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A Literature Review of Odor-driven Innate and Learned Behaviors

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by

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ABSTRACT OF THE THESIS

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The olfactory system is crucial for survival in mice, supporting a variety of behaviors including foraging, social interaction, and predator avoidance. Bypassing the thalamus, the olfactory bulb acts as the first sensory processing center for odors, then sending olfactory information throughout the olfactory cortex and other higher order structures. Within these networks, there also are genetically preserved pathways that support odor-driven innate attractive and aversive behaviors, connecting to structures such as the nucleus accumbens and posterolateral cortical amygdala. Moreover, structures in

the olfactory cortex like the piriform cortex displays a level of plasticity that allow olfactory learning, which is further supported by reciprocal connections with higher order structures. This review focuses on providing an understanding of the olfactory system as well as odor-driven innate and learned behaviors, with a final focus on providing an evidence-based hypothesis that the orbitofrontal cortex is involved with an odor-learning based override of innate behavior.

1. Olfactory system in Mice

1.1. Brief Overview

The olfactory system in mice is an evolved sensory-based circuit that incorporates the olfactory bulb as its first processing center and multiple other brain structures essential for rodent behavioral responses to odorants (Huilgol & Tole, 2016) (Figure 1). This system is critical in distinguishing between odors for survival-based behaviors such as finding and evaluating food, avoiding predators, and marking territory. Additionally, in conjunction with the olfactory system, the vomeronasal system assists in processing non-volatile odors and carrier associated molecules like pheromones in the environment, ensuring a high level of fitness in finding a mate and for reproducing (Martínez-Marcos & Halpern, 2009).

The olfactory system is different from other sensory systems (e.g., vision or taste) in that its neuronal projections are not gated by the thalamus. The olfactory system is the only sensory system not processed by the thalamus, but instead processed directly by the olfactory bulb, which sends projections to the primary olfactory cortex, including areas such as the piriform cortex and cortical amygdala (Courtiol & Wilson, 2015).

1.2. Odor Detection and Receptor Genes

Odorants are typically hydrophobic and volatile molecules, often found in an aerosolized form (Peterlin et al., 2008; Su et al., 2009). The detection of these odors is dependent on two main specific features: their molecular structure and concentration (Khan et al., 2007; Laing et al., 2003).

Through normal respiration or airflow during food consumption, odors travel through the nares or mouth to reach the olfactory epithelium, an aqueous fluid covered pseudostratified epithelium that is lined with many cell types, including olfactory sensory neurons (OSNs) (Choi & Goldstein, 2018). In a concentration-dependent manner, these odor molecules enter the aqueous fluid and bind to specific olfactory receptors (ORs) located on the dendritic cilia of an OSN (Jenkins et al., 2009; Sharma et al., 2019). This binding leads to a change from chemical energy to electrical energy, developing a cascade of neuronal action-potentials from the OSNs to the main olfactory bulb (Sharma et al., 2019).

It should be noted that there are about 1000 different types of ORs that are produced in a one-to-one allelic manner by the OSNs in the nasal epithelium, meaning that each neuron expresses only one OR (Buck & Axel, 1991; Takeuchi & Sakano, 2014). These ORs are majority G-protein coupled receptors (GPCRs) where specific odor binding dissociates the G protein that activates adenyl cyclase and opening of an adenyl cyclase gaged ion channel, thus generating action potentials. A minority of ORs are non-GPCRs with differing mechanisms of action (Weis & Kobilka, 2018). These ORs are also one to one with the pairs of glomeruli in the olfactory bulb, thereby, the OSNs exclusively converge to a unique pair of glomeruli, the first synapse of the olfactory pathway, creating a topographic map of odor response in the olfactory bulb (Sakano, 2010). However, with high specificity and sensitivity, the olfactory system is still able to respond to at least 1 trillion odor stimuli by having the OSNs act in combination (Bushdid et al., 2014). Multiple specific ORs will be responsive to odor creating a unique combination of activation both at the OSN level and at the glomeruli

level. The glomeruli in the OB form a map of activated OSNs. Therefore, even in the case of multiple ORs binding an odorant molecule, there is always a unique combination of specific glomeruli receiving the projections.

The vomeronasal system is specialized to detect mostly non-volatile molecules such as peptides or small proteins, which can act as pheromones or non-pheromones (Pérez-Gómez et al., 2014). Unlike the main olfactory system, detection of molecules requires direct contact between the nose and the odor source. These molecules travel into the nares and are pumped into the vomeronasal organ where they act on the vomeronasal neuroepithelium (Boehm, 2006; Pérez-Gómez et al., 2014). This neuroepithelium in mice has a non-overlapping apical layer expressing vomeronasal sensory neurons (VSNs) with vomeronasal receptor 1 (V1Rs) and a basal layer expressing VSNs with vomeronasal receptor 2 (V2Rs), which are both distinct superfamilies of pheromone GPCRs each >100 genes per family (Dulac & Axel, 1995; Herrada & Dulac, 1997; Keverne, 2002; Matsunami & Buck, 1997; Ryba & Tirindelli, 1997; Thorne & Amrein, 2003). Additionally, four different formyl-peptide receptors (VNO-FPRs) are expressed on the apical layer, while only one unique member is expressed in the basal layer (Pérez-Gómez et al., 2014). The mechanism of transduction for VSNs is different from that of ORNs, in that the G protein signal leads activation of TRPC2 ion channels to generate action potential firing.

As odorants lead to a cascade of action potentials in the VSNs, the potentiation runs parallel to the olfactory system, traveling from the vomeronasal organ to the accessory olfactory bulb (AOB) rather than the main olfactory bulb. Additionally, this signal keeps the same segregation as the neuroepithelium where apical VSNs project to

glomeruli at the anterior AOB and basal VSNs to the glomeruli at the posterior AOB, which is fundamental to discrimination (Boehm, 2006; Pérez-Gómez et al., 2014).

1.3. Olfactory Bulb

In multiple sets of literature, the olfactory bulb is divided into six layers by cell body location: the olfactory nerve layer (ONL), the glomerular layer (GL), the external plexiform layer (EPL), the mitral cell layer (MCL), the internal plexiform layer (IPL), and the granule cell layer (GCL) (Imamura et al., 2020). As the OSNs travel from the olfactory epithelium through the cribriform plate, their axons at the surface of the olfactory bulb make up the ONL. From the ONL, the OSNs' axon terminals synapse with the primary dendrites of olfactory bulb projection neurons (mitral cells and tufted cells), with additional synapses to the dendrites of periglomerular cells (GABAergic interneurons). These synapses, as well as interneurons make up sets of circular neuropil that define each glomerulus and the GL (Imamura et al., 2020; Purves et al., 2001). For the 1800+ glomeruli in the mouse olfactory bulb, however, OSN's with the same OR will input to a select few glomeruli with odor concentration also modulating how many glomeruli are activated and to what intensity (Nagayama et al., 2014; Purves et al., 2001). Additionally, the GL is topographically mapped such that the left and right hemispheres of the brain are symmetric due to genetic axonal pathfinding of the OSNs (Albeanu et al., 2018).

