--- Chapter 1 ---

Introduction

Overview of olfaction

A sauté of garlic and onions in butter, the finishing of chocolate-chip cookies in the oven, a lover's perfume, the wretchedness of an urban public bathroom: these are examples of the powerful ways our sense of smell (olfaction) elaborates our experiences. Terrestrial mammals possess an olfactory sense that can detect theoretically between ~10,000 and ~10,000,000 airborne odors at nanomolar concentration. The detection of airborne odors begins in the posterior region of the nasal cavity. The main olfactory epithelium (MOE) houses several million olfactory sensory neurons (OSNs) that protrude into the airspace. The inhalation of odors, which are typically hydrophobic organic molecules, and their dissolution into a superficial mucus allow the odors to make contact with OSNs. The OSNs express olfactory receptor (OR) proteins (Buck and Axel 1991) that bind to the odors and begin a signal transduction process that culminates in membrane depolarization and the propagation of action potentials along axons towards the olfactory bulb (OB). Axons of OSNs converge into discrete units called glomeruli in the superficial layer of the OB (Firestein 2001). Olfactory information is received by mitral cells that synapse with the glomeruli in the OB; the information is subsequently passed to the anterior olfactory nucleus, amygdala, and piriform cortex in the deeper parts of the brain (Miyamichi et al. 2011).

The discriminatory power of the olfactory sense is related to the number of functional OR genes collectively expressed in OSNs. Rodents, which rely on olfaction for survival (Brunet et al. 1996), have ~1,000 distinct intronless OR genes. Humans possess ~200-400 functional OR genes, but have many more OR pseudogenes. In contrast, nonmammalian vertebrates have only ~1/10 the number of OR genes found in mammals (Mombaerts 1999). The platypus, one of the earliest-evolved mammals (Venditti et al. 2011), has ~300 functional OR genes and ~400 additional OR pseudogenes (Warren et al. 2008). Thus, the large OR repertoire appears to be a uniquely mammalian property. A single OSN is thought to express

a single OR gene, and the axons of OSNs expressing the same OR gene contribute to a singular set of 2 ipsilateral glomeruli (Mombaerts 2004). Hence, one can determine which ORs are activated by determining which neurons or glomeruli are activated. Individual ORs are tuned to detect several odors, and an individual odor is recognized by several ORs (Malnic et al. 1999). The combinatorial code of odor detection means that individual odors produce "maps" of activated glomeruli in the OB (Rubin and Katz 1999; Wachowiak and Cohen 2001).

The rodent OB is composed of different domains that receive and process input from specialized sets of primary olfactory neurons located in anatomically distinct regions of the nasal cavity. The largest domain is the main OB (MOB), which handles input from the aforementioned OSNs in the MOE. A subset of the glomeruli in the MOB is additionally targeted by sensory neurons whose somata reside in the septal organ of Masera (Ma 2011). In contrast, the accessory OB (AOB) is innervated by axons from vomeronasal sensory neurons (VSNs), located in the vomeronasal (Jacobson's) organ (VNO) anterior of the MOE. VSNs express G-protein coupled receptors known as pheromone receptors (VRs) (Dulac and Axel 1995) and are involved in the detection of pheromones, cues that induce innate both behavioral responses such as freezing, aggression, flight, feeding, and copulation, and physiological responses such as the accleration of reproductive development (Brennan and Zufall 2006). VSNs respond to conspecific cues such as male bedding, as well as heterospecific cues such as snake bedding (Isogai et al. 2011). Removal of VSNs in mice results in the loss of gender identity (Kimchi et al. 2007; Stowers et al. 2002) and a deficit in courtship ultrasonic vocalizations (Wysocki et al. 1982).

