Olfactory subsystems associated with the necklace glomeruli in rodents

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ABSTRACT

The necklace glomeruli are a loosely defined group of glomeruli encircling the caudal main olfactory bulb in rodents. Initially defined by the expression of various immunohistochemical markers, they are now better understood in the context of the specialized chemosensory neurons of the main olfactory epithelium and Grueneberg ganglion that innervate them. It has become clear that the necklace region of the rodent main olfactory bulb is composed of multiple distinct groups of glomeruli, defined at least in part by their afferent inputs. In this review, we will explore the necklace glomeruli and the chemosensory neurons that innervate them.

Keywords: GC-D; Trpc2; Grueneberg ganglion; cGMP; main olfactory bulb

INTRODUCTION

The mammalian main olfactory bulb (MOB) is a three-layered cortical structure and the central nervous system target of peripheral chemosensory neurons within the main olfactory system (MOS). In mice, each olfactory sensory neuron (OSN) axon passes from its peripheral origin in the nose, through the skull, and along the surface of the MOB before entering and terminating in one of ~2000 glomeruli – spherical neuropil near the pial surface – where they will synapse with second order projection neurons and local interneurons. While each glomerulus has the same basic structure and cellular components, each varies based on the type of OSN innervating it. In this review, we will focus on glomeruli in the most caudal aspects of the MOB, the so-called “necklace” glomeruli. Though they have been known for decades, the necklace glomeruli remain minimally studied and poorly understood. However, we have begun to better understand the chemosensory neurons that target the necklace glomeruli as well as the functions those sensory neurons subserve. This understanding provides us with important tools to investigate this obscure but important domain of the olfactory brain.

OLFACTORY SUBSYSTEMS

Mammalian olfactory systems can be divided into several subsystems based on the anatomical location of their sensory neurons, the type of olfactory receptors they express, the signaling mechanisms they employ to transduce chemosensory stimuli, the chemosensory stimuli they respond to, and the axonal targets of their sensory neurons to regions of the olfactory forebrain (Munger *et al.* 2009b). The principal division in rodents is between the main and accessory olfactory systems (**Figure 1A**). The accessory olfactory system (AOS) includes a distinct peripheral sensory structure, the vomeronasal organ, and a circumscribed target region in the olfactory forebrain, the accessory olfactory bulb (AOB). Three subpopulations of

vomeroneural sensory neurons (VSNs) can be distinguished based on the family of chemosensory receptors they express: type 1 vomeronasal receptors (V1Rs), type 2 vomeronasal receptors (V2Rs), or formyl peptide-like receptors (FPRs). Each group of VSNs maintains a distinct projection pattern to the AOB.

The MOS includes the MOB as well as three distinct sensory organs: the main olfactory epithelium (MOE), the septal organ of Masera, and the Grueneberg ganglion (**Figure 1A**). In the rodent nasal cavity, the MOE covers the nasal septum in its most superior and posterior aspects, the nasal turbinates on each lateral wall, and the dorsal recesses that connect them. The MOE contains several distinct subpopulations of OSNs. These include canonical OSNs that express G protein-coupled odorant receptors (ORs) first described by Buck and Axel; OSNs expressing trace amine-associated receptors (TAARs); guanylyl cyclase-D (GC-D)-expressing OSNs (GC-D+ OSNs); and several populations of transient receptor potential (TRP)-channel expressing neurons. Each of the OSN subpopulations innervate the MOB. OSNs located within the septal organ, an anteriorly displaced patch of epithelium that is similar in composition to the MOE, also innervate the MOB, as do chemosensory neurons found at the most anterior aspect of the nasal cavity within the Grueneberg ganglion.

The rodent olfactory bulb can be divided into several anatomical domains based on the molecular identity and anatomical origin of the chemosensory neurons that innervate them. For example, the anterior AOB receives projections from V1R-expressing VSNs, while the posterior AOB is innervated by VSNs expressing V2Rs (**Figure 1A**). The MOB contains even more subdivisions. OSNs within the septal organ primarily innervate glomeruli on the ventromedial aspect of the caudal MOB. Canonical OSNs expressing Class I ORs (which are most closely related to ORs found in fish) and TAAR-expressing OSNs from the MOE innervate adjacent regions of the dorsal MOB, while canonical MOE OSNs expressing Class II ORs innervate glomeruli along the lateral, medial and ventral surfaces (**Figure 1B**). The caudal-most aspects of the MOB comprise the “necklace” region, so named because early histochemical and

immunohistochemical staining revealed a ring of glomeruli encircling the posterior MOB like beads on a chain (**Figure 1C**). This region contains necklace glomeruli innervated by several subpopulations of chemosensory neurons including GC-D+ OSNs from the MOE and septal organ, Trpc2+ OSNs from the MOE, and ciliated sensory neurons emerging from the Grueneberg ganglion (**Figure 1D**).