Following the dendrites of the tufted cells (glutamatergic neuron) leads to the next deepest layer of the olfactory bulb, the EPL where the cells' somata lies. For a tufted cell, their primary dendrites extend to a single glomerulus, while their secondary dendrites extend within the EPL and have reciprocal synapses with granule cell

dendrites (axon-less interneuron) (Imamura et al., 2020; Nagayama et al., 2014). Further, prior to outputting to the olfactory cortex, these tufted cells are directly innervated by the strong projections of OSNs, thereby increasing their sensitivity to odors (Jones et al., 2020). However, within the EPL, these tufted cells are often sparsely packed and far apart from each other (Nagayama et al., 2014).

The next deepest layer of the olfactory bulb is the MCL where the somata of the mitral cells are densely packed around each other (Nagayama et al., 2014). These mitral cells (glutamatergic neuron) synapse at a single glomerulus and act similarly to the tufted cells in that their secondary dendrites extend in the EPL and have reciprocal synapses with granule cells. However, prior to outputting to the olfactory cortex, while some mitral cells do receive input directly from OSNs, most receive odor signals through a multi-step process utilizing different types of tufted cells, lowering their sensitivity to odor (Gire et al., 2012).

The axons of both the mitral and tufted cells, along with the axon collaterals of external tufted cells that surround the glomeruli then make up the deeper IPL (Nagayama et al., 2014; Shipley & Puche, 2014). From this layer, the mitral and tufted cells' axons form the lateral olfactory tract projecting to other areas of the central nervous system (Shipley & Puche, 2014). Additionally, mitral cells can project to the whole olfactory cortex and have excitatory glutamatergic effects, while tufted cells mainly have excitatory glutamatergic projections to the anterior portion of the olfactory cortex (Imamura et al., 2020).

The somata of the granule cells define the final deepest layer of the olfactory bulb: the GCL. Although there are multiple subtypes of these cells, those that extend their

dendrites to the EPL are GABAergic and cause lateral inhibition of the mitral and tufted cells (Nagayama et al., 2014). Thereby, these granule cells provide feedback inhibition and modulate sensitivity. In addition to granule cells, deep short-axon cells (interneuron) exist in the GCL and are able to project to other layers of the olfactory bulb as well as olfactory cortices (Nagayama et al., 2014).

1.4. Accessory Olfactory Bulb

At the dorsoposterior region of the olfactory bulb lies the accessory olfactory bulb, which receives projections from the VSNs of the vomeronasal organ. Similar to the main olfactory bulb, the accessory olfactory bulb can be described using cell-body defined layers. For the accessory bulb these layers include the accessory olfactory nerve layer, the accessory glomerular layer, the accessory external plexiform layer, the accessory mitral layer, the accessory internal plexiform layer, and the accessory granule cell layer (Leinders-Zufall & Zufall, 2009; Martín-López et al., 2012; Nagayama et al., 2014). However, since this layering is less pronounced than the main olfactory bulb, some literature has defined the accessory bulb as having an external and internal cellular layer separated by the lateral olfactory tract (Larriva-Sahd, 2008).

Starting from the accessory olfactory nerve layer, the VSNs from the vomeronasal organ travel segregated in rostral and caudal segments. At the junction of these segments lies the glomeruli which are distinct from those in the main olfactory bulb (Halpern & Martínez-Marcos, 2003). Notably, contrasting OSNs, VSNs of the same receptor type will extend into multiple glomeruli rather than only a few. Additionally, the mitral cells below the accessory glomerular layer have multiple primary dendrites that extend into multiple glomeruli. The significance of this networking may be for long-term

sensory integration, processing complex blends of odors, increased sensitivity, or robust signaling (Imamura et al., 2020; X. Zhang & Meeks, 2020; Zylbertal et al., 2017). However, similar to the OSNs, the mitral cell's secondary dendrites also make dendrodendritic synapses with the granule cells, furthering the complexity of the bulb's signaling pathway. Lastly, to leave the accessory olfactory bulb, the mitral cell axons converge at the lateral olfactory tract to reach higher order brain structures (Imamura et al., 2020).

It is believed that the mitral cells are the only main projection neuron in the accessory olfactory bulb, however there are conflicts in the literature (Imamura et al., 2020; Kang et al., 2011; Larriva-Sahd, 2008). For instance, at least three types of projection neurons with different characteristics from mitral cells have been identified in the accessory olfactory bulb (Imamura et al., 2020). However, there is also evidence for mitral cells having highly heterogeneous subpopulations (Imamura et al., 2020; Zylbertal et al., 2017)

1.5. Convergence of Main and Accessory Olfactory Bulb

Although the accessory olfactory bulb is distinct from the main bulb, these structures do work together, and their projections meet at many locations. One of the earliest sites noted is located at the most rostral part of the accessory bulb where efferent neurons from the main bulb converge with principal cells and bulbar interstitial neurons of the accessory bulb (Larriva-Sahd, 2008; Mucignat-Caretta et al., 2012). Another higher order area is the amygdala where the medial amygdala is a major target for the accessory olfactory bulb projections, however it also receives input from the main bulb (Mucignat-Caretta et al., 2012).

1.6. Olfactory Cortex and Further Projections

As odor information is processed and glomeruli are activated, a topographic map is formed (Mori & Sakano, 2024). This map makes the olfactory bulb the first significant relay station to the olfactory cortex, which leads to higher order processing and behavior through multiple intermediate structures (Chon et al., 2020; Igarashi et al., 2012). Starting from the anterior cortex where both tufted cells and mitral cells can project to, the areas included in this region are the anterior olfactory nucleus, the anterior piriform cortex, and the olfactory tubercle. The posterior cortex that only mitral cells project to are the Tenia tecta, the posterior piriform cortex, the lateral entorhinal cortex, peri-amygdaloid cortex, and the cortical nucleus of the amygdala. Additionally, the olfactory cortex further projects to the striatum, pallidum, orbitofrontal cortex, thalamus, hypothalamus, and hippocampus. Each one of these projections helps elicit important perceptual features and behaviors to odor, and below in the next chapters a few of these pathways will be discussed further.

In regards to the accessory olfactory bulb, this structure bypasses many of the intermediates the main bulb goes through, and instead makes direct projections to higher order brain structures. For instance, the accessory bulb sends axons to the medial and posterior medial cortical amygdala, barely utilizing the piriform cortex (Kang et al., 2011).

2. Innate Response to Odor

2.1. Brief Overview

Prior to learning and other experiences, naïve animals will still have species-specific patterns of responses to stimuli in the environment that can be crucial for survival. The sensory experience of these animals to the environment leading to an unconditioned response are called innate behaviors. For mice, these innate behaviors include attractive (e.g., self-grooming or reproduction), appetitive (e.g., food seeking or avoiding), and aversive (e.g., fear or freezing) responses. As these behaviors are embedded in a mice's biology, it is likely that they are mediated by evolutionarily conserved neural circuits that are genetically determined during development. Further, these "hard wired" neural circuits are likely distinct and carry a robust signal from receptor to specific brain structures (Figure 2). Since smell is essential for feeding, social behavior, and predator avoidance, these odor-driven innate behaviors suggest that the olfactory system has developed similar preserved circuitry, utilizing both the accessory and main olfactory bulb.

It should be noted that although this chapter is focused on innate behaviors, learned behaviors can have major impacts on the performance of innate behaviors.