Though VSNs and OSNs are broadly similar in that they reside in the epithelia in the nasal cavity, protrude into the nasal airspace, and relay information to glomeruli in the OB, they possess different olfactory signal transduction mechanisms. In the protruding cilia of OSNs, odor binding by the OR activates G_{olf} protein and the adenylate cyclase ACIII, resulting in the production of cAMP (Firestein 2001). The increase in [cAMP] opens a heterotetrameric cAMP-gated cationic channel composed of 2 CNGA2, 1 CNGA4, and 1 CNGB1b subunits (Biel and Michalakis 2007; Frings et al. 1992). The influx of ions, many of which are Ca^{2+}, not only depolarizes the neuronal membrane but also activates a Ca^{2+}-

activated Cl^- channel, whose activity amplifies the underlying depolarization due to the abnormally high intracellular [Cl^-] in OSNs (Kaneko et al. 2001; Lowe and Gold 1993). The depolarization increases the frequency of spontaneous action potentials. In the protruding microvilli of VSNs, pheromone binding to the VR activates G_i/G_o proteins. The intermediate steps of signal transduction seem to involve the subsequent activation of phospholipase C and its hydrolysis of PIP_2 into IP_3 and diaglycerol (DAG) (Zufall and Munger 2001). The electrical transduction process is initiated with the opening of the DAG-gated TRPC2 cationic channel, which depolarizes the neuronal membrane (Lucas et al. 2003). VSNs respond to nanomolar concentrations of pheromones with increases in spontaneous action potential frequency (Holy et al. 2000; Leinders-Zufall et al. 2000).

Necklace glomeruli

In a specialized region of the caudal superficial rodent OB, between the glomerular fields of the MOB and AOB, there are a set of 20-50 "atypical" glomeruli that can be distinguished by their labeling with acetylcholinesterase (AChE) histochemistry (Zheng et al. 1987). These atypical glomeruli include the modified glomerular complex in the dorsomedial OB that is active in newborn mice, suggesting a role in suckling (Greer et al. 1982). Using an aromatase antibody (later found to bind to huntingtin associated protein (Fujinaga et al. 2007)), Shinoda and colleagues demonstrated that the AChE+ atypical glomeruli form a domain in which each glomerulus resembles a bead on a necklace; thus, the atypical glomeruli are also collectively referred to as the "necklace domain" of the OB. The aromatase antibody also identified a sparsely distributed set of neurons embedded with OSNs in the caudal MOE that potentially innervated the necklace glomeruli (Shinoda et al. 1993; Shinoda et al. 1989).

These neurons, interspersed throughout the caudal MOE and having a similar morphology as OSNs, were found to express key components of a cGMP signal transduction cascade. Thus, the necklace glomeruli differ from the glomeruli of the MOB and AOB in that they represent a zone of primary cGMP transduction. These neurons express a guanylate cyclase, pGC-D, a single-pass transmembrane receptor that catalyzes the production of cGMP when stimulated (Fulle et al. 1995; Juilfs et al. 1997). The

necklace olfactory system is thereby also called the GC-D system. GC-D neurons express a cGMP-stimulated phosphodiesterase (PDE2A) and a cGMP-gated heterotetrameric cationic channel composed of 2 CNGA3 and 2 CNGB3 subunits (Meyer et al. 2000). In addition, GC-D neurons express high levels of the carbonic anhydrase CAII. Functional studies have demonstrated that GC-D neurons respond to CO_2 (Hu et al. 2007), bicarbonate (Guo et al. 2009; Sun et al. 2009), and uroguanylin (Duda and Sharma 2008; Leinders-Zufall et al. 2007) with elevations in cytosolic [Ca^{2+}] and increased spontaneous firing frequency.

Grueneberg ganglion

In 1973, Hans Grüneberg reported the startling identification of clusters of neuronal cell bodies in the most anterior region of the mouse nasal cavity, where the nasal septum is cartilaginous. Possessing large spherical nuclei, these neurons were readily discerned in hematoxylin-and-eosin-treated sections of the nasal vestibule. The neuronal clusters were sparse, appearing intermittantly in transverse serial sections spanning an anterior-posterior distance of 250 μm in the nose of the newborn mouse. Because the neuronal cell bodies were more anterior than other cranial nerves and could be distinguished from the surrounding fibers of the anterior ethmoidal nerve, Grüneberg concluded that these ganglionic cell bodies belonged to the enigmatic cranial nerve zero, *nervus terminalis*, believed to have a chemosensory function but separate from the olfactory system (Bojsen-Moller 1975; Wirsig and Leonard 1986). In the other examined mammalian samples of the adult rat, adult hamster, fetal cat, fetal anteater, and 5-month-old human embryo, Grüneberg's neurons exhibited a similar clustered morphology and likewise bilaterally occupied the dorsal septal corners of the nasal vestibule (Grüneberg 1973).

