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AVERSIVE LEARNING EFFECT ON ODOR CODING IN RAT'S PIRIFORM CORTEX

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A DISSERTATION APPROVED FOR
DEPARTMENT OF ZOOLOGY

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ABSTRACT

Olfaction, a sense for detecting and discriminating chemical molecules in the environment, is critical for animal survival, reproduction and other adaptive behaviors. The olfactory system is organized in three major stations (a sensor sheet, an initial processing and projection unit, and a central processing unit) that are shared across phyla, and has been functioning for millions of years. Since Buck and Axel identified a multigene family for coding the olfactory receptors, knowledge of the olfactory system has quickly accumulated in the last 20 years. This allows us to investigate fundamental questions in olfaction, including how odor percepts are formed, how olfactory information is used and stored, and how experiences shape olfactory perception in our daily life.

Aversive events involving olfactory information are commonly experienced in nature. In the lab, aversive olfactory experiences have been shown to modify odor responses in rodents behaviorally and physiologically. Traditionally, studies regarding olfactory aversive learning were conducted by using odor-shock conditioning. Here, I explored the possibility of using 2-way active avoidance conditioning for awake unit recording in rats. The results confirmed previous findings that the rats can learn to actively avoid both auditory and olfactory cues that are associated with a dangerous event. Interestingly, the rats appeared to have rapid acquisition but poor behavioral retention. After comparing between the two paradigms, I decided to use odor-shock conditioning for chronic unit recording in awake rats.

Three different odor-shock conditioning paradigms were used to investigate how aversive learning affects odor processing in the olfactory cortex. We first found that odor-evoked fear responses were training paradigm-dependent and each induced different levels of fear responses and odor generalization. In addition, we observed a decrease in spontaneous firing rate in the olfactory cortical neurons after conditioning and that was associative learning dependent. The results also suggested that generalized fear is associated with an impairment of olfactory cortical

discrimination. In conclusion, changes in sensory processing are dependent on the nature of training, and can predict the behavioral outcome of the training.

CHAPTER 1

Functional Organization of the Olfactory System

The olfactory system is organized in three major stations that have been shown to be similar across phylogeny (Hildebrand and Shepherd, 1997; Davis, 2004). In mammals, the three stations are: the olfactory epithelium of the nose, which has olfactory sensory neurons for detecting airborne molecules (odorants); the olfactory bulb, where olfactory information from the olfactory epithelium is first processed and relayed to more central structures; and the olfactory cortex, which includes several brain regions and is responsible for further odor processing, odor perception and odor memory. In insects, the functional counterparts of the three stations are the antenna, the antennal lobe, and the mushroom body. Given that my research focus has been on rodent central olfactory processing, in this chapter, I will mainly review the mammalian olfactory system.

Olfactory Epithelium

Anatomical and Synaptic Organization

Olfaction in mammals begins in the nasal cavity where odorants meet with olfactory sensory neurons in the olfactory epithelium. The

olfactory epithelium is a tissue specialized for odorant detection that in humans is located deep in the nasal cavity and is about 1—2 cm^2 in size (Nolte, 2002). The epithelium has three major cell types: olfactory sensory neurons (OSNs), supporting cells, and basal stem cells (Graziadei and Graziadei, 1979) (**figure 1A**). Each cell type contributes differently to olfaction at the peripheral level. Olfactory sensory neurons are responsible for transducing odorants into neural signals. These neurons are bipolar cells. Each has a single apical dendrite extending to the epithelial surface and an axon on the opposite pole which innervates the glomerular layer of the olfactory bulb (OB). The dendrite terminal has cilia that extend above the epithelial surface into the mucus layer where dissolved odorants are adsorbed (Morrison and Costanzo, 1990). The cilia have specific receptor proteins for odorant detection (Chen and Lancet, 1984); binding of the receptor and its suitable odorants leads to a cascade of biochemical reactions that eventually result in changes in membrane potential of the OSN (Firestein and Werblin, 1989; Restrepo et al., 1990; Firestein, 1992; Reed, 1992). OSNs are relatively short-lived (30-60 days) and are continuously replaced by newly differentiated cells from the basal stem cells (Graziadei and Graziadei, 1979; Caggiano et al., 1994).

The supporting cells, along with Bowman's glands in the olfactory epithelium, secrete the mucus layer that helps to maintain the optimal ionic environment for the cilia (Getchell et al., 1984; Carr et al., 1990). The mucus layer contains odorant-binding proteins (OBPs), which are soluble proteins and have been shown to have binding specificity to a number of odorant molecules (Bignetti et al., 1985; Pevsner et al., 1986). OBPs are thought to be part of the ligand-binding carrier family that is known to bind and transport hydrophobic ligands (Lee et al., 1987; Pevsner et al., 1988). The function of OBPs is still not clear. However, they are proposed to enhance odorant detection in several ways, including concentrating the odorant in the hydrophilic mucosal environment, transporting odorants to their suitable olfactory receptors, and removing odorants from the receptor environment after transduction (Pevsner et al., 1986; Lee et al., 1987; Pevsner et al., 1988; Pevsner and Snyder, 1990). Several proteins functionally similar to OBPs have been reported in different species, including maltose-binding protein in *E. coli* (Manson et al., 1985) and pheromone-binding protein in the wild silkmoth, *Antheraea polyphemus* (Vogt et al., 1991). Their existence suggests a commonly used mechanism

prior to and after odorant-receptor association for enhancing odorant sensitivity in chemical sensing systems.

Olfactory Transduction

Olfactory receptors (ORs) are embedded in the membrane of the cilia and are G protein-coupled receptors sensitive to odorants (Pace et al., 1985; Sklar et al., 1986; Jones and Reed, 1989; Boekhoff et al., 1990). In 1991, a multigene family was identified in rats that appeared to code for the ORs (Buck and Axel, 1991). Numbers of OR genes varied among species; rodents contain $\sim$1000 genes whereas humans have less than 400 functional ones (Zhang and Firestein, 2002; Olender et al., 2008). Binding of odorants and the G protein-coupled receptors is believed to activate second messenger pathways involving cAMP (Pace et al., 1985; Sklar et al., 1986; Nakamura and Gold, 1987; Boekhoff et al., 1990) and IP_3 (Huque and Bruch, 1986; Boekhoff et al., 1990) (**figure 1B**). The cAMP opens cyclic nucleotide-gated cation channels and causes depolarization of membrane potential (Firestein et al., 1991a; Firestein et al., 1991b). On the other hand, IP_3 is proposed to open IP_3-gated Ca^{2+} channels and induce Ca^{2+} influx, which might lead to either excitation or inhibition of an OSN (Restrepo et al., 1990; Firestein, 1992; Reed, 1992; Buck, 1996). While the two pathways

work independently (Boekhoff et al., 1990), they may have cooperative effects on changes in membrane potentials of OSNs (Firestein, 1992; Reed, 1992; Buck, 1996).

