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Parallel processing of social signals by the mammalian main and accessory olfactory systems

M. Spehr, J. Spehr, K. Ukhanov, K. R. Kelliher, T. Leinders-Zufall and F. Zufall*

Department of Anatomy and Neurobiology, University of Maryland School of Medicine, 20 Penn Street, Baltimore, MD 21201 (USA), Fax: +1 410 706 7068, e-mail: fzufa001@umaryland.edu

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Abstract. The mammalian olfactory system has evolved complex mechanisms to detect a vast range of molecular cues. In rodents, the olfactory system comprises several distinct subsystems. Current interest has focused on the exact role that each of these subsystems plays in detecting molecular information and regulating chemosensory-dependent behaviors. Here, we summarize recent results showing that the mouse main and accessory olfactory systems detect, at least in part, overlapping sets of social chemosignals. These findings give rise to a model that involves parallel processing of the same molecular cues in both systems. Together with previous work, this model will lead to a better understanding of the general organization of chemical communication in mammals and give a new direction for future experiments.

Keywords. Main olfactory epithelium, vomeronasal organ, TRPC2 channel, major histocompatibility complex, CNGA2 channel, nonvolatile chemosignal, social behavior, pheromone.

Introduction

A complementary article in this issue by Breer and colleagues [1] has summarized the growing complexity of the organization of the mammalian olfactory system, showing that the mouse nose is organized into an ever increasing number of subsystems. These include a main olfactory system, an accessory olfactory system (which itself is segregated into two distinct subsystems), a GC-D cell system (consisting of sensory cells expressing type D guanylyl cyclase), a septal organ of Masera, a Grueneberg ganglion, as well as several other functionally distinct subpopulations of chemosensory cells located within the nose (Fig. 1) [1–10]. A central problem in olfaction remains in examining the exact role that each of these subsystems plays in detecting olfactory information and regulating chemosensory-dependent behaviors. For instance, what are the consequences of the anatomical segregation of these subsystems for chemical communication and social behavior? And what types of social recognition signals are detected by each of these subsystems and how are these signals processed in higher centers to influence behavior? These topics have been intensely discussed recently with respect to the relative roles of the main and accessory olfactory system, where new findings are accumulating rapidly [11–18]. New concepts derived from these results will also be a critical starting point for addressing similar problems in nasal subsystems with yet unknown biological functions [e.g. 6–10].

Here, we review unexpected findings showing that the main and accessory olfactory systems detect, in part, overlapping sets of social chemosignals, based on the results of our own studies and those of others. In this context, we will particularly focus on the sensing of a family of immune system molecules by the mouse nose: peptide ligands of major histocompatibility complex (MHC) molecules. Recent work has implicated these ligands in the chemosensory evaluation of genetic relatedness (i.e. MHC genotype) among conspecifics [19–22]. Together, these findings have important implications for our understanding of the general organization of chemical communication in mammals. Five key results, as summarized below, emerge from this work: (i) The main and accessory olfactory systems can both detect and process social chemosignals of volatile and nonvolatile

* Corresponding author.

nature. (ii) Both systems are highly sensitive, exhibiting detection thresholds in the subnanomolar range. (iii) System-specific signal transduction pathways are used for the detection of these cues, indicating that diverse olfactory mechanisms have evolved to assess the structural diversity of social chemosignals. (iv) Coding strategies and tuning properties of individual sensory neurons in the main olfactory epithelium (MOE) and vomeronasal organ (VNO) differ significantly, suggesting that different receptors could be employed in both systems for the detection of the same ligands. (v) In behavioral tests using mice with genetic and surgical lesions, stimulation of each system by the same social signal can lead to distinct behavioral outcomes. Therefore, it appears that the same chemosignals can mediate different sexual and social behaviors through differential activation of each system.

