e-Neuroforum 2010 · 1:9–16
DOI 10.1007/s13295-010-0002-1

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Sniffing out social signals

Chemical communication and the vomeronasal organ

"*Odors have a power of persuasion stronger than that of words, appearances, emotions, or will. The persuasive power of an odor cannot be fended off, it enters into us like breath into our lungs, it fills us up, imbues us totally. There is no remedy for it.*" – In his novel *Perfume: The Story of a Murderer*, Patrick Süskind [65] skillfully couches the profound emotional depth that can be evoked by odor perception. Understanding how the mammalian nose detects and simultaneously discriminates thousands of different scents, how odor information is decoded in different areas of the brain, and how perception of a specific odor frequently becomes a trigger of long forgotten memories – both pleasant and repulsive – is one of the most fascinating areas in modern sensory neuroscience. Therefore, the nose has become a busy place for neuroscientists these days, with a multitude of recent discoveries now adding to an emerging conceptual view of the architecture of the olfactory system that appears, at least in part, fundamentally different from previous conceptions.

The theoretical range of olfactory stimuli is virtually infinite. To meet the bewildering complexity of such structurally diverse chemical signals, several distinct populations of sensory cells have evolved within the mammalian nose. Each sensory cell type is identified by a unique receptor expression profile and characteristic central projection patterns. This cellular diversity has given rise to the organizational concept of olfactory subsystems – or noses within noses [45] – each dedicated to a particular role in chemosensation (**Fig. 1**).

Not too long ago, it was believed that the mammalian olfactory system had only two anatomical and functional divisions: a main and an accessory (vomeronasal) olfactory system. The main olfactory system was thought to predominantly detect general environmental odors (to probe, e.g., the type and quality of foods), whereas the accessory olfactory system and its peripheral sensory structure – the vomeronasal organ (VNO) – was considered to play a critical role in the detection and communication of social chemosignals (pheromones) that impact stereotyped social and sexual behaviors or hormonal responses among conspecifics. It has now become clear that the organization of the sense of smell is much more complex, revealing a diversity of subsystems that was not anticipated even a few years ago [1, 45]. With this newfound appreciation of functional diversity, several new and exciting questions now rank high on the research agenda of (chemo)sensory neuroscientists: Which different receptor structures have evolved to confer and maintain a sufficient degree of stimulus selectivity for each subsystem? Which signaling strategies are implemented by the different cell populations? What functional logic underpins the anatomical segregation of the different subsystems in the nasal cavity? How is subsystem-specific (parallel) information segregated, processed, and integrated by higher-order brain centers?

Subsystem organization of the mammalian nose

Nearly two decades ago, the discovery of the odorant receptor (OR) multigene family in rodents by Linda B. Buck and Richard Axel [7] marked the beginning of the molecular era in olfactory research. Since that watershed event for understanding olfactory function, the past years have seen an explosion of studies on the neurobiology of the main olfactory system.

The main olfactory neuroepithelium lines the dorsocaudal regions of the nasal septum and the endoturbinates within the nasal cavity of most mammals. Here, the 'classical'/canonical olfactory sensory neurons (OSNs) express a single type of receptor from a massive gene repertoire of OR genes (>1000 functional OR genes in rodents; ~350 in humans). In an individual OSN, this monogenic (indeed, monoallelic) receptor gene expression is tightly regulated by the gene products – the OR proteins – themselves, which exert a negative feedback effect on OR gene choice [60, 33]. ORs share various hallmarks of typical G protein-coupled receptors (GPCRs), e.g., seven putative membrane-spanning α-helices and the highly conserved DRY amino acid motif [43]. A group of hypervariable residues within transmembrane domains 3–6 likely builds the OR ligand binding pocket. It took seven years from publication of the OR discovery for the first unambiguous OR-ligand pair to be reported [72]. Though some ORs have since been matched to cognate ligands, the great majority of mammalian OR genes are yet to be deorphanized. Numerous laborato-

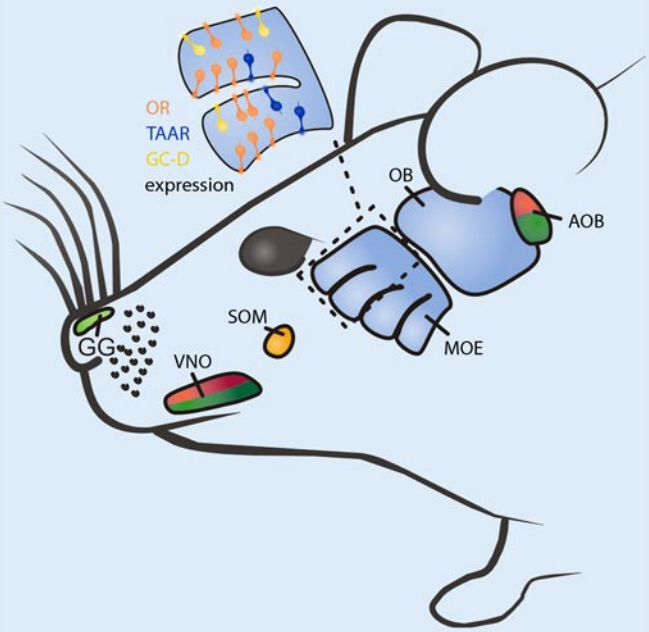

Fig. 1 ▲ Schematic drawing of the subsystem organization of the rodent olfactory system. Peripheral sensory structures consist of at least four anatomically separated tissues: the main olfactory epithelium (MOE), the septal organ of Masera (SOM), the Grueneberg ganglion (GG) and the vomeronasal organ (VNO). Sensory neurons located in either the MOE, the SOM or the GG project their axons to glomeruli within the main olfactory bulb (OB), whereas vomeronasal neurons make synaptic contacts with mitral cell dendrites in the accessory olfactory bulb (AOB). In the MOE, sensory neurons can be divided into at least three subpopulations according to individual receptor expression profiles. The great majority of neurons express one member of the large superfamily of canonical odorant receptors (ORs). Smaller groups of sensory cells are characterized by the expression of either a receptor guanylyl cyclase D (GC-D) or trace amine-associated receptors (TAARs)