One Receptor-One Neuron Hypothesis

Genetic tools have allowed investigation of the olfactory system since Buck and Axel identified the OR gene superfamily in 1991. As a result, one interesting finding, which is now widely accepted, is that an OSN only expresses one OR, which is also called the one-receptor-one-neuron hypothesis. Evidence supporting this hypothesis was collected mainly from rodents by using genetic techniques. First, by using *in situ* hybridization of OR RNA probes, OR gene expression was observed in individual OSNs in mouse olfactory epithelium (Ressler et al., 1993; Vassar et al., 1993). OR gene expression is confined to one of the four specific areas or "zones" on the olfactory epithelium of mice, but is widely distributed within the zone (Ressler et al., 1993, 1994a). *In situ* hybridization on OB sections also suggested the possibility that OSNs expressing the same receptor send their axons to the same glomeruli (mostly 2 glomeruli, one medial and one lateral glomerulus in each bulb) (Ressler et al., 1994b; Vassar et al., 1994). The convergent axonal projections were confirmed by genetic labeling

studies in which the reporter (tau-lacZ or tau-GFP) was co-expressed with the targeted OR gene, enabling visualization of trajectories of axonal projections (Mombaerts et al., 1996; Treloar et al., 2002). The relative bulbar positions of the targeted glomeruli appeared to be fixed in all mice, indicating a topographic map in the OB (Ressler et al., 1994b; Vassar et al., 1994; Mombaerts et al., 1996). Moreover, the OR is a determinant of OSN axon targeting: replacing the coding sequence of an OR gene with another resulted in changes in targeted glomeruli (Mombaerts et al., 1996; Wang et al., 1998; Bozza et al., 2002). Together, these data form a strong argument against dual or multiple expression of OR genes. If there was dual or multiple OR expression, it would be unlikely to lead to fixed and precise glomerular targets as seen in the *in situ* hybridization and the gene labeling studies noted above.

However, a direct test of this hypothesis is to look for dual or multiple OR expression in the OSNs. By using two-color *in situ* hybridization, Tsuboi and colleagues demonstrated that the selected OR genes are not expressed simultaneously in an OSN even though they have highly similar amino acid sequences (Tsuboi et al., 1999). Moreover, a genetic targeting study using two genetic markers (lacZ and GFP) in mice also excluded co-

expression of the two targeted OR genes (Strotmann et al., 2000). Finally, single-cell reverse-transcription polymerase chain reaction (RT-PCR) data indicated that individual mouse OSNs express only one OR gene (Malnic et al., 1999; Touhara et al., 1999). All together, these findings strongly support the one-receptor-one-neuron hypothesis.

Molecular Receptive Range of the OSNs

Early electrophysiological data has shown that a given OSN can respond to a wide range of odorants and an odorant can activate multiple types of OSNs simultaneously (Sicard and Holley, 1984). As the one-receptor-one-neuron hypothesis has become generally accepted, the focus has shifted from molecular receptive range (MRR) of single OSNs to ligand-receptor interactions. More recent studies have combined single-cell RT-PCR and calcium imaging to further identify relationships between odorants and ORs (Malnic et al., 1999; Touhara et al., 1999; Kajiya et al., 2001). In addition, Firestein and colleagues used an adenovirus vector to induce expression of the I7 receptor gene in the OSNs and used both electro-olfactograms (EOGs) and calcium imaging to detect odorant responses (Araneda et al., 2000). The findings from these studies can be summarized as follows: 1) a given OR can have high specificity for certain molecular

features (e.g., functional groups); 2) an OR type can have high tolerance for certain molecular features (e.g., structural variations at the tail of the molecule); 3) different odorants are recognized by distinct combinations of ORs; 4) there is a concentration effect of odorant on the OR response.

In summary, current evidences suggest that an OSN only expresses one OR. Odorant specificity of an OR appears to be affected by the functional group, the molecular structure, and the concentration of an odorant. Therefore, an odorant may activate different types of OSNs, depending on odorant specificity of the OR they express.

Olfactory Bulb

Anatomical and Functional Organization

The olfactory bulb (OB) is a neural structure protruding from the forebrain, specialized for processing olfactory information (Nolte, 2002). In most mammals except humans and higher primates, the olfactory bulb is composed of two subregions, the main olfactory bulb (MOB) and the accessory olfactory bulb (AOB) (Mori, 1987). The MOB receives sensory input from the olfactory epithelium and sends its output to the olfactory

cortex (Shepherd et al., 2004). The AOB, on the other hand, receives input from the vomeronasal organ, the function of which is believed to be pheromone detection (Mori, 1987). The following sections will focus on functional organization of the MOB since the vomeronasal organ appears to be absent in adult humans.

The MOB is a laminated; each lamina is composed of distinct cell types and organization (Allison, 1953). The laminae are termed, from the surface inward: the olfactory nerve layer (ONL); the glomerular layer (GL), which is composed of approximately 2000 glomeruli (spherical neuropils) and numerous periglomerular (PG) cells (a local interneuron type); the external plexiform layer (EPL), where the cell bodies of middle tufted cells (T_m, a principle neuron type) and dendrites of mitral/tufted cells (a principle neuron type) are distributed; the mitral cell layer (MCL), which is a thin layer formed by somata of mitral cells; the internal plexiform layer (IPL), which contains fibers and dendrites of granule cells (a local interneuron type); the granule cell layer (GRL), where cell bodies of granule cells are distributed; and the subependymal layer (SEL, or periventricular zone, PVZ), which is the innermost layer surrounding the intrabulbar part of the lateral ventricle (Mori, 1987) (**figure 2**).

Principle neurons in the MOB are mitral and tufted cells, which are responsible for processing and relaying olfactory information to the olfactory cortex. In mammals, somata of mitral cells are 15—30 μm in diameter; tufted cells are slightly smaller in size, with somata 15—20 μm in diameter. Tufted cells have three subpopulations: external (T_e), middle (T_m), and internal (T_i) tufted cells, and each of which has a distinct morphology and laminar distribution. Both mitral and tufted cells have a primary dendrite that extends and arborizes inside specific glomeruli, where it synapses with OSN axons and PG dendrites (Pinching and Powell, 1971; Mori et al., 1999). Secondary (basal) dendrites of the mitral cells, T_m and T_i extend laterally in the EPL, forming dendrodendritic reciprocal synapses with granule cells (Price and Powell, 1970a). The basal dendrites make excitatory synapses onto granule cell dendrites and the excitatory neurotransmitter is glutamate (Wellis and Kauer, 1994; Isaacson and Strowbridge, 1998; Schoppa et al., 1998). Finally, mitral cells, T_m and T_i and partial T_e extend their axons deep into the olfactory bulb, where they come together to form the lateral olfactory tract, which terminates in the olfactory cortex (Mori et al., 1999; Wilson and Stevenson, 2003).

There are two main types of local interneurons in the MOB: the PG cells and the granule cells. PG cells are distributed within the glomerular layer of the OB. The cell body of a PG cell is 6—8 μm in diameter, among the smallest neurons in the brain (Pinching and Powell, 1971; Shepherd et al., 2004). Dendrites of PG cells arborize into one glomerulus (or at times two glomeruli), where they make synaptic connections with OSN axons (axodendritic synapses) and with primary dendrites of the mitral/tufted (M/T) cells (reciprocal dendrodendritic synapses) (Pinching and Powell, 1971). PG cells are believed to be GABAergic (Ribak et al., 1977; Halasz et al., 1979) and dopaminergic (Halasz et al., 1977) and are postulated to form inhibitory synapses onto M/T cells (Trombley and Shepherd, 1997).