THE NECKLACE GLOMERULI

The necklace glomeruli have been differentially defined. The term was first coined by Shinoda and colleagues, who identified a small number of OSNs in the rat that were immunoreactive for human placental antigen X-P2 (PAX) and which converged on 7-9 glomeruli that ringed the caudal MOB (Shinoda *et al.* 1989). These glomeruli overlapped with a subset of “atypical” glomeruli that displayed acetylcholinesterase (AChE) reactivity (Shinoda *et al.* 1993; Zheng *et al.* 1987). Subsequent immunohistochemical studies with two monoclonal antibodies of unclear immunoreactivity – 2C6 and MAb213 – also labeled axons innervating subsets of glomeruli in the necklace region (Ring *et al.* 1997). However, while MAb213+ glomeruli were typically also AChE+, 2C6+ glomeruli were not. This suggests that the 2C6 and MAb213 antigens are found in two distinct groups of OSNs that target glomeruli of the necklace region. Ring and colleagues also suggest that the PAX+/AChE+ glomeruli are a subset of the MAb213+/AChE+ glomeruli. The antigens recognized by the PAX, 2C6 and MAb213 antibodies were not restricted to OSN axons, but were also abundant in the somata and dendrites of those neurons. Immunoreactive OSNs were typically found in the dorsal and lateral recesses (a.k.a., cul-de-sacs) between the turbinates. Although no colocalization studies were done, it does appear that 2C6+ and MAb213+ OSNs are distinct subpopulations (Ring *et al.* 1997), a finding consistent with the lack of overlap in the MOB. Together, these studies indicate that glomeruli in

the necklace region are molecularly diverse. Therefore, it is probably best to think of this MOB domain as containing more than one chain of necklace glomeruli.

Studies have identified other subpopulations of chemosensory neurons innervating glomeruli in the necklace region. Approximately 15 glomeruli in the necklace region are innervated by OSNs expressing the phosphodiesterase isoform Pde2A (Juilfs *et al.* 1997). In the MOE, Pde2A colocalizes with the atypical olfactory receptor guanylyl cyclase type D (GC-D) (Juilfs *et al.* 1997), indicating that this OSN type innervates at least a subset of necklace glomeruli. OSNs expressing the transient receptor potential channel Trpc2 innervate two ventral MOB glomeruli within, or adjacent to, the necklace. And neurons of the Grüneberg ganglia innervate dorsomedial necklace glomeruli (Fuss *et al.* 2005) that are closely apposed to, but distinct from, those innervated by GC-D+ OSNs (Matsuo *et al.* 2012) (**Figure 1D**). We will focus on these three sensory neuron populations for the rest of this review.

CHEMOSENSORY NEURONS INNERVATING THE NECKLACE GLOMERULI

GC-D-expressing olfactory sensory neurons

GC-D+ OSNs were first identified in the mouse by their expression of the type D isoform of the receptor guanylyl cyclase family (Fulle *et al.* 1995; Kuhn 2009). They are ciliated, bipolar neurons similar in morphology to canonical OSNs (Fulle *et al.* 1995). Surprisingly, they do not express olfactory marker protein (OMP) (Cockerham *et al.* 2009), broadly considered a conserved marker for vertebrate OSNs. GC-D+ OSNs are scattered throughout the rodent MOE but represent less than 1% of OSNs. They are produced throughout the lifespan, and terminal maturation coincides with the expression of GC-D, MS4A putative chemoreceptors (Greer *et al.* 2016), and the neuropeptide CART (cocaine and amphetamine regulated transcript) (Bloom *et al.* 2020). Most GC-D+ OSNs are found singly or in clusters within the dorsal recesses of the

ectoturbينات, but they can also be found on the endoturbينات and the septum and in the septal organ (Fulle *et al.* 1995; Hu *et al.* 2007; Ma *et al.* 2003). The axons of GC-D+ OSNs project through the cribiform plate of the skull and synapse in ~15 necklace glomeruli within the caudal MOB (Juilfs *et al.* 1997; Leinders-Zufall *et al.* 2007; Walz *et al.* 2007). While GC-D+ OSN axons have innervated the MOB by postnatal day one, their target glomeruli do not appear for a few days more (Walz *et al.* 2007).