ries have been puzzled by inherent difficulties in recombinant OR expression as these receptor proteins are frequently retained in ER/Golgi membranes and hardly translocate to the plasma membrane [40]. All ORs deorphaned to date detect volatile odorants of diverse chemical classes and are broadly tuned to multiple stimuli. *Vice versa*, different receptors can respond to the same odor molecule. Thus, odor information is encoded by combinatorial activation of multiple ORs [6, 37]. Highly enriched in the apical ciliary compartments of OSNs (the site of odor interaction), OR activation triggers a complex biochemical signaling cascade leading to adenylate cyclase activity and transiently increased cAMP levels. Opening of cyclic nucleotide-gated (CNG) channels and successive activation of Ca^{2+}-gated Cl^- channels results in a depolarizing receptor potential that is transformed into axonal trains of action potentials [14]. Convergent OR-specific axonal projection patterns to a few distinct glomeruli in the main olfactory bulb confer an 'OR identity' to each glomerulus and, thus, underlie processing of odor information by odotopic activity 'maps' [44].

Aside from canonical ORs, a second family of chemosensory GPCRs in the main olfactory epithelium was identified in 2006 by Linda B. Buck's laboratory [34]. In a broad screen of OSN-enriched murine cDNA, members of the trace amine-associated receptor (TAAR) family were found selectively expressed by sparse, nonoverlapping subset of OSNs. Furthermore, Liberles and Buck reported that TAAR and OR expression appear mutually exclusive, thus, suggesting a distinct olfactory function for TAAR-expressing OSNs. However, their exact functional role in chemosensory signaling remains to be determined.

Both TAARs and ORs are typical rhodopsin-like class A GPCRs that signal via a G protein-mediated cAMP-dependent transduction pathway [46]. A third group of neurons in the main olfactory epithelium, however, is likely to transduce olfactory stimuli independent of OR or TAAR expression and cAMP signaling. As a common molecular marker, these neurons express an orphan receptor guanylyl cyclase (GC), denoted as GC-D [15]. About 0.1% of OSNs share expression of GC-D in concert with other proteins reminiscent of a cGMP-mediated transduction cascade (e.g., phosphodiesterase PDE2 and the CNG channel subunit A3 [23, 41]. Another distinctive feature of GC-D-expressing OSNs is their clustered distribution within rather dorsal areas of the main olfactory epithelium. What, if any, chemosensory role is fulfilled by these cells? Ever since their discovery, the common structure of receptor GC proteins [16] – an extracellular peptide binding domain coupled to an intracellular catalytic domain by a single transmembrane α-helix – has fueled speculation about GC-D-positive OSNs as peptide sensors that regulate intracellular cGMP levels. At present, a conclusive picture of how these cells are functionally involved in olfaction is lacking. In 2007, two parallel studies suggested different and somewhat controversial functions for GC-D-expressing OSNs. Hu et al. [21] provided evidence for CO_2-mediated, carbonic anhydrase type II (CAII)-dependent Ca^{2+} signals in GC-D-expressing neurons. By contrast, functional data from both wild-type and gene-targeted mice strongly support a role of GC-D-positive OSNs as sensitive and selective sensors for two natriuretic peptides – uroguanylin and guanylin [31]. Are these findings necessarily contradictory? The pharmacological profiles of both CO_2- and peptide-dependent Ca^{2+} responses in GC-D-expressing neurons indicate that both chemosignals could share a final common transduction pathway. Future studies on this still enigmatic OSN subpopulation will eventually elucidate whether both pathways can be combined in an integrated signaling model or if either mechanism serves a predominant physiological function.

In addition to the main olfactory epithelium and its cellular heterogeneity, many mammals possess at least three further olfactory tissues – the VNO, the Grueneberg ganglion (GG), and the septal organ of Masera (SOM) – adding a whole new layer of complexity to an already complex organization (**Fig. 1**). Compared to our detailed knowledge of the main olfactory system and, to a lesser extent, the vomeronasal system, our functional understanding of both the GG and the SOM is still in its infancy.

Located near the entrances to the nasopalatine ducts [53], the rodent SOM is a small, relatively flat, isolated patch of neuroepithelium that is composed of roughly 10,000 ciliated sensory neurons that appear to largely resemble canonical OSNs of the main epithelium with respect to OR and downstream signaling protein expression. Interestingly, the vast majority of SOM neurons (>90%) choose one member of a group of only nine ORs for monogenic expression [67]. An unconventionally abundant receptor, SR1, is expressed in ~50% of SOM neurons and has recently been physiologically scrutinized in great detail in gene-targeted mice [19]. These experiments revealed an unusually broad odor response profile over a wide concentration range in SR1 expressing neurons. Given the observed correlation between SR1-dependent broad responsiveness and mechanosensitivity [17, 18] the authors discuss the hypothesis that the SOM could function as a strategically placed outpost of the main olfactory epithelium that might signal general changes in airflow and/or odor environment and, thus, prime the main system for overall sensitivity adjustment.

Similar to the history of the septal organ, the Grueneberg ganglion [19] made a comeback in chemosensory research activity just a few years ago. GG cell bodies are bilaterally located at the dorsal tips of each nasal cavity, in close proximity to the opening of the naris. Each ganglion comprises 300–500 cells that project single axons along the dorsal roof of the nasal cavity to dorsocaudal regions of the main olfactory bulb. This area somewhat overlaps with the bulb region harboring the so-called necklace glomeruli, which receive input from GC-D expressing neurons. GG cells share the characteristic expression of the olfactory marker protein (OMP; [38]) with canonical OSNs, TAAR- and GC-D-expressing neurons of the main olfactory system, vomeronasal neurons, and sensory cells of the SOM. By contrast, GG cells seem to lack direct access to the lumen of the nasal cavity. In light-microscopic images, GG cells show no prominent cellular processes such as dendrites, cilia, or microvilli. Using scanning electron microscopy, however, Brechbühl et al. [3] recently demonstrated that mouse GG neurons bear primary cilia that can be accessed by water-soluble chemostimuli via a water-permeable keratin layer. The authors of the same study also reported transient cytosolic Ca^{2+} elevations in GG neurons in response to chemical cues that are secreted under stress to signal danger to conspecifics. The molecular nature of such 'alarm pheromones,' however, has not been identified.

