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Neural basis for pheromone signal transduction in mice

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Pheromones are specialized chemical messengers used for inter-individual communication within the same species, playing crucial roles in modulating behaviors and physiological states. The detection mechanisms of these signals at the peripheral organ and their transduction to the brain have been unclear. However, recent identification of pheromone molecules, their corresponding receptors, and advancements in neuroscientific technology have started to elucidate these processes. In mammals, the detection and interpretation of pheromone signals are primarily attributed to the vomeronasal system, which is a specialized olfactory apparatus predominantly dedicated to decoding socio-chemical cues. In this mini-review, we aim to delineate the vomeronasal signal transduction pathway initiated by specific vomeronasal receptor-ligand interactions in mice. First, we catalog the previously identified pheromone ligands and their corresponding receptor pairs, providing a foundational understanding of the specificity inherent in pheromonal communication. Subsequently, we examine the neural circuits involved in processing each pheromone signal. We focus on the anatomical pathways, the sexually dimorphic and physiological state-dependent aspects of signal transduction, and the neural coding strategies underlying behavioral responses to pheromonal cues. These insights provide further critical questions regarding the development of innate circuit formation and plasticity within these circuits.

KEYWORDS

pheromone, vomeronasal system, hypothalamus, innate behavior, neural circuit

Introduction

Animals utilize chemosensory signals, which are crucial for mediating a range of behaviors involved in survival and reproduction (Wyatt, 2014). These signals are encapsulated in biochemical compounds known as pheromones, which facilitate intraspecific communication (Karlson and Lüscher, 1959). Pheromones are principally detected by two sensory systems: the main olfactory system and the vomeronasal system (Baum and Cherry, 2015; Tirindelli, 2021). The main olfactory system is responsible for detecting volatile pheromones, while the vomeronasal system is particularly attuned to decoding social cues embedded within these signals (Holy, 2018). Such cues encompass a range of biological information, including species, sex, developmental stage, health status, and reproductive condition of conspecifics. A variety of vomeronasal ligands carrying this intricate information have been identified, indicating a complex and diverse chemosensory communication network among animals (Murata and Touhara, 2021). Vomeronasal receptors (VRs), which are a subset of the G protein-coupled receptor (GPCR) superfamily, are specialized for the detection of these chemosensory signals (Silva and Antunes, 2017).

These receptors translate the chemical information from ligands into a biological response through dedicated signal transduction pathways, ultimately leading to behavioral or physiological responses.