Granule cells are axonless and small, with somata 6—8 μm in diameter (Price and Powell, 1970b). The cell bodies form a thick lamina (GRL) deep in the OB. Each granule cell gives rise to a process (peripheral dendrite) toward the OB surface, arborizing within the EPL, and its deep dendrites are distributed in the GRL. The granule cell-to-M/T part of the dendrodendritic synapse is inhibitory (Mori and Takagi, 1978) and the neurotransmitter used at this synapse is GABA (Ribak et al., 1977; Halasz et al., 1979), which is received by GABAa receptors of M/T cells (Isaacson and

Strowbridge, 1998) leading to increased membrane permeability to Cl^- ions (Nowycky et al., 1981). Finally, recent data indicate that voltage-gated calcium channels play an essential role in GABA release at the dendrodendritic synapse during reciprocal feedback inhibition (Isaacson, 2001).

Most PG and granule cells are generated postnatally and continue to be generated and replaced in the adult (Bayer, 1983). They are generated in the anterior horn of the lateral ventricle and travel to the MOB via the rostral migratory stream (Doetsch et al., 1997; Coskun and Luskin, 2002). This continuous replacement and incorporation of local interneurons in the circuits has been suggested to be important for adaptive behaviors and olfactory memory in adult animals (Rochefort et al., 2002).

Glomeruli come in different sizes, from 20—40 μm in diameter in fish and amphibians to 100—200 μm in diameter in rabbits and cats (Shepherd et al., 2004). Glomeruli are suggested to be functional units for odor processing in the MOB (Mori and Yoshihara, 1995; Hildebrand and Shepherd, 1997), glomerular modules (Friedrich and Korsching, 1997; Mori et al., 1999). There is increasing evidence supporting this suggestion. First, individual glomeruli show responses to specific sets of odors (Leveteau and

MacLeod, 1966; Rubin and Katz, 1999; Fried et al., 2002; Wachowiak et al., 2004). Second, M/T cells that innervate the same glomerulus have similar odor tuning or molecular receptive range (Buonviso and Chaput, 1990; Imamura et al., 1992; Mori et al., 1992; Katoh et al., 1993). Third, there is interaction between neighboring glomerular modules, such as lateral inhibition (Meredith, 1986; Wilson and Leon, 1987; Yokoi et al., 1995; Isaacson and Strowbridge, 1998).

The MOB has two major inputs: sensory input from OSNs in the olfactory epithelium and centrifugal (top-down) input from the higher brain regions. Axons of the OSNs fasciculate to form the olfactory nerve that passes through the cribriform plate and forms the outermost layer of the olfactory bulb, the ONL (Shepherd, 1972a). Individual axons that express the same OR gene then further coalesce into a bundle before terminating in glomeruli (Treloar et al., 2002). These axons form excitatory synapses on dendrites of the M/T and PG cells using glutamate as the neurotransmitter (Berkowicz et al., 1994). As mentioned earlier, OR expression is confined within zones on the olfactory epithelium, and this zonal expression corresponds to zonal glomerular innervations in the MOB (Mori et al., 1999).

Centrifugal inputs to the MOB come from several regions of the brain, including the anterior olfactory nucleus (AON), the piriform cortex, the horizontal limb of the diagonal band (HDB), the locus coeruleus and the raphe nucleus (Broadwell and Jacobowitz, 1976; Davis and Macrides, 1981; Mori, 1987). Axons of pyramidal cells in the AON and piriform cortex form excitatory synapses mostly with granule cells, suggesting inhibitory feedback loops back to the MOB. Fibers from the locus coeruleus provide noradrenergic (NA) input and are distributed in the granule cell layer (GRL), the IPL, and the glomerular layer (GL). Serotonergic input is from the dorsal raphe nucleus, the fibers of which terminates in the GRL and the GL. Cholinergic input is sent from the HDB and terminate in all portions of the OB, with particularly heavy termination in the IPL and GL (Nickell and Shipley, 1993). In summary, each MOB input has a distinctive laminar projection within the MOB, implicating different effects and power of modulation from the higher centers.

Cell Populations

The convergence ratios of OSNs to the glomerular module are very high: OSNs to glomeruli, 25000:1; OSNs to mitral cells, 1000:1; OSNs to tufted cells, 500:1, based on the estimation of cell numbers in (Allison,

1953). The high OSNs to M/T cells ratios may have functional significance in temporal synchronization and noise reduction in odor sampling. The ratios of local interneurons to principle neurons are also high: PG to mitral is estimated to be 20:1, and granule to mitral to be 50—100:1, suggesting an important role of intrinsic circuits for information processing in the MOB (Shepherd, 1972b). Although estimations of these ratios vary in different species and developmental stages (Meisami, 1989; Royet et al., 1998), the fact remains that there are high convergence ratios of OSNs and local interneurons onto the glomerular module.

Odor Processing in the Olfactory Bulb

Odor processing in the OB has been mainly investigated at two different levels: global odor representation in the glomerular layer and odor coding of the principle (mitral/tufted) cells (Hildebrand and Shepherd, 1997). Studies of the former utilized different techniques to visualize odor-evoked activity patterns of the glomerular sheet, including [^{14}C]2-deoxy-D-glucose (2-DG) (Sharp et al., 1975), voltage-sensitive dye (Kauer et al., 1987), *c-fos* mRNA (Guthrie et al., 1993), Ca^{2+} sensitive dye (Friedrich and Korsching, 1997), high-resolution functional magnetic resonance imaging (fMRI) (Yang et al., 1998), and intrinsic optical signal imaging (Rubin and

Katz, 1999). Glomerular activity detected by these techniques reflects changes in energy consumption within its neighboring areas and/or neuronal activity of the underlying neural circuits. These studies found spatially overlapping but distinct patterns elicited by different odorants, which are commonly termed odor images, odotopic maps or epitopic maps. Several principles have emerged from these studies. First, odorants tend to evoke activity patterns in which activated glomeruli are often observed in the medial and lateral OB. Second, different odorants evoked distinct odor images. Third, the odor image for a given odorant is constant across animals. Fourth, glomerular activity encodes both odor identity (e.g., functional groups) and odor concentration. A given odor stimulus may activate more glomeruli with higher concentration. Fifth, patterns evoked by odorants having the same functional group (e.g., aldehyde) are correlated.

Interestingly, a recent study suggested that even glomerular modules that together form a specific odor map might not be functionally equal (Kobayakawa et al., 2007). Kobayakawa and colleagues showed that genetically wiping out glomeruli in the dorsal domain of the mouse OB abolished innate fear responses to a fox odor. However, the mutant mice

were capable of detecting the odor using glomeruli in the ventral domain of the OB. In other words, this sub-population of glomerular modules might have hard-wired connections to the emotional center (amygdala) of the mice. The modular odor processing in the OB is evolutionarily reasonable, as natural selection would favor those individuals who are able to avoid odors of natural enemies without experience, which is often fatal. Evidence for this hypothesis has been provided by two recently published studies (Miyamichi et al., 2011; Sosulski et al., 2011). Miyamichi and colleagues showed that the cortical amygdala preferentially receives input from the dorsal OB. In addition, Sosulski and colleagues presented evidence that specific groups of glomeruli have stereotypical projection patterns in the cortical amygdala.

In summary, the topographic odor maps not only represent intrinsic properties of odorant molecules but also provide an excellent spatial reference for investigating odor encoding and processing by the principle (mitral/tufted) cells in the OB. In addition, recent studies reveal that odor processing is not just dependent on experience. Instead, there are hard-wired odor processing channels that are necessary for innate odor responses.