New methodological approaches enable direct functional comparison of the two systems

A powerful set of methods is being developed to define the molecular nature of social chemosignals in mammals and subsequently to compare the functions of these molecules with respect to their relative role in the main and accessory olfactory systems (Fig. 2). This analysis begins in the periphery where whole-organ preparations of the mouse MOE [21, 23–25] and VNO [19, 26–29] are being used, in combination with recordings of local field potentials, to examine large sets of chemosignal candidates and define the molecular receptive range, sensitivity and pharmacological properties of neuronal populations in each sensory epithelium. Potentially, this approach can be applied for the screening of entire chemical libraries. These methods are also useful for initial characterization of sensory phenotypes of mice exhibiting targeted deletions in specific olfactory genes [21, 24, 25, 27–29]. Submerged field potential recording techniques were developed for this analysis. Compared with conventional electro-olfactogram recording [30], a main advantage of this approach is that it is independent of the volatility of a given stimulus. It also allows for application of pharmacological agents without altering the thickness of the aqueous layer covering the epithelium. Results can therefore be compared directly with those obtained at the single-cell level (see below). These experiments can be performed using highly localized, aqueous micropulse stimuli that prevent prestimulation of the epithelial tissue and allow for spatial mapping of sensitive epithelial spots [e.g. 21].

The next step in this analysis involves the preparation of acute tissue slices of mouse VNO [19, 26] or MOE [21, 31] (Fig. 2). These slice preparations permit optical or electrophysiological recordings from large numbers of individual sensory neurons that retain their spatial location and microenvironment within the epithelial sheet. Furthermore, the use of slice preparations allows us to superimpose different kinds of sensory maps within the same experiment. For example, VSN activation maps were overlayed on protein expression maps by the use of molecular markers specific for either of the two major VNO expression zones, showing that responsive neurons expressed receptors of the V2R family [19]. This methodology is currently being extended to be employed in combination with gene-targeted mice in which specific subsets of OSNs or VSNs have been genetically labeled, allowing for superposition of neuronal response maps onto receptor gene expression maps. To achieve a more detailed analysis of signaling mechanisms at the subcellular level, neurons can also be freshly dissociated and recordings made directly from the transduction compartments in distal dendrites [e.g. 32].

The finding that MOE and VNO detect overlapping sets of chemosignals (see below) predicts that such stimuli should cause simultaneous neural activation of both the main and accessory olfactory bulbs (MOB and AOB). Indeed, recent work employing high-resolution functional magnetic resonance imaging (fMRI) succeeded in testing this hypothesis by obtaining recordings of simultaneous responses in MOB and AOB evoked by the same chemosignals [33]. It should be interesting to extend these findings by applying other *in vivo* MOB and AOB recording techniques with enhanced spatial and temporal resolution [e.g. 34–37].

Information about the same stimuli derived from each system is probably integrated in higher brain centers such as amygdala and hypothalamic nuclei [38, 39]. Accordingly, there is a new focus on establishing neural connections between chemosensory neurons of the MOE and VNO and neural circuits involved in hormone regulation [18, 40, 41]. Gonadotropin-releasing hormone (GnRH) is the key regulator of reproductive function and regulation of sex hormones in vertebrates. GnRH neurons are primarily distributed throughout the rostro-caudal extent of the medial-basal hypothalamus, but neurons and fibers are also found in the amygdala and olfactory bulbs [42, 43]. While it has been previously suggested that the VNO influences GnRH secretion [39, 44], recent studies in the mouse found that GnRH neurons seem to receive input from both MOE and VNO [40, but see 41]. Substantial progress could be achieved in the future by directly recording from these cells and determining their response properties to social cues that stimulate both systems.

Finally, the consequences of encoding the same chemosignals in MOE and VNO need to be examined at the level of species-typical behaviors. In at least one example this has now been reported, results that will be summarized below [19, 45].