The vomeronasal organ – a key player in chemical communication and social interaction

Probably the most well characterized olfactory subsystem division is between the main olfactory and vomeronasal systems. The latter's peripheral sensory structure, the VNO – first described in 1813 by Ludvig L. Jacobson [22] – consists of two bilaterally symmetrical blind-ended cigar-shaped tubes which lie within the vomer bone at the anterior nasal septum. Vomeronasal sensory neurons (VSNs) reside medially in a crescent-shaped sensory neuroepithelium. Each bipolar VSN extends a single apical dendrite that ends in a microvillous knob which is bathed in mucus secreted by vomeronasal glands. Upon intimate contact with a pheromone source, stimuli access the VNO lumen via autonomically controlled pulsative vascular contractions of a large lateral blood vessel [27]. This mechanism enables the VNO to take up relatively nonvolatile cues from urine deposits, vaginal secretions, scent gland secretions, or saliva [30].

The rodent vomeronasal system is organized in at least a bipartite manner. Two topographically segregated VSN subpopulations express distinct repertoires of receptors and other putative signaling mol-

Abstract

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Abstract
In most mammals, conspecific chemical communication strategies control complex social and sexual behavior. Just a few years ago, our concept of how the olfactory system is organized to ensure faithful transmission of social information built on the rather simplistic assumption that two fundamentally different classes of stimuli – 'general' odors *versus* 'pheromones' – are exclusively detected by either of two sensory structures: the main olfactory epithelium or the vomeronasal organ. A number of exciting recent findings, however, revealed a much more complex and functionally diverse organizational structure of the sense of smell. At least four anatomically segregated olfactory subsystems, some remarkably heterogeneous in their cellular composition, detect distinct, but partially overlapping populations of sensory stimuli. Discerning how subsystem-specific receptor architectures and signaling pathways orchestrate the coding logic of social chemosignals, will ultimately shed new light on the neurophysiological basis of social behavior.

Keywords
Olfaction · Social chemosignals · Sensory neurophysiology · Homeostatic plasticity · Chemoreceptor

