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Mouse Nasal Organ
for Cold Emergencies



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Our Cover: The Grueneberg ganglion is a recently discovered unique chemosensory organ with a specialized function allowing increased sensitivity to specific odorants in cool temperatures. The cover image shows the head of a neonatal mouse in a sagittal section, in which neurons of the Grueneberg ganglion in the anterior nasal region (to the left) are stained (in green) by immunohistochemistry with an antiserum against the olfactory marker protein (OMP). In more posterior regions, olfactory sensory neurons of the main olfactory epithelium including their axons which project to the olfactory bulb of the brain are labeled. The section was counterstained in blue with 4',6-Diamidino-2-phenylindole (DAPI). [Data from Fleischer et al. (2006) J. Neurochem. 98: 543-554; with kind permission of John Wiley and Sons]

ChemoSense (ISSN 1442-9098)

Web: www.chemosense.net

Published by **E-Nose Pty Ltd**

P.O. Box 488

Gladesville, NSW Australia 2111

Ph. (+61 2) 9209 4083

Fax (+61 2) 9209 4081

www.e-nose.info

Production Team

Editor & Advertising:

Graham Bell, g.bell@e-nose.info

Graham Bell and Associates Pty Ltd Centre for ChemoSensory Research

Elke Weiler, elke.weiler@uni-ulm.de

University of Ulm, Germany

Design and Layout:

Lawton Design Pty Ltd,

jodi@lawtondesign.com.au

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Graham Bell and Elke Weiler, Co-Editors of ChemoSense

EDITORIAL

Another Olfactory Organ Discovered in Mammals

Graham Bell

E-Nose Pty Ltd

g.bell@e-nose.info

Elke Weiler

University of Ulm

elke.weiler@uni-ulm.de

Please excuse my ebullience at the discovery of another olfactory organ in the nose of mammals (well, in mice for the time being).

The Grueneberg ganglion is a recently discovered unique chemosensory organ with a specialized function allowing increased sensitivity to specific odorants in cool temperatures. In this issue, Joerg Fleischer presents a brief but comprehensive review of research which identifies the ganglion as a functional organ of the sense of smell, with very interesting properties.

It lies underneath the skin at the junction of the roof of the nasal cavity and nasal septum. Its olfactory cells are compacted and do not protrude into the nasal space. Here the air entering the nose will be at its coldest. The sensing organ has the prime position for early detection of molecules signalling danger. Specific mouse alerting compounds can penetrate the covering of the organ which is amplified in its response to them by cold air.

We are seeing an exquisite survival mechanism for mammals which must live in warm burrows or nests, yet be capable of flight or fight the instant they emerge into a cold world.

Other news comes from the commercial domain where sensory matters can mean life or death for human entrepreneurs and investors, if not consumers themselves.

These articles owe much to the enthusiasm of our new co-editor, Elke Weiler.

Graham Bell



The Grueneberg Ganglion: A Cool Chemodetector



Joerg Fleischer

*Institute of Physiology
University of Hohenheim
D-70599 Stuttgart
Germany*

email: joergf@uni-hohenheim.de

Introduction

In the mammalian olfactory system, an almost unlimited number of odorous and pheromonal compounds is detected by olfactory sensory neurons. These highly specialized cells reside in various nasal compartments, including the main olfactory epithelium (MOE), the vomeronasal organ (VNO), and the septal organ (SO) (Breer et al. 2006). While the MOE is responsible for the detection of general odorants, the VNO is supposed to mediate detection of pheromonal compounds which play a crucial role for intraspecific communication regulating essential behaviors, such as aggression and mating (reviewed by Keverne 1999; Dulac 2000; Zufall et al. 2002; Gaillard et al. 2004; Mombaerts 2004). Compared to the MOE and the VNO, the precise function of the SO is so far only poorly understood (Weiler and Farbman 2003; Breer et al. 2006).

In the above mentioned olfactory organs, mature sensory neurons are characterized by the expression of the olfactory marker protein (OMP). In recent studies, it has been found that neurons of the so-called Grueneberg ganglion (GG) also express OMP (Fig. 1), eliciting speculations that

the GG might be another olfactory subsystem (Fuss et al. 2005; Koos and Fraser 2005; Fleischer et al. 2006a; Roppolo et al. 2006; Storan and Key 2006). Albeit the GG was first described by Hans Grüneberg in 1973 (Grüneberg 1973), it has been studied in more detail only recently. The key findings of these studies are summarized in the present review which focuses on the murine GG since this organ has been studied so far almost exclusively in mice although it apparently exists in a number of other mammalian species (Grüneberg 1973; Tachibana et al. 1990).

Morphology of the GG and its cell types

The GG is a bilateral organ found in the vestibule of the nasal cavity (Fig. 1A-B). With a rostral-caudal extent of a few hundred micrometers, the GG is situated between the levels of the orifices of the lateral nasal gland (glandula nasalis lateralis or Steno's gland) and the glandula nasalis medialis. The GG is embedded in connective tissue surrounded by the nasal roof, the septum, and a thin and keratinized epithelial layer lining the lumen of the nasal cavity (Fig. 1B-C) (Grüneberg 1973; Fuss et al. 2005; Koos and Fraser 2005; Fleischer et al. 2006a;