By using *in vivo* extracellular recording, Mori and colleagues showed that M/T cell activities encode the stereochemical structure of odor molecules, including the length or structure of a hydrocarbon chain, a functional group, and the position of the functional group in the structure (Imamura et al., 1992; Mori et al., 1992; Katoh et al., 1993). Like the OSN, a mitral or tufted cell has its molecular receptive range (MRR), which to some extent reflects odor tuning of the OSNs that express the same OR gene (Malnic et al., 1999; Touhara et al., 1999). However, in contrast to the MRR of an OSN, the odor repertoire that excites an OSN, M/T cells also have inhibitory MRRs (Mori and Yoshihara, 1995). The suppressive odor responses may be produced by different layers of inhibitory interneurons through feed-forward, feedback, and lateral inhibition (Nowycky et al., 1981; Mori, 1987; Yokoi et al., 1995; Isaacson and Strowbridge, 1998).

Odor-evoked excitation and suppression of M/T cells have also been shown in studies using *in vivo* intracellular recording (Hamilton and Kauer, 1985, 1989; Cang and Isaacson, 2003). Hamilton and Kauer showed that an odor response of a cell can be a complex temporal combination of suppression and excitation. The combination is sensitive to stimulus

concentration. Cang and Isaacson also observed intensity-dependent odor responses in the M/T cells.

Using intracellular recording, Friedrich and Laurent observed complex temporal firing patterns in zebrafish M/T cells in response to 16 amino acid odorants (Friedrich and Laurent, 2001). They showed that the average correlation between M/T cell responses within the initial and the following time windows (400-ms long; 100-ms increments) decreased over time, suggesting that variability of single-cell temporal odor responses increased in the course of odor stimulation. In addition, they used principle component analysis (PCA) to analyze the neural ensembles (treating the cell population as a whole). Their results showed an initial (at 200 ms) clustering of similar odorants in principle component space and de-clustering of ensemble responses over time (until 2000 ms). These results suggested that the cell population may initially recognize the 16 odorants as several groups but may gradually treat them as single odorants during odor stimulation. Together, this study suggested that temporal activity patterning of the M/T cells may play an important role in encoding and discrimination of odors.

In summary, current evidence suggests that odor information is processed by glomerular modules of the OB that appear to utilize both spatial and temporal coding mechanisms. As temporal coding is absent in the OSNs, it may be mainly contributed by inhibitory circuits of the OB.

Odor Learning in the Olfactory Bulb

Evidence for olfactory perceptual learning has been shown by Fletcher and Wilson wherein prolonged novel odor exposure (50 seconds) caused a shift in M/T cell optimal molecular receptive range (MRR) (Fletcher and Wilson, 2003). These experience-induced changes are interesting and may be related to an enhancement of odor discrimination. Additional evidence for perceptual learning in 2nd-order neurons was found in the antennal lobes of the locust (Stopfer and Laurent, 1999). Stopfer and Laurent discovered that repeated odor stimulation can induce gradual synchronization of antennal lobe projection neuron spikes and local field potential (LFP) oscillations. This synchronization during odor sampling, once established, can last for several minutes, and thus may be related to short-term odor memory. Learning-induced changes in mitral cell synchrony have also been demonstrated in rodents (Doucette et al., 2011).

Operant olfactory conditioning can also induce changes in odor-evoked LFP oscillations in the OB (Ravel et al., 2003; Martin et al., 2004). Ravel and colleagues trained the rats to discriminate odor pairs using the go-no go task and simultaneously recorded LFP in the OB. They found that the odor-evoked beta (15-40 Hz) oscillation was stronger in the expert (well-trained) rats than in the naïve rats, while the gamma (60-90 Hz) oscillation depressed after training (Ravel et al., 2003). Because the operant conditioning paradigms (e.g., a go-no go task) may take animals weeks to master, the changes in LFP oscillations may be related to long-term olfactory memory.

Odor learning can be facilitated by important biological events, such as giving birth (Kendrick et al., 1992), and forming mother-infant attachment (Moriceau et al., 2010). Kendrick and colleagues found that after a lamb giving birth, there is a significant increase in the number of M/T cells that are tuned to lamb odors, with a subset of cells specifically tuned to the odor of its own lamb. This effect is associated with increased release of acetylcholine and norepinephrine in the OB. Furthermore, only own lamb odor induces a significant increase in local glutamate and GABA concentrations, suggesting that inhibitory mechanisms in the OB might play

a role in enhancing odor discrimination between complex odor mixtures (alien versus own lamb odors).

Olfactory Cortex

Anatomy and Synaptic Organization

The olfactory cortex is usually defined as the set of cortical areas that receive direct synaptic input from the MOB, including the anterior olfactory nucleus (cortex), the olfactory tubercle, the piriform cortex, the cortical amygdaloid nucleus, the anterior rudiment of the hippocampus and the lateral entorhinal area (Price, 1973). With the exception of the lateral entorhinal cortex, each of these is composed of three layers, instead of the six layers that commonly constitute a neocortex. A three-layer cortex is considered a phylogenetically older type and thus the olfactory cortex has been termed paleocortex (Neville and Haberly, 2004). Among all the subregions, the piriform cortex is the largest component of the olfactory cortex. It receives a large portion of synaptic input from the M/T cells and relays olfactory information to a number of brain regions (Price, 1973; Haberly and Price, 1978). Moreover, fibers from higher brain centers and

neuromodulatory systems also terminate in the piriform cortex, theoretically providing top-down modulation (Haberly, 2001). These properties together make the piriform cortex the major target for investigating central odor processing and odor learning. Given the importance of the piriform cortex in the olfactory pathway, in this section, I will mainly focus on reviewing piriform cortex anatomy, physiology, and functionality.

The piriform cortex is a laminated structure with three basic layers: a superficial plexiform layer (layer I), a compact cell body layer (layer II), and a deep polymorphic cellular layer (layer III) (Price, 1973) (**figure 3**). Layer I contains dendrites of pyramidal cells, fiber systems and a small number of interneurons. It has two distinct subdivisions: Ia and Ib. Axons from the M/T cells terminate exclusively in layer Ia, while association fibers of pyramidal cells from the whole olfactory cortex heavily distribute in layer Ib (Price, 1973; Luskin and Price, 1983). Layer II contains cell bodies of the principle neurons – semilunar cells (in layer IIa) and superficial pyramidal cells (in layer IIb) (Price, 1973). It only receives minor association fiber projections from subregions of the olfactory cortex (Luskin and Price, 1983). Layer III is composed of deep pyramidal cells and several non-pyramidal cell types.

Density of the pyramidal cells in layer III is moderate but decreases with increasing depth. Like layer Ib, layer III also receives heavy association fiber projections from several intracortical regions (Haberly and Price, 1978; Luskin and Price, 1983).

The piriform cortex can be subdivided into anterior and posterior piriform cortex (aPCX and pPCX, respectively), based on differences in neuronal organization and fiber systems (Price, 1973; Haberly and Price, 1978). Functional differences between aPCX and pPCX have been recently reported in rodents and humans (Gottfried et al., 2006; Kadohisa and Wilson, 2006). Both indicated that aPCX is responsible for odor identity encoding (e.g., rose or jasmine odor), and pPCX codes for odor similarity (e.g., floral or wood odors).