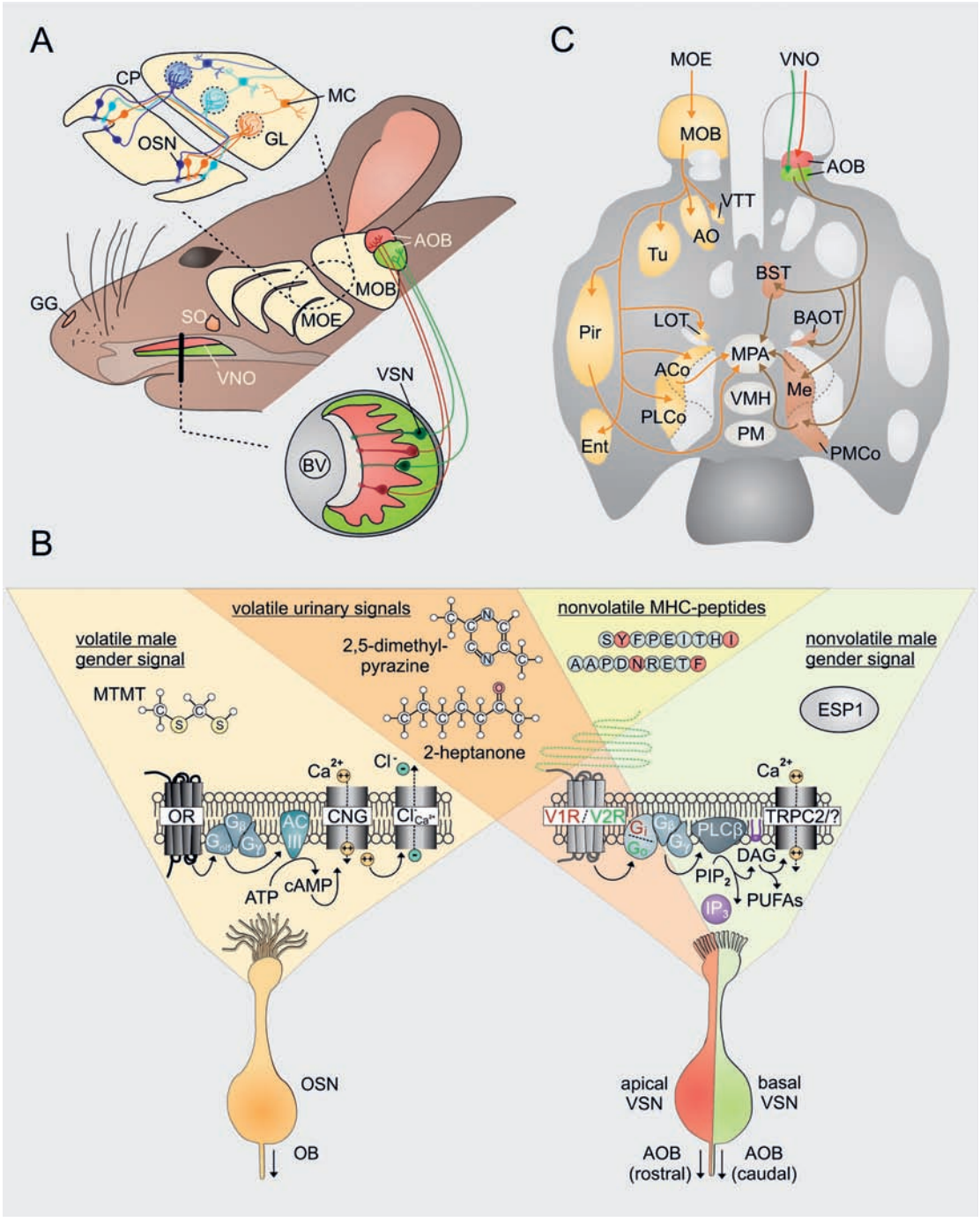


Figure 1. Parallel processing of overlapping sets of social chemosignals by the main and accessory olfactory systems. (*A*) Schematic diagram showing the organization of the rodent nose into chemosensory subsystems with the main olfactory epithelium (MOE), vomeronasal organ (VNO), septal organ (SO) and Grueneberg ganglion (GG). Not shown are the GC-D cell system, trigeminal nerve or nervus terminalis. The MOE lines the posterior part of the nasal septum as well as the dorsolateral surface of the endoturbinates. Bipolar OSNs extend their apical ciliary dendrites into the olfactory mucus. OSN axons pass through small foramina in the cribriform plate (CP) and converge in a receptor-dependent pattern onto mitral cell (MC) dendrites in the glomerular layer (GL) of the main olfactory bulb (MOB). The VNO consists of bilaterally symmetrical blind-ended tubes at the anterior base of the nasal septum. Stimuli are sucked into the VNO lumen following vascular contractions of lateral blood vessels (BV). Two subpopulations of microvillous VSNs reside medially in either the apical (red) or basal (green) layer of a crescent-shaped sensory epithelium. This organizational dichotomy is maintained in the AOB. Apical VSNs project their axons to the rostral part of the AOB, whereas basal VSNs innervate the caudal AOB. (*B*) Partially overlapping sets of social chemosignals are detected by MOE and VNO. Both volatile (e.g. 2-heptanone, 2,5-dimethylpyrazine) and nonvolatile social signals (e.g. MHC peptides; anchor residues shown in red) are potent sensory stimuli for OSNs and VSNs. Shown are also two male-specific signals, a urinary volatile, MTMT [(methylthio)methanethiol] [36], and a 7-kDa peptide named ESP1 (exocrine gland-secreting peptide 1) that is found in mouse tears and activates basal VSNs [51]. Effects of these two molecules have not yet been compared directly in both systems. OSNs and VSNs employ distinct signaling mechanisms. In the