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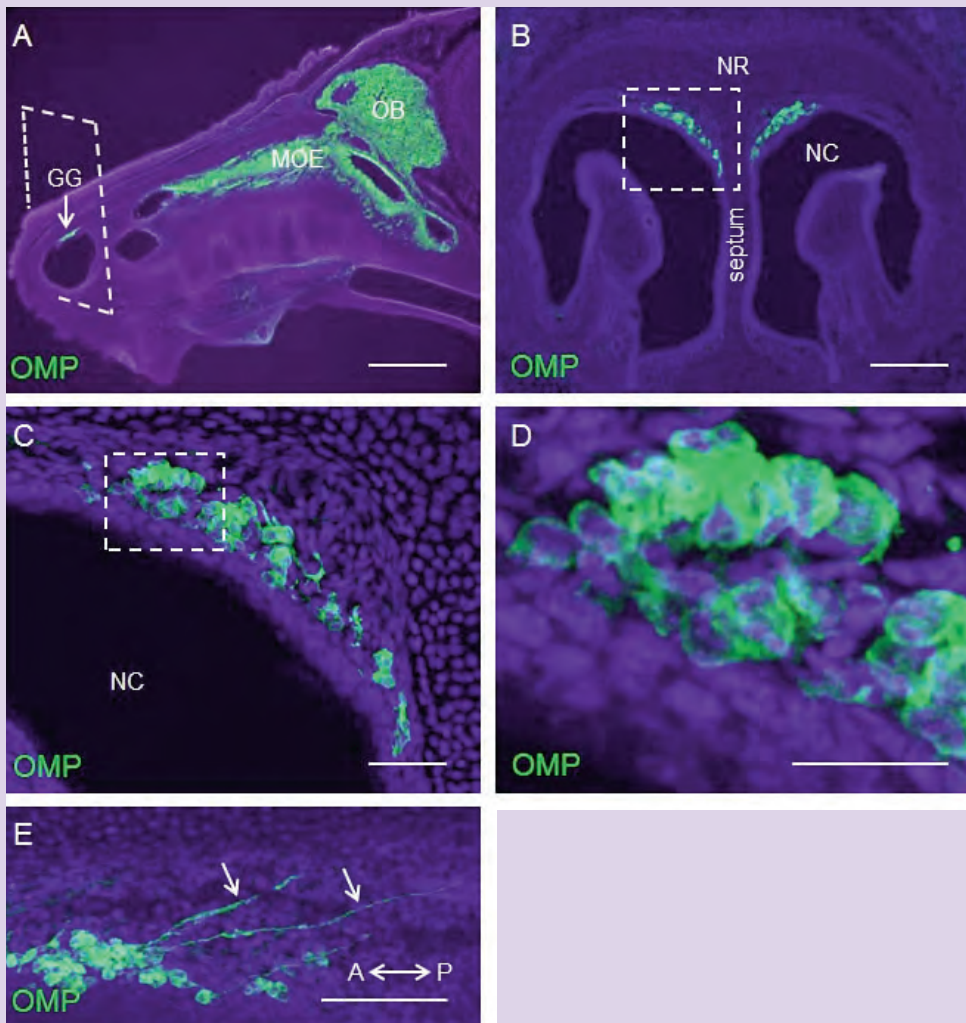


Fig 1. Immunohistochemical visualization of OMP-expressing neurons in the GG.

(A) Sagittal section through the head of a neonatal mouse incubated with an antiserum against OMP. In the anterior nasal region, OMP-positive neurons of the GG are labeled (arrow); in a more posterior area, olfactory sensory neurons of the MOE including their axons projecting to the OB of the brain are stained. **(B)** Coronal section through the anterior nasal region according to the section plane indicated by the dashed rectangle in (A). The GG is a bilateral organ comprising clusters of OMP-positive neurons which reside between the nasal roof (NR), the nasal cavity (NC) and the septum. **(C)** Higher magnification of the boxed area in (B). Clusters of OMP-positive GG are embedded in the surrounding tissue and separated from the lumen of the nasal cavity (NC) by a thin epithelial layer. **(D)** High power magnification of the framed area in (C) visualizing the morphology of GG neurons. **(E)** High magnification image of a sagittal section through the anterior part of the murine nose incubated with an OMP antiserum shows axons (arrows) emanating from GG neurons which project in a posterior direction (A, anterior; P, posterior). The sections depicted in (A)-(E) were counterstained in blue with 4',6-Diamidino-2-phenylindole (DAPI) or Toto-3. Scale bars: A 500 μm ; B 200 μm ; C 50 μm ; D 25 μm ; E 100 μm . (Data from Fleischer et al. 2006b; with kind permission of John Wiley and Sons).

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Roppolo et al. 2006; Storan and Key 2006). Hence, unlike other chemosensory compartments in the nose, the GG is not part of the nasal epithelium.

The GG comprises neuronal and non-neuronal cells. The neurons of the GG (commonly designated as GG cells) have a round to oval or elliptical shape (Fig. 1C-D) (Fuss et al. 2005; Koos and Fraser 2005; Roppolo et al. 2006; Storan and Key 2006) and express OMP as well as the neuronal marker β III tubulin (Fleischer et al. 2006a; Brechbühl et al. 2008). GG neurons extend about 30 to 40 cilia arranged into three to four bundles which are localized in discrete cellular regions. The cilia are about 15 μ m long and 0.2 μ m thick and have an unusual structure: their base is located deep in the cell body. Consequently, the cilia are partially invaginated into the cytoplasm of the soma (Tachibana et al. 1990; Brechbühl et al. 2008). The ciliary axoneme is also exceptional since it comprises three distinct regions along its extent: the basal body area comprises 9 triplets of microtubules, the proximal portion of the cilia is composed of a (9+0) microtubule doublet and the more distal regions harbor an (8+1) doublet (Brechbühl et al. 2008). GG neurons and their cilia are apparently largely enveloped by the second cell type of the GG: the so-called satellite or ensheathing cells (Tachibana et al. 1990; Brechbühl et al. 2008). Based on the expression of molecular markers such as the glial fibrillary acidic protein (GFAP), these cells are supposed to have a glia-like phenotype (Brechbühl et al. 2008). Being largely enwrapped by the satellite cells and lacking dendritic processes reaching the epithelial surface,

GG neurons seem to be devoid of any direct access to the nasal lumen.

Each GG neuron extends a single axon that projects to the olfactory bulb (OB) of the brain (see below; Fuss et al. 2005; Koos and Fraser 2005; Fleischer et al. 2006a; Roppolo et al. 2006; Storan and Key 2006). During development, the first GG axons emerge at about embryonic day 16 (E16) (Fuss et al. 2005). Similar to OMP-positive neurons in other nasal compartments, axonal lesion elicits degeneration of GG neurons; however, unlike other neurons in the nose, there is no regeneration of GG neurons following axotomy (Roppolo et al. 2006).