Pyramidal cells in the piriform cortex are similar to those found in other primary sensory cortices and are about 15-25 μm in diameter (Price, 1973). Both superficial and deep pyramidal cells have an apical dendrite that shoots upward perpendicularly to the surface. Their basal dendrites arborize downward into deep layer II and layer III. And both pyramidal cell types have myelinated deep-directed axon. The axon has unmyelinated branches that give rise to association fibers that terminate both within the

olfactory cortex and back into the OB. Like pyramidal cells, single semilunar cells have a spiny apical dendrite and a deep-directed axon that gives rise to associational projections. In contrast to pyramidal cells, they lack basal dendrites and do not project back to the OB (Haberly and Price, 1978). Interestingly, cell death of semilunar cells occurs within 24 hours following removal of the OB (Heimer and Kalil, 1978; Leung and Wilson, 2003).

Physiological properties of the two principle neuron types in layer II have been investigated by Suzuki and Bekkers using *in vitro* patch-clamp recordings (Suzuki and Bekkers, 2006). They showed that superficial pyramidal (SP) cells are likely to fire bursts of action potentials in response to *in vivo*-like stimuli, whereas semilunar (SL) cells only fire nonbursting action potentials. Moreover, synapses between M/T cell axons and SP cells dendrites show greater paired-pulse facilitation than those between M/T cells and semilunar cells. Finally, these two kinds of neurons show different latencies in response to naturalistic stimuli. The differences between these two principle neuron types in firing rates and latency may suggest two coding strategies for odor processing in the piriform cortex. Recently, Suzuki and Bekkers indicated that SP and SL cells belong to two laminarly segregated subcircuits in the piriform cortex: SP cells (in layer IIb) receive

stronger associational (intracortical) excitatory inputs, while SL cells (in layer IIa) receive stronger afferent input from the OB.

Odor Processing in the Piriform Cortex

In contrast to topographic odor representation in the OB, odor information in the piriform cortex is represented by sparse and spatially distributed sets of cells (Illig and Haberly, 2003; Rennaker et al., 2007; Stettler and Axel, 2009). This distributed cortical representation results from diffuse excitatory projections from individual glomeruli in the OB (Apicella et al., 2010; Sosulski et al., 2011) and convergence of synaptic inputs from multiple glomeruli to single neurons in the piriform cortex (Apicella et al., 2010; Davison and Ehlers, 2011; Miyamichi et al., 2011). Although prominent spatial coding is absent in the piriform cortex, temporal coding seems to be preserved in the neural ensemble (Rennaker et al., 2007).

In addition to non-topographic odor representation, principle neurons in layer II/III piriform cortex exhibit several distinct features that separate them from M/T cells in the OB. First, odor habituation is stronger in layer II/III aPCX neurons than in the M/T cells (Wilson, 1998). With either repeated short (2 s) or prolonged (50 s) odor stimulation, odor-evoked

activity of aPCX neurons greatly decreased, whereas M/T cell activity remained. As we are often habituated to odors in the environment soon after initial encounter, these results imply that conscious odor perception might be formed in the cortical areas.

Second, in contrast to neighboring M/T cells that often have continuous MRR (Yokoi et al., 1995), neighboring neurons in aPCX do not necessarily have the same or similar MRRs (Rennaker et al., 2007). In fact, individual neurons in the piriform cortex respond to several structurally distinct odorants (Rennaker et al., 2007; Poo and Isaacson, 2009; Stettler and Axel, 2009). These results may reflect spatially distributed axonal projections along with specific axonal convergence from homogeneous M/T cells to the piriform cortex.

When habituated to a binary odor mixture, the M/T cells show stronger habituation to the components than aPCX neurons do (Wilson, 2000b), suggesting that odor processing is more synthetic in the piriform cortex than in the OB. This difference is even more prominent when animals process complex (10-component) odor mixtures (Barnes et al., 2008). Individual M/T cells are sensitive to minor changes in mixture components, whereas neurons in the piriform cortex appear to tolerate the

absence of minor components (pattern completion). Together, these results reflect bulbar convergence projections on neurons in the piriform cortex and suggest that neurons in aPCX have different MRR properties than the M/T cells do.

Neurons in the piriform cortex also encode odor intensity, as more neurons can be activated by odor stimulation with higher concentration (Stettler and Axel, 2009).

Neuromodulation in the Piriform Cortex

Neural modulation is one of the major mechanisms underlying long-term changes in nervous circuits and, the olfactory system is no exception. There is a large body of *in vitro* studies about cholinergic synaptic modification (Linster and Hasselmo, 2001) and its role in learning-induced intrinsic property changes of neurons in the piriform cortex (Barkai and Saar, 2001). Acetylcholine is important for olfactory associative learning and memory (De Rosa and Hasselmo, 2000; Saar et al., 2001); it causes a depolarization of the membrane potential of pyramidal cells (Tseng and Haberly, 1989; Barkai and Hasselmo, 1994) and increases the excitability of piriform cortex neurons *in vivo* (Zimmer et al., 1999). In the piriform circuit, acetylcholine has a stronger suppressing effect on excitatory intrinsic fiber

synapses than afferent synapses (Hasselmo and Bower, 1992) and also suppresses inhibitory synapses (Patil and Hasselmo, 1999). Although it suppresses both excitatory and inhibitory synapses, acetylcholine enhances associative long-term potentiation (LTP) in the piriform cortex (Hasselmo and Barkai, 1995; Patil and Hasselmo, 1999). This synaptic modification may result from suppression of inhibitory synapses, as this NMDA-dependent associative LTP requires blockade of GABAergic innervations of the piriform cortex (Kanter and Haberly, 1993). Blockade of acetylcholine by the muscarinic cholinergic antagonist scopolamine impairs rule learning of olfactory discrimination tasks (Saar et al., 2001). However, scopolamine does not affect already formed single-unit receptive fields in rats' piriform cortex (Wilson, 2001). Like acetylcholine, norepinephrine also has greater suppressive effects on intrinsic than afferent synaptic transmission (Hasselmo et al., 1997) and may result in an enhancement of neuronal responses to the afferent input relative to internal activity – an enhancement of signal-noise ratio (Servan-Schreiber et al., 1990).

Olfactory Learning in the Piriform Cortex

Non-associative odor learning (odor habituation) has been shown in layer II/III aPCX neurons by using *in vivo* single-unit recording in

anesthetized rats (Wilson, 2000a). Wilson showed no cross-habituation (reduction in odor response to a second odor after previous habituation to the first odor) in aPCX neurons between closely related alkane hydrocarbons, suggesting that aPCX neuron responses are more odor-specific than odor-feature specific. The odor specificity may be modulated by acetylcholine, as scopolamine (a muscarinic receptor antagonist) enhances odor generalization (Wilson, 2001).

Operant conditioning can also induce plasticity in piriform cortical odor responses. Schoenbaum and colleagues recorded aPCX (Roesch et al., 2007) and pPCX (Calu et al., 2007) single-unit activity while the rats were learning to discriminate a new odor pair (~50 trials) and after the criterion had been achieved (60-100 trials). The behavioral paradigm (a go-no go task) involved association of an odor pair with positive (go) and negative (no go) rewards. Results indicated that aPCX and pPCX single units can become tuned to the cue odors in the course of the training, although at different rates. Interesting, these units appeared to code not only for odor quality but also for odor meaning (positive or negative), as several single units stopped responding when the odor association values had been reversed. As olfactory operant conditioning involving odors has been shown

to induce plasticity in multiple cortical areas (Mouly et al., 2001; Mouly and Gervais, 2002), the associative encoding in the piriform cortex might be contributed by inputs from associational projections from the orbitofrontal cortex (Illig, 2005) and the amygdala (Majak et al., 2004).