MOE and VNO detect overlapping sets of chemosignals

A widely held view is that nonvolatile cues are detected by the accessory olfactory system, whereas volatile social cues are processed by the anatomically separate main olfactory system. On the basis of the results summarized here, it will be necessary to revise this notion. Three key results have emerged: (i) Both MOE and VNO are involved in the detection of pheromones as well as in the detection of social cues that cannot be classified as pheromones. (ii) MOE and VNO detect in part overlapping sets of social chemosignals. (iii) Both systems are involved in detecting volatile as well as nonvolatile molecules.

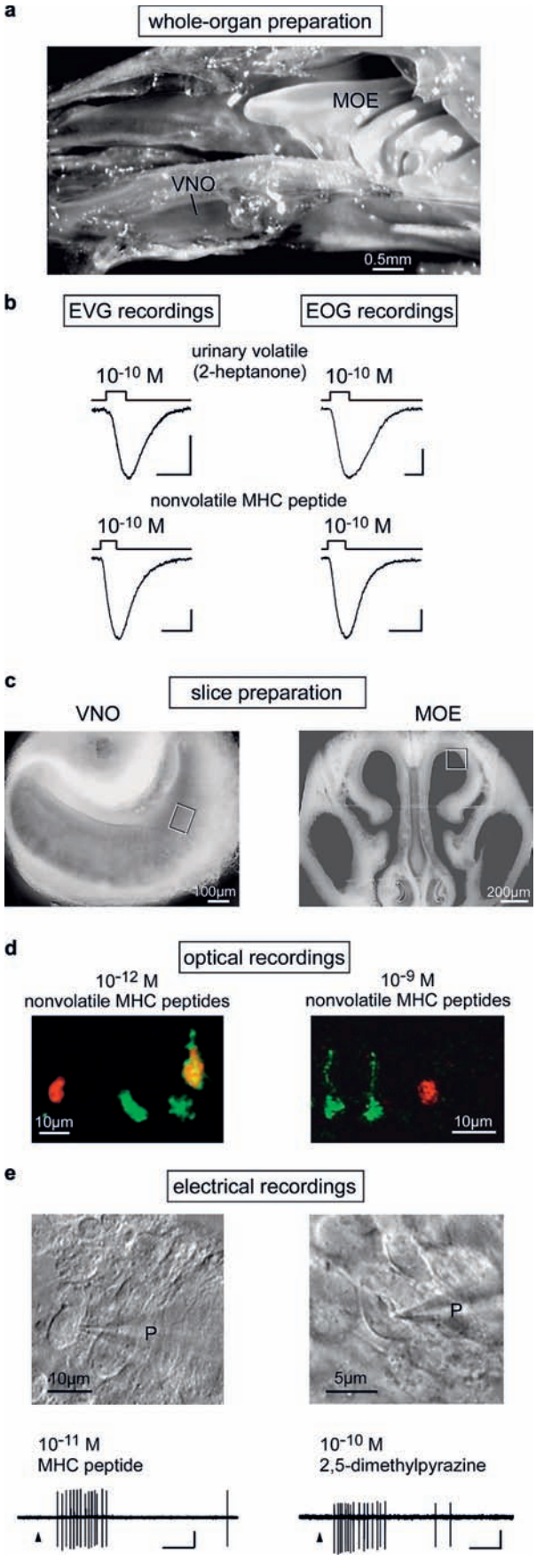
With respect to VSNs, there is strong evidence that activation by appropriate stimuli occurs following direct physical contact of the nose with a stimulus source [19, 34]. For example, recordings from single neurons in the AOB of mice that were engaged in natural behaviors showed increased neuronal firing after physical contact with a conspecific [34]. Bodily secretions such as urine contain a plethora of chemosignals, from small volatile organic molecules to short nonvolatile peptides to larger proteins [46–48]. Once the suction mechanism that transports such fluids into the lumen of the VNO is activated [49], all of these molecules will gain access to the VSNs and potentially can serve as chemosignals (i.e. the vomeronasal pump does not discriminate for molecular weight). Excellent evidence has established that mammalian VSNs are indeed capable of recognizing a set of structurally diverse urinary volatiles that have pheromonal activity [26, 28, 33, 50], as well as at least two distinct families of nonvolatile peptide ligands [19, 51]. The accessory olfactory system is also activated by a fraction of odorants without known pheromonal functions [29, 33, 52, 53]. For nonvolatile cues such as peptides, physical contact is required to reach the VSNs. Whether the VNO can detect volatiles in the absence of direct physical contact remains debated [29, 34], but in at least one example, clear activation of the mouse AOB was imaged *in vivo* in response to volatile stimuli that were delivered via the airstream [33].