Canonical MOB glomeruli receive homogenous input from OSNs expressing the same OR. By contrast, individual necklace glomeruli innervated by GC-D+ OSNs (GC-D+ necklace glomeruli) are also innervated by a second, noncanonical OSN subtype that is OMP+ but PDE4A- (Cockerham *et al.* 2009). The specific identity of this other OSN population remains unknown. However, the heterogeneous afferent input would seem to preclude the combinatorial coding scheme observed in the canonical MOS. GC-D+ OSN-innervated necklace glomeruli are tightly integrated into MOB circuitry, as both neuronal tracing and functional imaging studies have shown that interneurons associated with individual GC-D+ necklace glomeruli have extensive interglomerular connectivity with both other necklace and non-necklace glomeruli (Cockerham *et al.* 2009). However, the implications for the intrabulbar connections for odor processing by GC-D+ necklace glomeruli remain unclear.

Canonical OSNs express proteins associated with a cAMP-mediated olfactory transduction cascade, including the G protein subunit $G\alpha_{olf}$, type III adenylyl cyclase (ACIII), the phosphodiesterase isoforms PDE1C or PDE4A, and the cyclic nucleotide-gated (CNG) channel subunits Cnga2, Cnga3 or Cngab1b (reviewed in (Munger *et al.* 2009a)). Indeed, while Cnga2 null mice show a loss of afferent activity to canonical glomeruli, it is retained to Pde2A+ glomeruli (Baker *et al.* 1999). Instead, GC-D+ OSNs express proteins implicated in cGMP-mediated signaling, including the cGMP-stimulated phosphodiesterase Pde2A, the Ca^{2+} -calmodulin stimulated PDE isoform PDE1A and the cGMP-specific CNG channel subunits

Cnga3 and Cngb3, in addition to GC-D itself (Bloom *et al.* 2020; Fulle *et al.* 1995; Hu *et al.* 2007; Huang *et al.* 2018; Juilfs *et al.* 1997). Treatment with the cyclic nucleotide analog 8-bromo-cGMP increases action potential firing in GC-D+ OSNs (Leinders-Zufall *et al.* 2007), while pharmacologic inhibition or genetic deletion of Cnga3 renders the cells incapable of activation by chemosensory stimuli (Leinders-Zufall *et al.* 2007; Munger *et al.* 2010).

GC-D+ OSNs respond to a limited repertoire of diverse chemosensory stimuli. Two guanylin family peptides, guanylin and uroguanylin, are highly potent and specific stimuli for these cells. These peptides are also excreted in urine and feces, two rich sources of semiochemicals for rodents, and are thus available as chemosignals in natural environments. Guanylin and uroguanylin activate the MOE of wildtype mice at $K_{1/2}$ values as low as 66 pM, but responses are completely absent in the MOE of either *Gucy2d* or *Cnga3* null mice (Leinders-Zufall *et al.* 2007). However, individual GC-D+ OSNs do not show identical response profiles to the guanylin peptides: approximately half of these cells respond to either uroguanylin or guanylin alone, while the remaining GC-D+ OSNs respond to both peptides (Leinders-Zufall *et al.* 2007). The basis of these functional differences is not known. It is also unclear if these functionally disparate GC-D+ OSN subpopulations project to different subsets of necklace glomeruli. GC-D+ OSNs also respond to carbon disulfide (CS₂) (Munger *et al.* 2010), a volatile semiochemical that is found in mammalian breath (Galef *et al.* 1988). CS₂ activation of the MOE at submicromolar concentrations is dependent on GC-D and Cnga3, and identified neurons in *Gucy2d* null mice are unresponsive to this compound (Munger *et al.* 2010). Carbon dioxide (CO₂) can also activate GC-D+ OSNs (Hu *et al.* 2007), albeit only at concentrations greater than 7 mM (Munger *et al.* 2010).