Axonal projection of GG neurons

Contrary to the initial concept that the GG belongs to the trigeminal nerve system (Grüneberg 1973), the expression of OMP strongly suggests that the GG is part of the olfactory system. Usually, olfactory neurons project their axons to the OB where they synapse on dendritic processes of downstream projection neurons in round-shaped structures termed glomeruli. Axonal processes from GG neurons form a single or a few bundles (with a diameter of \sim 20 μ m) that run ipsilaterally in posterior direction (Fig. 1E). Reaching the area of the MOE, these bundles are associated with main olfactory bundles but do not appear to intermingle with them. Then, they pass through the cribriform plate and approach the OB. Subsequently, the axon bundles project along the medial side of the bulb to the caudal bulbar region. There, they diverge into two (or sometimes more) smaller bundles. While one portion of the fibers takes a medial to ventral route, the other projects

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across the dorsal aspect of the bulb before reaching lateral bulbar areas. Finally, fibers from both trajectories seem to reach the ventral bulbar region; thus GG axonal projections in the caudal region of the bulb are ring-shaped surrounding the so-called accessory olfactory bulb (AOB) (Fig. 2A) (Fuss et al. 2005; Koos and Fraser 2005; Fleischer et al. 2006a; Roppolo et al. 2006; Storan and Key 2006; Matsuo et al. 2012). Along these ring-shaped axonal projections, GG fibers form ~10 glomerular structures (Fig. 2A-D) (Matsuo et al. 2012). Since there is almost nothing known about these structures, it is even still elusive whether they indeed represent glomeruli. However, the most recent discovery that these structures are endowed with the synaptic marker synaptotagmin strongly suggests that they are glomeruli enabling synaptic contact between GG axons and downstream neurons (Matsuo et al. 2012). Yet, in contrast to other glomeruli in the bulb, the glomerular structures innervated by the GG are interconnected by fibers (Fig. 2A-B). The nature of these interconnections is so far unclear; in this context, it is tempting to speculate that some axons traverse given glomeruli and terminate in successive ones (Fig. 2C-D). Based on this peculiar arrangement resembling "beads on a string" (inset in Fig. 2A), these glomeruli are designated as necklace glomeruli (Fuss et al. 2005; Koos and Fraser 2005; Roppolo et al. 2006; Storan and Key 2006; Matsuo et al. 2012). Necklace glomeruli in the OB – initially described by Shinoda and co-workers (1989) – are also innervated by axons of the so-

called GC-D neurons from the MOE (Juilfs et al. 1997; Leinders-Zufall et al. 2007; Walz et al. 2007). A recent study (Matsuo et al. 2012) has shown that the two distinct axonal populations do not converge on the same glomeruli; thus, there are (at least) two different subsets of necklace glomeruli: one exclusively innervated by neurons from the GG, the other by GC-D neurons from the MOE. This differential projection pattern of GG and GC-D neuron axons is also reflected by the expression of distinct axonal guidance factors: while targeting of GC-D axonal processes depends on neuropilin-2 (Walz et al. 2007), neuropilin-1 is relevant for establishing accurate wiring of the GG (Matsuo et al. 2012).

Development of the GG

The GG as a cluster of OMP-positive cells in the very anterior nasal area becomes evident for the first time around embryonic days 15 and 16 (E15/E16). Already at this early stage, GG cells are embedded in a connective tissue. Yet, the GG neurons are not born in this connective tissue. Instead, it appears that they originate at E14 from a relatively thick epithelium in the anterior nasal region. From this epithelial tissue, they seem to bud off before penetrating the subjacent connective tissue; accordingly, in contrast to other OMP-positive neurons in the nose, GG cells do not reside in the nasal epithelium. In the subsequent perinatal stages, GG cells form smaller groups or larger clusters embedded in this connective tissue of the rostral nose. Throughout the postnatal development, the morphology of the GG changes: in juveniles and adults,

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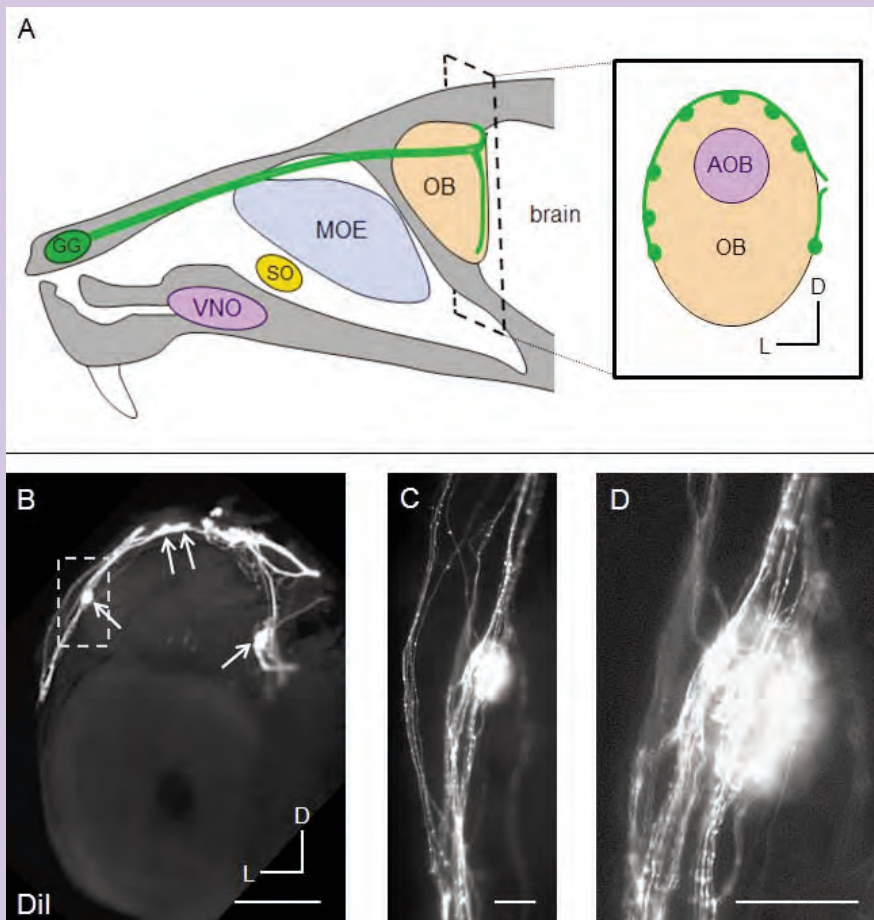


Fig 2. Axonal projection of GG neurons.