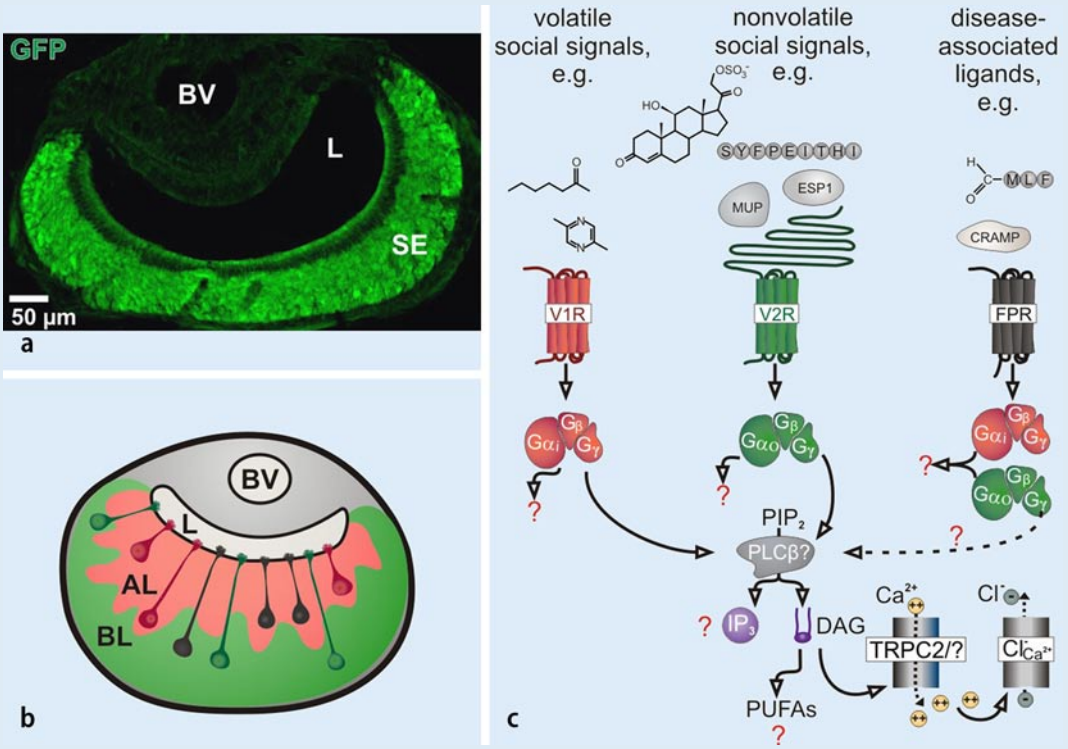

Fig. 2 ▲ Tissue architecture and putative signal transduction pathways in the mouse VNO. **a**) Confocal photomicrograph of a coronal VNO cryosection. Mature VSNs are labeled by fluorescence of a green fluorescent protein (GFP) expressed under the control of the regulatory sequences of the olfactory marker protein (omp) gene (OMP-GFP). SE, sensory epithelium; L, lumen; BV, blood vessel. **b**) Schematic view of the VNO sensory neuroepithelium that is divided into an apical (AL) and basal layer (BL). **c**) Putative signaling cascades implemented in VSNs. Volatile urinary compounds (e.g., 2-heptanone or 2,5-dimethyl pyrazine) activate V1R- and $G_{\alpha i2}$-expressing neurons, whereas V2R- and $G_{\alpha o}$-expressing neurons are likely activated by non-volatile social cues such as major urinary proteins (MUPs), exocrine-gland-secreting peptides (ESPs) or major histocompatibility complex (MHC) class I peptides (e.g., SYFPEITHI). Sulfated steroids (e.g., corticosterone 21-sulfate), rather non-volatile stress signals, have also been described as vomeronasal stimuli. However, the receptors detecting these ligands are still unknown. FPR-rs-expressing VSNs are activated by disease-associated ligands like the formylated peptide fMLF. Current downstream signaling models hypothesize G-protein-dependent activation of phospholipase C (PLC), cleavage phosphatidylinositol-4,5-bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), and final gating of a Ca^{2+} current that is, at least for some stimuli, critically dependent on the TRPC2 ion channel subunit. A number of interactions, however, are still elusive. Future investigations will have to pinpoint the exact function of, e.g., $G_{\alpha i2}$ and $G_{\alpha o}$ subunits, the recently described Ca^{2+}-gated Cl^-channel, IP_3 or polyunsaturated fatty acids (PUFAs) that have also been shown to induce a Ca^{2+} responses in VSNs

ecules (**Fig. 2**). VSNs located in the apical layer of the sensory epithelium express $G\alpha_{i2}$ in concert with one member of a multigene family that encodes 137 intact G protein-coupled receptors (GPCRs) – the V1Rs [13, 54]. Like OR genes, their coding regions show no introns, they are located in genomic clusters and expressed in a tightly controlled monoallelic fashion [55]. However, OR and V1R genes share no significant sequence homology. With one prominent exception (V1Rb2; [2]), all vertebrate V1R proteins still represent orphan receptors whose putative chemosensory function is only indirectly inferred from their tissue distribution, expression pattern, organizational commonalities with other chemoreceptors, and, notably, severe behavioral deficits observed in mice deficient for a gene cluster that encodes for 16 V1R proteins [12].

Neurons of the basal $G\alpha_o$-positive zone express members of an unrelated class C GPCR family – the V2Rs [39, 56]. The hallmark of all ~120 apparently functional V2R receptor proteins [70] is a large hydrophobic amino (N)-terminal extracellular domain, sharing sequence similarity with metabotropic glutamate, Ca^{2+}-sensing, and sweet/umami-sensing T1R taste receptors. Based on this observation, this extracellular domain has been proposed to form the V2R ligand binding site [43].

Today, we believe that both V1Rs and V2Rs are activated by a structurally diverse group of semiochemicals that have frequently been collectively referred to as pheromones – a term whose definition is currently in flux [5, 64]. Pheromonal cues are typically embedded in complex bodily secretions such as urine or sweat and range from small volatile molecules ([29, 51] to steroids [47], complex peptides [27, 30, 32], and proteins [9]. A blunt categorization of the VNO as a specialized pheromone detector and the main olfactory system as a general sensor of 'conventional' odors would, however, be simplistic [50, 62].

Important aspects of V1/2R function and downstream signal transduction pathways remain elusive. Based on layer-specific coexpression, a role of $G\alpha_{i2}$ and $G\alpha_o$ in V1R- and V2R-mediated signaling pathways, respectively, represents an attractive model. However, functional evidence supporting this hypothesis is lacking. Knockout models failed to demonstrate a critical role of $G\alpha_{i2}$ and $G\alpha_o$ subunits in pheromone sensing [48, 66]. Far better consensus is achieved on a role of phospholipase C (PLC). Inositol-1,4,5-trisphosphate (IP_3), diacylglycerol (DAG) as well as polyunsaturated fatty acids (PUFAs) have all been implicated in gating a Ca^{2+} permeable transduction channel [63]. Efforts to identify this channel have focused on a distinct transient receptor potential channel subunit, TRPC2 [35]. *TRPC2*$^{-/-}$ mice show severe defects in social and sexual behaviors. Yet, there are significant differences between *TRPC2* deletion and surgical VNO ablation [25]. A DAG-activated TRPC2-dependent current [36] not only appears to be activated as a downstream effector in VSN signaling, but also functions in vomeronasal sensory adaptation and gain control [61]. Likely, this current also provides the initial Ca^{2+} influx that has recently been proposed to trigger a Ca^{2+}-activated Cl^-current that could boost membrane depolarization via Cl^- efflux [69].

Compared to the many substantial advances in our understanding of canonical OSN signaling, our present conception of sensory signaling in the VNO is still fragmentary. Given the prime biological importance of intraspecific social communication, current research in my laboratory focuses on the basic physiological concepts underlying chemical communication in conspecific mammals. Thus, in the long term, we aim to gain detailed functional insight into the neuronal mechanisms that link chemosensation and social behaviors.

