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Review

The Grueneberg ganglion: a novel sensory system in the nose

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Summary. Within the nasal epithelium of mammals, there are several compartments which are populated with neuronal cells. One of them – the so-called Grueneberg ganglion – is composed of ciliated neurons residing in the anterior region of the nose. Although cells of the Grueneberg ganglion lack direct contact with the lumen of the nasal cavity, they are endowed with features indicative of olfactory sensory neurons, such as the olfactory marker protein and distinct olfactory receptors, as well as projection of axonal processes to the olfactory bulb of the brain. These findings have led to the notion that the Grueneberg ganglion might be a novel olfactory subsystem; a concept which was lately supported by the observation that chemical cues activate Grueneberg ganglion neurons. Unexpectedly, it was recently found that these cells also respond to cool ambient temperatures, presumably via a signaling pathway mediated by second messengers. Thus, the Grueneberg ganglion may operate as a dual sensory organ involved in the detection of both chemical and thermal stimuli.

Key words: Olfaction, Thermosensation, Necklace glomeruli, Cilia, cGMP

Introduction

The mammalian olfactory system is capable of detecting an almost indefinite number of chemical substances. This enormous chemosensory capacity is based on a large number of olfactory sensory neurons (OSNs) which reside in different nasal compartments (Fig. 1), including the main olfactory epithelium (MOE), the vomeronasal organ (VNO), and the septal organ (SO). Although the OSNs in these nasal subsystems are distinct, they share a series of common features, such as axonal projection to the olfactory bulb (OB) as well as expression of olfactory receptors and the olfactory marker protein (OMP) (reviewed by Breer et al., 2006; Spehr et al., 2006; Ma, 2007; Munger et al., 2009). Recently, a novel compartment was found in the apical region of the nose which comprises cells with these characteristics of OSNs (Fuss et al., 2005; Koos and Fraser, 2005; Fleischer et al., 2006a,b, 2007; Roppolo et al., 2006; Storan and Key, 2006). It turned out that this ganglion-like structure had already been discovered in 1973 by Hans Grüneberg (Grüneberg, 1973). Accordingly, this small organ is now commonly designated as Grueneberg (also spelled Grüneberg) ganglion (GG). The notion that the GG may serve a sensory function has been supported by recent studies demonstrating that environmental stimuli activate GG neurons (Brechbühl et al., 2008; Mamasuew et al., 2008). In this review, the current knowledge regarding the structure, development, axonal wiring and intracellular signaling of the GG is updated and its potential sensory functions are discussed.

Morphology and cell types of the GG

The GG has been identified in a series of mammalian species, including humans; however, it is yet unclear whether all mammals are endowed with a functional GG (Grüneberg, 1973; Tachibana et al.,

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Abbreviations: AChE, acetylcholine esterase; AOB, accessory olfactory bulb; cGMP, cyclic guanosin monophosphate; CNG, cyclic nucleotide-gated; E, embryonic day; GFP, green fluorescent protein; GG, Grueneberg ganglion; GnRH, gonadotropin-releasing hormone; MOE, main olfactory epithelium; OB, olfactory bulb; OMP, olfactory marker protein; OR, odorant receptor; OSNs, olfactory sensory neurons; SO, septal organ; TAARs, trace amine-associated receptors; TRP, transient receptor potential; V1Rs, class I vomeronasal receptors; V2Rs, class II vomeronasal receptors; VNO, vomeronasal organ