(A) Schematic representation of a sagittal section through the head of a mouse. The positions of the GG, the VNO, the SO, the MOE, and the OB are denoted (adapted from Fleischer et al. 2007). Axons from GG neurons (green) reach the OB where they project along the medial side of the bulb to the caudal bulbar region. There, one portion of the fibers takes a medial to ventral route, while the other ones project across the dorsal region of the bulb before reaching lateral bulbar areas. The inset shows a drawing of the OB corresponding to the coronal section plane indicated by the dashed rectangle (L, lateral; D, dorsal). GG axonal projections (green) in the caudal region of the bulb are ring-shaped surrounding the OB [including the accessory olfactory bulb (AOB)]. Along their trajectory encircling the OB, GG fibers form several glomerular structures (indicated by green dots) which appear to be interconnected. (B) Coronal section through the posterior region of the OB of a mouse in which the GG and its axons have been labeled by the fluorescent tracer substance Dil (white staining). The Dil-stained GG axonal fibers encircle the OB and form a smaller number of glomerular structures (arrows) (L, lateral; D, dorsal). (C)-(D) Higher magnifications of the boxed area in (B) showing a glomerular structure. Some GG axons appear to traverse this glomerulus heading for a subsequent one. Scale bars: B 500 μm ; C 50 μm ; D 50 μm .



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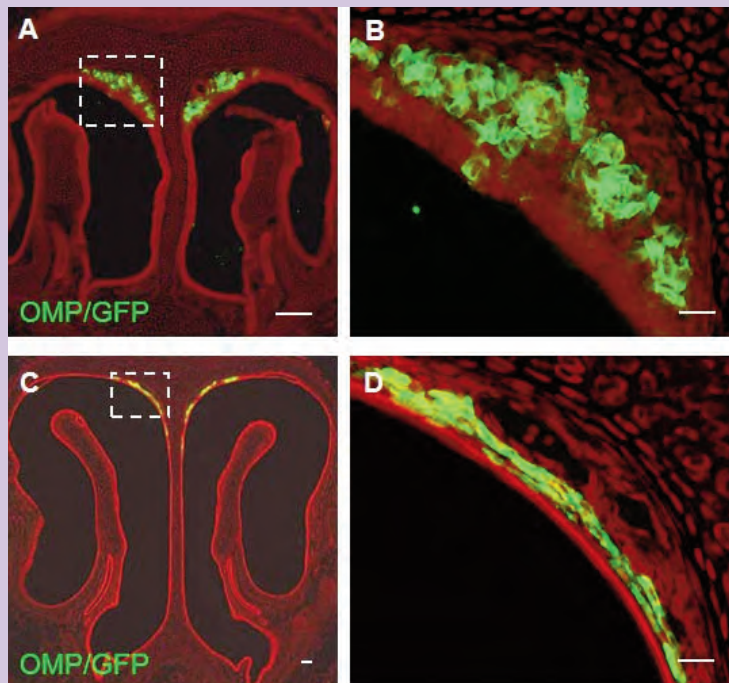


Fig 3. Arrangement of GG neurons during distinct developmental stages.

(A), (C) Coronal sections through the anterior nasal region of a neonatal (A) and an adult (C) mouse from the transgenic line OMP/GFP in which OMP-positive GG neurons fluoresce in green. (B) and (D) represent higher magnifications of the boxed areas in (A) and (C). GG neurons are arranged in ganglionic cell clusters in neonates (B). In adults, by contrast, they form a rather filiform layer directly beneath the thin epithelium (D). The sections were counterstained with propidium iodide (red). Scale bars: A and C 50 μm ; B and D 20 μm . (Data from Fleischer et al. 2006a; with kind permission of Springer Science and Business Media).

the ganglion-like structure is converted into a filiform arrangement of the GG neurons (Fig. 3) (Fleischer et al. 2006a); in addition, the number of the approximately 800 GG neurons in perinatal stages is reduced to less than 700 in adults (Fleischer et al. 2007). The relatively high number of GG neurons in early postnatal stages might indicate that the GG serves an important function in this early phase. This concept is supported by the observation that the presynaptic marker synaptotagmin is already present in GG glomeruli at birth, suggesting an early maturation of the GG circuitry and indicating that the GG might be functional in newborn animals (Matsuo et al. 2012).

Chemosensation in the GG

Expression of the olfactory marker protein OMP as well as axonal projection to the OB indicates that the GG is part of the olfactory system. Accordingly, GG neurons should respond to chemical stimuli. Testing a larger number of odorants, Mamasuew and co-workers (2011a) found that most of them do not activate GG neurons whereas some pyrazine derivatives strongly stimulate these cells in vivo as monitored by the expression of the activity-dependent gene *c-Fos*. In this regard, more detailed analyses have revealed that the three pyrazine derivatives 2,3-dimethylpyrazine (2,3-DMP), 2,5-dimethylpyrazine, and 2,3,5-trimethylpyrazine activate GG neurons while even closely related chemical compounds, such as 2-methylpyrazine, 2,3-diethylpyrazine, and *o*-xylene do not (Mamasuew et al. 2011a). These findings indicate that the ligand spectrum of the GG is rather limited to

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given substances. Electrophysiological recordings have shown that 2,3-dimethylpyrazine does not only elicit c-Fos expression in the GG but also evokes electrical responses (Hanke et al. 2013), indicating that electrical signals might be conveyed to the brain following exposure to appropriate chemicals.

The functional relevance of GG activation by pyrazine derivatives is so far unclear. Two potentially relevant natural sources for dimethylpyrazines have been described. First, dimethylpyrazines have been found in the urine of grouped females that are neither pregnant nor lactating (Jemiolo et al. 1989). Second, dimethylpyrazines are present in the urine of some predators of mice, such as ferrets (Zhang et al. 2005). Consequently, these compounds may have an alerting function for rodents. In this context, a most recent study has shown that dimethyl- and trimethylpyrazines are the chemical constituents in wolf urine that elicit avoidance and fear-associated freezing behavior in mice (Osada et al. 2013). Thus, the GG might indeed be involved in the detection of alerting substances. This notion first received support by calcium-imaging experiments suggesting that murine GG neurons are also activated by so-called alarm pheromones that have been reported to induce freezing behavior in conspecifics (Brechtbühl et al. 2008). The chemical nature of murine alarm pheromones has been elusive for a long time; however, some of these substances have been identified recently (Brechtbühl et al. 2013a). One of these compounds [2-sec-butyl-4,5-dihydrothiazole (SBT)] did not only

evoke systemic stress responses characteristic of alarm pheromones but also stimulated a substantial number of GG neurons further substantiating the concept that the GG is dedicated to the detection of alerting substances in mice (Brechtbühl et al. 2013a). However, SBT has been also reported as a chemical that is present in mouse urine in a testosterone-dependent manner and which elicits inter-male aggression (Novotny et al. 1985). Thus, further studies are required to decipher the functional role of this compound and to elucidate the relevance of its detection via the GG.