The GC-D protein itself is likely the principal chemoreceptor in these cells. Many members of this protein family function as peptide receptors, and the closely related mammalian GC-C is also activated by guanylin and uroguanylin (Kuhn 2009). One *in vitro* study directly

supports the model that GC-D is similarly a receptor for uroguanylin (Duda and Sharma 2008). However, more systematic studies were initially hampered by a surprising characteristic of GC-D: the enzymatic activity of this protein is activated by intracellular bicarbonate (Guo *et al.* 2009; Sun *et al.* 2009), and thus at maximal activity when expressed in heterologous cells cultured in elevated CO₂. Interestingly, this bicarbonate sensitivity is likely the mechanism by which both CS₂ and CO₂ can activate GC-D+ OSNs. Indeed, inhibition of carbonic anhydrase 2 (Car2) expressed in GC-D+ OSNs nearly abolishes CS₂-dependent responses in these cells, and similar results are seen in Car2-deficient mice (Munger *et al.* 2010). While Car2-deficient mice also fail to avoid elevated environmental CO₂ (Hu *et al.* 2007), the real-world relevance of this GC-D+ OSN-mediated CO₂ avoidance is unclear given the high CO₂ concentrations needed to activate these cells.

Members of another protein family have been proposed to play a role as chemosensory receptors in GC-D+ OSNs, the membrane-spanning 4A (MS4A) proteins (Greer *et al.* 2016). MS4A proteins are four-pass transmembrane proteins, many of which have are expressed in leukocytes where they play a role in cell activation, growth and development (Eon Kuek *et al.* 2016). In the MOE, MS4A proteins are expressed in the distal dendrites of GC-D+ OSNs, though whether they are localized to dendritic cilia (the principal site of olfactory transduction) is less clear. In both *in vitro* and *ex vivo* experiments, MS4As confer responses to ethologically relevant ligands including pheromones and fatty acids with half maximal effective concentrations (EC50) values in the micromolar range, show no functional implications of these MS4A proteins as receptors and fail to show ciliary localization of the MS4A proteins. Future work should clarify the roles of MS4As in GC-D+ OSNs, including whether there is intracellular crosstalk with the GC-D-mediated sensory transduction cascade.

GC-D+ OSNs play a central role a type of odor-driven associative learning known as the social transmission of food preference (STFP) (Munger *et al.* 2010). First described decades

ago (Galef 1985; Galef and Wigmore 1983; Posadas-Andrews and Roper 1983), STFPs occur when a recipient rodent (the observer) simultaneously encounters a general odor – such as a food odor – and a relevant semiochemical – such as CS₂ (Galef *et al.* 1988) – from a conspecific (the demonstrator). When the observer rodent is presented with a choice of two otherwise identical foods that contain either the demonstrated odor or a novel odor, it will display a strong preference for food containing the demonstrated odor. GC-D+ OSNs are required for STFP acquisition: *Gucy2d* and *Cnga3* null mice, as well as mice receiving an intranasal injection of a carbonic anhydrase inhibitor, fail to acquire an STFP from either a live demonstrator or from a surrogate demonstrator presenting the general odor plus a GC-D+ OSN stimulus (CS₂, guanylin or uroguanylin) (Arakawa *et al.* 2013; Kelliher and Munger 2015; Munger *et al.* 2010). This preference acquisition does not require either a food choice or consummatory behavior, as mice will exhibit a GC-D-dependent odor preference when undergoing the same conditioning paradigms (Zimmerman *et al.* 2020). These GC-D-dependent odor preferences can be acquired by neonates and are maintained for weeks (Zimmerman *et al.* 2020). GC-D-dependent preferences can even be acquired for odors to which mice are made conditionally averse, suggesting that GC-D+ OSNs have a privileged role in odor learning (Zimmerman *et al.* 2020).

Trpc2-expressing olfactory sensory neurons

Although broadly expressed in chemosensory neurons of the vomeronasal organ, transient receptor potential channel 2 (Trpc2) is also found in a small number of OSNs (Trpc2+ OSNs) of the MOE (Liman *et al.* 1999). Trpc2+ OSNs are ciliated neurons that express the ion channel subunit Cnga2 and the G protein subunit Gα_o (Choi *et al.* 2016; Omura and Mombaerts 2014). The Trpc2+ OSNs can be further divided into two subtypes: Type A, which expresses ACIII, and Type B, which does not. Approximately one-third of Trpc2+ Type A cells coexpress

canonical ORs Olfr68 and Olfr69. By contrast, Trpc2⁺ Type B OSNs may rely on a cGMP signaling cascade. They express the soluble guanylyl cyclase Gucy1b2 as well as phosphodiesterases Pde1c, Pde4a and Pde6d (Omura and Mombaerts 2015; Saraiva *et al.* 2015).

