthetic system and the organ of Masera are also primarily responsive to chemicals introduced from outside the body. We should now add the gut to the list, and while pausing to remember that the gut is a tube which functions to introduce chemical material (food), from the outside world into our bodies, it may not surprise the reader to learn from Paul Bertrand's review in this issue, that the wall of the intestine includes the machinery to perform olfactory and gustatory chemoreception. The significance of these mechanisms and their role in health, nutrition, and experience, will no doubt become the subject of important discoveries.

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Chemosensory transduction in the gastrointestinal tract

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Introduction

A wide variety of stimuli - chemical, mechanical and others - occur in the gastrointestinal (GI) tract and many of these demand an immediate response. Stimuli include potentially harmful chemicals, toxins and tissue damage as well as 'normal' stimuli which include nutrients, the presence of good bacteria and the mechanical deformation of the epithelium. In order for the GI tract to respond to these stimuli, they must be transduced into the activation of neurons. This information is then used by the enteric nervous system (ENS) to generate reflexes within the GI tract and by the CNS *via* the vagal and dorsal root afferents to affect behavioural change.

In the GI tract, the mechanisms of sensory transduction seem to be as plentiful as are the potential number of stimuli. This review looks at the sensory transduction

Abbreviations used in this paper: enteric nervous system (ENS), enteroendocrine cell (EE cell); enterochromaffin cell (EC cell); gastrointestinal (GI); serotonin (5-HT, 5 hydroxytryptamine).

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Your indulgence is craved to read herein and consider two more offerings from Australia: a technology for concerned communities, based on artificial olfaction, to address the costly problem of graffiti vandalism; and an opportunity to join Australasian chemosensory scientists at their annual meeting at Heron Island on The Great Barrier Reef, this coming December.

Chemosensory transduction in the gastrointestinal tract
continued

mechanisms present in the GI tract and examines some of the theories proposed to account for these mechanisms. The focus is on more recent data demonstrating taste transduction machinery and on the types of cells which respond to stimuli including the transmitter containing enteroendocrine cells (EE cells). In the end, the idea is put forward that sensory transduction mechanisms in the GI tract utilise many overlapping and complementary mechanisms for detecting and transducing stimuli into reflex action. For other recent reviews on this and related topics please see Grundy, (2005); Blackshaw *et al.*, (2007); Dyer *et al.*, (2007); Sternini *et al.*, (2008); Bertrand & Bertrand, (2009).

Dual afferent innervation of the GI tract

The intestine has two separate but equal forms of sensory (afferent) innervation. The first, and better known, is the primary afferent nerve terminals arising from cell bodies in the dorsal root ganglia and the nodose ganglia; these fibres run predominately in the spinal and vagal tracts, respectively (Blackshaw *et al.*, 2007). Some vagal nerve terminals form specialised mechanosensitive endings (Zagorodnyuk & Brookes, 2000) but most are probably unspecialised and terminate under the epithelial cell layer (Smid, 2009) (Figure 1). The cell bodies of these sensory nerves originate outside of the intestine, and thus from the view of the intestine, they can be referred to as extrinsic sensory neurons (or more correctly, extrinsic primary afferent neurons).

This distinction would be superfluous were there no other sensory neurons to distinguish them from. In the gut, however, the nervous system resident within its wall, the ENS, also contains the cell bodies of sensory neurons with projections to the mucosa and other enteric ganglia (Furness *et al.*, 1998) (Figure 1). The intrinsic sensory neurons are also called intrinsic primary afferent neurons (IPANs). Some subserve a chemosensory role: they are sensitive to acid, base and to short-chain fatty acids at a neutral pH (Bertrand et al., 1997). They also serve a mechanosensory role (Kunze *et al.*, 1998). A large proportion of these sensory nerves are also activated by GI hormones such as 5-HT acting via 5 HT_3 receptors (Bertrand *et al.*, 1997) and ATP acting at P2X receptors (Bertrand & Bornstein, 2000).

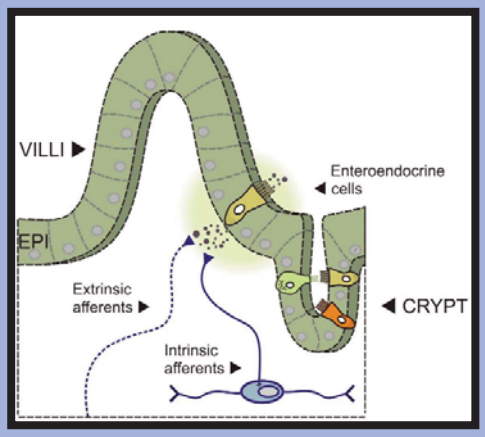

Figure 1. Side view of the intestinal wall showing a villus/crypt unit. A diagram showing a section of intestine with a villus/crypt unit shown in detail. The mucosal epithelium (EPI) contains the enterocytes and the enteroendocrine (EE) cells specialised epithelial cells that contain neuroactive substances located in secretory granules. Several types of EE cell are depicted (different colours) including the 5-HT containing enterochromaffin (EC) cell (depicted releasing 5-HT near to afferent nerve terminals into the underlying lamina propria). Afferent nerve terminals are from extrinsic sources (vagal and dorsal root ganglia) and from intrinsic sources (myenteric and submucosal afferent/sensory neurons).

Sensory transduction in the GI tract

The initiation and modulation of GI reflexes relies on transduction of chemical and mechanical information from the lumen of the gut to the extrinsic and intrinsic sensory nerve terminals. There are several ideas about how the GI tract transduces mechanical, chemical and nutrient stimuli. Stimulants cross the epithelium where they then interact with specialised receptors on the nerve terminals (Figure 2A). This is the mechanism hypothesised by Liu *et al* (1999) for glucose which would utilise specific transport proteins to be ferried across the epithelium. This is also likely to be the mechanism for capsaicin activating afferents through TRPV1 receptors (transient receptor potential ion channels) where capsaicin passively diffuses through the epithelium. Alternatively, and I believe more importantly, receptors and transduction machinery may exist on the luminal aspect of specialised epithelial cells within the mucosal epithelium (Figure 2B). The EE cell is one class of epithelial cell which much research has focused on in particular. The EE cells have luminal

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Chemosensory transduction in the gastrointestinal tract