Tachibana's ultrastructural study in 1990 reported ciliated cell bodies with a ganglionic arrangement in the anterior nasal cavity of the musk shrew (Tachibana et al. 1990). However, over 30 years elapsed between Grüneberg's study and the next published report that directly addressed the so-called Grueneberg ganglion (GG) in the mouse. In 2005, a series of 5 papers, beginning with the study of Koos and Fraser, independently reclassified the mouse GG as part of the primary olfactory system

(Fleischer et al. 2006a; Fuss et al. 2005; Koos and Fraser 2005; Roppolo et al. 2006; Storan and Key 2006). This conclusion was based on 2 pieces of evidence: (1) the expression in the GG of olfactory marker protein (OMP), a protein found in all mature primary olfactory neurons, including OSNs and VSNs; and (2) the final destination of GG axons in the posterior (caudal) region of the OB. With regard to the former, GG neurons exhibit a strong green fluorescence in transgenic mice expressing green fluorescent protein (GFP) from the *Omp* locus. With regard to the latter, GG axons form 8-12 glomeruli that resemble pearls on a necklace, near the previously described GC-D necklace domain in the OB. However, the GG necklace glomeruli appear to be distinct from the classic GC-D necklace (Cockerham et al. 2009); we have termed the domain formed by GG glomeruli to be the "necklace-like" domain.

Besides their far-forward location, GG neurons are anatomically distinct in several facets from OSNs and VSNs. Present at embryonic day 16 (Fuss et al. 2005; Roppolo et al. 2006), the GG organ forms a discontinuous arrowhead shape, in contrast to the continuous pseudostratified epithelial sheets found in the MOE and VNO. GG neurons, numbering 300-600 per side, are tightly packed in clusters and are ensheathed by glial cells (Grüneberg 1973). No consistent protrusions have been found to extend from subsurface GG somata through a thin keratinized epithelium into the nasal cavity (Roppolo et al. 2006). This differs from bipolar OSNs and VSNs, which each project a dendritic process that terminates as a ciliated (in OSNs) or microvillar (in VSNs) knob, at which ORs and VRs are localized, in the nasal cavity. Each GG neuron has a single axonal projection. These projections collect into a single fascicle that extends caudally and crosses the bony cribriform plate on the medial sides of the olfactory bulbs (Koos and Fraser 2005).

GG neurons are likely to have a chemosensory function. The report of GG responses to alarm pheromones by Brechbuhl and colleagues marked a major advance in the functional characterization of the neurons (Brechbuhl et al. 2008). It was demonstrated that GG neurons exhibit increases in cytosolic $[Ca^{2+}]$ when exposed to saline droplets collected from cages of recently asphyxiated mice. Curiously, the cytosolic $[Ca^{2+}]$ rise did not require extracellular Ca^{2+}. The $[Ca^{2+}]$ increase was not observed with exposures to CO_2-infused, cold, or acidified saline. No responses were observed with exposures to a

nipple wash or urine of conspecific animals. As the water-soluble characteristic of the stimulus agreed with previously described properties of mammalian alarm pheromones (Kiyokawa et al. 2005), whose exact chemical identities have not been elucidated, and the responses were observed in GG neurons in acute slice preparations of the mouse nasal vestibule, it was claimed that the GG directly detects and mediates behavioral responses to alarm pheromones. In support of this claim, surgical ablations of the GG resulted in the disappearance of animal freezing-immobility responses in behavioral assays that exposed mice to the putative alarm pheromone (Brechbuhl et al. 2008).