2.2. Odor Receptors for Innate Responses

Odors often lead to highly distributed activity in the main olfactory bulb where receptors in the nares are redundant, allowing odors to be represented in many unique ways. In the context of innate behavior, several experiments have been conducted to understand if olfactory receptors on OSNs are utilized the same way as other odors.

In Dewan et al., the team focused specifically on the 15 Trace Amine-Associated Receptors (TAAR) genes in mice, where 14 of them are involved in the main olfactory system. These genes encode olfactory receptors that have trace amines as ligands, and in the context of innate behavior, bind to volatile amines found in predator urine, sweat, and other secretions. Further, post-binding, recognition of many of these amines should lead to innate aversion behaviors. In Dewan et al., the 14 TAAR genes involved in the olfactory system were knocked out, which eliminated aversion to a diverse set of high-sensitivity structural amines and ethologically relevant predator urine (Puma), while non-amine odor responses were unaffected (Dewan et al., 2013). Additionally, the team examined the effect of genetically removing a single TAAR gene (sensitive to PEA and predator cat urine) rather than all 14, and saw that when the TAAR4 gene was replaced with YFP, aversion was eliminated to predator urines (Puma and Lynx), while other amine odor responses were unaffected. The results of this study suggest that at least for amines implicated in innate aversive behavior, their receptors act in a non-redundant manner differentiating them from other odor receptors (Dewan et al., 2013). A few limitations of this study however are that certain silenced TAAR genes have been implicated in attractive behavior rather than aversion and the TAAR olfactory subsystem has less variety of receptors than other subsystems.

In Jiang et al., the team further investigated OSNs in the context of innate behaviors to ethologically relevant predator odors (2,5-dihydro-2,4,5-trimethylthiazoline [DMP]), focusing specifically on 4-transmembrane protein CD20 (MS4A1), which encodes non-GPCR receptors for compounds (e.g., nitrogenous heterocyclic compounds, long-chain fatty acids, and steroids) in mouse predator secretions. When CD20 was knocked out in

mice, the team found that these mice responded differently to predator odor DMP than wild type mice (Jiang et al., 2024). Since other GPCR olfactory receptors respond to similar MS4A1 receptor ligands, the team believes that this finding suggests the MS4A1 pathways evolved to promote certain innate behaviors, rather than bind specific compounds (Jiang et al., 2024).

Certain studies have also looked at the combinatorial effects of odor leading to innate behavior rather than focusing on individual olfactory receptors. In Saraiva et al., further working with TAARs, where different ligands lead to different behavioral responses (neutral, aversive, attractive), the team showed that in binary combinations of attractive and aversive odors, the individual effect of each odor on the mouse's behavior can be cancelled out and without receptor antagonism. Additionally, the team showed that a TAAR ligand or a common odorant causing activation of a specific TAAR can lead to behavioral blocking of a mouse's aversive response to predator odors, suggesting that innate odor responses can be context-dependent and other receptor signals can impact its "hard wired" circuitry (Saraiva et al., 2016). The work in Horio et al., instead focusing on the effect of multiple olfactory receptors activating, is in line with these findings as their team utilized Olfr288 receptors and (Z)-5-tetradecen-1-ol (Z5–14:OH) odor to further understand attractive (low Z5–14:OH concentration) and aversive (high Z5–14:OH concentration) behavior in mice. In this study, Olfr288 knockout mice did not show attraction in their respective odor concentration, however the mice still showed aversion, meaning activation of the other low-affinity Z5–14:OH receptors caused this behavior (Horio et al., 2019). These findings suggest that the final response to odorants,

even in the case of innate behavior, is influenced by the summation of attractive/aversive valences from respective olfactory receptors.

2.3. Glomeruli in Innate Responses

For most odors, each glomerulus corresponds to a unique odor receptor and activation of receptors elicits a unique combination of glomeruli activation. In Kobayakawa et al., additional research was done to understand how innate versus other behaviors' odor processing was done with a focus on the glomeruli level. In this study, the dorsal-zone specific O-MACS promoter was used to express the diphtheria toxin gene at specific locations to ablate class I odorant receptors in the dorsal region of the olfactory epithelium. After confirmation of ablation via in situ hybridization and RT-PCR, the absence of glomeruli in the anterodorsal glomeruli, which the class I receptors project to, was confirmed via immunohistochemistry (posteroventral region of the olfactory bulb was still occupied). With this mouse group, the team saw a lack of innate avoidance behavior during olfactory preference and avoidance tests, including no avoidance to predator odor (Kobayakawa et al., 2007). Further, the mice were still able to discriminate between predator odors and other odorants and were able to associate predator odor to a sugar reward, while wild-type mice could not. Additionally, these mice could be conditioned for aversion, although no innate aversion was observed. This research suggests, that at least for aversive innate behaviors, there are distinct glomeruli that process this information that are separate from glomeruli implicated in learned behaviors. This finding also further specifies dorsal subdomains 1 (D1) and 2 (D2) where D2 has distinct glomeruli for predator odors and D1 has distinct glomeruli for

spoiled food odor, suggesting functional compartmentalization (Igarashi et al., 2012; Mori & Sakano, 2011).

In line with Kobayakawa et al., other research has also shown that there are distinct glomeruli in the ventral domain of the olfactory bulb for innate attractive behaviors that are distinct from those for learned behavior (Mori & Sakano, 2024).

2.4. Olfactory Amygdala (Posteromedial and Anterior Medial Cortical Amygdala)

After odor travels from OSNs to the glomeruli, olfactory output neurons will carry the signal to higher order brain structures. For innate behaviors, many of these responses are carried from the ventral and dorsal domains of the olfactory bulb where a set of mitral cells will project to specific amygdaloid structures (Mori & Sakano, 2021). Further, although the olfactory bulb utilizes both mitral and tufted cells as the main projection neurons, for odor decisions innate behaviors utilize mitral cells while learned behaviors utilize mitral and tufted cells. A possible explanation for this may be that because mitral cells are the earliest forming projection neuron in the olfactory bulb, they lay the groundwork for innate behaviors and odor-valence (Mori & Sakano, 2024). Additionally, from the dorsal-ventral axis of the olfactory bulb, the Nrp2/Sema3F system guides mitral cells from glomeruli to specific amygdaloid nuclei (Nrp2- for aversive; Nrp2+ for attractive).

For aversive behaviors, the mitral cell pathways go from the dorsal region of the olfactory bulb to the posteromedial cortical amygdala (CoA). At the CoA, synapses are formed with a specific set of neurons that lead to aversive innate behavior, commonly named negative-valence cells. For attractive social behaviors, the mitral cell pathways

instead go from the posteroventral region of the olfactory bulb to the anterior medial amygdala (MeA). At the MeA, the synapses formed also differ from the aversive pathway in that they are formed with a specific set of neurons leading to attractive innate behaviors, commonly named positive-valence cells. Additionally, this nucleus of the amygdala is a major center receiving input from both the olfactory bulb and the accessory bulb in distinct circuitry (Keshavarzi et al., 2015).

In regards to the respiration cycle, which is functionally related to odor perception, both aversive and attractive odor-based processing occur during the later stages of respiration, while processing such as for learned behavior occurs during the earlier stages (Mori & Sakano, 2024). This likely is related to how, for both nuclei of the amygdala, there are specific networks of these valence cells (positive/negative) for innate and learned behaviors (Mori & Sakano, 2021). Further, there likely is a level of cross-talk between specific networks, leading to outputted behavior.