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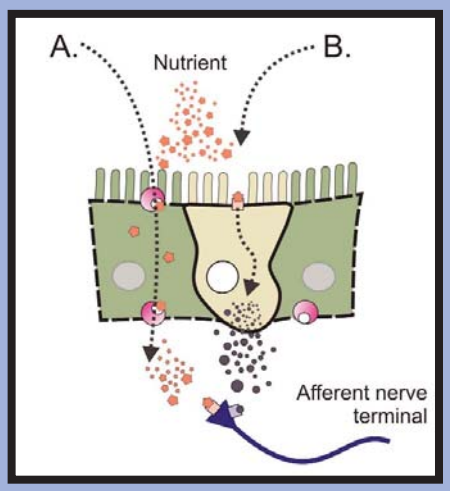

Figure 2. Nutrients may have two distinct routes to active nerve terminals. A diagram showing epithelial cells (either side) and an enteroendocrine (EE) cell. Nutrients present in the lumen of the intestine (depicted as star-shapes) may signal to afferent nerve terminals in the underlying lamina propria by two different routes. A. Stimulants may be ferried across the epithelium by specific transport proteins where they then interact with specialised receptors on the nerve terminals. B. Receptors and transduction machinery may exist on the luminal aspect of specialised epithelial cells within the mucosal epithelium.

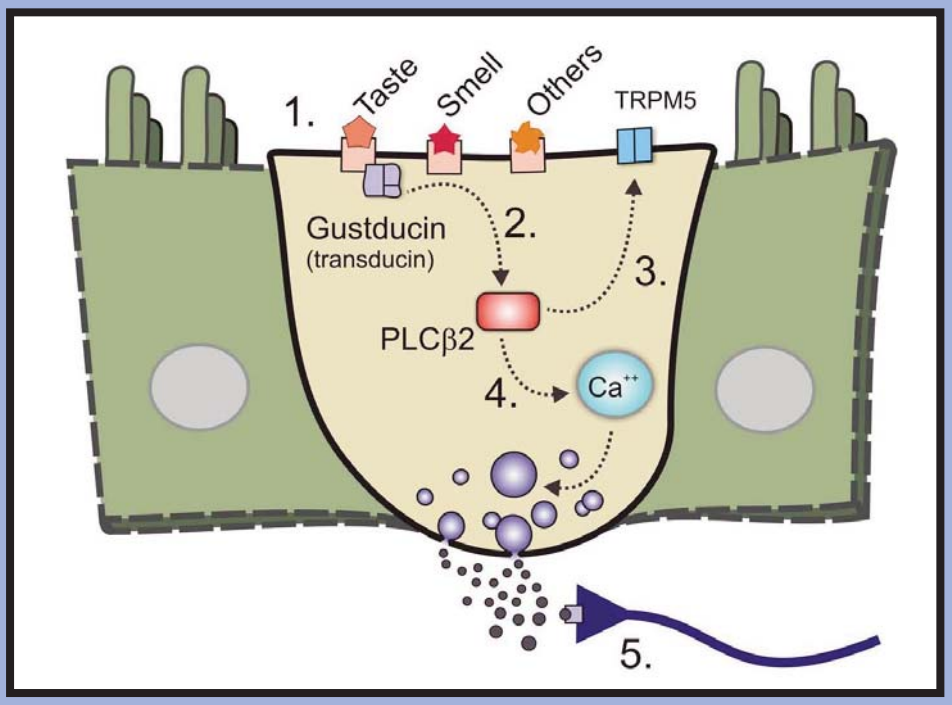

Figure 3. Sensory transduction machinery and the enteroendocrine (EE) cell. A diagram showing an enteroendocrine (EE) cell with transduction machinery. 1. Some of the more important components of the taste transduction machinery are the taste receptors, T1Rs which respond to sweet and umami tastants, and T2Rs which responds to bitter tastants. Receptors for olfactory receptors as well as others exist. 2. These receptors couple to the taste G-protein (gustducin, or in some cases transducin). 3. These in turn couple to taste specific second messenger systems such as phospholipase Cß2 (PLCß2). 4. PLCß2 generates second messengers such as diacylglycerol which can activate specialised ion channel receptors like transient receptor potential ion channel M5 (TRPM5) which is required for the transduction of bitter, sweet and umami tastes. 5. Second messengers such as inositol triphosphate are also generated and can release calcium (Ca^{++}) from intracellular stores and cause transmitter release and nerve terminal activation.

microvilli and granules full of a variety of neuroactive transmitters and hormones. It is known that many of these transmitters can act on receptors on the afferent nerve terminals and it is generally believed that this is the sensory transduction mechanism through which many stimulants act. Nonetheless, the molecular machinery that allows EE cells to transduce sensory stimuli are not well known despite intense interest by many research groups, especially on the 5-HT containing enterochromaffin (EC) cell (e.g., Grundy, 2008).

Work in the last 15 years has focused on the idea that the GI tract uses much of the same transduction machinery as do other senses. Models of how the GI tract senses mechanical or chemical stimuli have been based on hearing transduction (Gershon, 1995), on glucose sensing by the ß-cells of the pancreas (Raybould *et al.*, 2004) and on the internal mechano- and chemosenses (e.g., carotid bodies). However, what is exciting is the recent evidence that shows that machinery for taste (e.g., Sutherland *et al.*, 2007) and smell (e.g., Braun *et al.*, 2007) are localised to the EE cells in the GI tract. Since these discoveries there has been an explosion of interest in transduction machinery with studies using selective antibodies and animals with genetic deficiencies to provide evidence for their presence, if not the function, in the GI tract. Some of these many new findings are highlighted below.

Taste machinery in the GI tract

Taste is perhaps the best used analogy for explaining GI sensory transduction, even before the discovery of the taste transduction machinery in the GI tract (e.g., Rozengurt & Sternini, 2007). It is natural to extend lingual taste down into the GI tract. Anatomically, the EE cells and other specialised epithelial cells resemble the taste receptor cells in the taste buds. Both have apical microvilli that are exposed to ingested contents, and both contain granules filled with transmitters that can be released. Further, it has been assumed that similar sorts of ingested chemical stimuli would activate both taste receptors and EE cells (e.g., sugars). It is now clear that sub-sets of EE cells have many of the elements that make up the taste transduction machinery (Figure 3). Some of the more important components of the taste transduction machinery are the taste receptors, T1Rs, which respond to sweet tastant, and T2Rs which responds to bitter tastants (for review, see Sternini, 2007). These receptors couple to the taste G-protein (gustducin) and to taste specific second

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messenger systems (phospholipase C; PLCß2). There are also specialised ion channel receptors such as TRPM5 which is required for the transduction of bitter, sweet and umami tastes (Liman, 2007).