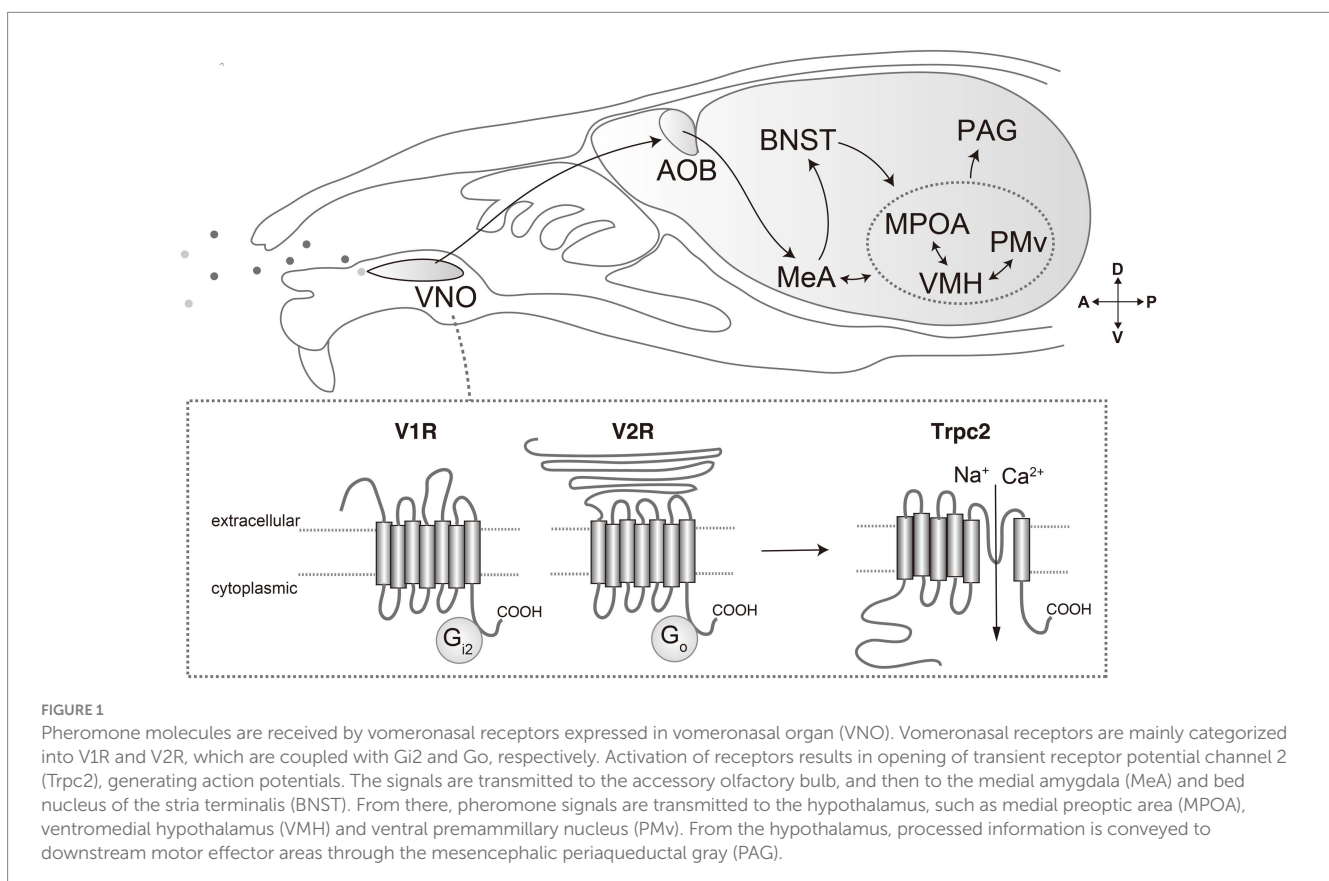
Vomeronal ligands, receptors, and functions

VRs are expressed in vomeronasal sensory neurons (VSNs) of the vomeronasal organ (VNO): a tubular organ located in the base of the nasal septum (Døving and Trotier, 1998). These receptors are categorized into two principal classes: V1Rs and V2Rs (Figure 1). Mice have approximately 240 V1R genes and 120 V2R genes (Young and Trask, 2007; Miller et al., 2020). Each of these receptor types has a distinct structure and function. V1Rs are members of the rhodopsin-type GPCR family and are typically coupled with the G protein G_{i2} (Trouillet et al., 2019). They are generally involved in detecting small molecule ligands, such as volatile compounds and steroid derivatives (Lee et al., 2019; Wong et al., 2020). In mice, V1Rs are expressed in the apical layer of the VNO and are responsible for recognizing a variety of urinary molecules that convey information about sex and physiological states (Doyle and Meeks, 2018). V2Rs belong to the class C GPCR family and are associated with the G protein $G_{\alpha o}$ (Chamero et al., 2011). V2Rs have a large extracellular domain that is thought to be involved in ligand recognition. They are primarily expressed in the basal layer of the VNO and are tuned to detect larger molecules such as peptides and proteins (Pérez-Gómez et al., 2014).

Each VSN expresses only one or a restricted few VRs, which allows for a highly specific response to particular chemosensory cues (Nikaido, 2019). When a VR binds to its ligand, it initiates a cascade of intracellular events that lead to the opening of ion channels, and ultimately results in the generation of action potentials (Yu, 2015). These electrical signals are then transmitted to the brain, where they are processed into behavioral responses.