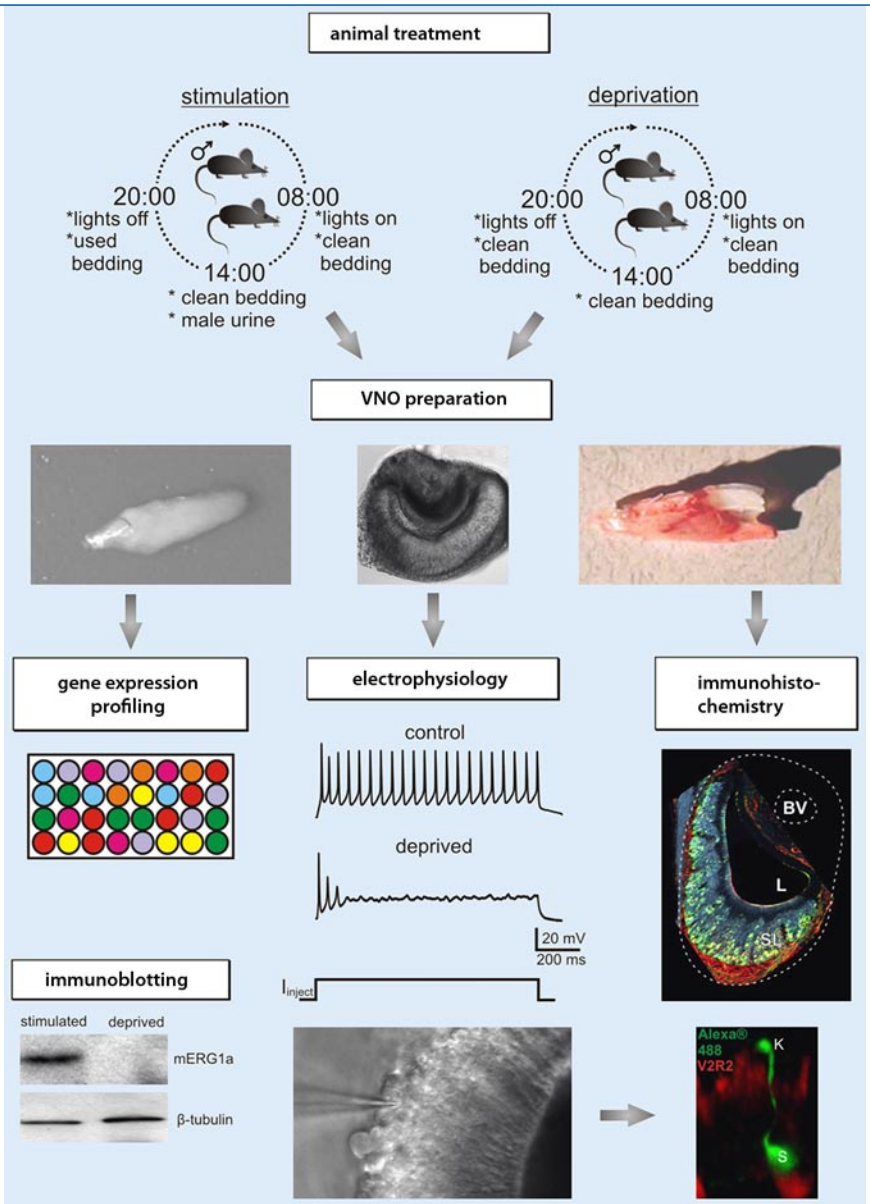

Fig. 3 ▲ Illustration of the VNO expression profiling approach used to uncover ion channel proteins involved in homeostatic VSN plasticity and activity-dependent maintenance of output stability. Top) Schematic diagram illustrating the experimental strategy used for pheromone exposure (left) and stimulus deprivation (right) of singly housed C57Bl/6 mice. Middle) Typical VNO preparations used for gene expression profiling (left), patch-clamp recordings in acute tissue slices (middle), and cryosections for immunochemistry (right). Bottom) Western blot analysis demonstrating increased levels of vomeronasal ERG1a expression in stimulated versus deprived mice. Blots were additionally probed for β-tubulin as loading control (left). IR-DIC photomicrograph shows a patch pipette used for dye (Alexa®488) loading of VSNs during recordings (middle). The molecular identity of the neuron can later be determined by post-hoc immunocytochemistry against layer-specific marker proteins (e.g. V2R2; right)

Homeostatic plasticity in vomeronasal neurons

To better understand the complex mechanisms involved in pheromone sensing, we recently engaged in a high-throughput whole-genome search for vomeronasal transcripts that are regulated in an activity-dependent fashion. We designed an expression profiling paradigm that integrates various levels of analysis (◘ **Fig. 3**) by combining microarray-based quantitative determination of activity-dependent 'transcriptomes' with 'manual' confirmation of candidate signaling genes/proteins by means of RT-PCR and immunocytochemistry, respectively [20]. We initially hypothesized that, analog to non-synaptic homeostatic plasticity in many brain areas primarily associated with learning and memory [11, 68], the dynamic range and stability of VSN input-output relationships is constantly adjusted within mean-

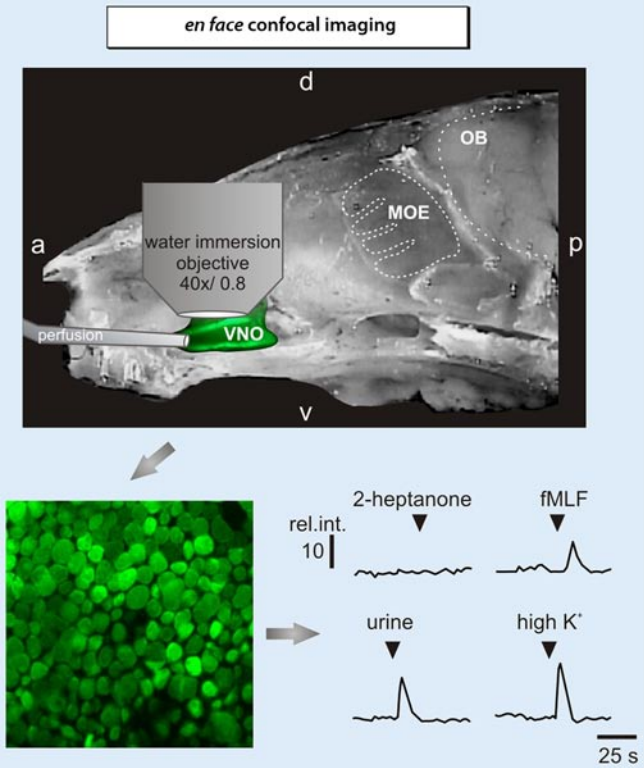

Fig. 4 ▲ Formyl-peptides evoke Ca^{2+} signals in VSN dendritic tips. Top) Merged macroscopic bright field and fluorescence images of the hemisected rostral head of an OMP-GFP mouse, illustrating the en face confocal Ca^{2+} imaging approach recently established in our laboratory. Bottom) High magnification confocal section of the dendritic surface of fluo-4/AM-loaded VSNs (left). Representative original recordings of cytosolic Ca^{2+} signals in response to fMLF (900 nM), 2-heptanone (1 µM), diluted urine (1:500), and elevated extracellular K^+ (40 mM; right)

ingful firing rate limits in response to altered sensory input. One mechanism that ensures such homeostatic plasticity on a longer time scale, i.e., hours to days [71], is compensatory feedback regulation of *de novo* protein synthesis. This concept provided the temporal framework for investigating the consequences of stimulus deprivation in the mouse VNO (◘ **Fig. 3**).