1990). In the murine nose, the GG resides bilaterally in the most anterior nasal region – also designated as the nasal vestibule – close to the opening of the naris (Fig. 2A,B). Along the anterior/posterior axis, the GG has an extent of a few hundred micrometers only and is situated between the levels of the orifices of the lateral nasal gland (glandula nasales lateralis I or Steno's gland) and the glandula nasales medialis. In this area, the GG is circumscribed by the nasal roof, the septum, and a thin epithelial layer bordering the lumen of the nasal cavity (Fig. 2C). Thus, the ganglion cells are not part of the epithelium lining the anterior nasal cavity; instead, they are embedded in the underlying connective tissue. Here, the GG is located in the vicinity of large blood vessels; nevertheless, GG cells are not in direct contact with them. The ganglion is not a compact structure as its cells are mainly arranged in small groups or larger clusters (Fig. 2C,D). Typically, GG cells have an oval or elliptical shape; some of them are rather round. Neighboring GG cells tend to flatten against each other (Fig. 2D). GG cells apparently lack a direct access to the nasal lumen; instead, they are covered by connective tissue which is superimposed by a keratinized epithelial layer (Grüneberg, 1973; Fuss et al., 2005; Koos and Fraser, 2005; Fleischer et al., 2006a; Roppolo et al., 2006; Storan and Key, 2006). More detailed analyses performed on the GG of musk shrews (*Suncus murinus*) and mice (Tachibana et al., 1990; Brechbühl et al., 2008) disclosed that GG cells seem to be largely covered by a sheath of satellite cells. In mice, these ensheathing cells express molecular markers of glia cells, such as the calcium-binding protein S100, and the glial fibrillary acidic protein (GFAP), indicating that these cells have a glia-like phenotype (Brechbühl et al., 2008). GG cells (not the ensheathing cells) protrude a single axon (Fig. 2E), leading to the notion that they are neurons (Grüneberg, 1973; Tachibana et al., 1990). This concept was substantiated by the finding that they express the neuronal marker ßIII tubulin (Fleischer et al., 2006a; Brechbühl et al., 2008). Thus, the GG comprises two distinct cell types: neuronal cells (commonly designated as GG neurons or GG cells) and glia-like cells (called ensheathing or satellite cells) which envelop the GG neurons. The GG neurons - but not the ensheathing cells - bear multiple cilia. The structure of these cilia is unusual; however, they are somewhat related to the so-called primary cilia (Tachibana et al., 1990; Brechbühl et al., 2008). The ciliary processes of GG neurons arise from the deep portion of the soma. Consequently, a substantial portion of the cilia is invaginated into the cytoplasm of the soma. The plasma membrane which encloses the invaginated parts of cilia forms a complex intracellular network including desmosome-like membrane specializations (Tachibana et al., 1990). The microtubular arrangement of the ciliary axoneme is also quite exceptional (Brechbühl et al., 2008). The basal body area comprises 9 triplets of microtubles, the proximal portion of the cilia is composed of a (9+0) microtuble doublet and the more distal regions harbor an (8+1) doublet. The cilia are mostly enveloped by satellite cells; in some cases, however, the distal portion of the cilia was found to be situated in close contact with the surrounding connective tissue. Each GG neuron harbors about 30 to 40 cilia with a length of about 15 μm and a diameter of approximately 0.2 μm; the cilia are grouped into three to four bundles which are localized to discrete cellular regions. Importantly, the cilia of GG cells do not reach the surface of the nasal epithelium (Brechbühl et al., 2008). Thus, GG cells apparently lack any direct access to the nasal lumen.

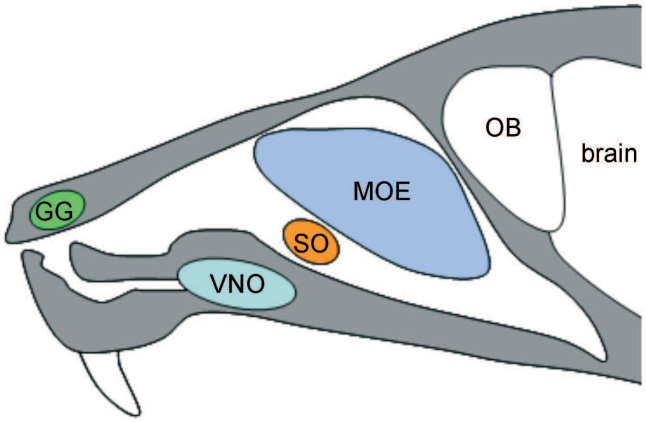

Fig. 1. Distinct neuronal compartments in the nose. Schematic representation of a sagittal section through the head of a mouse (adapted from Fleischer et al., 2007). The localization of the GG, the MOE, the SO, and the VNO is given. Sensory neurons in these nasal compartments project their axons to the OB of the brain.