The transient receptor potential channel 2 (Trpc2) is specifically expressed in the VNO and plays a critical role in signal transduction (Figure 1). The disruption of Trpc2 significantly diminishes the responsiveness of VSNs, leading to marked alterations in a spectrum of social behaviors, such as inter-male and maternal aggression, basically because of impairment of sex recognition (Leypold et al., 2002; Stowers et al., 2002; Kimchi et al., 2007). The role of Trpc2 in parental behavior is somewhat controversial. In one report, Trpc2-KO virgin male mice show marked reduction in pup-directed aggression, and even exhibit parental care (Wu et al., 2014). Conversely, other research underscores the relevance of Trpc2 in maternal behaviors (Fraser and Shah, 2014). Moreover, it is currently unclear whether the phenotypic manifestations observed in Trpc2-KO models are solely attributable to compromised vomeronasal signaling or whether they may also be a consequence of perturbations in the developmental processes. Indeed, some studies have implied the significance of the vomeronasal input during the development in terms of gene expression, anatomy, and behaviors (Cross et al., 2021; Pfau et al., 2023).

The ligands for vomeronasal receptors are diverse and are often species-specific molecules involved in social communication. A



prominent class of these ligands is the exocrine gland-secreting peptide (ESP) family (Kimoto et al., 2005). This group comprises species-specific peptide ligands that have undergone evolutionary divergence from the α -globin gene in rodent species (Niimura et al., 2020). ESPs are synthesized and secreted by various exocrine glands and are subsequently detected by a subset of the V2R receptor class (Kimoto et al., 2007). The differential secretion patterns of ESPs by sex and genetic strain enable the transmission of critical information regarding an individual's sex and unique identity. One notable peptide, ESP1, secreted by the extraorbital lacrimal gland of male mice, is received by Vmn2r116 and enhances female sexual receptivity and inter-male aggression (Haga et al., 2010; Hattori et al., 2016). Conversely, ESP22, found in the tear fluid of juvenile mice, acts through Vmn2r115—a receptor closely homologous to Vmn2r116—and suppresses sexual behavior in both adult male and female mice (Ferrero et al., 2013; Osakada et al., 2018).

Another significant class of vomeronasal ligands is Major Urinary Proteins (MUPs), primarily found in the urine of rodents (Hurst and Beynon, 2004). MUPs bind to and gradually release volatile molecules that are detected by the V1Rs (Leinders-Zufall et al., 2000). Concurrently, MUPs themselves act as agonists for V2Rs (Chamero et al., 2007). Since each mouse strain secretes specific patterns of MUPs and males release more MUPs than females, MUPs are implicated in behaviors associated with territorial demarcation and individual recognition (Roberts et al., 2012). Notably, MUP3 and MUP20 have been identified as having an aggression-promoting effect in mice (Kaur et al., 2014). Although the V2Rs for MUPs have yet to be identified, electrophysiological recordings from putative V2R-expressing VSNs have suggested that a diverse array of V2R subtypes interact with MUPs in a complex manner: some demonstrating specificity to individual MUPs, while others respond to multiple MUP isoforms. The mechanism by which this combinatorial receptor coding is interpreted by the downstream neural circuits is not understood.

Urine is a major source of vomeronasal ligands. The urine of female mice is rich in sulfated steroids, which are hormonal derivatives that act as ligands for V1Rs (Nodari et al., 2008; Isogai et al., 2011). Some VRs have been identified to bind sulfated steroids in a combinatorial manner. The presence of these V1R ligands in female urine may convey information regarding the female's reproductive status, such as estrous cycle phase, thereby enhancing sexual behavior in the male mouse (Haga-Yamanaka et al., 2014). On the other hand, the identification of VRs responsive to male urine has not been as fruitful. Neurons expressing Vmn2r53 in the VNO are activated by urine from males across different mouse strains (Itakura et al., 2022). Activation of Vmn2r53 has been associated with the promotion of inter-male aggressive behavior. The consistent activation of Vmn2r53 by urine from various strains of male mice suggests that its ligand is a robust marker of 'maleness' within the species.