Concrete interest in taste transduction machinery in the GI tract began in the late 90's when Hofer *et al* demonstrated a-gustducin mRNA and protein in a sub-set of epithelial cells (Hofer *et al.*, 1996; Hofer *et al.*, 1998). Interestingly, this first study identified cells that were not EE cells, but a poorly characterised cell called a brush cell (also called a tufted cell). One major issue with accepting that these cells participated in sensory transduction was the lack of evidence for granules or other evidence of transmitter storage. Luckily, Hofer et al had recently shown that nitric oxide synthase (NOS) was also expressed by the brush cells (Kugler *et al.*, 1994), suggesting at least a plausible sensory transduction pathway via the generation of nitric oxide.

Some of the first work to look beyond gustducin was by Dyer *et al*, who showed that T1R sweet taste receptor mRNA and protein (as well as α-gustducin) were expressed in the STC-1 enteroendocrine cell line (Dyer *et al.*, 2005). These data were recently confirmed by studies showing a-gustducin in brush cells from mouse stomach (Hass *et al.*, 2007) and in brush cells and some EE cells from mouse small intestine (Sutherland *et al.*, 2007). For example, Sutherland *et al* used immunohistochemical techniques to localise α-gustducin and found over half of the cells were brush cells while the remainder were EE cells (of these, 25% were 5-HT containing EC cells and about 15% were GLP containing L cells) (Sutherland *et al.*, 2007).

The presence of taste transduction machinery has also been examined in human tissues. When the mucosal epithelium from biopsies were analysed using PCR methods, taste receptors, α-gustducin, PLCß2 and TRPM5 were found to be expressed along the GI tract (Bezençon *et al.*, 2007). Bezençon et al also used the promoter for the Trpm5 gene to express eGFP in a transgenic mouse and then used antibodies to colocalise TRPM5 protein with some morphologically identified EE cells (containing PLCß2 and TRPM5) and brush cells (containing TRPM5, α-gustducin, T1Rs). Unfortunately, there was no colocalisation of Trpm5-eGFP expression with EE cell transmitters such as ghrelin, orexin, peptide YY (PYY), glucagon-like peptide (GLP-1), or cholecystokinin (CCK). On the other hand, Jang *et al* (2007) in human and mouse found immunohistochemical colocalisation of α-gustducin and GLP-1 containing L cells. Using a cell line, they also showed that sugars caused the release of GLP-1 which was blocked by inhibition of taste receptors or α-gustducin (Jang *et al.*, 2007).

The expression levels of the taste transduction machinery are not static but seem to respond dynamically to nutrient conditions. For example, Young *et al* (2009) have shown in human and mouse that mRNA expression for sweet taste receptors (T1R2 and T1R3 subunits), TRPM5 and α-gustducin were inversely related to blood glucose levels. Similarly, the Na+/glucose co-transporter 1 (SGLT-1) has been shown to be upregulated via a taste receptor mediated pathway. Margolskee et al have shown that sweet taste receptors (T1R3 subunit) and α-gustducin are coupled to the levels of SGLT mRNA expression in mouse small intestine and an endocrine cell line (GLUTag) (Margolskee *et al.*, 2007). Similarly, ingested sugars have been found to increase expression of glucose transporter-2 (GLUT2) in rat small intestine with the taste receptors (T1R2 and T1R3 subunits) and α-gustducin being implicated in this process (Mace *et al.*, 2009). Finally, molecules involved in the control of fat metabolism have been shown to upregulate T2R bitter receptors on STC-1 cells and in EE cells from mouse intestine (Jeon *et al.*, 2008).

Smell and glucose sensing in the GI tract

Taste transduction machinery are not the only sensory transduction elements localised to the GI tract. Molecules involved in vision and smell transduction have also been found. Hofer et al (1999) showed that the vision G-protein, transducin, was present in GI epithelium. More recently, Mace *et al* have provided support for this study by showing that transducin could couple through the taste machinery present in rat jejunum (Mace *et al.*, 2007; Mace *et al.*, 2009). Smell transduction machinery is also present in the GI tract with a recent study by Braun *et al* (2007) showing odorants, such as those from roses and raspberries, evoked 5-HT release from an EC cell model (BON cells). In particular, a subset of olfactory receptors was detected, a finding supported by Kidd et al (2008) who showed that activation of class II olfactory receptors stimulated 5-HT release from an EC cell model (KRJ-I cells).

Glucose sensing has been well reviewed recently: please see Dyer *et al* (2007). In brief, sugar sensing has been linked to taste machinery (Mace *et al.*, 2009) and to mechanisms of glucose sensing used by the pancreatic ß-cells (Raybould, 2002; Raybould *et al.*, 2004). Pancreatic ß-cells use the ATP activated potassium channel (K_{ATP}) to monitor glucose concentrations indirectly. In the GI tract glucose evokes enteropancreatic reflexes which are reduced by a K_{ATP} blocker (Kirchgessner et al., 1996). The GI tract uses the Na^+/glucose co-transporter 1 (SGLT) to transport simple sugars into the enterocyte, and these have also been found on enteric neurons (Liu *et al.*, 1999). Perhaps more importantly, Kidd et al (2008) have shown mRNA for GLUT1/3 and SGLT1 transporters are enriched in human EC cells and have demonstrated release of 5-HT evoked by sugars. Similarly, sweet taste receptors (T1R3 subunit) and α-gustducin have been shown to be coupled to SGLT expression in mouse and the GLUTag cell line (Margolskee et al., 2007) and GLUT2 (Mace *et al.*, 2007).

EE cells and their transmitters are the common theme in GI sensory transduction

The common question in most studies of GI sensory transduction is whether the EE cells contain any of this transduction machinery. This is because the EE cells are known to release GI hormones/transmitters and receptor antagonists for these transmitters are known to modulate intestinal reflexes. The following is a closer look at the types of EE cells in the GI tract with a particular focus on the 5-HT containing enterochromaffin (EC) cell.