A nearly simultaneous report by Mamasuew and colleagues proposed a second GG stimulus modality (Mamasuew et al. 2008). It was found that a modest lowering of ambient temperature (e.g., from 25˚C to 15˚C) induced the new production of c-Fos mRNA, a marker of neuronal activity, in the GG. Neonatal mice displayed the greatest sensitivity to cold, as visible c-Fos transcription in the GG of these mice could be observed with the lowering of ambient temperature to 22˚C. In adult mice, no c-Fos transcription in the GG was evident when the ambient temperature was lowered to 15˚C. In a later study, Mamasuew and colleagues demonstrated that coolness-induced GG c-Fos transcription depended on a cyclic nucleotide-gated cationic channel subunit, CNGA3, that is primarily found in cone photoreceptor cells in the eye but is also expressed in the GG (Mamasuew et al. 2010). Schmid and colleagues supported and extended the *in vivo* work by showing *in vitro* TTX-dependent increases in cytosolic [Ca^{2+}] could be induced by exposing acute slices of the GG to cold saline (Schmid et al. 2010), with an ET_{50} of ~16˚C. Curiously, *in vitro* the GG responses to cold were unaffected by the deletion of *Cnga3*.

Outline of chapters

Due to their unusual far-anterior location, their presence at birth and throughout life, and their apparent exclusiveness to mammals, GG neurons are interesting objects of study for researchers of various specializations. The following chapters describe original contributions to the molecular, electrophysiological, and functional characterization of mouse GG neurons.

Chapter 2 reports the identification of proteins and small molecules involved in a potential odor-evoked transduction pathway in mouse GG neurons. In contrast to OSNs, GG neurons do not express ORs and lack the transduction machinery of OSNs and VSNs (Roppolo et al. 2006). GG neurons express one type of pheromone receptor, V2r83, and several trace-amine associated receptors (TAARs) that have olfactory functions, but these expression patterns are either restricted to animals younger than p21 (TAARs) or have unknown functional significance (V2r83 and TAARs) (Fleischer et al. 2006b; Fleischer et al. 2007). Chapter 2 demonstrates ciliary localization of a membrane-bound receptor guanylate cyclase and a cGMP-gated cationic channel subunit in GG neurons. These results were independently obtained and in general agreement with those simultaneously reported by Fleischer and colleagues (Fleischer et al. 2009). The molecular similarities between the GG and GC-D neurons are discussed.

Chapter 3 describes the electrophysiological characterization of voltage-dependent ionic conductances in mouse GG neurons. Similar to OSNs (Lynch and Barry 1991; Ma et al. 1999; Vargas and Lucero 1999; Weiss et al. 2011) and VSNs (Dibattista et al. 2008; Liman and Corey 1996; Ukhanov et al. 2007), GG neurons exhibit two fast-inactivating Na^+ conductances, a delayed-rectifier K^+ conductance, a non-inactivating Ca^{2+} conductance, and hyperpolarization-activated "funny" currents. This chapter focuses on the unique relationship between the two Na^+ conductances and presents a Hodgkin-Huxley computer model that predicts how the Na^+ conductances may contribute to the heterogeneity of spontaneous firing patterns in GG neurons.

Electrophysiological studies of chemosensory function in the mouse GG are presented in Chapter 4. These experiments were performed on the acute slice preparation of the mouse nasal vestibule, presented in Chapter 3, and report on the odorant modulation of spontaneous firing. Chapter 4 supports and extends previous *in vivo* work that demonstrated the CNGA3-dependent responsiveness of GG neurons to certain isoforms of dimethylpyrazine (Mamasuew et al. 2011a; Mamasuew et al. 2011b), a pheromone capable of altering reproductive cycles in mice (Jemiolo and Novotny 1994; Ma et al. 1998). The work presented here also shows excitatory responses to cGMP and inhibitory responses to general

lipophilic molecules in individual GG neurons. Moreover, it is suggested that serum and cortisol serve as biologically relevant GG stimuli.