With respect to OSNs, it came as a surprise that some of the chemosignals established earlier as sensory stimuli of VSNs are also detected by the MOE [21, 25, 33, 54]. In this context, it is perhaps most astonishing that the MOE is even involved in the detection of nonvolatile chemosignals such as MHC peptide ligands [21]. Given that social investigation in mice involves periods of robust physical contact between conspecifics during which intense sniffing and licking of facial and anogenital areas occurs, it was hypothesized that nonvolatile chemostimuli may gain access to the MOE during such behaviors [21]. Recent work tested this idea and has indeed shown that nonvolatile MHC peptides are detected by the MOE after direct physical contact. These cues stimulate a subset of OSNs and mediate specific, MOE-dependent behaviors [21]. Thus, MOE and VNO detect partially overlapping sets of chemosignals. It is because of this unexpected finding that we can now directly compare the functional attributes (sensitivity, selectivity, detection mechanism and role in species-typical behaviors) for each of these systems.

Ultrasensitive detection of chemosignals by both VNO and MOE

The mammalian VNO and MOE not only detect overlapping sets of social chemosignals, but both can do so with surprisingly high sensitivity, a result that pertains to volatile as well as nonvolatile signals [21]. Analysis of dose-response curves of single VSNs to defined stimuli first revealed an exquisite sensitivity of VSNs of the apical layer to volatile urinary pheromones, with stimulus-specific activation thresholds near or below the nanomolar range [26, 50]. Even lower thresholds were later found for responses to MHC peptide ligands in basal VSNs, with values near 10^{-12} M [19]. By contrast, OSNs have frequently been described as general chemodetectors with relatively elevated stimulus-response EC_{50} (half-maximum effective concentration) values, in the range of 10–100 μM [55]. Recent evidence, however,

main population of OSNs [2, 3, 4, 55], odorant receptors (ORs) as well as other canonical signaling proteins are densely packed in apical ciliary membranes. Ligand binding in ORs activates type III adenylate cyclase (AC III) via the G protein $G_{\alpha olf}$. In turn, an increase in ciliary cAMP opens a cyclic nucleotide-gated (CNG) channel consisting of three subunits (CNGA2, CNGA4, and CNGB1b). Cation influx and successive activation of Ca^{2+}-gated Cl^- channels (Cl^-Ca^{2+}) result in a depolarizing receptor current. This primary signal is transformed into trains of action potentials. By contrast, apical VSNs coexpress members of a multigene GPCR family, the V1Rs, with $G\alpha_{i2}$, whereas unrelated V2Rs and $G\alpha_o$ are found in basal VSNs [2, 4, 5]. Downstream products of phospholipase C (PLC) such as inositol 1,4,5-trisphosphate (IP_3), diacylglycerol (DAG) and polyunsaturated fatty acids (PUFAs) all have been implicated in VSN signaling [32, 68]. The primary transduction channels are formed, in part, by the transient receptor potential channel TRPC2 [27, 32, 62]. (*C*) Central pathways involved in the processing of chemical signals activating the MOE or VNO. Note that information from both pathways is potentially integrated in hypothalamic GnRH neurons to serve a regulatory function in endocrine control of social and reproductive behavior [15, 16, 18, 39, 40]. ACo, anterior cortical amygdaloid nucleus; AO, anterior olfactory nucleus; BAOT, bed nucleus of the accessory olfactory tract; BST, bed nucleus of the stria terminalis; Ent, entorhinal cortex; LOT, nucleus of the lateral olfactory tract; Me, medial amygdala; MPA, medial preoptic area; Pir, piriform cortex; PLCo, posterolateral cortical amygdaloid nucleus; PM, premammilary nucleus; PMCo, posteromedial cortical amygdaloid nucleus; PPC, posterior piriform cortex; Tu, olfactory tubercle; VTT, ventral tenia tecta; VMH, ventromedial hypothalamus.