Types of EE cells

The EE cells contain and release secretory granules into the sub-epithelial space - near where the sensory nerve terminals are. The cells are generated in the crypts and migrate to the

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tip of the villus; as a result they cannot form lasting appositions with these nerve terminals (Berthoud & Patterson, 1996) so transmission is likely to be paracrine. There is a variety of transmitters found in EE cells. For example, CCK is found in the I cells and is widely believed to participate in signalling the presence of lipids to the extrinsic sensory nerves (e.g., Grundy *et al.*, 1998) and to the intrinsic innervation (e.g., Gwynne *et al.*, 2004). Other sub-types of EE cell contain a host of putative GI hormones\transmitters such as serotonin (5-HT), neurotensin, somatostatin (SOM) and GABA. Some transmitters are co-stored, while others like 5-HT and CCK represent distinct populations of EE cells. Newly discovered gut hormones such as orexin and ghrelin are also important for nutrient signalling and are contained within specific sub-populations of EE cell. Despite this variety of transmitters, it is the 5-HT containing EC cell that has received the most experimental attention.

The EC cell, 5-HT and intestinal reflexes

The EC cells receive the bulk of the attention for good reason. The release of 5-HT from the EC cell appears to one of the critical steps in the transduction of chemical and mechanical information from the gut lumen to intrinsic and extrinsic sensory neurons (for reviews see Bertrand, 2003; Grundy, 2006). EC cells are responsible for the production and storage of the largest pool of 5-HT in the body (Erspamer, 1954). 5-HT is stored in dense core granules located predominately in the basal part of the EC cell.

Studies over the past 50 years have shown that the EC cells release 5-HT during physiologically meaningful stimuli (e.g., Bülbring & Crema, 1959; Kirchgessner *et al.*, 1992; Foxx-Orenstein *et al.*, 1996; Grider *et al.*, 1996; Linden *et al.*, 2003; O'Hara *et al.*, 2004). Similarly, nutrients - such as short or long chain fatty acids, peptides, glucose - or chemical stimuli (e.g., acid, base) can release 5-HT from the EC cells (Bertrand & Bertrand, 2009). Once in the laminae propria, high concentrations of released 5-HT can activate sensory nerve terminals via 5-HT_3 receptors (Bertrand *et al.*, 2000). Many in vitro studies have shown that 5-HT_3 receptors are important for initiation or propagation of motor reflexes (Kadowaki *et al.*, 1996; Tuladhar *et al.*, 1997; Grider *et al.*, 1998; Jin *et al.*, 1999) or secretory reflexes (Sidhu & Cooke, 1995; Cooke *et al.*, 1997a, b). Despite this keen interest in the EC cell, studies have yet to firmly nail down the transduction machinery that the EC cell uses to detect luminal stimuli or to couple this to release of 5-HT.

Control of 5-HT release from the EC cell

Much of the mechanistic data on how EC cells work has come from Racké, Schwörer and colleagues who over the years have described 5-HT overflow from *in vitro* segments of intestine from a variety of small and large animals (for review, see Racké *et al.*, 1996). They found that 5-HT release is generally via an external Ca^{++}-dependent process via L type calcium channels (Forsberg & Miller, 1983); however, upon muscarinic receptor activation release can occur via calcium from internal stores. Release of 5-HT could be evoked by agonists at several receptors such as adrenoceptors and 5-HT_3 autoreceptors while release was inhibited by activation of $GABA_A$, nicotinic or somatostatin (SST) 2 receptors or 5-HT_4 autoreceptors (Gebauer *et al.*, 1993).

The 5-HT measured using these traditional overflow methods is the sum of many EC cells measured many minutes after release occurs and far from the site of action; thus, temporal and spatial information are lost. It is not surprising then that other techniques have been used in an attempt to look at the activity of only a few EC cells at a time. Studies of calcium transients in small numbers of EC cells show clearly that apparently identical EC cells respond to transmitter or calcium channel agonist differently (Satoh *et al.*, 1995; Lomax *et al.*, 1999; Satoh *et al.*, 1999); although for the most part the pharmacological features found by Racké and Schwörer have been supported. A further attempt to look at single cells has utilised an EC cell model, the BON cell which is derived from a metastatic human carcinoid tumour of the pancreas (Evers *et al.*, 1994). BON cells have been used as a model of EC cell function (Christofi *et al.*, 2004; Tran *et al.*, 2004) and to investigate the release of 5-HT by D glucose or mechanical stimulation (Kim *et al.*, 2001a; Kim *et al.*, 2001b).

Studying single EC cells *in vitro* has been difficult as they are a relatively small proportion of the epithelial cells, perhaps 1 - 3% (Bose *et al.*, 2000; Coates *et al.*, 2004). In addition, as we have seen, there are many other types of EE cells with similar structural properties. A successful approach at enriching the EC cells has used techniques developed for the histamine containing enterochromaffin-like cell of the stomach (Oh *et al.*, 2005). Using successive sucrose gradients, Schafermeyer *et al* were able to enrich the EC cell fraction (Schafermeyer *et al.*, 2004). Building upon this work, Kidd *et al* has succeeded in enriching the EC cells using fluorescence-activated cell sorting techniques to

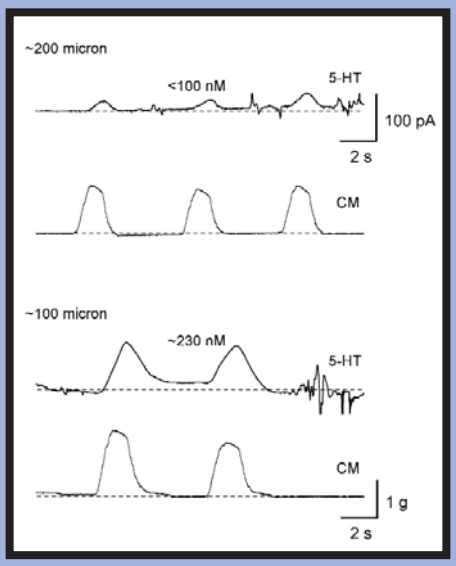

Figure 4. Effect of electrode position on 5-HT concentration in guinea pig ileum. A. Spontaneous contraction of the circular muscle (CM) and release of 5-HT from guinea pig ileum. In the top traces, the electrode was held ~200 mm above the mucosa. Only threshold concentrations of 5-HT were detected (<100 µM). In the same preparation, when the electrode was lowered to within ~100 mm, 5-HT concentrations more than doubled. B. Stretch activated release of 5-HT with the electrode in contact with the mucosa yielded ~10 µM 5-HT. In contrast, with the electrode ~200 or ~100 µm away, 5-HT concentrations fell to 200 or 100 nM respectively (traces from A., scale is the same for all traces in B.). Adapted from Bertrand PP & Bertrand RL. (2009). Serotonin release and uptake in the gastrointestinal tract. Autonomic Neuroscience, *in press*, Figure 5, with permission of Autonomic Neuroscience.