SBT and the above mentioned pyrazine derivatives are both heterocyclic sulfur- and/or nitrogen-containing chemicals. Investigating other compounds with a similar chemical structure revealed that these substances also activate the GG (Brechtbühl et al. 2013a). These chemicals include 2,4,5-trimethylthiazoline (TMT), a component of fox feces which is known to elicit fear in rodents (Vernet-Maury et al. 1984; Wallace and Rosen 2000; Kobayakawa et al. 2007), and 2-propylthietane (2-PT) that is secreted by predators such as stoats (*Mustela erminea*) and evokes avoidance responses in rodents (Heale and Vanderwolf 1994). Based on these observations, it has been proposed that the GG is dedicated to the detection of substances indicating danger which are either released by predators (kairomones) or by conspecifics (alarm pheromones) (Brechtbühl et al. 2013a). In fact, in experiments in which the GG was lesioned by axotomy, the fear- and/or aversion-inducing effects of these chemicals were clearly reduced

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(Brechtbühl et al. 2008, 2013a). However, such alerting substances do not only activate cells in the GG but also in other olfactory compartments; moreover, these other chemosensory organs are also essential for the behavioral effects of these compounds (Kobayakawa 2007; Matsumoto et al. 2010; Bepari et al. 2012; Kiyokawa et al. 2013). Accordingly, future studies have to clarify the precise role of the GG for the detection and the subsequent behavioral consequences of such chemicals.

In view of the above mentioned observation that GG neurons (in contrast to other chemosensory cells in the nose) lack direct contact to the nasal lumen, it is unclear how chemicals from the environment might reach these cells *in vivo*. In this regard, it has been found that the epithelium covering the GG is permeable for at least some chemical substances, suggesting that such compounds have access to GG neurons (Brechtbühl et al. 2008). This concept is consistent with the finding that given substances indeed activate GG neurons *in vivo* (Mamasuew et al. 2011a, b).

Olfactory signaling proteins in GG neurons

The responsiveness of the GG to odorous substances such as 2,3-DMP, SBT, TMT, and 2-PT suggests that its neurons might be endowed with odorant receptors (ORs). Yet, expression of ORs appears to be restricted to a few GG neurons in prenatal stages; instead, most GG neurons (~80%) express the receptor type V2r83 (also called Vmn2r1 or V2R2) (Fleischer et al. 2006b, 2007). This receptor belongs to the V2R

family of olfactory receptors and is also expressed by a substantial portion of the sensory neurons in the VNO (Martini et al. 2001). V2r83-negative GG neurons express members of the trace amine-associated receptors (TAARs); thereby, only one subtype is expressed in each cell (Fleischer et al. 2007). Accordingly, similar to OSNs in the MOE (reviewed in Mombaerts 2004), only one olfactory receptor type seems to be present in each GG neuron. Expression of olfactory receptors (V2r83 and TAARs) in the GG appears to be highest in perinatal stages and diminishes in consecutive phases (Fleischer et al. 2007). In summary, based on the expression of olfactory receptors, GG neurons can be classified into two major groups: V2r83- and TAAR-positive cells. However, due to their spontaneous firing pattern in extracellular recording experiments, GG neurons have been subdivided recently into three categories with each of them comprising about 30 to 40% of these cells (Liu et al. 2012). Thus, the spontaneous activity of a GG neuron might not be dependent on its olfactory receptor type. Instead, it might be substantially influenced by other signaling elements which are not equally distributed among these cells. Although our knowledge about signaling proteins in the GG is rather limited, in line with the presence of (putatively) G protein-coupled olfactory receptors, GG cells have been described to abundantly express G proteins, in particular the G protein subunits Gi and Go (Fleischer et al. 2006b), both of which are also present in vomeronasal neurons (Berghard and Buck 1996; Jia and Halpern 1996). Yet, it is unclear whether the above

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mentioned olfactory receptors and G proteins are indeed involved in the detection of chemical substances such as 2,3-DMP or TMT by GG neurons.

Recent attempts to characterize the molecular mechanisms underlying the responsiveness of GG neurons to chemical stimuli have focused on cyclic nucleotide-gated (CNG) ion channels since the CNG subtype CNGA2 is crucial for olfactory signaling evoked by odorants in the MOE (Brunet et al. 1996). These studies have revealed that CNGA2 is (almost) absent from the GG (Mamasuew et al. 2010). Instead, most GG neurons express the related CNG channel subtype CNGA3 (Fig. 4A-B) (Liu et al. 2009; Mamasuew et al. 2010). In contrast to CNGA2 which is stimulated by cyclic adenosine monophosphate (cAMP), CNGA3 is poorly responsive to cAMP but is strongly activated by cyclic guanosine monophosphate (cGMP) (Kaupp and Seifert 2002). This aspect has stimulated the search for further cGMP-associated signaling proteins expressed in GG neurons and the assessment of their potential involvement in sensory processes in these cells. These studies have shown that the transmembrane guanylyl cyclase subtype GC-G (Fig. 4C-D) and the cGMP-hydrolyzing phosphodiesterase PDE2A are also expressed in numerous GG neurons (Fleischer et al. 2009; Liu et al. 2009). In the GG, these three cGMP-associated signaling elements (CNGA3, GC-G, and PDE2A) seem to be exclusively expressed in the V2r83-positive neurons (Fleischer et al. 2009; Mamasuew et al. 2010; Matsuo et al. 2012). Since only this subpopulation of GG neurons is stimulated by the above