The piriform cortical circuit is hypothesized to be the place where analytical odor inputs become synthetic olfactory perception (Wilson, 2000a; Haberly, 2001; Wilson and Stevenson, 2003). This hypothesis is gaining more support from recent anatomical and physiological findings (Davison and Ehlers, 2011; Miyamichi et al., 2011; Sosulski et al., 2011). Other than sensory processing features, a number of studies have revealed associative features of the piriform cortex (Schoenbaum and Eichenbaum, 1995; Johnson et al., 2000; Calu et al., 2007; Roesch et al., 2007), which has not been seen in other primary sensory cortices.

SUMMARY

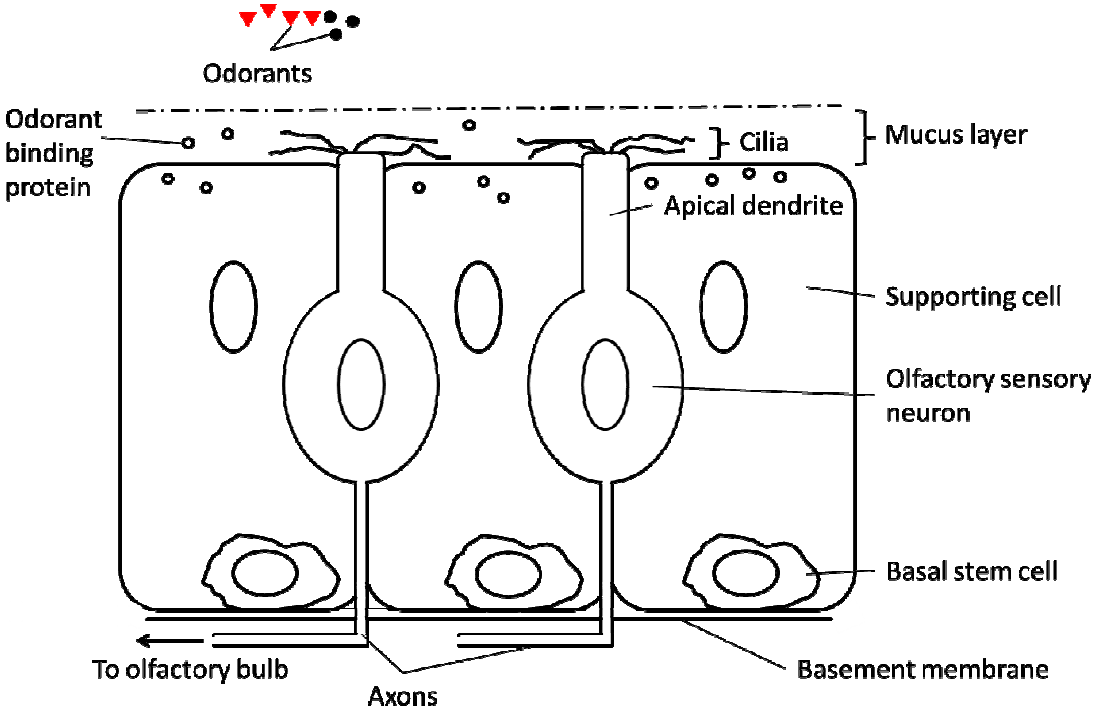
Olfaction begins when airborne molecules enter the nasal cavity, where they dissolve in the mucus layer of the olfactory epithelium, perhaps with the help of odorant binding proteins. Combinations of odorant

molecules with suitable olfactory receptors on the cilia activate a cascade of reactions that depolarize membrane potentials of the olfactory sensory neurons. This is when chemical signals are transduced into electrical signals in the olfactory pathway. Electrical signals that contain odor information are conveyed to the glomerular layer of the olfactory bulb where they are further processed by circuits of glomerular modules using spatial and temporal coding strategies. The output neurons in glomerular modules then send electric signals to the olfactory cortex, where the odor percept is believed to be synthesized and tagged with value. Therefore, when we smell an odor, we smell not only the odor quality (e.g., new house or new car) but also intensity, familiarity, and emotional meanings (e.g., pleasantness or aversiveness) of the odor.

As noted earlier, perceptual learning and operant conditioning can induce plasticity in the piriform cortex. However, effects of aversive classical conditioning on cortical odor processing have not been reported. Rosenkranz and Grace have shown enhanced cued odor-evoked firing in neurons in the lateral nucleus of the amygdala after odor-shock conditioning (Rosenkranz and Grace, 2002). Because their recordings were performed in anesthetized rats, it raised a question as to how we can

translate the results into awake state conditioning. Therefore, it may be better to investigate effects of classical conditioning on cortical odor processing by recording piriform single-unit activity in awake animals.

A



B

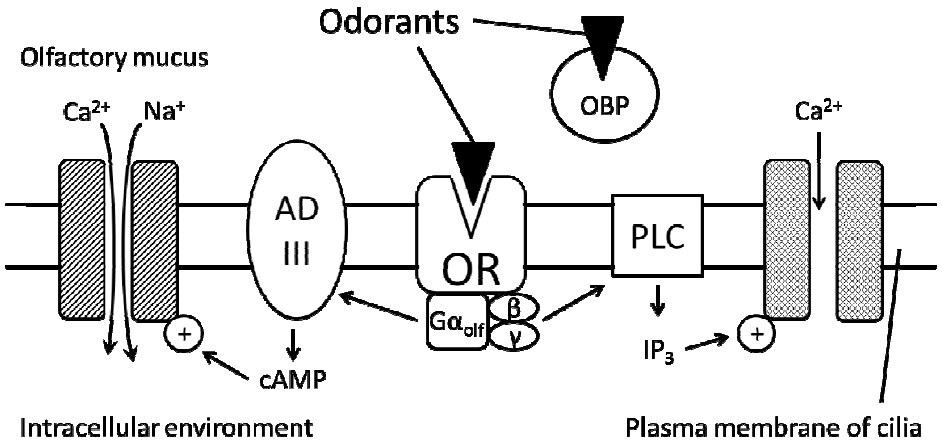


Figure 1. Olfactory epithelium. **A,** Schematic olfactory epithelium. **B,** Olfactory transduction models. (cAMP and IP_3 pathways). **AC III**, adenylyl cyclase type III. **$G\alpha_{olf}$, β, γ**, subunits of the olfactory G-protein. **IP_3**, Inositol trisphosphate. **OBP,** odorant binding protein. **OR,** odorant receptor. **PLC**, phospholipase C. [Adapted from Buck and Axel, 1991; Buck, 1996].