A functional role for Trpc2⁺ Type A OSNs has not been described, but their expression of two ORs and the cAMP-producing enzyme ACIII suggests that they may transduce olfactory stimuli similarly to canonical OSNs. By contrast, mouse Trpc2⁺ Type B OSNs have been implicated in the detection low oxygen levels (Bleymehl *et al.* 2016). Low oxygen levels generate a rapid and non-adaptive Ca²⁺ response in Trpc2⁺ Type B, but not Type A, OSNs that is abolished in both *Gucy1b2* and *Trpc2* null mice. However, *Cnga2* null mice showed wildtype responses, indicating that Trpc2 but not Cnga2 is the critical ion channel in for low oxygen responses in Type B cells. Low oxygen also produced a Gucy1b2-dependent increase in cFos⁺ cells around glomeruli innervated by Trpc2⁺ OSNs as well as a Gucy1b2- and Trpc2-dependent behavioral aversion to low environmental oxygen.

Grueneberg ganglion neurons

The Grueneberg ganglia (GG), bilateral clusters of neurons (GGNs) at the most anterior aspect of the nasal cavity, were first described nearly 50 years ago (Gruneberg 1973). The GG were “rediscovered” in the mouse many years later when this patch of OMP⁺ neurons were recognized by several groups nearly simultaneously (Fleischer *et al.* 2006a; Fuss *et al.* 2005; Koos and Fraser 2005; Roppolo *et al.* 2006; Storan and Key 2006). A subsequent study found the GG in rats, gerbils and hamsters, as well, albeit with some morphological and physiological variations (Brecht *et al.* 2014). GGNs lie within a fibroblast meshwork between the nasal septum and a keratinized epithelium (Fleischer *et al.* 2006a; Fuss *et al.* 2005; Roppolo *et al.*

2006; Storan and Key 2006). While GGNs do not have prominent dendrites or microvilli, and they do not directly access the nasal lumen, they do possess numerous cilia that are speculated to play a role in sensory transduction (Breer *et al.* 2006). Glial cells wrap around GGNs, entrapping the cilia within the ganglion. Initially thought to be associated with the nervus terminalis (Gruneberg 1973), later studies showed that GGNs project to the caudal MOB. GGNs fasciculate into a single or a few nerve bundles that project to about 10 necklace glomeruli (Fuss *et al.* 2005; Koos and Fraser 2005; Roppolo *et al.* 2006). These glomeruli are distinct from those innervated by GC-D+ OSNs (Matsuo *et al.* 2012) (**Figure 1D**).

It is clear that GGNs are heterogeneous in their molecular signatures. One subpopulation of GGNs express the type 2 vomeronasal receptor V2r83 (Fleischer *et al.* 2006b). Another expresses members of the TAAR family, though this expression seems restricted to late embryonic and neonatal time points (Fleischer *et al.* 2007). Numerous signal transduction proteins are expressed in GGNs, including $G\alpha_o$, $G\alpha_{i2}$, $G\alpha_{olf}$ and ACIII (Fleischer *et al.* 2006b). Of particular note, many GGNs express components of a cGMP signaling cascade (Fleischer *et al.* 2009; Liu *et al.* 2009; Mamasuew *et al.* 2011; Schmid *et al.* 2010). V2r83+ GGNs coexpress transmembrane guanylyl cyclase subtype G (GC-G) (Fleischer *et al.* 2009). Pde2a and Cnga3 are also expressed in GGNs, but not GC-D or Car2 (Fleischer *et al.* 2009; Liu *et al.* 2009; Schmid *et al.* 2010). The neuropeptide CART is not expressed in GGNs (Huang *et al.* 2018).

While no peptide ligands have been identified for GC-G (unlike GC-D), this protein is responsive to intracellular bicarbonate (Chao *et al.* 2010) and to cold temperatures (Chao *et al.* 2015). This latter function is consistent with a thermosensory role for GGNs. Indeed, multiple studies find that GGNs function as cold sensors (Mamasuew *et al.* 2008; Schmid *et al.* 2010). While, the exact mechanism by which these cells transduce cold temperature is unclear, it does appear to be independent of Cnga3. Furthermore, at least one study found that thermosensory responses are more pronounced in juvenile mice (Mamasuew *et al.* 2008).