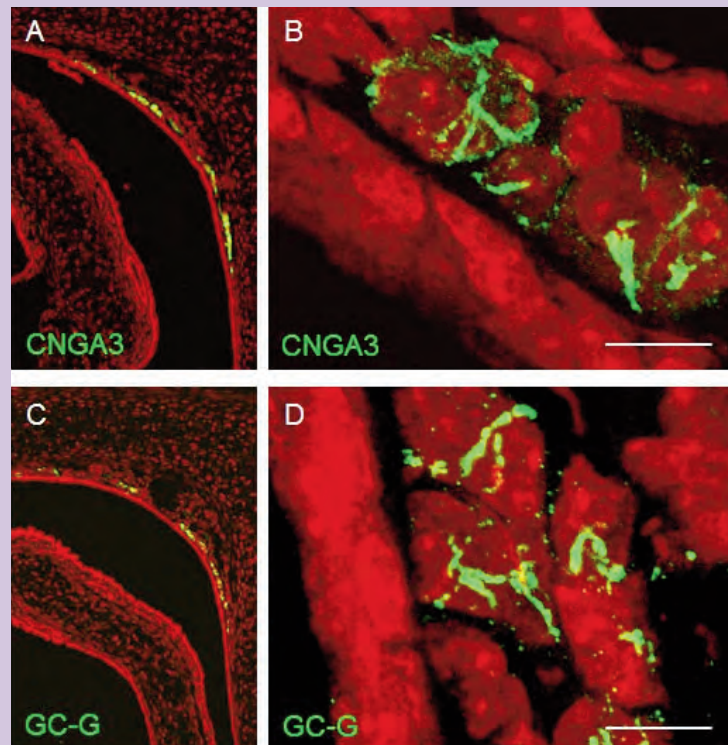


Fig 4. Expression and localization of the signal transduction proteins CNGA3 and GC-G in the GG.

(A), (C) Immunohistochemical staining on coronal sections through the anterior nose of adult mice incubated with antibodies (green) against the cGMP-regulated ion channel CNGA3 (A) or the guanylyl cyclase GC-G (C). (B), (D) High magnification images demonstrate that the GC-G and CNGA3 proteins are localized to fiber-like subcellular domains representing the cilia of GG neurons. The sections were counterstained with propidium iodide (red). Scale bars: B and D 10 μ m.

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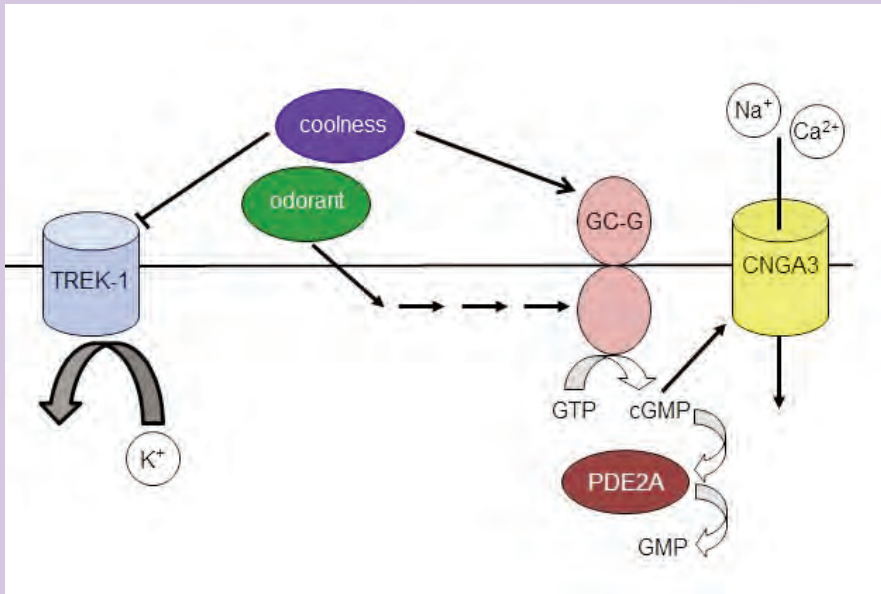


Fig 5 Chemo- and thermosensory signaling pathways in the GG.

Activation of GG neurons by odorants is mediated by cGMP-associated signaling elements, including the transmembrane guanylyl cyclase GC-G and the cGMP-activated ion channel CNGA3. Yet, it is unclear how odorous substances can stimulate these signaling elements.

Cool temperatures appear to inhibit the thermosensitive potassium channel TREK-1, thus evoking a reduced efflux of K⁺ ions. In addition, coolness seems to directly activate GC-G, leading to a cGMP-induced opening of CNGA3. The cGMP synthesized upon exposure to appropriate odorants and/or coolness could be hydrolyzed by the phosphodiesterase subtype PDE2A.

mentioned dimethylpyrazine ligands (Mamasuew et al. 2011a), it was tested whether cGMP signaling might mediate the responses evoked by these compounds in the GG. In fact, in c-Fos experiments (Mamasuew et al. 2011b) and in electrophysiological recordings (Hanke et al. 2013), responsiveness to these odorants was significantly diminished in mice deficient in CNGA3 or GC-G compared to wild-type conspecifics. Moreover, the CNG channel inhibitor substance L-cis diltiazem clearly reduces odorant-evoked calcium signals in the GG (Brechtbühl et al. 2013b). Taken together, these observations strongly indicate that activation of the V2r83-positive subset of GG neurons by given odorants is (at least partially) mediated by cGMP signaling proteins, including CNGA3 and GC-G (Fig. 5). The latter protein has been reported to be activated by bicarbonate, a finding leading to speculations that the GG might respond to CO₂ (Chao et al. 2010). However, in calcium-imaging (Brechtbühl et al. 2008) and in c-Fos (J. Fleischer; unpublished results) experiments, CO₂ did not stimulate GG neurons.

Activation by cool temperatures

In addition to their responsiveness to given chemical cues, GG neurons are also activated by cool temperatures. In fact, about 75% of the GG neurons respond to coolness (Mamasuew et al. 2008, 2010; Schmid et al. 2010; Brechtbühl et al. 2013b). Thus, its responsiveness to cool temperatures might be the reason why the GG is localized to the very anterior region of the nose whose temperature undergoes substantial changes when the environmental temperature is altered (Brechtbühl et al. 2013b). In the GG, only the V2r83-positive neurons are stimulated by cool temperatures while the TAAR-expressing GG cells do not respond to coolness (Mamasuew et al. 2008). Consequently, the V2r83-positive GG cells are dual sensory neurons which respond to chemical and thermal stimuli. Interestingly, as

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already mentioned, receptor V2r83 is also expressed in numerous sensory neurons in the VNO (Martini et al. 2001) which are not activated by cool temperatures (Mamasuew et al. 2008; Schmid et al. 2010).