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continued

the point that genechips could be used to show expression of some taste machinery message (Kidd *et al.*, 2006; Modlin *et al.*, 2006; Kidd *et al.*, 2008). Finally, Braun *et al* have purified human EC cells isolated by laser micro-dissection (Braun *et al.*, 2007).

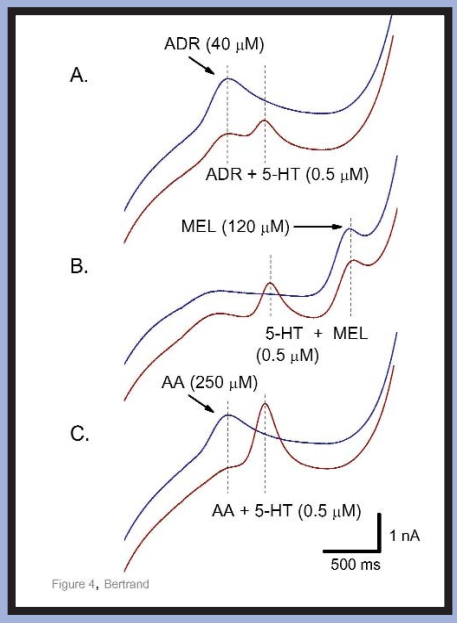

Figure 5. 5-HT can be detected selectively in the presence of other transmitters. A C are cyclic voltammetry traces taken with the carbon fibre electrode touching the base of an organ bath during equilibration of drugs. All traces were taken with a scan rate of 0.47 V/s and are from the same electrode: background current has not been subtracted. The bath solution was flushed with fresh physiological saline between panels. The region of the ramp from 350 mV to +920 mV is shown. The scale in panel C. applies to all traces and the vertical dotted lines denote the time/voltage at which the peak current was produced. A. Two traces taken with adrenaline (ADR 40 μM) alone (upper) with a peak current at +200 mV, and (lower) adrenaline with the addition of 5-HT (0.5 μM) with a peak current at +360 mV. B. Melatonin (MEL 120 μM) alone (upper) with a peak current at +710 mV and with the addition of 5-HT (0.5 μM; lower) with a peak current at +380 mV. C. Ascorbic acid (AA 250 μM) alone with a peak current at +200 mV (upper) and with the addition of 5-HT (0.5 μM; lower) with a peak current at +350 mV. Note that oxidation potentials will be offset when different styles of electrochemical electrodes are used (e.g., Bian *et al.*, 2007; Patel, 2008). Reproduced from Bertrand PP & Bertrand RL. (2009). Serotonin release and uptake in the gastrointestinal tract. Autonomic Neuroscience, *in press*, Figure 4, with permission of Autonomic Neuroscience.

Detecting 5-HT direct from the EC cell

Another successful approach is to use real time electrochemical methods, which preserves both temporal and spatial information. The chemical structure of 5-HT is such that it is readily oxidized at low voltages; thus, it is particularly well-suited to electrochemical detection methods. When a compound is oxidized, the transfer of electrons can be detected, and quantified. This provides a direct and accurate measure of the number of molecules (i.e., the concentration) at the tip of the electrode. Using electrochemical techniques in healthy mucosa we have shown that it is possible to record 5-HT release close to the site of release and close to the site of action (Bertrand, 2004; Grundy & Schemann, 2004). 5-HT release has been shown to be a dynamic and highly regulated process that is dependent on disease state.

5-HT levels have been measured near the mucosa from a variety of animal models (Bertrand, 2004, 2006b; Bian *et al.*, 2007; Patel *et al.*, 2007; Bertrand *et al.*, 2008a; Bertrand *et al.*, 2008c), human surgical specimens (Bertrand *et al.*, 2008b) and from BON cells (Braun *et al.*, 2007). Generally, carbon fibre electrodes are used, but boron doped diamond coated electrodes have also been used successfully (Bian *et al.*, 2007; Patel *et al.*, 2007). The concentration of 5-HT detected depends on how close the electrode is to the EC cell. The minimal concentrations detected away from the mucosa are less the 100 nM. As the electrode is brought close to or touching the mucosal epithelium, 5-HT concentrations rise to over 1 μM (Figure 4). Although this makes for a great signal, there are several problems and criticisms inherent in actually touching the epithelial surface (see Vanden Berghe, 2008).

Is it really 5-HT?

Given the number of substances that could be released from EE cells in general, it is notable that 5-HT is the only electro-active substance recorded at low oxidation voltages. This was determined using cyclic voltametric techniques which systematically alter the voltage while recording current versus time (Figure 5). In each species tested, it has been shown that 5-HT and not catechols or dopamine are released from the mucosa (e.g., Bertrand, 2004).

Another way to ensure that 5-HT, and not metabolites, are detected is to coat the electrode with a thin film of Nafion; a anionic compound that repels anionic species such as ascorbic acid and the metabolite 5-HIAA (Gerhardt *et al.*, 1984). Under these conditions, the large peak at the 5-HT oxidation potential is unchanged, suggesting that 5-HIAA does not contribute to the 5-HT signal. High performance liquid chromatography coupled to an electrochemical detector has also been used to confirm that, under the conditions used during electrochemical experiments, large amounts of 5-HT are released from the mucosa (e.g., Bian *et al.*, 2007).

What information do electrochemical recordings give?

The recent studies using electrochemical methods have measured steady state levels of 5-HT at the mucosal surface, and mechanically evoked 5-HT release from the EC cells.