2.5. Posterolateral Cortical Amygdala

In addition to the previous amygdaloid nuclei, the posterolateral cortical amygdala (plCoA) has also been a source of interest for its significance in innate behaviors. This structure is stereotypically mapped where glomeruli, preferentially in the dorsal region of the olfactory bulb, project to the plCoA in a spatially-segregated manner (Iurilli & Datta, 2017; Sosulski et al., 2011). Further, projections from the olfactory bulb to the plCoA are done in glomeruli-specific subdomains, suggesting genetically determined wiring.

For certain aversive and attractive odorants, the plCoA is required for innate behavior output, including 2, 3, 5-trimethyl-3-thiazoline (TMT) from fox secretions and

2-phenylethanol (2PE) in rose water (Root et al., 2014). Across the anterior-posterior axis of the plCoA, aversive odors such as TMT activate neurons across the entire plCoA (majority in anterior plCoA), while activation by appetitive odors are only seen in the posterior plCoA (majority in caudal third plCoA). Additionally, neutral odors not ethologically relevant show activation similar to appetitive odors.

In the Root lab, prior research showed that the anterior portion of the plCoA under select activation is enough to elicit aversive behavior, while the posterior portion cannot (Lee, 2019). These results suggest a level of functional organization for the plCoA when driving innate behavior. Further, research in the Root lab has shown that the anterior plCoA has projections to the medial amygdala, which has been implicated in aversive behavior, and the posterior plCoA has projections to the nucleus accumbens, which has been implicated in appetitive behaviors. This circuitry likely contributes to odor processing and final output of innate behaviors.

3. Olfactory Learning

3.1. Brief Overview

Throughout a rodent's life span, learning is essential to survival, helping learn social cues and adapt to the environment. For instance, although mice show innate behaviors in mating and fighting, there is a social-experience based component that is required for promoting the attack response to male mice intruders versus female mice intruders (Remedios et al., 2017). Another example would be in the case of finding resources where a mouse would learn and create memories regarding where food is, then later adapt based on changes in the season and weather.

As mice rely on their olfactory system throughout their lives, learning also is done through olfaction, where certain odors can hold different meanings or promote different behaviors. For example, although predator odor is innately aversive to mice, the physical act of avoiding the area can be a learned component of the fear response. Additionally for foraging, learned odor identification could lead to areas with safe food and keep the mouse from consuming harmful substances a second time.

In neuroscience experiments, there are many types of behavioral experiments that are used to perform olfactory learning prior to looking at the connectivity in the brain. These experiments include fear conditioning, appetitive conditioning, odor discrimination, sensitization, override of innate behavior, second-order conditioning, and contextual odor learning. It is believed that the olfactory cortex is the main area where odor memory is formed and that inputs from other brain structures lead to added valence (Figure 3).

3.2. Piriform Cortex Anatomy and Connectivity

The piriform cortex (PC) is the largest structure in the olfactory cortex and is divided into 3-layers, similar to other paleocortical or allocortical structures (Martin-Lopez et al., 2019). The most superficial layer is considered the input layer, which includes the lateral olfactory tract in the outermost regions, and is split into layer Ia and Ib. Below the lateral olfactory tract is layer Ia, which sparsely contain mitral and tufted cell axons that traveled from the olfactory bulb through the lateral olfactory tract, but the layer is mainly occupied by neuropil (K. R. Illig & Wilson, 2014; Martin-Lopez et al., 2019). Layer Ib is similar in that it is mainly occupied by neuropil, however it additionally contains horizontal, globular neurogliaform, multipolar cells, and associative axons from neurons in the PC, neurons from the ipsilateral cortical structures (e.g., anterior olfactory nucleus), and commissural axons from the contralateral olfactory cortical structures (K. R. Illig & Wilson, 2014; Martin-Lopez et al., 2019).

Moving deeper to layer II, this layer contains the majority of the PC's principal cells and they are densely packed throughout the layer. Further, the superficial half and the deeper half of layer II can be divided into sublayers IIa and IIb. In layer IIa, there are semilunar cells, which are pyramidal-type cells without basal-dendrites, while in layer IIb there are superficial pyramidal cells, which are similar to the pyramidal neurons found in the hippocampus (Suzuki & Bekkers, 2006). Other than morphological differences, these two principal cells also differ in strength and connectivity, where semilunar cells have stronger input and make synapses with superficial pyramidal cells without recurrent synapses, while superficial pyramidal cells have lower strength input and are recurrently connected (Mazo et al., 2017). The next deepest part of the PC is layer III

where the cell bodies of deep principal cells, GABAergic neurons, multipolar cells, and associational fibers from the PC and other areas fill the space (K. R. Illig & Wilson, 2014; Mazo et al., 2017). Throughout all layers, however, there are several classes of inhibitory interneurons that contribute to odor processing and are involved in feedforward/feedback synaptic inhibition of the semilunar and superficial pyramidal cells in layers II to III (Bekkers & Suzuki, 2013). In addition to these three layers, some call the endopiriform nucleus layer IV of the PC as it is occupied by multipolar neurons, is highly interconnected to the PC, and although it has no direct olfactory bulb input, it is a olfactory afferent to the thalamus (K. R. Illig & Wilson, 2014).

The PC, in addition to being divided into layers, is commonly divided into at least 5 different areas with the anterior piriform cortex (aPC) and posterior piriform cortex (pPC) being the most common split since the PC receives different inputs across anterior-posterior axis. The aPC receives more olfactory bulb inputs as compared to the pPC, however, the pPC has more associational inputs (Bekkers & Suzuki, 2013). Further, the pPC's minimal input from the olfactory bulb is majority mitral cells, while the aPC receives mitral cell and tufted cell input. However, regarding tufted cells, the ventral region of the aPC is unique in that it is one of the only PC areas that receives input from middle/internal tufted cells (K. R. Illig & Wilson, 2014; Mori & Sakano, 2021). These differences across the anterior-posterior axis are reflected in the PC layering as well since layer Ia has less width in the pPC compared to the aPC, while layer Ib is wider in the pPC.

The unique combination of glomeruli activation from the olfactory bulb as well as the specific projections to the PC convey spatial organization for odors, which is

subsequently removed once the mitral/tufted cells synapse at the PC. Recent research has suggested that after removing the original organization, the PC encodes odor representations in complex distributions instead (Bekkers & Suzuki, 2013; Martin-Lopez et al., 2019). One supporting finding comes from the spatial and temporal differences between mitral/tufted cells. Spatially, both the mitral/tufted cells project to different areas of the PC depending on the odorant and there is evidence of diffuse mapping throughout the PC after synapsing. Temporally, mitral/tufted cells activate at different times of the respiration cycle, have different frequencies and type (e.g., burst) of firing, and have different sensitivities to odor. In addition to mitral/tufted cells, *in vivo* calcium imaging showed that in response to odorants, pyramidal cells of the PC activate in a unique pattern and single pyramidal cells can activate to various types of odorants, further supporting odor representations through complex distributions (Bekkers & Suzuki, 2013; Stettler & Axel, 2009).