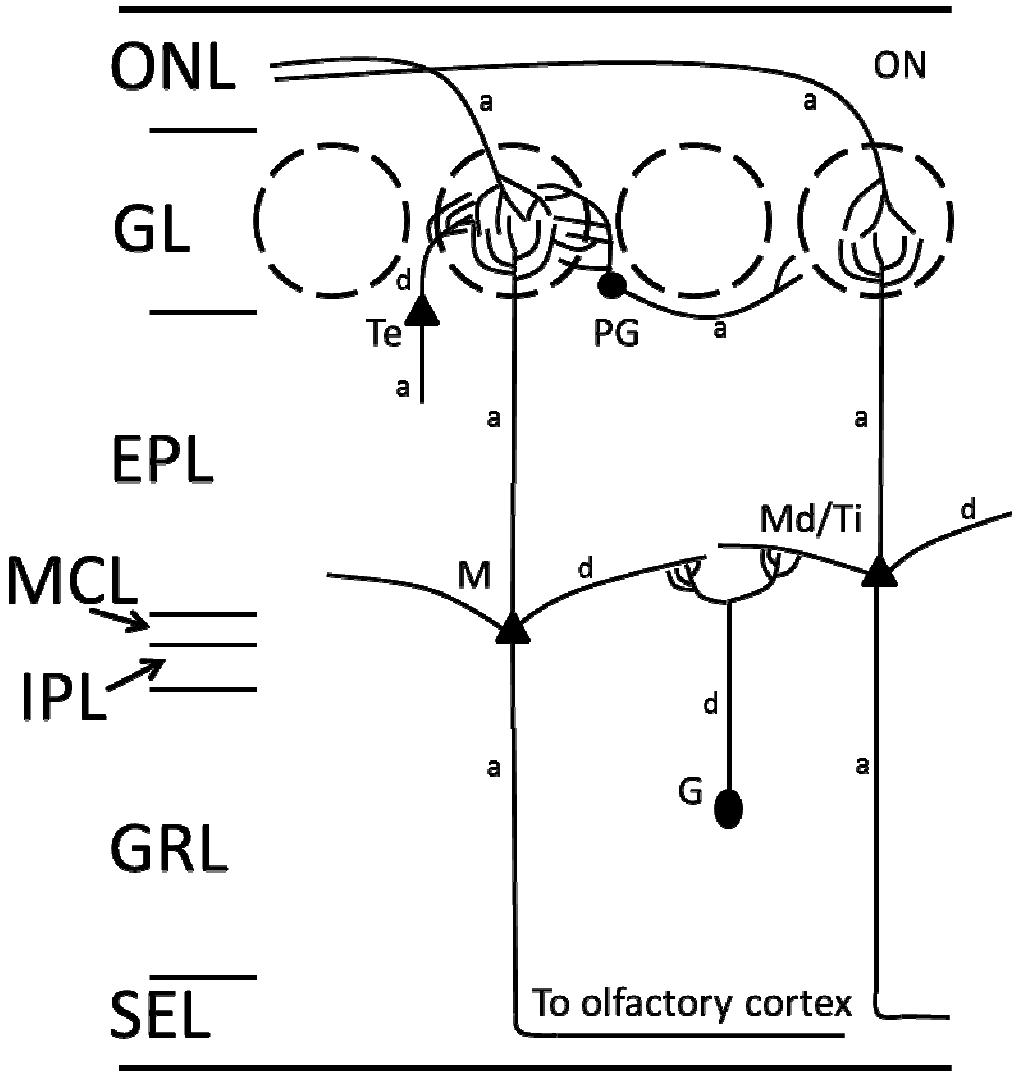


Figure 2. Lamination and components of mammalian olfactory bulb. Lamination from top to bottom: **ONL**, olfactory nerve layer. **GL**, glomerular layer. **EPL**, external plexiform layer. **MCL**, mitral cell layer. **IPL**, internal plexiform layer. **GRL**, granule cell layer. **SEL**, subependymal layer. Components: **a**, axon. **d**, dendrites. **G**, granule cell. **M**, mitral cell. $\mathbf{M_d}$, dorsal mitral cell. **ON**, olfactory nerve fivers. $\mathbf{T_e}$, external tufted cell. $\mathbf{T_i}$, internal tufted cell. **PG**, periglomerular cell. [Adapted from Mori, 1987].

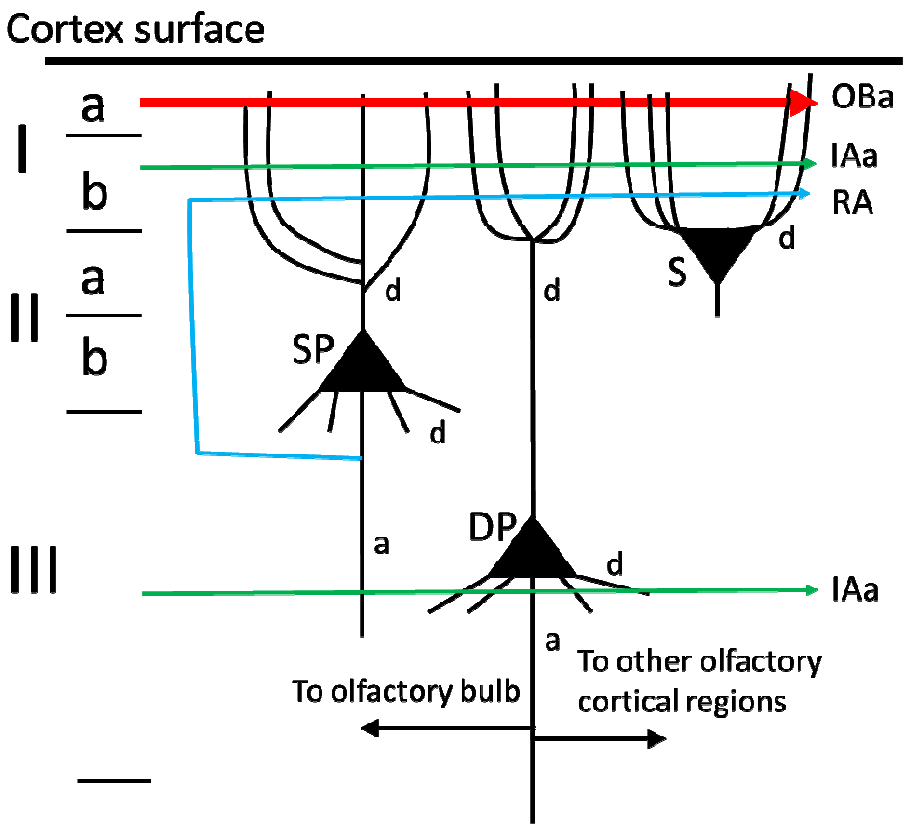


Figure 3. Lamination and principle neurons of piriform cortex. Excitatory inputs: **OBa**, afferent input from olfactory bulb. **IAa**, intercortical associational afferent input., **RA**, recurrent associational input. Principle neurons: **DP**, deep pyramidal cell. **S**, semilunar cell. **SP**, superficial pyramidal cell.

CHAPTER 2

Olfactory Aversive Learning and Behaviors in Rodents

INTRODUCTION

Learning from past aversive experiences is critical for animals to survive in the environment. An aversive, or fearful, event often involves multiple sensory modalities and different learning types, such as associative and contextual learning (LeDoux, 2000). Studies of aversive learning, especially in the auditory pathway of rats, have revealed that nuclei in the amygdala and their interconnections are crucial for aversive learning (LeDoux, 2000).

Aversive events involving a particular odor(s) are commonly experienced in nature, and fear learning in animals has been discovered across phylogeny (Davis, 2004). Traditionally, studies of olfactory aversive learning used odor-shock conditioning (Sullivan et al., 1989; Davis, 2004; Sevelinges et al., 2004). Odor-shock conditioning uses the freezing behavior of animals as an index to measure fear responses elicited by an olfactory cue, the conditioning stimulus (CS). Even though the freezing behavior can be easily observed, it can be difficult to accurately discriminate between freezing and motionlessness. In contrast, two-way active avoidance, which requires the subject to leave the occupied compartment and enter the

adjacent compartment in response to the CS, is much easier to evaluate behavioral responses (Darvas et al., 2011).