Ligands for the vomeronasal system are not limited to species-specific molecules. For instance, hemoglobin from diverse species acts as a ligand for Vmn2r88 and has been shown to elevate digging and rearing behaviors in lactating female mice, although the ethological significance of these behaviors remains undetermined (Osakada et al., 2022).

The submandibular gland protein C (Smgc) from pups and female mice has been identified as a ligand for Vmn2r65 (Isogai et al., 2018). In virgin male mice, Vmn2r65, alongside Vmn2r88, appears to

be partially required for the exhibition of infanticidal behavior. It is postulated that activation of certain vomeronasal receptor pairs is essential for the induction of infanticidal behavior, yet the precise receptor combinations that are sufficient for eliciting such behavior have not been fully elucidated.

The neural encoding of signals from VSNs expressing narrowly-tuned VRs is hypothesized to be interpreted by downstream neural circuits in a manner akin to a labeled-line model (Tye, 2018). The elucidation of this mechanism is anticipated to significantly enhance our understanding of the neural bases of innate behaviors. Further analysis of these neural circuits may also yield valuable insights into the general principles governing sensory information processing and the resultant behavioral manifestations.

Neural basis for vomeronasal signal transduction

The vomeronasal system is characterized by its distinct neural pathway through which sensory information from the VNO is transmitted to the hypothalamus (Figure 1) (Halpern, and Martínez-Marcos, 2003; Ishii and Touhara, 2019). The initial synaptic relay occurs at the accessory olfactory bulb (AOB), where the axons of VSNs responding to pheromonal stimuli converge upon multiple glomeruli (Mombaerts, 2004; Dulac and Wagner, 2006). Here, they establish synaptic connections with second-order neurons: mitral and tufted cells. These AOB neurons integrate VR signals to encode sexual and species-specific information (Hammen et al., 2014). Subsequently, this integrated signal is propagated to higher brain areas such as the medial amygdala (MeA) and the bed nucleus of the stria terminalis (BNST), which are pivotal in modulating socio-sexual behaviors through the processing of species- and sex-specific cues (Li et al., 2017; Yang et al., 2022).

The information is further transmitted from the MeA and BNST to the interconnected hypothalamic nuclei implicated in reproductive and aggressive behaviors: the ventromedial hypothalamus (VMH), medial preoptic area (MPOA), and ventral premammillary nucleus (PMv) (Choi et al., 2005). These hypothalamic neurons are characterized by distinctive patterns of gene expression and neural circuitry, which confer selective responsiveness to various stimuli. The functional specialization of these neuronal populations has been elucidated through various experimental methodologies, such as *in vivo* neural activity recording and the application of optogenetic or chemogenetic techniques, which have substantiated the unique role of these neurons in behavioral modulation. Notably, by leveraging Ca²⁺ imaging, neurons responsive to male and female signals are shown to be largely segregated from the VNO to the VMH (He et al., 2008; Hammen et al., 2014; Li et al., 2017; Remedios et al., 2017; Karigo et al., 2021; Yang et al., 2022).

The activity of the hypothalamic nuclei extends to the midbrain periaqueductal gray (PAG), a key structure in orchestrating various survival behaviors. The PAG integrates these signals and extends projections to additional brain regions responsible for the execution of motor functions (Falkner et al., 2020; Chen et al., 2021). This forms a comprehensive neural circuit that translates the detection of pheromonal signals into appropriate behavioral responses.

Recent studies have provided significant insights into the neurobiological underpinnings of intermale aggression. Specifically, a