Steady state levels of 5-HT detected electrochemically reflect the amount of 5-HT that is trapped in the unstirred layer. This level of 5-HT represents a steady state between escape into the bulk solution (the lumen) and reuptake by SERT into the epithelial cells (more about SERT below). As an example, the steady state levels of 5-HT in mouse colon are about 2 μM, lower than that found in the mouse ileum (Bertrand, 2006b) or in the ileum of other species such as rat (Bertrand *et al.*, 2008c) or guinea pig (Bertrand, 2004) and similar to known distributions of EC cells (Sjölund *et al.*, 1983). Mechanical compression of the epithelium is a challenge to the EC cell and so the mechanosensory function of the EC cell can be tested. When a glass rod (Bian *et al.*, 2007) or the electrode itself (Bertrand *et al.*, 2008c) is used to stimulate the EC cells an increase in the levels of 5-HT are detected. In addition, a contraction of the circular or longitudinal muscle is a mechanical stimulus for 5-HT release (Bertrand, 2006a). The peak concentration of mechanically evoked 5-HT release is usually 2-4 times that of the steady state levels. For example, peak levels of 5-HT released in mouse colon are approximately 7 μM in response to compression of the mucosa versus 2 μM at steady state (i.e., the steady state levels were approximately 30% of peak compression levels). In mouse ileum compression evoked 10 μM peak

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5-HT while steady state levels were 6 µM (Bertrand, 2006b).

5-HT uptake by SERT

The actions of 5-HT in the GI tract are terminated by uptake via the serotonin reuptake transporter (SERT) which is the same Na^+/Cl- dependent transporter found in the CNS. SERT has been localised to many if not all epithelial cells using immunohistochemistry (Wade *et al.*, 1996) and northern blots (Chen *et al.*, 1998). Steady state levels of 5-HT detected electrochemically reflect the amount of 5-HT that is trapped in the unstirred layer at the mucosal surface. This level is in a steady state between escape into the bulk solution and transport by SERT into the epithelial cells. Using electrochemical techniques, it is possible to infer SERT function in real time from GI tissues. Blockade of SERT with a reuptake inhibitor such as fluoxetine can increase peak levels of compression evoked 5-HT release and can prolong the decay time of these responses. Furthermore, there is an increase in steady state levels of 5-HT near the mucosa (Bian *et al.*, 2007; Bertrand *et al.*, 2008c) and an increase in the decay times of exogenously applied 5-HT (pressure ejected onto the surface of the mucosa) (Bertrand *et al.*, 2008c). In contrast, is seems that blockade of SERT does not increase the peak level of 5-HT detected following contraction evoked release (Bertrand *et al.*, 2008c). This suggests SERT may be located far from the nerve terminals, rather than close by where it could control 5-HT access to the receptors (Bertrand *et al.*, 2008c).

Absolute levels of 5-HT determine receptor activation

Electrochemical methods allow the accurate measure of the absolute concentrations of 5-HT near the mucosa and, by extension, near the afferent nerve terminals that control GI function and sensation (Keating *et al.*, 2008). The concentration of 5-HT near the nerve terminal is important because this may substantially alter the activation or desensitization of serotonin receptors on afferent nerve terminals, or on the EC cells themselves. For example, the peak concentration of 5-HT measured from mouse colon was approximately 7 µM, high enough to activate the 5-HT_3 receptor (Bertrand *et al.*, 2008a; Bertrand *et al.*, 2008d). The ligand gated 5-HT_3 receptor can only be activated by relatively high (> 1µM) concentrations of 5-HT and to be exposed to these high concentrations, the receptor must be close to the site of 5-HT release. Near the EC cell concentrations are high, while far from the receptor, concentrations are lower as a result of dilution and reuptake. However, these lower concentrations of 5-HT are not inactive as all other 5-HT receptors are G protein coupled requiring much lower (> 5nM) concentrations of 5-HT for activation. Thus, we can speculate that the actions of 5-HT through the clinically important 5-HT_4 receptor could be far from the site of 5-HT release.

Changes in 5-HT availability during development and disease

Recent studies have used electrochemical techniques to explore the availability of 5-HT in animal models of development and disease. Bian et al has shown that mechanically stimulated release of 5-HT is increased by blockade of SERT in adult but not neonatal guinea pig ileum (Bian *et al.*, 2007). This suggests that SERT levels are lower in neonatal ileum, a finding that was supported with western blot analysis (Bian *et al.*, 2007). The levels of 5-HT may increase during aging. Preliminary data has shown a 50% increase in 5-HT release from aged mice (21 months) compared to young mice (3-5 months)(P. Bertrand unpublished observations). Similarly, electrochemical determination of 5-HT availability has been seen to increase in a mouse model of inflammation (Bertrand *et al.*, 2008a). During DSS colitis, the levels of 5-HT detected by electrochemical methods were almost double that found in inflamed tissues. It will be interesting to see how functional measures of 5-HT availability, such as the electrochemical methods described here, compare to anatomical or genetic analyses in a variety of disease states. Taken together, electrochemical determinations appear to be a useful adjunct to traditional methods of measuring 5-HT availability.

New ideas in GI sensory transduction mechanisms

The sensory transduction needs of the GI tract are more varied than in many other systems. For example, no other organ has to communicate with the central, peripheral and enteric nervous system. A new model of GI sensory transduction should encompass the width and breadth of transmitters, the many specialised cell types and varied transduction machinery present in the GI tract.

The case has recently been made that the motility and secretion of the GI tract is highly interconnected with overlapping control mechanisms (Blackshaw *et al.*, 2007; Huizinga & Lammers, 2009). I propose that these ideas be extended to the transduction of sensory stimuli within the GI tract. The release of sensory mediators from EE cells most likely act in concert to produce motor and secretory patterns tuned to the contents and to the region of intestine. One way that this may work is by utilising many overlapping and complementary mechanisms for detecting and transducing stimuli into reflex action. For example, it is unlikely that only one method of glucose sensing operates in the GI tract. There is evidence for direct activation of afferent nerves by sugars as well as indirect activation via taste receptors on EE cells.

Conclusions

The GI tract senses the luminal contents and signals to the extrinsic and intrinsic nerves in the wall of the gut to produce motor and secretory patterns tuned to the contents and to the region of intestine. It is clear that the EE cells play a key role in helping to transduce these signals by converting chemical, nutrient or mechanical stimuli into the release of neuroactive transmitters. Proteins once thought specific for taste or other sensory transduction systems have been implicated in controlling release of these neuroactive transmitters from EE cells. New techniques have allowed the genetics of enriched EC cells to be studied and electrochemical techniques have provided new insight into the kinetics of 5-HT release. It is time to embrace the idea that many overlapping and complementary mechanisms of sensory transduction are present in the GI tract.