Searching for the mechanisms mediating coolness-evoked GG responses, it has been observed that the coolness-sensitive transient receptor potential (TRP) channel subtype TRPM8, which is crucial for the responsiveness to cool temperatures in neurons of the dorsal root and trigeminal ganglia (Bautista et al. 2007; Dhaka et al. 2007), is absent from GG neurons (Fleischer et al. 2009). For thermosensation in mammals, in addition to TRP channels, another family of temperature-responsive ion channels is important: the temperature-gated TREK ion channels which belong to the two-pore domain potassium (K2P) channels (Maingret et al. 2000; Kang et al. 2005; Zhang et al. 2008; Noël et al. 2009). TREK channels are inactivated at cooler temperatures, i.e., these potassium channels are closed (Maingret et al. 2000; Kang et al. 2005), inducing a depolarization of the cell membrane. Recently, it has been observed that among the three known TREK channel subtypes (TREK-1, TREK-2, and TRAAK), one of them (TREK-1) is expressed in the GG where its expression seems to be restricted to coolness-sensitive cells. Moreover, in TREK-1-deficient mice, the responsiveness to cool temperatures is clearly reduced in comparison to wild-type conspecifics, suggesting that TREK-1 serves as a

thermoreceptor in GG neurons (Stebe et al. 2014). Yet, even in mice lacking TREK-1, substantial GG responses following exposure to coolness are detectable, providing evidence for an additional thermosensory pathway in the GG (Stebe et al. 2014). It is so far unclear how GG neurons can be activated by cool temperatures even in the absence of coolness-sensitive thermoreceptors such as TREK channels (and TRPM8). Interestingly, in this context, it was found that in mice deficient for the ion channel CNGA3, coolness-evoked responses were also strongly reduced, suggesting that cGMP signaling might substantially contribute to GG responsiveness to cool temperatures (Mamasuew et al. 2010). This notion received support by the finding that the CNG channel inhibitor L-cis diltiazem significantly diminishes coolness-evoked signals in the GG (Brechtbühl et al. 2013b). Although involvement of a cGMP-mediated transduction pathway in coolness-induced GG signaling is still a matter of controversial discussion (Mamasuew et al. 2010; Schmid et al. 2010; Brechtbühl et al. 2013b), most recent findings support this concept since it has been observed that mice lacking the guanylyl cyclase GC-G are also impaired in their responsiveness to cool temperatures. Moreover, human embryonic kidney (HEK) cells become responsive to coolness upon heterologous co-expressing of GC-G and CNGA3. And finally, it has been found that the enzymatic activity of heterologously expressed GC-G is stimulated by cool temperatures, suggesting that GC-G might serve as an unusual

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thermoreceptor for coolness in the GG (Y.C. Chao, J. Fleischer, and R.B. Yang; unpublished results). Thus, it seems that there are two distinct thermoreceptors operating in GG neurons: TREK-1 and GC-G (Fig. 5).

GG cells as dual sensory neurons: interactions between coolness- and odorant-evoked responses and potential functional implications

Based on their responsiveness to chemical and thermal stimuli, GG neurons can be regarded as dual sensory neurons. However, while the chemosensory capacity of the GG seems to serve detection of chemical compounds associated with fear or danger (as discussed above), the implication of its responsiveness to cool temperatures is still elusive; in particular as the absence of a functional GG does not affect characteristic coolness-associated behaviors, such as thermotaxis and huddling (Brechtbühl et al. 2013b). Importantly, in this regard, it has been reported that cool temperatures substantially enhance odorant-evoked GG responses (Mamasuew et al. 2011a; Brechtbühl et al. 2013b); an observation which is in line with the concept that transduction of both chemical and thermal stimuli relies on a cGMP signaling cascade comprising GC-G and CNGA3. However, clear differences between chemo- and thermosensory signaling in the GG have been noted: in contrast to odorant-induced GG responses, coolness-evoked GG activation does not attenuate upon long-term exposure, indicating that the relevant transduction mechanisms are not identical in spite of sharing some signaling elements. This notion is also

substantiated by the finding that odorant-adapted GG neurons are still responsive to cool temperatures (Mamasuew et al. 2011b). Yet, in this context, it has to be pointed out that no reduction of odorant-evoked GG responses upon long-term exposure occurs when the ambient temperature is cool (Mamasuew et al. 2011b; Brechtbühl et al. 2013b). Thus, in summary, it appears that an important role for GG responsiveness to cool temperatures exists in the modulation of odorant-induced responses of this organ. This notion is further supported by the observation that odorant-induced GG activation is abolished at very warm temperatures (35 °C) (J. Fleischer; unpublished results). Concerning the latter aspect, the thermosensitive potassium channel TREK-1 might be an interesting molecule since it is closed at cool temperatures, leading to a membrane depolarization, whereas it is opened at higher temperatures, eliciting membrane hyperpolarization (Maingret et al. 2000).

What could be the functional importance of modulating odorant-evoked responses in the GG by thermal stimuli? Although the biological relevance of the dual sensory trait of GG neurons is so far uncertain, based on its activation by alerting chemical compounds, such as alarm pheromones and predator odors, it is tempting to speculate that cool temperatures might sensitize GG neurons for such components. Regarding this, one might assume that rodents are primarily exposed to alerting chemicals when they leave their warm nest and enter a more dangerous environment which is not

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only characterized by numerous hazards but (usually) also by lower ambient temperatures. According to such a scenario, a high sensitivity for the above mentioned substances is not essential in the relatively safe nest but vital outside of it. Therefore, the GG might use an ambient stimulus (temperature) to adjust its chemosensory sensitivity and to prevent untimely adaptation/desensitization.

Enhancement of the chemosensory sensitivity of the GG by coolness might be also important in view of a (potentially) reduced volatility of the relevant chemical ligands at cool ambient temperatures. Interestingly, a coolness-induced increase in olfactory sensitivity has been also reported for insects (Riveron et al. 2009). Although it is unclear whether integration of chemo- and thermosensory signals in insects occurs on the level of peripheral sensory neurons or in higher centers of the nervous system, dual sensory cells in the olfactory system of animals are clearly not restricted to the GG since olfactory neurons in the MOE and in the SO of rodents respond to chemical and to mechanical stimuli, using a shared molecular cascade mediated by cAMP (Grosmaître et al. 2007; Chen et al. 2012). Moreover, a dual sensory neuronal cell type responsive to given chemicals and temperatures has also been described for the nematode worm *Caenorhabditis elegans* (*C. elegans*): the so-called AWC neurons (Biron et al. 2008; Kuhara et al. 2008).