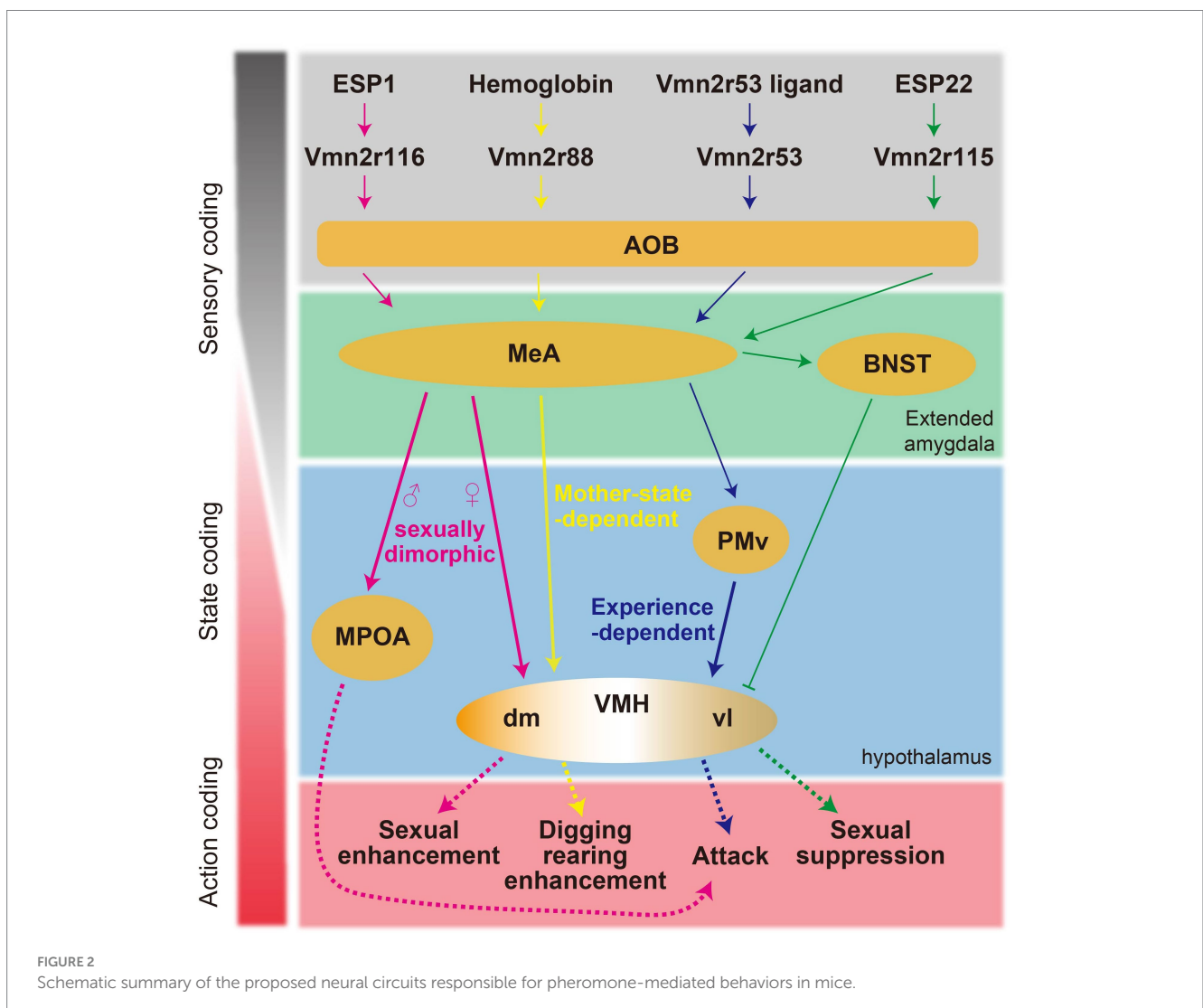
cluster of neurons located within the ventrolateral part of the VMH (VMHvl), which express estrogen receptor type 1 (Esr1) and are henceforth referred to as VMHvl^{Esr1} neurons, have been identified as critical mediators of intermale aggressive behavior (Lin et al., 2011; Lee et al., 2014). The activity and sensitivity of VMHvl^{Esr1} neurons are dynamically modulated by sexual experience, which in turn affects the neural coding of sexual cues and can lead to the onset of aggressive responses (Remedios et al., 2017).