Chapter 5 reports immunohistochemical experiments showing that specific behavioral manipulations induce the nuclear accumulation of c-Fos protein, a marker for neuronal activity, in GG neurons. These manipulations involve as their common element the separation of unweaned mice from their parents. Maternal separation is a highly anxiogenic stimulus, leading to an increase in pup ultrasonic vocalizations (Hofer and Shair 1978). This chapter discusses the potential relationship between this new work and the existing literature on the GG detection of alarm pheromones and cold.

A prevailing theme of this work centers on the mechanisms of information processing by GG neurons. These mechanisms are partially revealed, from the subcellular level to the organ level. At the end of the thesis, directions for future work are discussed.

REFERENCES

Biel M, and Michalakis S. Function and dysfunction of CNG channels: insights from channelopathies and mouse models. *Mol Neurobiol* 35: 266-277, 2007.

Bojsen-Moller F. Demonstration of terminalis, olfactory, trigeminal and perivascular nerves in the rat nasal septum. *J Comp Neurol* 159: 245-256, 1975.

Brechbuhl J, Klaey M, and Broillet MC. Grueneberg ganglion cells mediate alarm pheromone detection in mice. *Science* 321: 1092-1095, 2008.

Brennan PA, and Zufall F. Pheromonal communication in vertebrates. *Nature* 444: 308-315, 2006.

Brunet LJ, Gold GH, and Ngai J. General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron* 17: 681-693, 1996.

Buck L, and Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65: 175-187, 1991.

Cockerham RE, Puche AC, and Munger SD. Heterogeneous sensory innervation and extensive intrabulbar connections of olfactory necklace glomeruli. *PLoS One* 4: e4657, 2009.

Dibattista M, Mazzatenta A, Grassi F, Tirindelli R, and Menini A. Hyperpolarization-activated cyclic nucleotide-gated channels in mouse vomeronasal sensory neurons. *J Neurophysiol* 100: 576-586, 2008.

Duda T, and Sharma RK. ONE-GC membrane guanylate cyclase, a trimodal odorant signal transducer. *Biochem Biophys Res Commun* 367: 440-445, 2008.

Dulac C, and Axel R. A novel family of genes encoding putative pheromone receptors in mammals. *Cell* 83: 195-206, 1995.

Firestein S. How the olfactory system makes sense of scents. *Nature* 413: 211-218, 2001.

Fleischer J, Hass N, Schwarzenbacher K, Besser S, and Breer H. A novel population of neuronal cells expressing the olfactory marker protein (OMP) in the anterior/dorsal region of the nasal cavity. *Histochem Cell Biol* 125: 337-349, 2006a.

Fleischer J, Mamasuew K, and Breer H. Expression of cGMP signaling elements in the Grueneberg ganglion. *Histochem Cell Biol* 131: 75-88, 2009.

Fleischer J, Schwarzenbacher K, Besser S, Hass N, and Breer H. Olfactory receptors and signalling elements in the Grueneberg ganglion. *J Neurochem* 98: 543-554, 2006b.

Fleischer J, Schwarzenbacher K, and Breer H. Expression of trace amine-associated receptors in the Grueneberg ganglion. *Chem Senses* 32: 623-631, 2007.

Frings S, Lynch JW, and Lindemann B. Properties of cyclic nucleotide-gated channels mediating olfactory transduction. Activation, selectivity, and blockage. *J Gen Physiol* 100: 45-67, 1992.

Fujinaga R, Yanai A, Nakatsuka H, Yoshida K, Takeshita Y, Uozumi K, Zhao C, Hirata K, Kokubu K, Nagano M, and Shinoda K. Anti-human placental antigen complex X-P2 (hPAX-P2) anti-serum recognizes C-terminus of huntingtin-associated protein 1A common to 1B as a determinant marker for the stigmoid body. *Histochem Cell Biol* 128: 335-348, 2007.

Fulle HJ, Vassar R, Foster DC, Yang RB, Axel R, and Garbers DL. A receptor guanylyl cyclase expressed specifically in olfactory sensory neurons. *Proc Natl Acad Sci U S A* 92: 3571-3575, 1995.