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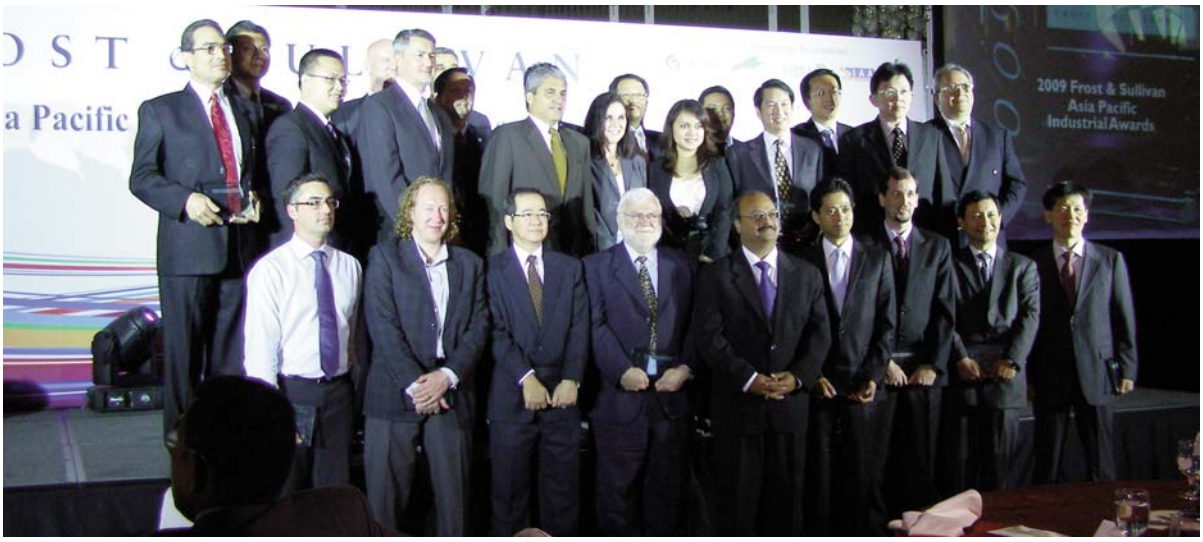
NEWS

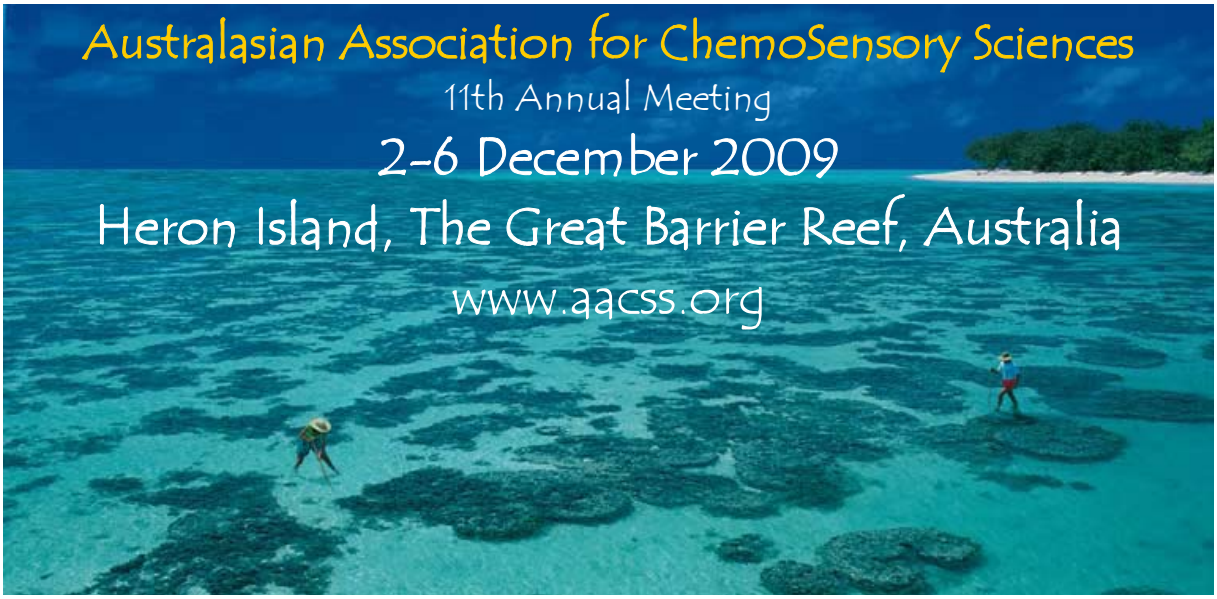
E-Nose HONOURED as "INNOVATOR of the Year"

At a recent ceremony in Singapore, the global consulting company, Frost and Sullivan, recognized E-Nose Pty Ltd as "Innovator of the Year in Odor Sensing Technologies". This unsolicited and independently assessed award was received by E-Nose Pty Ltd CEO, Dr Graham Bell, on behalf of the team of scientists and engineers who are the owners and creative force behind the company. E-Nose Pty Ltd is a 100% private company which spun out of the UNSW's Centre for ChemoSensory Research in 2003. Their research on artificial olfaction (e-noses) has included work in human and animal health, air pollution monitoring and security. They now have a portfolio of patents and several inventions which are being commercialized. For more information see www.e-nose.info.

The Innovator of the Year award trophy and some of E-Nose's inventions.



Graham Bell (front row, fourth from left) with Frost & Sullivan executives and award recipients at the gala presentation at Singapore's Intercontinental Hotel on 20th August 2009.



The conference will cover the society's broad range of chemosensory interests with sessions including:

- Neurobiology of Chemoreception
- Development of Chemosensory organs
- Marine/Aquatic Chemical Ecology
- Plant-Insect Interactions
- Chemosensory Genetic Evolution
- Human Olfaction, Taste and Flavour
- Therapeutic approaches
- Industrial Applications

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Organising committee:	Dr Coral Warr, *Monash University*
	Dr Jenny Ekerg. *Griffith University*
	Dr Graham Bell, *E-Nose Pty Ltd*
Conference website	www.aacss.org
Heron Island website:	www.voyages.com.au

NEWS

Artificial Olfaction CATCHES Graffiti Vandals

Graham Bell, Ph.D., CEO, E-Nose Pty Ltd

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It may not be the most prestigious application of artificial olfaction, but sniffing spray paint and ink is proving to be highly appreciated by Australian property owners and the wider community.