In female sexual behavior, a complex interaction between hormonal fluctuations and pheromonal cues is important. A subset of VMHvl^{Esr1} neurons, which express Cckar but not NPY2r, are both necessary for the initiation of and sufficient to induce sexual receptivity in females (Knoedler et al., 2022; Liu et al., 2022; Yin et al., 2022). This subset of neurons exists only in females and exhibits an increase in axonal projections to the anteroventral periventricular nucleus and changes neurophysiological properties during the estrous cycle (Inoue et al., 2019; Knoedler et al., 2022). These findings highlight the essential role of hormone-sensitive neurons within the VMHvl in regulating female sexual behaviors.

ESP1 has been shown to enhance female sexual receptivity, with its neural circuitry being extensively characterized (Figure 2) (Ishii

et al., 2017). ESP1 is detected by Vmn2r116-positive VSNs, leading to the activation of the caudal part of the AOB. This triggers a cascade of neural activity that results in the activation of glutamatergic neurons within the MeA. Notably, the propagation of this neural signal does not proceed to the VMHvl as might be expected, but rather to the VMH dorsomedial part (VMHdm)—a region typically implicated in fear response modulation. Within the VMHdm, a subpopulation of neurons expressing the nuclear receptor steroidogenic factor 1 (VMHdm^{SF1}) is preferentially responsive to ESP1. These neurons exhibit a response profile that is distinct from that of neurons responding to predator odors within the same neural cluster. Experimental optogenetic reactivation of the ESP1-responsive VMHdm^{SF1} neurons can induce an increase in sexual receptivity akin to that observed naturally in response to ESP1. Conversely, the genetic disruption of VMHdm^{SF1} neurons leads to a diminution of the ESP1-induced enhancement in sexual receptivity, although basal levels of receptivity are unaffected. In stark contrast, the ablation of VMHvl^{Esr1} results in the complete abolition of sexual receptivity.

Conversely, ESP22 suppresses female sexual behaviors. This suppressive influence of ESP22 is mediated through GABAergic projections from the BNST to the VMHvl (Osakada et al., 2018).



The signaling mechanisms of ESP1 and ESP22 exemplify a labeled-line neural circuit model; however, pheromonal neural circuitry does not universally adhere to such a linear pathway (Li et al., 2017; Li and Dulac, 2018).

Neural mechanisms of pheromone-mediated intermale aggression have also been characterized by focusing on specific pheromones and receptors. Exposure to a pheromonal component that activates Vmn2r53 invariably results in the activation of progesterone receptor (PR)-expressing neurons in the PMv (PMv^{PR}) in male mice (Itakura et al., 2022). This response occurs regardless of previous social interactions. It is noteworthy that although male urine is composed of a multitude of pheromonal substances in addition to Vmn2r53 ligands, the bulk calcium responses—serving as proxies for neural activity—elicited by this individual pheromonal component are quantitatively analogous to those triggered by the complete male urine. Contrastingly, after experiencing aggression, this pheromone fraction activates PR-expressing neurons in the VMHvl (VMHvl^{PR}), which substantially overlap with the VMHvl^{Esrl} neurons. In addition, the magnitude of this response is much smaller compared to the response elicited by exposure to complete male urine. Chemogenetic suppression of PMv^{PR} function abolishes VMHvl^{PR} responses to this specific pheromonal fraction and attenuates responses to male urine. This finding implicates PMv^{PR} neurons as a pivotal upstream mediator to the VMHvl^{PR} in the neural processing of male pheromone signals. Remarkably, Vmn2r53 knockout significantly diminishes PMv^{PR} responses to male urine, suggesting an overrepresentation of Vmn2r53-mediated signaling within the PMv^{PR}. In contrast, strain-specific male pheromones such as ESP1 and MUP3, which are also implicated in the induction of aggressive behavior, do not appear to activate PMv^{PR} or VMHvl^{PR} neurons. In males, ESP1 stimulates excitatory neurons in the MeA and downstream neurons in the BNST and MPOA (Ishii et al., 2017). However, the direct causal link between the activation of these neural pathways and the potentiation of aggressive behavior remains to be conclusively determined. These studies imply the existence of redundant or parallel pathways for pheromone-mediated aggression.