Similarities with dual sensory neurons from *C. elegans*

On the basis of several lines of evidence, it seems that GG neurons

share striking similarities with AWC neurons from *C. elegans*. Like GG neurons, AWC neurons are ciliated sensory cells responding to both olfactory and thermal stimuli (Bargmann 2006; Biron et al. 2008; Kuhara et al. 2008). Similar to most GG neurons, AWC neurons express elements of a cGMP pathway comprising transmembrane guanylyl cyclases and CNG channels which are critical for chemosensory signaling in these cells (reviewed by Bargmann 2006). Intriguingly, both types of neurons appear to have an overlapping ligand spectrum: murine GG neurons are activated by 2,4,5-trimethylthiazole (Brechtbühl et al. 2013b), a substance also detected by AWC neurons (reviewed by Bargmann 2006).

Yet, although murine GG neurons and AWC neurons from *C. elegans* share similar stimuli (temperature and given odorants), there are also considerable differences between these two cell types. First, GG neurons respond to coolness (Mamasuew et al. 2008, 2010; Schmid et al. 2010) whereas AWC neurons are activated by warm temperatures (Biron et al. 2008; Kuhara et al. 2008). And second, while the GG is activated in the presence of an appropriate odorant, AWC neurons have basal activity in the absence of odorants, are inhibited by odorants and are stimulated by odorant removal (Chalasan et al. 2007). Consequently, GG neurons cannot be simply regarded as mammalian counterparts of the AWC neurons from nematodes.

Conclusions

Recent studies have not only shown that neurons of the GG express OMP but have also demonstrated that these



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cells share characteristic features with olfactory sensory neurons in other nasal compartments, including the presence of olfactory receptors, axonal projection to the OB, and responsiveness to odorous substances. Moreover, its dual sensory character, i.e., activation by alerting compounds and by cool temperatures suggests that the GG is a unique chemosensory organ with a specialized function. This concept is further supported by its unusual position close to the entrance of the nasal cavity and the specific axonal projection to given glomeruli of the enigmatic "necklace system" in the OB. Future studies will (hopefully) further elucidate the molecular signaling pathways enabling the GG to respond to distinct environmental stimuli as well as its neuronal circuitry and its implications for the control of animal behavior in order to shed brighter light on this tiny but fascinating chemosensory organ ■

Abbreviations. 2,3-DMP, 2,3-dimethylpyrazine; 2-PT, 2-propylthietane; cGMP, cyclic guanosine monophosphate; CNG, cyclic nucleotide-gated; GG, Grueneberg ganglion; MOE, main olfactory epithelium; OB, olfactory bulb; OMP, olfactory marker protein; SBT, 2-sec-butyl-4,5-dihydrothiazole; SO, septal organ; TAAR, trace amine-associated receptor; TMT, 2,4,5-trimethylthiazoline; VNO, vomeronasal organ.



Acknowledgements. This work was supported by the Deutsche Forschungsgemeinschaft. J. Fleischer was supported by the Humboldt reloaded program of the University of Hohenheim financed by the Bundesministerium für Bildung und Forschung (01PL11003). The author would like to thank Heinz Breer for continuous and generous support.

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NEWS

SWEET TALK

Consumer Organisation Loses Fight Over Chocolate Flavour

By Elke Weiler elke.weiler@uni-ulm.de



GERMAN CHOCOLATE MANUFACTURER, Alfred Ritter Gmbh & Co KG, has won a court battle in a Munich court against an influential consumer organization, Stiftung Warentest (SW) over claims of "natural flavour" in its hazelnut chocolate which were challenged by SW. The flavour, piperonal (1,3-benzodioxole-5-carbaldehyde) is supplied by flavour house, Symrise. It has a floral odour, described as being similar to that of vanillin or cherry. Symrise argued that the flavourant is not "chemically manufactured" and is a plant extract, while SW claimed it cannot be derived in a "natural way." If so, the product might have been forced off the lucrative market during the current European winter. Instead, Alfred Ritter won the dispute and the product remains available to its consumers. The consumer organisation will have to pay a fine of Euro250,000 if it persists, and may yet face a commercial damages claim. It is likely to appeal the finding. ■

Links: <http://scmresearch.org/2013/12/>;

<http://www.tagesschau.de/wirtschaft/stiftungwarentest104.htm>;

<http://www.br.de/nachrichten/ritter-sport-stiftung-warentest-100.html>

World Grows E-noses

A MEETING OF THE INTERNATIONAL ELECTRONIC NOSE COMMUNITY (ISOEN) was held in Daegu, South Korea, from 2-5 July 2013.

The meeting attracted 200 participants and 150 presentations ■

Further Information: <http://www.isoen2013.kr/>

NEWS

Is Your Product Synesthesia-Ready?

By Graham Bell: g.bell@e-nose.info

YOUR SHARE OF THE MARKET MAY BE ABOUT to be undermined by competitors who are adding a synesthetic dimension to the sensory quality of their products. In an article reported in AdNews Online(24 March 2014), Thomasine Burnap reports a novel twist to product design: adding attributes that stimulate cross-modal sensory stimulation (synesthesia). A person with synesthesia (1-4 % of the population) may hear sounds when looking at colours or see colours while listening to music. Advertisers are interested in using synesthetic associations that are identified by the wider population. A well-known example is the association of the colour red and orange with sweetness, yellow and green with sourness and blue or purple with menthol or eucalyptus. Many people will report an association of low musical notes with dark or unsaturated colours, and high notes with bright and saturated colours.

Research is extending the repertoire of what may be possible, linking chemosensory experience to words, shapes and visual contexts. Burnap reports that psychologist Charles Spence of Oxford University has the attention of global advertiser, JWT London, and of major food clients (Unilever, Starbucks and Nestle) and even car companies (Toyota), all being alert to the value of sensory quality in gaining a competitive edge for their products■

Link: <http://www.adnews.com.au/news/opinion-synesthetic-marketing-the-next-dimension-in-sensory-marketing>



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- 30 July – 1 August 2014 **The Sensometric Society**
 Chicago, USA
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- 7-11 September 2014 **ECRO: European Chemoreception
 Organisation**
 Dijon, France
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- 18 November 2014
 then 20 – 23 November 2014
- Clinical topics course followed by Clinical
 CHEMOSENSATION 2014**
 Dresden, Germany
<http://www.uniklinikum-dresden.de>
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