Graffiti vandalism now costs Australian local governments (city councils) around $260 million a year (*LG Focus*, **25**(8) August 2009). That excludes private property. Whether you call it art or vandalism the cost is inescapable, and the cost is rising.

Cleaning and repair costs are now driving most efforts to eradicate graffiti vandalism in Australia. As an example, the City of Swan, WA, needs an annual graffiti budget of $1.2 Million (Graffiti Task Force, WA, 2009). Similar and greater costs are being borne elsewhere, in Australia and abroad.

There are also the hidden costs of graffiti vandalism, including loss of customers and business revenues, discouraged tourists, and diminished quality of life. Graffiti also directly increases disorder and crime, including stealing (Keiser et al, ***Science***, 2008, **322**, 1681-1685).

Annoyance levels in the community are very high, fuelled by attacks on private homes, schools and public buildings, road and safety signs, heritage buildings, sandstone in national parks and, recently in Sydney, a war memorial (*The Weekly Times*, NSW, April 2009).

A trawl through the Web reveals local governments and private property owners in crisis in Europe, the UK and the USA. It also reveals the level of communication within the vandal community: swapping photographs and information.

Programs to teach young people to do "aerosol art" instead of "tagging", and to use "legal walls" have not decreased "illegal" spraying and tagging. Of the dozen or so councils in NSW which introduced legal walls, only two still tolerate legal walls.

Vandals appreciate learning higher-skilled use of their materials. Having been taught "aerosol art" the vandal graduates from tagging walls to "bombing" railway carriages (Annon. Graffiti Vandal, personal communication, 2009).

The thrill of working illegally and in a peer group, often in dangerous places and with impunity, is a strong motivation to do and persist in doing acts of graffiti vandalism. Risks are low and the intrinsic rewards are high. One of the "best things" for the vandals, is to see (and photograph) their painted "piece" on the side of a moving train or bus (Annon. Graffiti Vandal, personal communication, 2009).

Two new tools are being deployed to counteract graffiti vandalism

CCTV cameras are becoming more widely deployed and sophisticated. They are augmented with movement sensors, low-light sensitivity, image recording capability and image analysis. They are much favoured by police agencies, because they have wide area coverage and are increasingly accepted as evidence in securing convictions for a wide range of offences (not just graffiti vandalism). Unfortunately, graffiti vandals present indistinct images to the CCTV cameras, through wearing of hooded jackets and dust-protective face-masks.

Artificial olfaction (e-noses) now offers a solution to making graffiti vandalism harder to get away with. E-Nose Pty Ltd, in Sydney, Australia, has developed an electronic nose and alarm system to detect graffiti vandals in action and to alert one or more relevant authorities to attend immediately to the vandalism while it is in progress.

The company's product, *graffit-e-nose*™ , silently

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Artificial Olfaction CATCHES Graffiti Vandals
continued

alarms, by sending SMS messages within a few seconds of sniffing graffiti paints, inks and solvents.

E-Nose Pty Ltd "spun-off " from UNSW's Centre for ChemoSensory Research in 2003. The e-nose research was supported by private enterprise and the Australian Meat industry, and close relationships were maintained with Sydney University and UNSW.

Their first product was a chemical sensor array optimised for monitoring smells in air at abattoirs and red meat processing plants. Then came an array for diagnosis of ailments in sheep, another for monitoring sewage treatment plants, and later one for detecting spray paint and wet ink. They also conducted research into breath diagnosis of diabetes and lung cancer.

Their recent invention, *graffit-e-nose*™ was shown on the ABC's "New Inventors" on 12 November 2008 and won "The Peoples Choice" in its round. In August 2009, the company was awarded "*Product Innovator of the Year for Odor Sensing Technologies*" by the global consulting company Frost & Sullivan.

In the process of inventing and developing the e-noses, several patents were filed and these now give international protection to several aspects of the devices, including real-time odour discrimination (Hibbert & Bell, 2007 PCT/AU2007/001214). Other patent families are growing based on Barnett et al.,(2005)PCT/AU2005/000564 and Barnett et al.,(2005) PCT/AU2005/000563.

The *graffit-e-nose*™ was deployed recently at a graffiti-plagued scout hall, resulting in the arrest of members of three gangs of graffiti vandals. Attacks on the hall have now diminished, as well as attacks on walls and fences in neighbourhoods where these vandals operated. Similar deterrence has occurred at a community centre in Newcastle and at a skate park in Campbelltown, protected by graffit-e-nose™. The silent alarm results in the vandals being apprehended or chased away "in mid-squirt." The result is a dramatic drop in attacks and cleaning costs. Local police and community leaders have expressed satisfaction (*LG Focus*, **2**5(8) August 2009).

New trials are under way in Sutherland Shire and Stirling and many more are being planned around Australia. The technology is about to cross the Tasman Sea to New Zealand.

The *graffit-e-nose*™ costs a tiny fraction of what is wasted on graffiti: money better spent elsewhere. E-Nose Pty Ltd offers a limited time, inexpensive trial package. Graffit-e-nose™ is available for sale, rent and lease. An active distributor network is growing in Australia and abroad.

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Upcoming Events

24-27 September 2009	**ECRO XIXth Congress** Villasimius-Cagliari, Italy www.ecro-online.info
17-21 October 2009	**Society for Neuroscience** 39th Annual Meeting Chicago, Ill., USA www.sfn.org/am2009/
2-6 December 2009	**Australasian Association for ChemoSensory Science (AACSS)** Annual Scientific Meeting Heron Island, Great Barrier Reef, Australia Contact: j.stjohn@griffith.edu.au
31 January – 3 February 2010	**Australian Neuroscience Society** and **Australian Physiological Society** Annual Scientific Meeting Sydney Convention Centre, Sydney www.ans.org.au/ans-annual-conference/
23-24 February 2010	**EcoForum Conference and Exhibition** Australian Technology Park, Sydney www.ecoforum.net.au/2010/
21-25 April 2010	**AChemS 32nd Annual Meeting** Tradewinds Resort, St Petersburg, Fl., USA www.achems.org

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