Pre- and postnatal development of the GG

In mice, the formation of the GG becomes apparent at embryonic day 14 (E14). At this stage, the epithelium is thickened in the anterior nasal region and neuronal cells seem to be budding off the epithelium and penetrate the subjacent connective tissue. At embryonic stages E15/E16, these prospective GG neurons no longer reside in the epithelium but are embedded in the underlying tissue, where they are arranged in a cluster-like manner; coincidentally, the thickness of the epithelial layer overlaying the ganglion is decreased (Grüneberg, 1973; Fleischer et al., 2006a). Although the ganglion is a constant feature of the murine anatomy (Grüneberg, 1973), several changes occur during the postnatal phase. Most strikingly, the morphology of the GG changes: the initially cluster-like structure of the GG is transformed into a filiform arrangement of the GG neurons in older animals (Fleischer et al., 2006a). Moreover, the total number of about 950 GG neurons in perinatal stages declines to less than 700 in adults (Fleischer et al., 2007).

Upon lesion of their axonal projections, OSNs usually degenerate. Similarly, lesion of the respective

axonal processes also leads to degeneration of GG neurons. However, in contrast to the MOE, after axotomy, there was no indication for regeneration in the ganglion (Roppolo et al., 2006). This observation is in line with experiments applying the thymidine analogue BrdU to label newly generated cells in the GG: the very small number of stained cells in these approaches (Roppolo et al., 2006) is supposed to be indicative of a rather poor capacity of the GG to regenerate.

Is the GG part of the terminal nerve or of the olfactory system?

The nose of mammals harbors several neuronal subdivisions, including the terminal nerve, the trigeminal nerve and a series of olfactory subsystems. Based on its localization within the anterior region of the nasal cavity, it was initially proposed that the GG might be an element of the terminal nerve (*Nervus terminalis*) (Grüneberg, 1973); an enigmatic neuronal network with multiple ganglionic structures that has been described in several vertebrate species (reviewed by Schwanzel-Fukuda and Pfaff, 2003). Acetylcholine esterase (AChE) activity, as well as the gonadotropin-releasing hormone (GnRH), are considered as markers for the Nervus terminalis system (Jennes and Stumpf, 1980; Schwanzel-Fukuda and Silverman, 1980; Silverman et al., 1982; Witkin and Silverman, 1983; Wirsig and Leonard, 1986; Witkin, 1987). The absence of both AChE activity (Tachibana et al., 1990) and GnRH (Fleischer et al.,