shows that mammalian OSNs can also detect social chemosignals with unexpected high sensitivity. For example, MHC peptides are recognized by mouse MOE at 10^{-10} M [21]. Likewise, urinary volatiles such as 2-heptanone elicit robust sensory responses in this concentration range in mouse MOE [21]. These findings are corroborated by results from several laboratories using a variety of approaches. For instance, the olfactory receptor OR912–93 recognizes 10 nM of 2-heptanone when expressed in a heterologous cell system [56]. Similarly, OSNs expressing the odorant receptor MOR23 respond to as little as 10 nM lyral [57], and *in vivo* imaging of glomerular activity in the mouse main olfactory bulb has revealed detection of short aliphatic odor molecules in the low nanomolar range [35]. These results at the cellular level are supported by a long line of psychophysical research done in rodents and primates [58]. Thus, accumulating evidence challenges a conceptual model of low sensitivity in the main *versus* high sensitivity in the accessory olfactory system, but rather shows that both systems are capable of detecting appropriate chemosignals at very low concentrations.

Detection of the same chemosignals by sensory cells using different second messenger pathways

What are the mechanisms underlying the detection of the same chemosignals by sensory neurons in MOE and VNO? It has been known for a while that OSNs and VSNs employ distinct sets of receptors, G proteins, enzymes, second messengers, and ion channels for signal detection and amplification (see Fig. 1) [2–5, 55]. Increasing

Figure 2 Detection of social chemosignals by the mouse main and accessory olfactory systems. (*a*) The first step in this analysis is the recording of local field potentials from the cilia or microvilli of submerged whole-organ preparations of the MOE and VNO. Adapted from [69]. (*b*) Representative EVG and EOG responses to focal, 500-ms stimuli reveal an exquisite sensitivity for both volatile and nonvolatile social cues in each system (vertical scale bar, 100 µV; horizontal scale bar, 1 s). (*c*) Both optical and electrophysiological recordings from individual sensory neurons are obtained in acute VNO and MOE tissue slices. Note that the VNO slice was prepared from adult VNO (adapted from [19]), whereas the MOE slice was obtained from a postnatal day 7 mouse (adapted from [21]). The white boxes are shown at higher magnifications in (*d*). (*d*) High-resolution pseudocolor images depict the relative increase in cytosolic Ca^{2+} ($\Delta F/F,\%$) in response to MHC peptides. In both systems, distinct subsets of neurons are sensitive to AAPDNRETF (green), SYFPEITHI (red) or both peptides (yellow; only observed in VSNs). Left panel adapted from [19], right panel adapted from [21]. (*e*) Infrared-differential interference contrast video microscopy permits targeting of distinct VSN or OSN somata by patch electrodes under visual control. Sensory neuronal output, i.e. stimulus-evoked action potential discharges, can be monitored with high temporal precision as capacitive membrane currents. Left panel: vertical scale bar, 40 pA; horizontal scale bar, 1 s (left image in *e* from [26]); right panel: vertical scale bar, 10 pA; horizontal scale bar, 1 s.

evidence has now shown that sensory cells of the mammalian MOE and VNO comprise heterogeneous populations, with groups of cells displaying different molecular markers and unique patterns of gene expression (e.g. see discussion in [10]). Of special interest in this context is the identification of new subpopulations of microvillous (i.e. VNO- or taste-like) cells in mammalian MOE [59, 60]. Hence, the signal transduction mechanism and molecular identity of sensory cells involved in the detection of an identified chemosignal must be evaluated independently in each system.