Fuss SH, Omura M, and Mombaerts P. The Grueneberg ganglion of the mouse projects axons to glomeruli in the olfactory bulb. *Eur J Neurosci* 22: 2649-2654, 2005.

Greer CA, Stewart WB, Teicher MH, and Shepherd GM. Functional development of the olfactory bulb and a unique glomerular complex in the neonatal rat. *J Neurosci* 2: 1744-1759, 1982.

Grüneberg H. A ganglion probably belonging to the N. terminalis system in the nasal mucosa of the mouse. *Z Anat Entwicklungsgesch* 140: 39-52, 1973.

Guo D, Zhang JJ, and Huang XY. Stimulation of guanylyl cyclase-D by bicarbonate. *Biochemistry* 48: 4417-4422, 2009.

Hofer MA, and Shair H. Ultrasonic vocalization during social interaction and isolation in 2-weeek-old rats. *Dev Psychobiol* 11: 495-504, 1978.

Holy TE, Dulac C, and Meister M. Responses of vomeronasal neurons to natural stimuli. *Science* 289: 1569-1572, 2000.

Hu J, Zhong C, Ding C, Chi Q, Walz A, Mombaerts P, Matsunami H, and Luo M. Detection of near-atmospheric concentrations of CO2 by an olfactory subsystem in the mouse. *Science* 317: 953-957, 2007.

Isogai Y, Si S, Pont-Lezica L, Tan T, Kapoor V, Murthy VN, and Dulac C. Molecular organization of vomeronasal chemoreception. *Nature* 478: 241-245, 2011.

Jemiolo B, and Novotny M. Inhibition of sexual maturation in juvenile female and male mice by a chemosignal of female origin. *Physiol Behav* 55: 519-522, 1994.

Juilfs DM, Fulle HJ, Zhao AZ, Houslay MD, Garbers DL, and Beavo JA. A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. *Proc Natl Acad Sci U S A* 94: 3388-3395, 1997.

Kaneko H, Nakamura T, and Lindemann B. Noninvasive measurement of chloride concentration in rat olfactory receptor cells with use of a fluorescent dye. *Am J Physiol Cell Physiol* 280: C1387-1393, 2001.

Kimchi T, Xu J, and Dulac C. A functional circuit underlying male sexual behaviour in the female mouse brain. *Nature* 448: 1009-1014, 2007.

Kiyokawa Y, Kikusui T, Takeuchi Y, and Mori Y. Alarm pheromone that aggravates stress-induced hyperthermia is soluble in water. *Chem Senses* 30: 513-519, 2005.

Koos DS, and Fraser SE. The Grueneberg ganglion projects to the olfactory bulb. *Neuroreport* 16: 1929-1932, 2005.

Leinders-Zufall T, Cockerham RE, Michalakis S, Biel M, Garbers DL, Reed RR, Zufall F, and Munger SD. Contribution of the receptor guanylyl cyclase GC-D to chemosensory function in the olfactory epithelium. *Proc Natl Acad Sci U S A* 104: 14507-14512, 2007.

Leinders-Zufall T, Lane AP, Puche AC, Ma W, Novotny MV, Shipley MT, and Zufall F. Ultrasensitive pheromone detection by mammalian vomeronasal neurons. *Nature* 405: 792-796, 2000.

Liman ER, and Corey DP. Electrophysiological characterization of chemosensory neurons from the mouse vomeronasal organ. *J Neurosci* 16: 4625-4637, 1996.

Lowe G, and Gold GH. Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells. *Nature* 366: 283-286, 1993.

Lucas P, Ukhanov K, Leinders-Zufall T, and Zufall F. A diacylglycerol-gated cation channel in vomeronasal neuron dendrites is impaired in TRPC2 mutant mice: mechanism of pheromone transduction. *Neuron* 40: 551-561, 2003.

Lynch JW, and Barry PH. Inward rectification in rat olfactory receptor neurons. *Proc Biol Sci* 243: 149-153, 1991.

Ma M. Multiple Olfactory Subsystems Convey Various Sensory Signals. 2011.