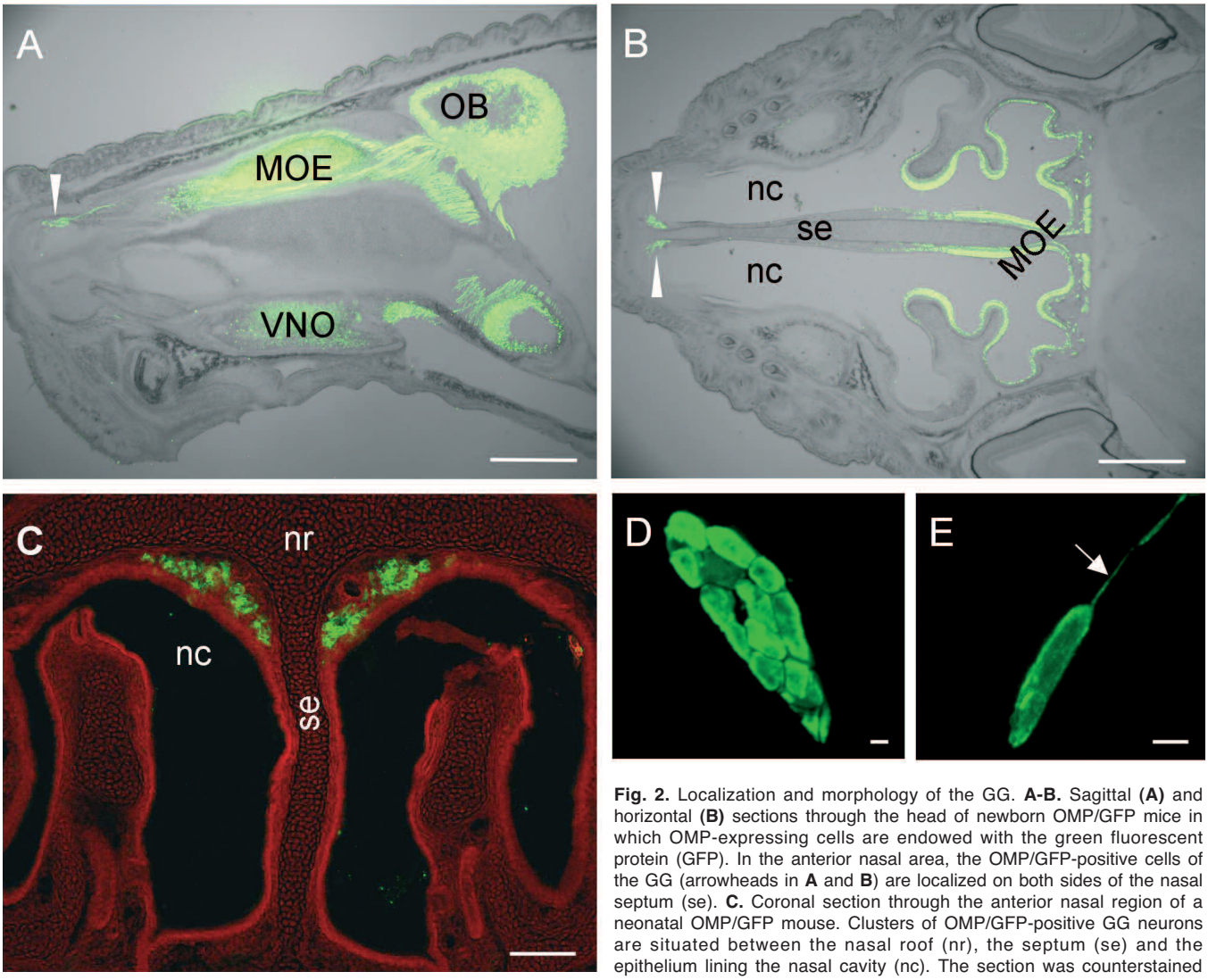

Fig. 2. Localization and morphology of the GG. **A-B.** Sagittal **(A)** and horizontal **(B)** sections through the head of newborn OMP/GFP mice in which OMP-expressing cells are endowed with the green fluorescent protein (GFP). In the anterior nasal area, the OMP/GFP-positive cells of the GG (arrowheads in **A** and **B**) are localized on both sides of the nasal septum (se). **C.** Coronal section through the anterior nasal region of a neonatal OMP/GFP mouse. Clusters of OMP/GFP-positive GG neurons are situated between the nasal roof (nr), the septum (se) and the epithelium lining the nasal cavity (nc). The section was counterstained with propidium iodide (red). **D.** High magnification image of the cluster-like arrangement of GG neurons. **E.** GG neurons extend an axonal process (arrow). Scale bars: A, B, 1 mm; C, 50 μm; D, E, 5 μm. (Data from Fleischer et al., 2006a; with kind permission of Springer Science and Business Media).

2006a) suggests that the GG is not part of the *Nervus terminalis*. The discovery that the olfactory marker protein (OMP) is expressed in GG neurons (Fuss et al., 2005; Koos and Fraser, 2005; Fleischer et al., 2006a; Roppolo et al., 2006; Storan and Key, 2006) has led to the hypothesis that the GG is a novel olfactory subsystem whose neurons serve a chemosensory function (Fuss et al., 2005; Koos and Fraser, 2005; Fleischer et al., 2006a; Roppolo et al., 2006; Storan and Key, 2006).

Axonal projection patterns of GG neurons

OSNs are generally supposed to project axonal processes to the OB of the brain. The concept that the OMP-expressing neurons in the GG share characteristic features of OSNs was further scrutinized exploring the projection pattern of their axons. The axonal trajectory of GG neurons was visualized making use of the lipophilic tracer DiI and of transgenic mice in which OMP-positive cells are labeled by means of the green fluorescent protein (GFP) or ß-galactosidase (Fuss et al., 2005; Koos and Fraser, 2005; Fleischer et al., 2006a; Roppolo et al., 2006; Storan and Key, 2006). It was found that axons emerge from the GG starting from embryonic day E16 (Fuss et al., 2005); they coalesce and form a few tracts which run ipsilaterally along the dorsal roof of the nose, penetrate the cribriform plate and approach the OB. They course along the dorsal part of the OB taking either a dorsal-lateral or a mid-medial route to reach its dorsal end. Here, they defasciculate into smaller bundles which innervate a small group of glomeruli (up to 10). These glomeruli surround the rostral part of the accessory olfactory bulb (AOB) which resides on the dorsal-posterior region of the OB (Fuss et al., 2005; Koos and Fraser, 2005; Fleischer et al., 2006a; Roppolo et al., 2006; Storan and Key, 2006). Formation of these glomeruli becomes evident at about postnatal day 5 (Koos and Fraser, 2005).