This has now been done for at least two of the chemosignals that are detected by both MOE and VNO: MHC peptides and 2-heptanone. In the VNO, field potentials to MHC peptides are inhibited by 2-aminoethoxydiphenylborate (2-APB) [19]. 2-APB had been shown previously to inhibit diacylglycerol-activated cation channels that depend on a functional *TRPC2* gene, but not Ins(1,4,5)P_3-activated currents present in a subset of mouse VSNs [32]. By contrast, 2-APB did not significantly reduce the size of MHC peptide-evoked field potentials in the MOE [21]. Hence, MHC peptides are detected in the MOE by sensory cells using transduction mechanisms that are distinct from those in the VNO. This notion was further extended by showing that MOE responses to MHC peptides are inhibited by adenylyl cyclase antagonists and critically depend on a functional *CNGA2* gene [21], which encodes a principal subunit of a cyclic AMP (cAMP)-sensitive CNG channel expressed in MOE but not VNO. Furthermore, MOE responses to MHC peptides were also impaired in *CNGA4*$^{-/-}$ mice [21], which lack a modulatory subunit of the olfactory CNG channel and exhibit specific defects in odor adaptation [24, 61]. Hence, MHC peptide ligands, in the MOE, are transduced by cells employing a cAMP-signaling pathway and the canonical olfactory CNG channel.

Comparable results were obtained with 2-heptanone. VNO potentials evoked by 2-heptanone were shown to critically depend on a functional *TRPC2* gene [27], whereas MOE responses evoked by the same stimulus were absent or strongly reduced in mice lacking *CNGA2* [21, 25]. Interestingly, residual responses to 2-heptanone present in *CNGA2*$^{-/-}$ mice have been postulated to be detected by another olfactory subsystem, the GC-D cell system [25], which is thought to employ a cGMP pathway for sensory transduction [6]. This could provide yet another layer of complexity with respect to parallel processing of the same signals within the mammalian olfactory system.

Different coding strategies for detecting the same ligands in VNO or MOE

Independent evidence that distinct mechanisms are employed in the mammalian main and accessory systems for the detection of the same ligands comes from the analysis of sensory responses in individual OSNs or VSNs capable of detecting the same chemosignals. Although these investigations are not yet complete, sensory neurons in both systems might employ distinct coding strategies for detecting the same ligands. Because sensory traits such as tuning and sensitivity of a given olfactory neuron depend, in part, on the receptor molecule(s) expressed in that cell, these data would suggest that distinct receptor coding mechanisms have evolved in the mammalian VNO and MOE for the detection of the same ligands.

A comparison of tuning properties of VSNs and OSNs to the same ligands was recently done using MHC class 1 peptides [19, 21]. In the VNO, peptides specific for a different MHC haplotype generate unique VSN activation patterns in the basal, V2R receptor-expressing zone of the VNO [19]. It is known that peptides specific for a given MHC molecule share common amino acid residues, so-called anchor residues [20]. Two particular findings indicated that these anchor residues play critical roles for VSN peptide discrimination: (i) A given VSN can be activated by different peptides that share the same anchor residues but differ substantially in the other positions [19]. (ii) Mutation of anchor residues, i.e. replacement with alanines, abolished the sensory response in VSNs, even when elevated concentrations of such peptides were used [19]. By contrast, peptides with mutated anchor residues were still eliciting responses in subsets of OSNs, although these responses were shifted to higher concentrations [21]. Thus, there are clear differences in the specificity of single, peptide-sensitive OSNs and VSNs.

Similar conclusions were reached by comparing the tuning profiles of individual VSNs and OSNs to volatile urinary pheromone candidates [26, 54]. VSNs located in the apical, V1R receptor-expressing zone of the VNO were capable of detecting these molecules in a highly selective manner, with tuning curves that were concentration-independent, i.e. did not broaden with increasing ligand concentrations [26]. Consistent with such narrow tuning profiles, targeted deletion of *V1R* genes produced discrete deficits in the ability of the VNO to detect some of these molecules, while leaving responses to others fully intact [28]. By contrast, OSNs detecting the same ligands were broadly tuned, and the selectivity profiles of these cells strongly depended on ligand concentration [54]. Furthermore, OSNs exhibited highly variable thresholds and concentration-response curves, suggesting a high degree of receptor heterogeneity [54]. It therefore appears that the same chemosignals encoded by a relatively homogeneous class of narrowly tuned, non-overlapping VSNs are also encoded by a heterogeneous combination of broadly tuned OSNs. A comparative structural analysis of receptor molecules in OSNs and VSNs recognizing the same ligands could reveal a structural basis for the molecular response range of a given receptor.