Ma M, Chen WR, and Shepherd GM. Electrophysiological characterization of rat and mouse olfactory receptor neurons from an intact epithelial preparation. *J Neurosci Methods* 92: 31-40, 1999.

Ma W, Miao Z, and Novotny MV. Role of the adrenal gland and adrenal-mediated chemosignals in suppression of estrus in the house mouse: the lee-boot effect revisited. *Biol Reprod* 59: 1317-1320, 1998.

Malnic B, Hirono J, Sato T, and Buck LB. Combinatorial receptor codes for odors. *Cell* 96: 713-723, 1999.

Mamasuew K, Breer H, and Fleischer J. Grueneberg ganglion neurons respond to cool ambient temperatures. *Eur J Neurosci* 28: 1775-1785, 2008.

Mamasuew K, Hofmann N, Breer H, and Fleischer J. Grueneberg ganglion neurons are activated by a defined set of odorants. *Chem Senses* 36: 271-282, 2011a.

Mamasuew K, Hofmann N, Kretzschmann V, Biel M, Yang RB, Breer H, and Fleischer J. Chemo- and thermosensory responsiveness of Grueneberg ganglion neurons relies on cyclic guanosine monophosphate signaling elements. *Neurosignals* 19: 198-209, 2011b.

Mamasuew K, Michalakis S, Breer H, Biel M, and Fleischer J. The cyclic nucleotide-gated ion channel CNGA3 contributes to coolness-induced responses of Grueneberg ganglion neurons. *Cell Mol Life Sci* 67: 1859-1869, 2010.

Meyer MR, Angele A, Kremmer E, Kaupp UB, and Muller F. A cGMP-signaling pathway in a subset of olfactory sensory neurons. *Proc Natl Acad Sci U S A* 97: 10595-10600, 2000.

Miyamichi K, Amat F, Moussavi F, Wang C, Wickersham I, Wall NR, Taniguchi H, Tasic B, Huang ZJ, He Z, Callaway EM, Horowitz MA, and Luo L. Cortical representations of olfactory input by trans-synaptic tracing. *Nature* 472: 191-196, 2011.

Mombaerts P. Molecular biology of odorant receptors in vertebrates. *Annu Rev Neurosci* 22: 487-509, 1999.

Mombaerts P. Odorant receptor gene choice in olfactory sensory neurons: the one receptor-one neuron hypothesis revisited. *Curr Opin Neurobiol* 14: 31-36, 2004.

Roppolo D, Ribaud V, Jungo VP, Luscher C, and Rodriguez I. Projection of the Gruneberg ganglion to the mouse olfactory bulb. *Eur J Neurosci* 23: 2887-2894, 2006.

Rubin BD, and Katz LC. Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* 23: 499-511, 1999.

Schmid A, Pyrski M, Biel M, Leinders-Zufall T, and Zufall F. Grueneberg ganglion neurons are finely tuned cold sensors. *J Neurosci* 30: 7563-7568, 2010.

Shinoda K, Ohtsuki T, Nagano M, and Okumura T. A possible functional necklace formed by placental antigen X-P2-immunoreactive and intensely acetylcholinesterase-reactive (PAX/IAE) glomerular complexes in the rat olfactory bulb. *Brain Res* 618: 160-166, 1993.

Shinoda K, Shiotani Y, and Osawa Y. "Necklace olfactory glomeruli" form unique components of the rat primary olfactory system. *J Comp Neurol* 284: 362-373, 1989.

Storan MJ, and Key B. Septal organ of Gruneberg is part of the olfactory system. *J Comp Neurol* 494: 834-844, 2006.

Stowers L, Holy TE, Meister M, Dulac C, and Koentges G. Loss of sex discrimination and male-male aggression in mice deficient for TRP2. *Science* 295: 1493-1500, 2002.

Sun L, Wang H, Hu J, Han J, Matsunami H, and Luo M. Guanylyl cyclase-D in the olfactory CO2 neurons is activated by bicarbonate. *Proc Natl Acad Sci U S A* 106: 2041-2046, 2009.