Olfactory phenotype of GG neurons

Based on the expression of OMP and the axonal projection to the OB, it was speculated that GG cells might also have a molecular phenotype related to OSNs in other nasal compartments. OSNs gain their chemosensory function through G protein-coupled olfactory receptors which render these cells responsive to odorous compounds (recently reviewed by Spehr and Munger, 2009; Fleischer et al., 2009a). The various receptor types include odorant receptors (ORs), vomeronasal receptors (V1Rs or V2Rs), trace amine-associated receptors (TAARs), and formyl peptide receptors (FPRs) (Buck and Axel, 1991; Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997; Liberles and Buck, 2006; Liberles et al., 2009; Riviere et al., 2009). Extensive studies exploring whether any of the OR types are expressed in the GG revealed that in prenatal stages of mice, a few cells (~10) are endowed with the OR subtype mOR256-17; in later stages, no evidence for mOR256-17 expression was obtained (Fleischer et al., 2006b). Moreover, there is no evidence that other ORs are expressed in the GG. Similarly, the V1Rs and V2Rs appear to be absent from the murine GG; however, a given V2R receptor - V2r83 - was found to be expressed in the overwhelming majority of the GG neurons (Fleischer et al., 2006b). In the V2r83-negative GG neurons, some of the TAARs are expressed; interestingly, only one distinct subtype in each cell (Fleischer et al., 2007). Thus, similar to OSNs in the MOE (reviewed by Mombaerts, 2004), each GG neuron seems to express one receptor type only. Expression of V2r83 and TAARs was highest in perinatal stages and diminished in juveniles and adults (Fleischer et al., 2007). Consistent with the presence of G protein-coupled receptors, distinct subtypes of G proteins - G_o and G_i - are abundant in GG neurons (Fleischer et al., 2006b); interestingly, the same G protein subtypes are also expressed in the chemosensory cells of the VNO (Berghard and Buck., 1996; Jia and Halpern, 1996). In summary, these findings indicate that GG cells share some characteristic features with OSNs; nevertheless, there are also considerable differences, in particular, the lack of a direct access to the nasal lumen (Tachibana et al., 1990; Fuss et al., 2005; Koos and Fraser, 2005; Fleischer et al., 2006a; Roppolo et al., 2006). This property, as well as the fact that GG neurons are largely enwrapped by ensheathing cells and overlaid by a keratinized epithelium (Tachibana et al., 1990; Fuss et al., 2005; Brechbühl et al., 2008) argues against an olfactory function. However, most recently, it was reported that murine GG neurons are activated *in vitro* by compounds called alarm pheromones which are supposed to be released by conspecifics under stressful conditions and whose chemical nature is unknown (Brechbühl et al., 2008). Moreover we found lately that GG neurons respond to low concentrations of defined chemical substances (our unpublished data), further supporting the view that GG neurons are in fact chemosensory cells.

Coolness-induced responses of GG neurons

Based on the finding that the GG is fully developed in early postnatal stages, it has been speculated whether it may serve a functional task crucial for this phase of life, e.g. a role related to mother/child interactions. To scrutinize this notion, neonatal mice were temporarily separated from their mother. It turned out that upon separation, GG neurons in pups were activated; after returning to the mother, this activation was abolished. Extensive studies revealed that these responses of GG neurons were not evoked by chemical stimuli but by cooler ambient temperatures in the absence of the warmth-giving mother (Mamasuew et al., 2008). This thermosensation is particularly interesting as neonatal mouse pups are poikilothermic since they cannot

regulate their body temperature (Wixson and Smiler, 1997). Furthermore, for them, the ambient temperature plays a key role for characteristic behaviors, such as huddling and vocalization (Alberts, 1978; Alberts and Brunjes, 1978; Oswalt and Meier, 1975; Blumberg et al., 1992; Szentgyörgyi et al., 2008). It is therefore conceivable that the GG might be involved in the regulation of these behaviors.