The PC's computational ability has been considered a result of its large network of synaptic connections with the olfactory bulb and many other cortical and limbic structures, which include inputting into the PC and receiving outputs from the PC. In addition to the dense inputs of the main olfactory bulb and the accessory olfactory bulb, the aPC receives dense cortical and thalamocortical inputs, including from the orbitofrontal cortex, anterior olfactory nucleus, frontal cortex, and medial pre-frontal cortex (integrates information from thalamus) (Russo et al., 2020; L. Wang et al., 2020) (Figure 4). Compared to the aPC, the pPC receives more inputs from the limbic system, which include the lateral entorhinal cortex (integrates information from hippocampus), hippocampal formation, and amygdala (Russo et al., 2020; L. Wang et al., 2020).

Regarding outputs, the aPC sends projections to many regions of the brain, including the dorsal aPC, the pPC, the anterior olfactory nucleus, the orbitofrontal cortex, the olfactory tubercle, the lateral entorhinal cortex, the insular cortices, and the cortical amygdala (Mazo et al., 2017; Mori & Sakano, 2021). The pPC is similar in that it has a very wide range of outputs throughout the brain, including many of the structures the aPC projects to (Johnson et al., 2000).

3.3. Piriform Cortex in Olfactory Learning

Based on the inputs to the PC, this structure is assumed to process information from direct olfactory input, while also integrating sensory information from indirect thalamic projections, emotional information from areas like the amygdala, and memory information from indirect hippocampal projections (L. Wang et al., 2020). Therefore, it is believed that the PC is a major center for olfactory learning.

As the olfactory bulb inputs move through the lateral olfactory tract, the odor information is randomly synapsed on the PC pyramidal neurons' dendrites. Research has shown that once the projection neurons from the lateral olfactory tract activate neurons of the PC, it strengthens intracortical excitatory recurrent synapses, forming "odor-specific ensembles" in the PC that is specific to the lateral olfactory tract inputs (Kumar et al., 2021). Further, through associative long-term potentiation and spike-timing-dependent plasticity (STDP) mechanisms, repeated exposure to the odor leads to stronger activation of these intracortical excitatory inputs. For these connections, there is also evidence of N-methyl-D-aspartate [NMDA]-spike-based plasticity where specific synapses are self-potentiated through an NDMA-spike mechanism leading to branch-specific odor encoding (Kumar et al., 2021). Evidence of both intracortical

excitatory synapse and NMDA-spikes support that the PC exhibits a level of plasticity that can lead to the generation of odor representations/odor memory, which are crucial in olfactory learning. This local network of branch-specific odor representation likely interacts with other brain structures to further integrate other facets of olfactory learning such as valence or contextual effects.

As the aPC and pPC have anatomical differences, research suggests that this difference is also related to each region's function for both odor memory and olfactory learning. For the aPC, this region is believed to be involved in odor identity/discrimination, while the pPC is involved in odor similarity or odor quality (L. Wang et al., 2020). Upon presentation of a complex mixture, the aPC works to encode the components of the mixture, however, as the mixture becomes more familiar the aPC instead becomes further tuned to the mixture identity rather than component identities, showing a decorrelated population response (Kadohisa & Wilson, 2006). The pPC differs in that on presentation to the complex odor mixture, it encodes odor quality of the components, however as the mixture becomes more familiar the pPC has a stronger correlated population response as the quality of the mixture and respective component are likely encoded similarly (Kadohisa & Wilson, 2006).

3.4. Olfactory Tubercle and Reward-based Learning

The olfactory tubercle along with the nucleus accumbens make up the ventral striatum, a region of the brain known to be involved with rewards and reward-based learning as well as having GABAergic principal neurons rather than glutamatergic (Haber, 2011). The olfactory tubercle receives strong inputs from the olfactory bulb, amygdala, and prefrontal cortex as well as many reciprocal connections with the

olfactory cortex (Murata et al., 2015). Further, this structure also receives strong dopaminergic input from other areas of the ventral tegmental area.

In Murata et al., the olfactory tubercle was shown to have large levels of c-fos activation post odor exposure for mice that were trained to an odor associated sugar reward or foot shock. For the mice that were trained to approach for the sugar reward, the team saw that regardless of odor type, medium spiny neurons with dopamine receptor 1 were highly activated in the anteromedial region of the olfactory tubercle. For mice that were trained to the foot shock and showed aversive behaviors, the medium spiny neurons with dopamine receptor 1 were highly activated in the lateral regions of the olfactory tubercle instead (Murata et al., 2015).

In Zhang et al., the connections between the ventral tegmental area and the medial olfactory tubercle were further explored. Through tracing experiments, the team first identified dopaminergic projections from the ventral tegmental area to the medial olfactory tubercle. These connections were then optogenetically stimulated with a neutral odor, where mice formed a learned preference. Upon inhibition of these connections, the mice then struggled to form learned preferences to new odors, and where unable to show prior conditioned odor-preference behaviors. These research findings suggest that the connections between the ventral tegmental area and the medial tubercle are involved in acquiring learned odor preferences and reward processing (Z. Zhang et al., 2017).

Millman et al. further supports these findings and suggests that rather than reward processing being completed within the olfactory cortex, it is done within the olfactory striatum, which include the olfactory tubercle and nucleus accumbens. Using single cell

recording, the team observed that there was an “explicit representation” for odor-reward conditioning in the olfactory tubercle that appears very quickly (several minutes). However, for the pPC, single cell recording did not show any explicit representation during both normal training and overtraining (several weeks) (Millman & Murthy, 2020).

3.5. Amygdala Nuclei and Fear Conditioning

The amygdala is a structure that has been highly implicated in valence coding with several nuclei that are essential for olfactory learning like fear conditioning. Within the MeA and CoA lies a set of positive-valence and negative-valence cells, respectively, that are specific for attractive (e.g., reward-based) and aversive (e.g., fear-based) behaviors. These networks of valence cells provide excitatory input into the aPC that is received by either positive-valence scene cells or negative-valence scene cells (Mori & Sakano, 2021). It is believed that since these scene cells only show activity in their respective attractive or aversive scene, they are integrated into PC odor processing to assign context-based valence.

Similar to the CoA, the basolateral amygdala (BLA) has been shown to be essential for fear conditioning (threat/safety) and production of the odor-based behaviors. Through field potential recordings, the BLA outputs to the pPC were shown to have stronger functional connectivity post fear conditioning (East et al., 2021). Then, through optogenetic silencing of the BLA, mice were no longer able to complete fear conditioning, and through lesions of the pPC similar results were seen (East et al., 2021). Silencing of the specific BLA-pPC connections also did not allow mice to produce conditioned behavior, and instead showed generalized behavior patterns.

The lateral entorhinal cortex is another structure that projects to the pPC and has been implicated in fear conditioning. Through chemogenetic inhibition of CaMKIIα+ of the lateral entorhinal cortex, mice were unable to encode negative valence through fear conditioning, while during inhibition of the pPC, conditioned mice were unable to recall fear memory and produce the conditioned behaviors (Liu et al., 2023).

3.6. Orbitofrontal Cortex

The orbitofrontal cortex has multiple functions and is sometimes called the secondary or tertiary processing center for sensory stimuli, including odor, taste, and vision. Further, these functions are compartmentalized with the most common division of the cortex being the medial, ventral, ventrolateral, and lateral orbitofrontal cortex as well as the anterior agranular insular cortex (Sarlitto et al., 2018). In total, the orbitofrontal cortex, as a higher order brain structure, has been implicated in an extremely large number of cognitive processes such as risk-assessment, value-assignment, reward processing, and emotional processing.