Behavioral consequences

What are the consequences of such parallel sensory processing by the main and accessory olfactory systems for the modulation of species-typical behaviors? The finding that distinct signaling mechanisms are employed by the MOE and VNO for the detection of the same ligands provides a basis for a genetic lesioning approach [11, 21, 25, 27–29, 62] to address this fundamental question. Surgical lesioning [63, 64] or a combination of surgical and genetic lesioning [21] can also be applied to examine this problem. However, a note of caution will be necessary here, because each method exhibits specific limitations. For example, surgical removal of the VNO (VNX) also leads to removal of the terminal nerve system [63], and lesioning of the MOE by application of $ZnSO_4$ is often incomplete. Similarly, the use of gene-targeted animals involves a number of limitations. Deletion of the TRPC2 channel causes a dramatic loss in VSN sensitivity rather than a total loss of function [27], and deletion of the CNGA2 subunit as a method to disrupt signaling in the MOE is likely to cause additional deficits [65]. Moreover, the role of these signal transduction molecules in additional olfactory subsystems such as the GC-D cell system [6], the septal organ [7] and the Grueneberg ganglion [8–10] is not yet clear. Thus, we must be careful in the interpretation of behavioral results based on the use of either surgical or genetic lesioning alone. In most cases, only a combination of methods, together with parallel analysis at the cellular level, will provide definitive answers.

Recent work has shown that while both the main and accessory systems can detect the same set of social signals, this is not just redundant processing but activation of each system leads to distinct behavioral outcomes [19, 21, 45]. For instance, processing of MHC peptides via the main system in male mice was required for decision making in the context of a social preference test [21]. Addition of disparate peptide ligands to urine enhanced the attractiveness of female urine, an effect that depended on a combination with an animal's own genotype. Importantly, no such preference was established in mice lacking the CNGA2 subunit and surgical removal of the VNO had no effect, demonstrating the MOE-dependence of this effect [21]. No preference was established under conditions that precluded direct physical contact with the stimulus source without posing a barrier for the volatiles, ruling out that the presence of peptides somehow affected the release of volatiles [21].

By contrast, processing of the very same peptide ligands via the accessory system provided a crucial signal in the pregnancy block paradigm of female mice (the Bruce effect), where disparate but not cognate peptides caused pregnancy block when added to otherwise familiar urine [19, 20]. This effect was not observed in mice in which the VNO was surgically removed [45]. This demonstrates that recognition of MHC peptides via the MOE does not replace VNO sensory input in the context of the Bruce effect. Thus, processing of the same chemosignals through the main or accessory systems can mediate different sexual and social behaviors. It remains to be seen whether this represents a general finding that is also applicable to the function of other molecularly defined chemosignals [e.g. 36, 51]. Alternatively, some social cues might be detected exclusively by either system. In any case, we have now available the tools to assess the role of each pathway in the modification of specific behaviors by molecularly defined chemosignals.

Conclusions

By comparing the current status of the field with a previous article that addressed a similar topic in 1991 [66], it becomes clear that tremendous progress has been made within this time frame. Beginning with the work of Winans and colleagues [67], the main and accessory olfactory systems have been viewed frequently as separate pathways presumably involved in the detection of distinct sets of olfactory cues. For example, the traditional distinction was that the accessory system detects nonvolatile pheromones that trigger behavioral and endocrine responses, while the main system functions as a nonselective molecular analyzer of volatile odorants. The advent of large-scale neurophysiological recording techniques in both systems has enabled a more systematic search for sensory stimuli, allowing the identification of molecularly defined chemosignals. The long-held notion that the VNO detects only nonvolatile molecules is an oversimplification of the systems' function. VSNs show responses to a wider range of stimuli, including molecules that would not normally be categorized as pheromones. Additionally, it is now clear that many social recognition signals including some pheromones can also be detected by OSNs in the MOE [17]. The mammalian main and accessory olfactory systems detect partially overlapping sets of social signals, giving rise to a model that involves parallel processing of the same molecules in both systems. Putatively, information about the same stimuli derived from each system is integrated in higher brain centers to control hormone production. Both systems play important roles in the regulation of mammalian social behavior. Consequently, the main and accessory olfactory systems should be viewed as complementary rather than separate pathways for chemical communication [11]. Taken together, these developments add up to what has been called 'a revolution in our understanding of the role of smell in controlling the neuroendocrine brain' [18].

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