The molecular mechanisms underlying coolness-evoked responses of GG neurons are unknown. Initial studies to characterize the cell types responding to coolness revealed that cool temperatures did not activate all GG neurons, but only those which express the receptor V2r83 (Mamasuew et al., 2008). However, it appears unlikely that the receptor itself contributes to the coolness-evoked responsiveness of these cells. In the somatosensory system of mammals, cool temperatures are detected by thermosensory neurons in the dorsal root ganglion or the trigeminal ganglion, which express coolness-activated transient receptor potential (TRP) channels, most notably the subtype TRPM8 (McKemy et al., 2002; Peier et al., 2002; Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007). However, the TRPM8 channel is not expressed by GG neurons in mice (Fleischer et al., 2009b). In the nematode *Caenorhabditis elegans* (*C. elegans*), detection of temperature is mediated by thermosensory neurons which employ a second messenger pathway mediated by cyclic guanosin monophosphate (cGMP). This transduction cascade includes transmembrane guanylyl cyclases that generate cGMP, which in turn activates cyclic nucleotide-gated (CNG) ion channels (Coburn and Bargmann, 1996; Komatsu et al., 1996; Inada et al., 2006). Alternative to TRP-mediated signaling, this cGMP cascade is considered as another mechanism to render cells responsive to temperature changes. In this context, it is of great interest that a given transmembrane guanylyl cyclase - subtype GC-G - is expressed in murine GG neurons (Fleischer et al., 2009b; Liu et al., 2009). In the GG, expression of GC-G is confined to those neurons responding to coolness; moreover, GC-G is absent from neurons in other olfactory subsystems (Fleischer et al., 2009b). Most recent findings demonstrate that GG neurons also express the cGMP-activated ion channel CNGA3 (Liu et al., 2009; Mamasuew et al., in press) whose expression in the GG is restricted to coolness-sensitive neurons (Mamasuew et al., in press). Transgenic mice with a deletion of CNGA3 showed significantly reduced responses of the GG to coolness (Mamasuew et al., in press). These observations suggest that a cGMP pathway may operate in GG cells, contributing to coolness-evoked responses; a mechanism reminiscent of the signaling processes in thermosensory neurons of *C. elegans*. In this regard, it is interesting to note that a given type of sensory cells in *C. elegans* - designated as AWC neurons - does not only respond to temperature changes, but also to chemical stimuli (Coburn and Bargmann, 1996; Komatsu et al., 1996; Kuhara et al., 2008; Biron et al., 2008). Thus, it is conceivable that some of the GG neurons may also be capable of sensing both modalities.

Does the GG belong to the "necklace" olfactory system?

The axons emerging from the GG project to a small number of interconnected glomeruli in the caudal OB which surround the anterior part of the AOB (Fuss et al., 2005; Koos and Fraser, 2005; Roppolo et al., 2006; Storan and Key, 2006). These "atypical" glomeruli, which are arrayed like "beads on a string", have been designated as "necklace" glomeruli (Shinoda et al., 1989). The "necklace" glomeruli are usually innervated by axons of the so-called GC-D neurons in the MOE (Juilfs et al., 1997; Leinders-Zufall et al., 2007; Walz et al., 2007). In contrast to other OSNs, GC-D neurons express the transmembrane guanylyl cyclase subtype GC-D and the phophodiesterase PDE2A; the latter enzyme is also present in the axons of GC-D neurons and consequently in the glomeruli innervated by these cells (Juilfs et al., 1997). Since all PDE2A-positive glomeruli in the OB appear to be innervated by axons of GC-D neurons (Leinders-Zufall et al., 2007), PDE2A immunoreactivity is considered as a marker for "necklace" glomeruli (reviewed by Luo, 2008). Interestingly, PDE2A is also present in axons of GG neurons (Fleischer et al., 2009b), indicating that certain "necklace" glomeruli might be concomitantly innervated by GC-D neurons of the MOE and by GC-G-positive neurons of the GG. This notion is in line with the observation that glomeruli innervated by GG neurons apparently also harbor axonal terminals from other nasal OSNs (Roppolo et al., 2006). Besides axonal projection to "necklace" glomeruli and expression of PDE2A, as well as distinct subtypes of transmembrane guanylyl cyclases (GC-D or GC-G) (Fülle et al., 1995; Juilfs et al., 1997; Fleischer et al., 2009b; Liu et al., 2009), both GC-D neurons and the majority of the GG neurons are endowed with the ion channel CNGA3 (Meyer et al., 2000; Liu et al., 2009; Mamasuew et al., in press). Thus, the GG might be indeed part of the "necklace" olfactory system.

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