Regarding primary olfactory information input, there have been many experiments done showing olfactory cortex input to the orbitofrontal cortex. Anterograde tracers injected into the orbitofrontal cortex showed that there were dense projections to the ventrolateral region from the rostral and caudal portions of the ventral aPC, and fewer projections from the caudal portion of the dorsal aPC (Kurt R. Illig, 2005). Retrograde tracing has also shown non-uniform projections across the anterior-posterior axis of the PC to the agranular insular cortex and the lateral orbitofrontal cortex, with some research further specifying that the PC input into the ventrolateral and lateral regions is only through the aPC (Chen et al., 2014; Clugnet & Price, 1987). Injection experiments

have shown evidence that the pPC has reciprocal connections with the ventral agranular insular cortex as well (Kurt R. Illig, 2005).

Prior lesion experiments of the orbitofrontal cortex have implicated this structure in olfactory learning. The earliest lesion experiments on this structure first suggested that the orbitofrontal cortex was important for response inhibition, and specifically for mice, simple discrimination learning. Upon further experimentation, it was found that animals with lesions showed deficits mainly in reversal learning phases, and could perform similarly to controls in new problems with the go, no go odor discrimination test (Schoenbaum et al., 2002). Through a later 2-odor discrimination problem applying the go, no go test (thirst-based), mice with cortex lesions were then found to have deficits from an initial reversal of their trained behavior, and a second reversal back to the original trained behavior (Schoenbaum et al., 2003). These findings suggest that the orbitofrontal cortex in mice is needed for learning reversals of conditioned behaviors, and possibly for changing behaviors to different variants of cues/outcomes.

Towards simple discrimination association in mice, recent research has suggested that the orbitofrontal cortex is required to establish odor value in mice. Through 2-photon imaging, strong activity was seen in the orbitofrontal cortex in response to a conditioned stimulus after learning (P. Y. Wang et al., 2020). Further, projections from the PC to the cortex could produce conditioned odor behaviors after training, implicating the orbitofrontal cortex in appetitive associations. Neuron recording for a go, no go odor discrimination test also supported these findings as during learning and the reversal phase, the orbitofrontal cortex showed plasticity in many cue-selective neurons (Roesch et al., 2007).

Given this information about the orbitofrontal cortex, we hypothesize that this structure could additionally function in the override of innate behaviors through learning. Firstly, the orbitofrontal cortex is in an optimal position of the olfactory circuit to incorporate olfactory information from its many reciprocal connections to the PC (Figure 6). Moreover, upon association learning, the cortex shows a large level of plasticity likely using the odor identity represented in the PC. Secondly, the orbitofrontal cortex has reciprocal connections to many structures in the limbic system, including the basolateral amygdala (Zimmermann et al., 2017) (Figure 6). Therefore, valence information from the amygdala could be incorporated and reassign odor value similar to the cognitive processes in reversal learning. Lastly, preliminary optogenetic experiments from the Root Lab have shown that upon inhibition of the orbitofrontal cortex, water deprived mice in a two-port water experiment (one port releasing TMT and large water reward, the other releasing 2PE and minimal water) that were conditioned to the TMT port revert to their innate attraction of the 2PE port (Figure 5).

Previously, I was investigating this hypothesis through several different experiments. The experimental plan was to first bilaterally inject retrograde tracers into the medial amygdala and nucleus accumbens to show a clear pathway from the orbitofrontal cortex to these areas that have been implicated in aversive and attractive innate behaviors, respectively (Figure 6). Then, using the two-port water experiment noted in the previous paragraph, I would train two groups of water deprived mice, where one group goes through the odor-based training (TMT large water reward, 2PE low water reward) and the other group is not exposed to odor from the ports, but learns that one port has a different probability of producing water (one large water reward, one low water reward).

This training would take one week, and afterward mice would be exposed to TMT odor within an individual housing cage and be processed for quantifying cfos expression as a proxy for neural activation. The brain regions of interest would be the medial amygdala and nucleus accumbens to see how they are involved in this training paradigm. After this experiment, we would then repeat the 2-port water experiment with water deprived mice, however instead both mice groups would go through odor-learning, with one group prepared for optogenetic silencing of the orbitofrontal cortex via halorhodopsin. Post-training (week) and recording performance, laser will be applied to the optogenetic mice group during the 2-port water experiment to confirm if the mice lose their preference to TMT (large water reward prediction) and revert to their innate attraction for 2PE (low water reward). Additionally, later both groups of mice will be placed within individual housing cages where the control group and laser-applied experimental group will be exposed to TMT odor, then processed for quantifying cfos expression. We hope to observe that the differences in orbitofrontal cortex activation, along with the medial amygdala and nucleus accumbens findings will suggest that the orbitofrontal cortex is involved in the override of innate behavior.

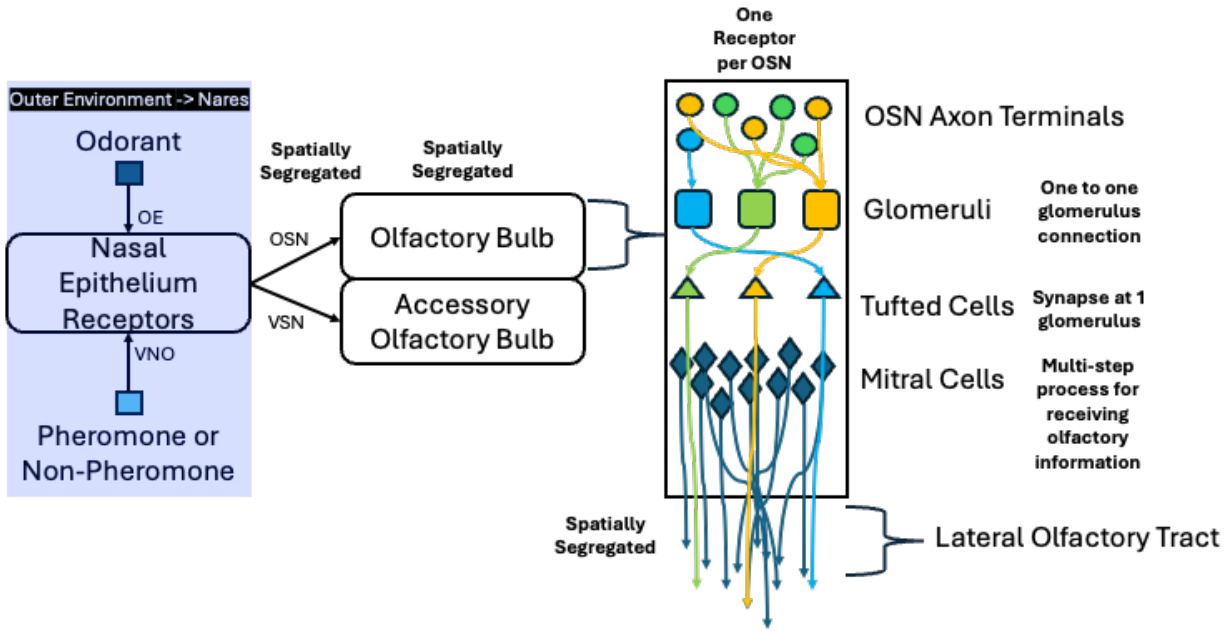

Figure 1. This figure is a summary of structure connectivity starting with the outside odor environment and going to the olfactory bulb.