State-dependent signal transduction of hemoglobin is observed. Hemoglobin activates Vmn2r88, mediated by its interaction site, Gly17, on the hemoglobin (Osakada et al., 2022). The hemoglobin signal reaches the MeA in mice, regardless of sex. However, VMHdm is selectively activated in lactating females. As a result, in lactating mothers, hemoglobin enhances digging and rearing behaviors. Manipulation of VMHdm^{SF1} neurons is sufficient to induce the hemoglobin-mediated behaviors.

Collectively, the identification of specific pheromone molecules and corresponding receptors responsible for behavioral regulations has contributed to delineating neural circuits from sensory input to behavioral output since this approach simplifies input–output relations (Figure 2).

Perspective

Research on pheromones and their behavioral impacts provides a rich field, particularly with regard to the formation and function of neural circuits inducing innate behaviors, known as ‘labeled line’ circuits. A viable starting point for dissecting these pathways is the study of VRs with identified pheromone ligands. For example, the

closely related receptors Vmn2r115 and Vmn2r116 bind to ESP22 and ESP1, respectively, yet exert opposite effects on female sexual behavior (Haga et al., 2010; Osakada et al., 2018). What variations exist in the spatial distribution of axonal terminals within the AOB’s glomerular array among VSNs expressing different VRs? Do VRs dictate projection patterns to the AOB in a manner similar to olfactory receptors? How do VSNs expressing individual VRs form synapses with mitral/tufted cells? This includes identifying whether there are distinct subtypes within the mitral/tufted cell populations based on their connectivity and gene expression. Furthermore, do mitral/tufted cells form specific synaptic connections with neurons in the MeA that correspond to discrete valences? If so, how is this multisynaptic ‘labeled line’ wired? Elucidating these mechanisms is crucial for a comprehensive understanding of the development of innate neural circuits.

The investigation into the mechanisms of sexually dimorphic circuit formation is a significant area of research within neurobiology (Yang and Shah, 2014). One promising avenue is the study of pheromone signal transduction pathways. Notably, sexually dimorphic processing of information is observed in response to ESP1, which elicits sexually divergent activations in the MeA. A critical question arises: at what developmental stages and by what processes does this sexual dimorphism manifest? Current evidence suggests that the sexually dimorphic responses observed in the MeA are heavily influenced by sex hormone signaling. This is supported by findings that male mice deficient in the enzyme aromatase, which is crucial for estrogen synthesis, exhibit disrupted sexually dimorphic responses (Bergan et al., 2014). It is hypothesized that the pathway from the MeA to the MPA or VMHdm in response to ESP1 may operate through a regulatory mechanism similar to that governed by sex hormone signaling. This pathway represents an excellent model for elucidating the precise mechanisms underlying sexually dimorphic neural circuit formation and pheromonal information processing.

How the pheromone molecule, its receptor, and subsequent signaling pathways co-evolved is an interesting question. The ESP and MUP gene families are pivotal examples, having undergone diversification predominantly via gene duplication—a well-documented mechanism in evolutionary biology (Sheehan et al., 2019; Niimura et al., 2020). Similarly, vomeronasal receptors have also evolved through this mechanism. Despite similarities within the ESP and MUP families and their receptors, there are significant functional divergences among their members, making them excellent models for studying pheromone evolution. In both insect pheromone systems and mammalian taste systems, which are well-known examples of labeled line signal transduction, the type of cell expressing the receptor determines the perceived value of the ligand (Mueller et al., 2005; Zhao and McBride, 2020). However, it remains uncertain whether a similar paradigm applies to the mammalian vomeronasal system, or whether ligand valuation is an intrinsic property of the receptor. Indeed, in the mammalian olfactory system, swapping one receptor gene for another affects the projection pattern of the given sensory neurons, which would affect the perception of the odorant, suggesting that the receptor gene itself has a deterministic factor of the value of the corresponding ligand (Mombaerts et al., 1996). This query could be empirically addressed for the vomeronasal system through targeted genetic experiments involving the Vmn2r115 and Vmn2r116 receptors. For instance, an experimental strategy might include the disruption of the Vmn2r116 gene and the substitution of its sequence