In this context, modulation of voltage-gated K^+ channel gene expression – major determinants of membrane excitability – represents a key molecular mechanism to orchestrate the output of an individual neuron [28]. By systematically comparing vomeronasal K^+ channel transcription levels in male mice strongly exposed to rich sources of pheromonal cues *versus* stimulus-deprived animals, we identified a member of the *ether-à-go-go* related gene (ERG) K^+ channel subfamily, mERG1, as both consistently and significantly up-regulated in stimulated mice. Characterized by rather unconventional gating kinetics (slow activation, fast inactivation), hERG, the human homolog of mERG1 and founding family member, has been intensely investigated because of the severe cardiac phenotype (long QT syndrome 2) caused by channel mutations [57, 59]. By contrast, the physiological roles of ERG channels in the nervous system are poorly understood [58]. Using immunochemistry, we showed that ERG1 channel proteins are selectively expressed in basal V2R-positive VSNs and substantially regulated upon pheromone exposure/deprivation. However, a key issue in interpreting these findings is to resolve whether the observed expression changes are reflected in VSN physiology. By combining whole-cell patch-clamp recordings from identified VSNs in acute vomeronasal slice preparations with *post-hoc* immunocytochemistry and three-dimensional reconstruction of fluorescently labeled neurons, we demonstrated that basal VSNs exhibit a fast ERG-mediated K^+ current that is significantly reduced after stimulus deprivation. When dissecting the 'internal anatomy' [1] of basal VSN spikes using the action potential (AP) clamp technique, our recordings reveal that ERG currents are critically involved in VSN spike repolarization. Consequently, AP discharge in basal VSNs is substantially impaired after ERG channel inhibition.

Together, our findings illustrate that, by regulating the expression level of ERG K^+ channels, basal VSNs are equipped to dynamically control/extend the range of their individual stimulus-response function. This novel example of homeostatic plasticity in the periphery of the accessory olfactory system is ideally suited to adjust VSNs to a target output range in a layer-specific and use-dependent manner [20].

A third family of vomeronasal chemoreceptors

Given the increasing diversity of neuronal subpopulations in the main olfactory epithelium, it is tempting to hypothesize that potentially still unidentified sensory cell populations and corresponding chemoreceptors might also exist in the VNO. In close collaboration with the laboratory of Ivan Rodriguez (University of Geneva), we therefore designed a screening strategy for putative mouse receptors which we expected to share the hallmarks of all previously identified chemosensory GPCRs, i.e., (a) showing a seven-transmembrane topology, (b) displaying a punctate expression pattern in the vomeronasal neuroepithelium, (c) excluding any coexpression of other olfactory chemoreceptor groups, (d) excluding cotranscription of other members of their own receptor family, (e) being located/enriched at the VSN dendritic tips, and (f) triggering neuronal activity in response to biologically relevant stimuli.

Screening mouse vomeronasal tissue for the expression of ~100 candidate GPCRs by RT-PCR [52], we identified five non-V1/2R GPCR genes [criterion (a)], all members of the formyl peptide receptor (FPR)-like genes (*Fpr-rs1*, *rs3*, *rs4*, *rs6* and *rs7*). Quantitative PCR experiments

showed that the presence of these transcripts indeed turned out highly VNO-specific. *In situ* analysis of FPR-rs1-7, as well as immunocytochemical localization studies of FPR-rs3, revealed both strong and punctate gene expression in the VNO sensory epithelium [criterion (b)], as well as protein translocation to the microvillous dendritic endings of VSNs [criterion (e)]. Furthermore, we demonstrated both *Fpr-rs* expression in VSN subsets that do not coexpress other known vomeronasal receptors [criterion (c)] and monogenic *Fpr-rs* expression within these neurons [criterion (d)]. Intriguingly, with the exception of FPR-rs1, FPR-rs proteins are restricted to VSNs found localized in the apical $G\alpha_{i2}$-expressing layer of the neuroepithelium.

Immune cells such as granulocytes or macrophages express FPR1 and FPR-rs2, two family members not transcribed in VSNs. These receptors show broad tuning profiles with ligand spectra rather defined by immunological function than by structural properties [10, 42]. These FPR agonists include peptides and lipids derived from pathogens (such as fMLF, the prototypical formylated peptide released by gram negative bacteria), or involved in acute inflammatory responses (such as the antimicrobial compounds CRAMP and Lipoxin A4). However, no ligands were described for FPR-rs3, rs4, rs6, or rs7 receptors. When we engineered human embryonic kidney (HEK) cells to express recombinant FPR-rs1–7 proteins, we were able to examine receptor activation. Surprisingly, VSN-specific FPR-rs proteins display distinct, but overlapping ligand profiles that, to a large extent, resemble those agonist spectra of immune-cell FPR proteins.

Are these results transferable to the *in vivo* situation, i.e., do these different disease- and inflammation-associated compounds actually activate vomeronasal neurons? To address this question, we established an *in situ* approach that combined whole mount vomeronasal preparations with dendritic Ca^{2+} imaging in the intact neuroepithelium (**Fig. 4**). This way, we were able to record responses from individual VSN knobs while the dendritic tips are kept covered by mucus and both the epithelial structure and the VSN axonal projections to the accessory olfactory bulb are left intact. Strikingly, we recorded exquisitely sensitive and concentration dependent responses to overlapping sets of FPR-rs agonists [criterion (f)], strongly suggesting that *Fpr-rs*-expressing VSNs represent a previously unrecognized type of chemosensory neuron.