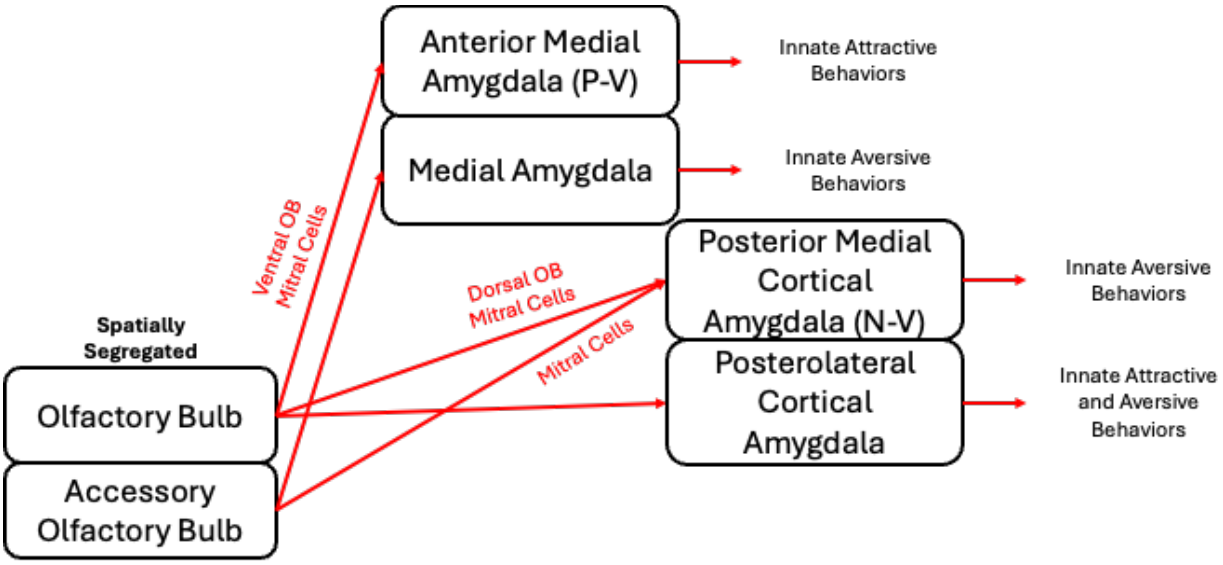

Figure 2. This figure is a summary of structure connectivity found during the literature review of odor-driven innate behaviors.

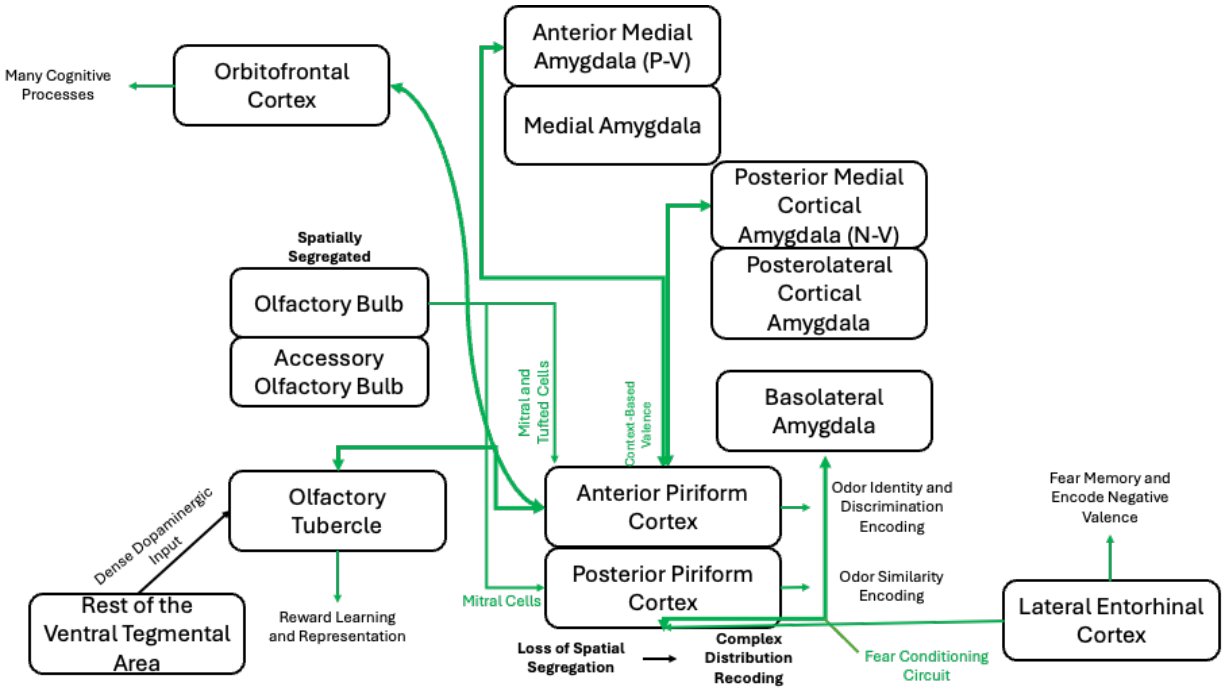

Figure 3. This figure is a summary of structure connectivity found during the literature review of olfactory learning.