at the *Vmn2r115* locus to assess the functional outcomes on ESP1-mediated behaviors—specifically, whether it continues to facilitate sexual behavior or shifts to inhibiting such behavior, akin to the effect observed with ESP22. Should the former occur, it would suggest a decisive role of receptor functionality; conversely, the latter outcome would indicate a locational influence on gene function. The identification and comprehensive characterization of receptors for ESPs and MUPs remain incomplete. Further elucidation of the evolutionary trajectories of these ligands and their corresponding receptors could significantly enhance our understanding of molecular evolution of pheromone-mediated communication. Additionally, investigating the mechanisms through which signals from these receptors interact and integrate with those from other sensory modalities to elicit specific behavioral responses presents an intriguing area of research.

The signal transduction pathways mediated by ESP1 and ESP22 appear to support a labeled line coding system; however, tracking the neural response toward conspecific sex across different social experiences revealed that pheromone signal processing involves complex mechanisms beyond this simple paradigm. Recent studies have indicated that sexual experiences can alter the neural coding of conspecific sex cues within the MeA and VMHv1^{Esr1}. The use of various VR and ligand pairs may be instrumental in elucidating the precise mechanisms involved, given that the inputs derived from these receptor-ligand interactions are typically simple and stable. For instance, the signal transduction pathway involving *Vmn2r53*, which is influenced by aggressive behavioral experiences, might serve as an excellent model for studying synaptic plasticity between the VMHv1 and the PMv.

A variety of aggression-inducing pheromones have been identified; however, the underlying mechanisms through which these pheromones collectively influence aggressive behaviors are not yet fully understood. Notably, the MUPs such as MUP3 and MUP20, as well as ESP1, exhibit secretion patterns that are distinct among mouse strains. In contrast, *Vmn2r53* is responsive to a male-specific pheromone present in the urine across various male mouse strains, indicating that *Vmn2r53* may detect a ubiquitous male signal within the species. These findings could be pivotal for the discussion on how differentially processed pheromone signals can orchestrate uniform behavioral responses. Moreover, the interaction complexity of pheromone signals is further exemplified by certain behaviors, such as infanticide, which are elicited only in the presence of multiple pheromonal signals. Identifying the precise pheromonal blend required to initiate such complex behaviors remains an outstanding challenge in the field. Furthermore, elucidating the specific sites and mechanisms through which the vomeronasal system processes and integrates these pheromone signals is an essential area of research that warrants further investigation.

The perception of pheromones alone typically does not elicit specific behavioral responses. The presence of conspecifics and the assimilation of diverse sensory inputs, including auditory, visual, and tactile information, is requisite. Elucidating how pheromone signaling is integrated with other sensory modalities is essential for a thorough understanding of behavioral expression. This intersection of sensory integration and behavior is a pivotal area for future investigation.

The framework of sensory input to aggressive and sexual behavioral output is emerging owing to the characterization of

pheromone molecules and corresponding receptors along with the detailed functional analysis within the central nervous systems. In contrast, we scarcely know how pheromones trigger physiological responses even though these phenomena are classically reported in rodents. Since the 1950s, several pheromone-mediated phenomena that modulate reproductive function in mice have been reported. Estrus cycle is suppressed in group housed females (van der Lee and Boot, 1955), and male urine induces estrus in those females (Whitten, 1956). Male urine also accelerates puberty in female mice (Vandenbergh, 1967). Exposure to a novel male induces pregnancy failure in female mice (Bruce, 1959). The advent of modern neuroscientific methodologies, including cell-type-specific imaging and sensors for neuromodulators, offers promising avenues for deciphering the central mechanisms governing these phenomena. In summary, ongoing research into pheromonal communication is poised to yield profound insights into the neural circuitry that orchestrates behavioral and physiological adaptations.

Author contributions

KM: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. TI: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. KT: Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

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