Tachibana T, Fujiwara N, and Nawa T. The ultrastructure of the ganglionated nerve plexus in the nasal vestibular mucosa of the musk shrew (Suncus murinus, insectivora). *Arch Histol Cytol* 53: 147-156, 1990.

Ukhanov K, Leinders-Zufall T, and Zufall F. Patch-clamp analysis of gene-targeted vomeronasal neurons expressing a defined V1r or V2r receptor: ionic mechanisms underlying persistent firing. *J Neurophysiol* 98: 2357-2369, 2007.

Vargas G, and Lucero MT. Dopamine modulates inwardly rectifying hyperpolarization-activated current (Ih) in cultured rat olfactory receptor neurons. *J Neurophysiol* 81: 149-158, 1999.

Venditti C, Meade A, and Pagel M. Multiple routes to mammalian diversity. *Nature* 479: 393-396, 2011.

Wachowiak M, and Cohen LB. Representation of odorants by receptor neuron input to the mouse olfactory bulb. *Neuron* 32: 723-735, 2001.

Warren WC, Hillier LW, Marshall Graves JA, Birney E, Ponting CP, Grutzner F, Belov K, Miller W, Clarke L, Chinwalla AT, Yang SP, Heger A, Locke DP, Miethke P, Waters PD, Veyrunes F, Fulton L, Fulton B, Graves T, Wallis J, Puente XS, Lopez-Otin C, Ordonez GR, Eichler EE, Chen L, Cheng Z, Deakin JE, Alsop A, Thompson K, Kirby P, Papenfuss AT, Wakefield MJ, Olender T, Lancet D, Huttley GA, Smit AF, Pask A, Temple-Smith P, Batzer MA, Walker JA, Konkel MK, Harris RS, Whittington CM, Wong ES, Gemmell NJ, Buschiazzo E, Vargas Jentzsch IM, Merkel A, Schmitz J, Zemann A, Churakov G, Kriegs JO, Brosius J, Murchison EP, Sachidanandam R, Smith C, Hannon GJ, Tsend-Ayush E, McMillan D, Attenborough R, Rens W, Ferguson-Smith M, Lefevre CM, Sharp JA, Nicholas KR, Ray DA, Kube M, Reinhardt R, Pringle TH, Taylor J, Jones RC, Nixon B, Dacheux JL, Niwa H, Sekita Y, Huang X, Stark A, Kheradpour P, Kellis M, Flicek P, Chen Y, Webber C, Hardison R, Nelson J, Hallsworth-Pepin K, Delehaunty K, Markovic C, Minx P, Feng Y, Kremitzki C, Mitreva M, Glasscock J, Wylie T, Wohldmann P, Thiru P, Nhan MN, Pohl CS, Smith SM, Hou S, Nefedov M, de Jong PJ, Renfree MB, Mardis ER, and Wilson RK. Genome analysis of the platypus reveals unique signatures of evolution. *Nature* 453: 175-183, 2008.

Weiss J, Pyrski M, Jacobi E, Bufe B, Willnecker V, Schick B, Zizzari P, Gossage SJ, Greer CA, Leinders-Zufall T, Woods CG, Wood JN, and Zufall F. Loss-of-function mutations in sodium channel Nav1.7 cause anosmia. *Nature* 472: 186-190, 2011.

Wirsig CR, and Leonard CM. Acetylcholinesterase and luteinizing hormone-releasing hormone distinguish separate populations of terminal nerve neurons. *Neuroscience* 19: 719-740, 1986.

Wysocki CJ, Nyby J, Whitney G, Beauchamp GK, and Katz Y. The vomeronasal organ: primary role in mouse chemosensory gender recognition. *Physiol Behav* 29: 315-327, 1982.

Zheng LM, Ravel N, and Jourdan F. Topography of centrifugal acetylcholinesterase-positive fibres in the olfactory bulb of the rat: evidence for original projections in atypical glomeruli. *Neuroscience* 23: 1083-1093, 1987.

Zufall F, and Munger SD. From odor and pheromone transduction to the organization of the sense of smell. *Trends Neurosci* 24: 191-193, 2001.