What might be the physiological function of these cells? It has been known for quite some time that mice use the olfactory system to discern pathogenicity or the health status of a conspecific [24]. However, no olfactory subsystem dedicated to the identification of pathogens, or pathogenic states, has yet been identified in mammals [45]. Since FPR-rs agonists are found in bodily secretions at various stages of diseases [8], our results could provide the link to understand how animals identify pathogens or unhealthy potential partners.

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Acknowledgement. Work in the author's laboratory is generously supported by the *Emmy Noether-Program* of the Deutsche Forschungsgemeinschaft (SP724/2–1), the Mercator Foundation (*Junges Kolleg*), and within the funding initiative *Lichtenberg Professorships* of the Volkswagen Foundation.

Conflict of interest. No statement made.

References

1. Bean BP (2007) The action potential in mammalian central neurons. Nat Rev Neurosci 8:451–465
2. Boschat C, Pelofi C, Randin O et al. (2002) Pheromone detection mediated by a V1r vomeronasal receptor. Nat Neurosci 5:1261–1262
3. Brechbuhl J, Klaey M, Broillet MC (2008) Grueneberg ganglion cells mediate alarm pheromone detection in mice. Science 321:1092–1095
4. Breer H, Fleischer J, Strotmann J (2006) The sense of smell: multiple olfactory subsystems. Cell Mol Life Sci 63:1465–1475
5. Brennan PA, Zufall F (2006) Pheromonal communication in vertebrates. Nature 444:308–315
6. Buck LB (2000) The molecular architecture of odor and pheromone sensing in mammals. Cell 100:611–618
7. Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65:175–187
8. Casella R, Shariat SF, Monoski MA et al. (2002) Urinary levels of urokinase-type plasminogen activator and its receptor in the detection of bladder carcinoma. Cancer 95:2494–2499
9. Chamero P, Marton TF, Logan DW et al. (2007) Identification of protein pheromones that promote aggressive behaviour. Nature 450:899–902
10. Chromek M, Slamova Z, Bergman P et al. (2006) The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. Nat Med 12:636–641
11. Davis GW (2006) Homeostatic control of neural activity: from phenomenology to molecular design. Annu Rev Neurosci 29:307–323
12. Del Punta K, Leinders-Zufall T, Rodriguez I et al. (2002) Deficient pheromone responses in mice lacking a cluster of vomeronasal receptor genes. Nature 419:70–74
13. Dulac C, Axel R (1995) A novel family of genes encoding putative pheromone receptors in mammals. Cell 83:195–206
14. Firestein S (2001) How the olfactory system makes sense of scents. Nature 413:211–218
15. Fulle HJ, Vassar R, Foster DC et al. (1995) A receptor guanylyl cyclase expressed specifically in olfactory sensory neurons. Proc Natl Acad Sci U S A 92:3571–3575
16. Gibson AD, Garbers DL (2000) Guanylyl cyclases as a family of putative odorant receptors. Annu Rev Neurosci 23:417–439
17. Grosmaitre X, Santarelli LC, Tan J et al. (2007) Dual functions of mammalian olfactory sensory neurons as odor detectors and mechanical sensors. Nat Neurosci 10:348–354
18. Grosmaitre X, Fuss SH, Lee AC et al (2009) SR1, a mouse odorant receptor with an unusually broad response profile. J Neurosci 29:14545–14552
19. Gruneberg H (1973) A ganglion probably belonging to the N. terminalis system in the nasal mucosa of the mouse. Z Anat Entwicklungsgesch 140:39–52
20. Hagendorf S, Fluegge D, Engelhardt C, Spehr M (2009) Homeostatic control of sensory output in basal vomeronasal neurons: activity-dependent expression of ether-a-go-go-related gene potassium channels. J Neurosci 29:206–221
21. Hu J, Zhong C, Ding C et al. (2007) Detection of near-atmospheric concentrations of CO2 by an olfactory subsystem in the mouse. Science 317:953–957
22. Jacobson L (1813) Anatomisk Beskrivelse over et nyt Organ i Huusdyrenes Næse. Veterinær=Selskapets Skrifter [in Danish] 2:209–246