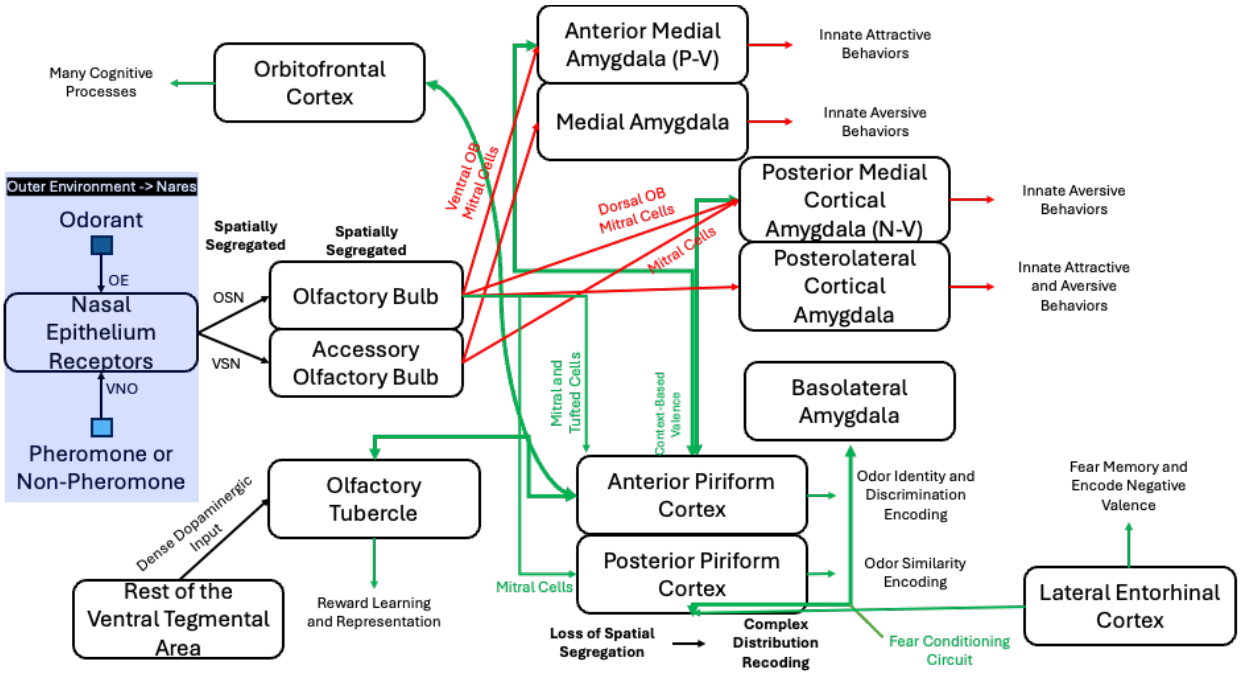

Figure 4. This figure is a combination of all structural connectivity found during this literature review, going from odorant exposure to innate and learned pathways.

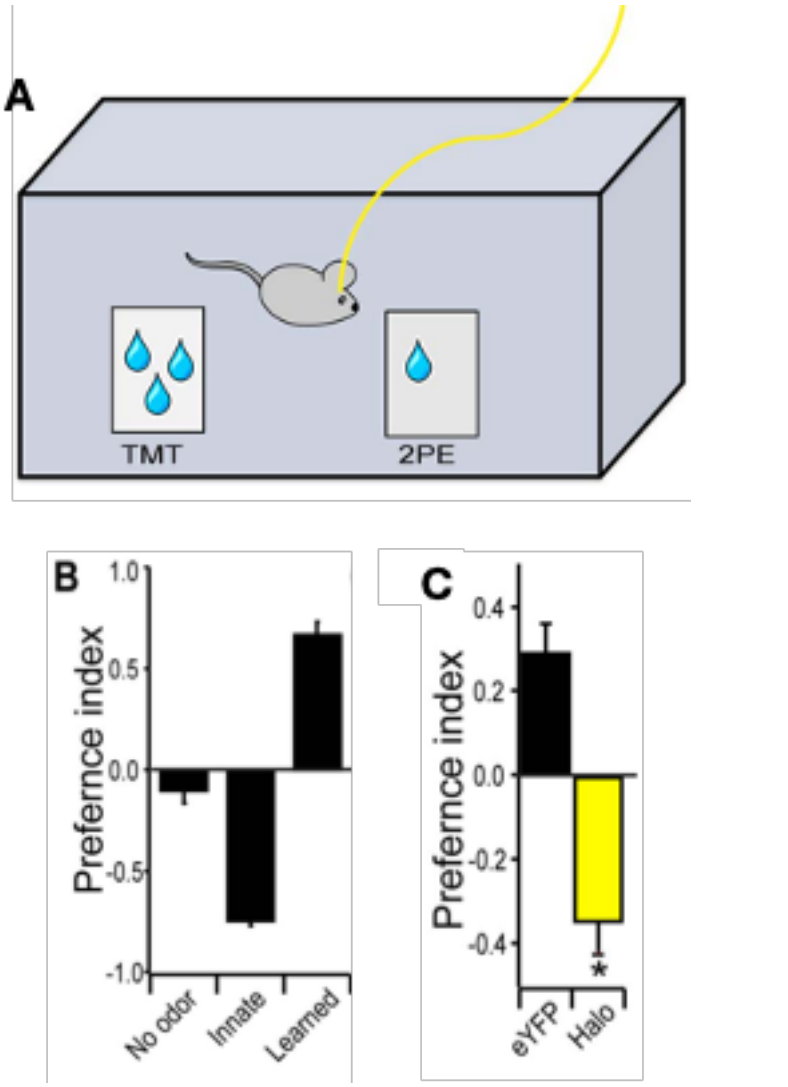

Figure 5. The top panel represents the 2-port water experiment with odors TMT and 2PE. Performance index is higher when approaching the TMT port. Halorhodopsin used for optogenetic silencing of orbitofrontal cortex in panel C.

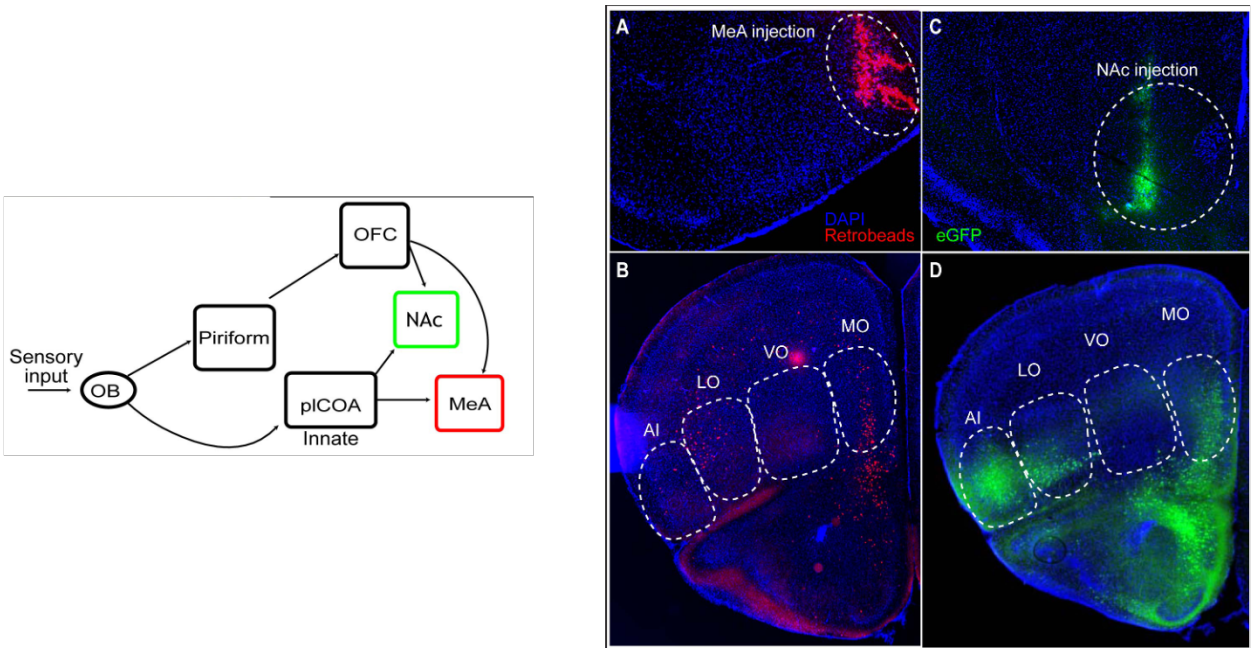

Figure 6. The left panel is a hypothetical circuit for how the orbitofrontal cortex (OFC) is involved in the override of innate behavior. The right panel shows retrograde tracing injected at the medial amygdala and nucleus accumbens, highlighting projections from the orbitofrontal cortex to these structures.

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