Despite the fact that GGNs are not directly exposed to the nasal lumen, there is good evidence that they also function as chemosensors, particularly for different types of semiochemicals. Alarm pheromones – a poorly defined chemosignal that is produced when animals are stressed or injured – will induce responses in GGNs (Brechtbühl *et al.* 2008). Additionally, GGN axotomy will abolish alarm pheromone-dependent freezing behaviors (Kikusui *et al.* 2001). A number of monomolecular putative semiochemicals have also been linked to activation of the GG. These include the predator odorant 2-propyl thietane (2-PT) (Brechtbühl *et al.* 2013; Perez-Gomez *et al.* 2015), some pyridine analogs (Brechtbühl *et al.* 2015), 2-sec-butyl-4,5-dihydrothiazole (SBT) (Brechtbühl *et al.* 2013) and 2,3-dimethylpyrazine (Hanke *et al.* 2013; Mamasuew *et al.* 2011). Together with the responses to cold temperatures, these findings suggest that the GG is important for avoiding potential dangers. Intriguingly, a recent study indicates that activation of the GG by predator odors in the context of food choices can impact those choices and even modify GC-D-dependent preferences (Brechtbühl *et al.* 2020).

TARGETS OF THE NECKLACE GLOMERULI

Compared to the afferent inputs to the necklace glomeruli, relatively little is known about their efferent targets in other areas of the brain. Activation of either GC-D+ OSNs or GGNs during behavioral exposure to their cognate stimuli leads to activation of subsets of necklace glomeruli, as assessed by an increase in nearby cFos-immunopositive juxtaglomerular cells (Perez-Gomez *et al.* 2015; Zimmerman and Munger unpublished). The ventral subiculum of the hippocampus is activated during acquisition of an STFP (Munger *et al.* 2010; Ross and Eichenbaum 2006), but the (likely) multisynaptic connections linking this region to the necklace glomeruli is unknown. Unpublished neuronal tracing studies from our group indicate that projection neurons associated with GC-D+ necklace glomeruli send axons to regions of the

olfactory peduncle including the anterior olfactory nucleus (AON) and tenia tecta. Two new lines of evidence support a role for the AON in STFP acquisition. Disruption of AON projections to the MOB impair the acquisition, but not the recall, of an STFP (Wang *et al.* 2020). Consistent with this, we have found that certain AON subdivisions show a GC-D-dependent increase in cFos+ cells after the acquisition phase of an STFP. Thus, it appears that the AON is a prime candidate for a key role in this odor-mediated social learning.

CONCLUSIONS

While the necklace glomeruli, their afferent inputs and their efferent targets remain poorly understood – at least compared to the components of the canonical main olfactory system – progress is being made. Critically, powerful molecular genetic tools, specific chemostimuli and distinct odor-mediated behaviors have been developed or identified for each of the three subsystems most clearly associated with the necklace region. It is now important to use these tools to understand how the olfactory information carried by each of these subsystems is processed in the brain. Of particular interest will be efforts to illuminate the neural mechanisms mediating interactions between these and with other subsystems, interactions that are critical if animals are to form a complete picture of, and develop appropriate behavioral responses to, a complex chemosensory world.

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ETHICS STATEMENT

No human or animal studies were conducted for this paper.

Figure 1: (A) Schematic illustrating the mouse olfactory system. VNO, vomeronasal organ (apical region, light green; basal region, dark green); GG, Grueneberg ganglion (red); MOE, main olfactory epithelium (yellow); MOB, main olfactory bulb (yellow); NG, necklace glomeruli (blue circles); AOB, accessory olfactory bulb (anterior region, light green; posterior region, dark green). Blowouts show confocal micrographs of (top) GC-D+ OSNs in a dorsal recess of the MOE, and (bottom) GG neurons (GGNs) within the GG. Modified from (Munger *et al.* 2010), with permission. (B) Whole mount mouse olfactory bulbs (dorsal view; rostral towards top) showing how the glomerular targets of OSNs expressing different classes of olfactory receptor – including canonical odorant receptors (ORs: Class I member, yellow; Class II member, red) or trace amine-associated receptors (TAARs; cyan) – are segregated in the MOB. OSNs were labeled with different genetically encoded fluorescent reporters. Modified from (Pacifico *et al.* 2012), with permission. (C) Necklace glomeruli innervated by axons of GC-D+ olfactory sensory neurons (green; arrows for examples of the necklace glomeruli). Lateral view of the olfactory bulb and other forebrain areas of a *Gucy2d*-EGFP reporter mouse, including the anterior olfactory nucleus (AON) and lateral olfactory tract (LOT). R, rostral; C, caudal; D, dorsal; V, ventral. Modified from (Uytingco *et al.* 2016), with permission. (D) Confocal micrograph of necklace glomeruli innervated by GC-D+ OSNs (red) or GGNs (green). Modified from (Matsuo *et al.* 2012), with permission.

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