23. Juilfs DM, Soderling S, Burns F et al. (1999) Cyclic GMP as substrate and regulator of cyclic nucleotide phosphodiesterases (PDEs). Rev Physiol Biochem Pharmacol 135:67–104
24. Kavaliers M, Choleris E, Pfaff DW (2005) Genes, odours and the recognition of parasitized individuals by rodents. Trends Parasitol 21:423–429
25. Kelliher KR, Spehr M, Li XH et al (2006) Pheromonal recognition memory induced by TRPC2-independent vomeronasal sensing. Eur J Neurosci 23:3385–3390
26. Keverne EB (2002) Mammalian pheromones: from genes to behaviour. Curr Biol 12:R807–R809
27. Kimoto H, Sato K, Nodari F et al. (2007) Sex- and strain-specific expression and vomeronasal activity of mouse ESP family peptides. Curr Biol 17:1879–1884
28. Lai HC, Jan LY (2006) The distribution and targeting of neuronal voltage-gated ion channels. Nat Rev Neurosci. 7:548–562
29. Leinders-Zufall T, Lane AP, Puche AC et al. (2000) Ultrasensitive pheromone detection by mammalian vomeronasal neurons. Nature 405:792–796
30. Leinders-Zufall T, Brennan P, Widmayer Pet al (2004) MHC class I peptides as chemosensory signals in the vomeronasal organ. Science 306:1033–1037
31. Leinders-Zufall T, Cockerham RE, Michalakis S et al (2007) 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
32. Leinders-Zufall T, Ishii T, Mombaerts P et al (2009) Structural requirements for the activation of vomeronasal sensory neurons by MHC peptides. Nat Neurosci 12:1551–1558
33. Lewcock JW, Reed RR (2004) A feedback mechanism regulates monoallelic odorant receptor expression. Proc Natl Acad Sci U S A 101:1069–1074
34. Liberles SD, Buck LB (2006) A second class of chemosensory receptors in the olfactory epithelium. Nature 442:645–650
35. Liman ER, Corey DP, Dulac C (1999) TRP2: a candidate transduction channel for mammalian pheromone sensory signaling. Proc Natl Acad Sci U S A 96:5791–5796
36. Lucas P, Ukhanov K, Leinders-Zufall T, Zufall F (2003) A diacylglycerol-gated cation channel in vomeronasal neuron dendrites is impaired in TRPC2 mutant mice: mechanism of pheromone transduction. Neuron 40:551–561
37. Malnic B, Hirono J, Sato T, Buck LB (1999) Combinatorial receptor codes for odors. Cell 96:713–723
38. Margolis FL (1982) Olfactory marker protein (OMP). Scand J Immunol Suppl 9:181–199
39. Matsunami H, Buck LB (1997) A multigene family encoding a diverse array of putative pheromone receptors in mammals. Cell 90:775–784
40. McClintock TS, Sammeta N (2003) Trafficking prerogatives of olfactory receptors. Neuroreport 14:1547–1552
41. Meyer MR, Angele A, Kremmer E et al (2000) A cGMP-signaling pathway in a subset of olfactory sensory neurons. Proc Natl Acad Sci U S A 97:10595–10600
42. Migeotte I, Communi D, Parmentier M (2006) Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. Cytokine Growth Factor Rev 17:501–519
43. Mombaerts P (2004) Genes and ligands for odorant, vomeronasal and taste receptors. Nat Rev Neurosci 5:263–278
44. Mombaerts P, Wang F, Dulac C et al (1996) Visualizing an olfactory sensory map. Cell 87:675–686
45. Munger SD (2009) Olfaction: noses within noses. Nature 459:521–522
46. Munger SD, Leinders-Zufall T, Zufall F (2009) Subsystem organization of the mammalian sense of smell. Annu Rev Physiol 71:115–140
47. Nodari F, Hsu FF, Fu X et al. (2008) Sulfated steroids as natural ligands of mouse pheromone-sensing neurons. J Neurosci 28:6407–6418
48. Norlin EM, Gussing F, Berghard A (2003) Vomeronasal phenotype and behavioral alterations in G alpha i2 mutant mice. Curr Biol 13:1214–1219
49. Pankevich DE, Baum MJ, Cherry JA (2004) Olfactory sex discrimination persists, whereas the preference for urinary odorants from estrous females disappears in male mice after vomeronasal organ removal. J Neurosci 24:9451–9457
50. Restrepo D, Arellano J, Oliva AM et al. (2004) Emerging views on the distinct but related roles of the main and accessory olfactory systems in responsiveness to chemosensory signals in mice. Horm Behav 46:247–256
51. Restrepo D, Lin W, Salcedo E et al. (2006) Odortypes and MHC peptides: Complementary chemosignals of MHC haplotype? Trends Neurosci 29:604–609
52. Riviere S, Challet L, Fluegge D et al (2009) Formyl peptide receptor-like proteins are a novel family of vomeronasal chemosensors. Nature 459:574–577
53. Rodolfo-Masera T (1943) Su l'estizenza di un particulare organo olfacttivo nel setto nasale della cavia e di altri roditori. Arch Ital Anat Embryol 48:157–212
54. Rodriguez I, Del Punta K, Rothman A et al. (2002) Multiple new and isolated families within the mouse superfamily of V1r vomeronasal receptors. Nat Neurosci 5:134–140
55. Roppolo D, Vollery S, Kan CD et al. (2007) Gene cluster lock after pheromone receptor gene choice. EMBO J 26:3423–3430
56. Ryba NJ, Tirindelli R (1997) A new multigene family of putative pheromone receptors. Neuron 19:371–379
57. Sanguinetti MC, Jiang C, Curran ME et al. (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell 81:299–307
58. Sanguinetti MC, Tristani-Firouzi M (2006) hERG potassium channels and cardiac arrhythmia. Nature 440:463–469
59. Schwarz JR, Bauer CK (2004) Functions of erg K+ channels in excitable cells. J Cell Mol Med 8:22–30
60. Serizawa S, Miyamichi K, Nakatani H et al (2003) Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. Science 302:2088–2094
61. Spehr J, Hagendorf S, Weiss J et al (2009) Ca2+-calmodulin feedback mediates sensory adaptation and inhibits pheromone-sensitive ion channels in the vomeronasal organ. J Neurosci 29:2125–2135
62. Spehr M, Munger SD (2009) Olfactory receptors: G protein-coupled receptors and beyond. J Neurochem 109:1570–1583
63. Spehr M, Spehr J, Ukhanov K et al (2006) Parallel processing of social signals by the mammalian main and accessory olfactory systems. Cell Mol Life Sci 63:1476–1484
64. Stowers L, Marton TF (2005) What is a pheromone? Mammalian pheromones reconsidered. Neuron 46:699–702
65. Süskind P (1985) Perfume: The story of a murderer.
66. Tanaka M, Treloar H, Kalb RG et al. (1999) G(o) protein-dependent survival of primary accessory olfactory neurons. Proc Natl Acad Sci U S A 96:14106–14111
67. Tian H, Ma M (2004) Molecular organization of the olfactory septal organ. J Neurosci 24:8383–8390
68. Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci 5:97–107
69. Yang C, Delay RJ (2010) Calcium-activated chloride current amplifies the response to urine in mouse vomeronasal sensory neurons. J Gen Physiol 135:3–13
70. Young JM, Trask BJ (2007) V2R gene families degenerated in primates, dog and cow, but expanded in opossum. Trends Genet 23:212–215
71. Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat Rev Neurosci 4:885–900
72. Zhao H, Ivic L, Otaki JM et al. (1998) Functional expression of a mammalian odorant receptor. Science 279:237–242