The goal of this chapter is to explore two-way active avoidance conditioning and evaluate whether this paradigm could be used for future ensemble recordings from awake, behaving animals. To my knowledge, there is only one other description of olfactory cued active avoidance (Owens et al., 1996), and thus the data are compared with the more traditional auditory cued active avoidance. In this chapter, I will present our data on two-way active avoidance with a tone or an odor cue as the CS and discuss the advantages and disadvantages of using this paradigm in chronic unit recording in freely behaving rats.

MATERIALS AND METHODS

Subjects. 10 male Long-Evans hooded rats (250-450g) were used as subjects (Charles River Laboratories, Wilmington, MA). Animals were housed individually in polypropylene cages on a 12-hour light/dark cycle, with food and water available *ad libitum*. Animal care protocols and all experiments were approved by the Nathan S. Kline Institute IACUC and in accordance with National Institutes of Health guidelines.

Odor Stimulation. A monomolecular odorant, propyl butyrate, was used in the behavioral training (Sigma-Aldrich, Inc.). Odor stimuli were presented for 5 sec from an odor port in the center of the plastic cover of the behavioral chamber via a flow-dilution olfactometer (1 LPM) and were manually operated (**Fig. 1A**). Odors were presented with an interval of at least 60 seconds and at random distances from the animals' noses given their free mobility.

Tone stimulation. A 5-second continuous tone (75 dB max; 27 Hz) was produced by two speakers mounted at two ends of the behavioral chamber (**Fig. 1A**) and each was manually controlled by the experimenter.

2-way active avoidance paradigm. Behavioral experiments were conducted in a custom chamber with a shock grid floor (56 cm x 20 cm x 21.5 cm, Lafayette Instrument, Lafayette, IN) (**Fig. 1A**). The shock grid floor comprised two independent grid systems (left and right) and each was connected to a DC stimulator. A transparent plastic cover was made for the chamber to allow easy observation of the behavioral responses and prevent rats from jumping out during training. A green plastic barrier was placed in the middle of the chamber with its height about 5 cm above the shock grid level. Different color papers with distinct patterns were glued onto the

inner plastic walls to create two visually distinct compartments (left and right) in the chamber.

10 naïve rats were randomly separated into two groups, 5 in each, and were trained with either a tone as the CS (the rats were named from T1 to T5, T refering to tone) or an odor as the CS (from O1 to O5, O refering to odor). All rats were allowed to explore the chamber for 15 minutes on the day before the first training session. Daily training began with a rat placed on the metal grid in one compartment of the chamber with the cover on. During a trial, the CS (tone or odor stimulus) was given to the rat continuously for 5 seconds and was followed by 3-second 1-mA electric foot shock. Since the electric shock was only delivered to the floor of the compartment a rat was in, to escape or avoid foot shock, a rat had to jump over the barrier to the adjacent compartment of the chamber. There were three possible behavioral responses in each trial: a successful avoidance response, which is defined as a rat actively jumping in response to the CS to the safe side before shock was delivered; an escape response in which a rat jumped to the safe side after shock onset; and a failed trial where a rat did not move to the opposite side before or during delivery of foot shock. Behavioral training was consisted of 40 trials a day for 5 consecutive days.

Animals were allowed to have at least 24 hours of rest after the last trial on the previous training day. The avoidance rate of each rat was measured as the number of successful avoidance responses observed in 10 trials (a block).

Data analysis. Behavioral data were analyzed using SPSS (IBM Corporation). A mixed ANOVA was conducted to access whether there were paradigm (a between-subjects variable) and training block (a within-subjects variable) differences in avoidance rates. The homogeneity assumption, known as sphericity, is required for mixed ANOVA. Sphericity requires equal variances and covariances for levels of the within-subjects variable at each level of between-subjects variable. When this assumption is violated, depending on the degree of violation, a corrected analysis result can be acquired using the Greenhouse-Greisser, the Huynh-Fildt, or the Lower-bound correction. Sphericity was also tested for data analysis using repeated-measures ANOVAs.

RESULTS

Two active avoidance paradigms (tone and odor) were conducted on 10 rats (2 groups, 5 in each group) for behavioral training.

Rats can learn to actively avoid auditory and olfactory signals that are associated with danger

We first looked at the results on the first training day. In the rats that were trained with tone signals, there was a significant main effect of training blocks on avoidance rates (repeated-measures ANOVA; F (3, 12) = 9.755, $p < 0.01$) (**Fig. 1B**), indicating that in general, the rats performed different tone avoidance behavior in different training blocks. Pair-wise comparisons indicated that block 1 was significantly different from block 3 ($p < 0.05$) and 4 ($p< 0.05$). In addition, there were significant linear (F (1, 4) = 17.442, $p < 0.05$) and cubic (F (1, 4) = 8.182, $p < 0.05$) trends in the data, suggesting that the more blocks the rats had, the more likely the rats performed avoidance.

Similarly, in the odor training rats, there was a significant main effect of training blocks (repeated-measures ANOVA, F (3, 12) = 4.567, $p < 0.05$) (**Fig. 1C**). Thus, we compared individual blocks via pair-wise comparisons and found that that block 1 was significantly different from block 2 ($p < 0.05$) and block 4 ($p < 0.01$). Furthermore, tests of within-subject contrast indicated a significant linear trend (F (1, 4) = 16.732, $p < 0.05$), suggesting that the rats had better performance as the training went on.

Together, the results suggested that even on day 1, the rats were able to associate both CS types with upcoming dangerous events, as shown by animals actively jumping into the opposite (safe) side of the chamber in response to the CS. Furthermore, the results indicated a linear relationship between avoidance rates and training blocks, suggesting that the more training the rats had, the better they performed. Finally, pair-wise comparisons of the training blocks indicated that significant training effects could be observed in the rats in the first two blocks (20 trials) in the tone avoidance rats and in the first block (10 trials) in the odor avoidance rats (**Fig. 1B&C**).

We then looked at learning performances of the rats in the course of the five-day training period. First, we found that each rat in both groups had a unique learning curve during the training (**Fig. 2A&B**). A mixed ANOVA with Huynh-Feldt correction was conducted to accesses whether there were paradigm and block differences in avoidance rates during the training. Results indicated a significant main effect of training blocks, F (15,391, 123.131) = 7.137, $p < 0.001$, but not of paradigm, F (1, 8) = 0.203, $p = 0.664$. In addition, the blocks main effect was not qualified by a significant interaction between blocks and paradigm, F (15,391, 123.131) = 1.503, p =

0.112. Together, the results suggested that even though, as a general rule, rats in both groups performed differently among blocks, tone training rats did not perform differently from odor training rats.

Dichotomy of the rats in training performances

As mentioned earlier, each rat had distinct abilities in learning active avoidance. We noted that several rats appeared to have limited learning capacity for avoidance conditioning. For example, among tone training rats, T-4 never performed over 20% avoidance in a block and T-3 only reached 60% once during the training (**Fig. 2A**). The majority of the rats were able to continuously improve or maintain their daily best avoidance rate per block up to 70% or better (T-1, T-2, T-5, O-2, O-4, and O-5). However, more training did not necessarily lead to higher avoidance rates in every rat, as T-3 and T-4 showed declines in their performances in the course of the training (**Fig. 2A**). A dichotomy in performance of the rats was most apparent by day 3, when we were able to clearly separate them into good (T-1, T-2 and T-5; O-2, O-4 and O-5) and poor (T-3 and T-4; O-1 and O-3) learners, based on whether they had reached 50% avoidance in any block on that day (**Fig. 2A&B**).

Among the poor tone learners, T-3 and T-4's avoidance rates deteriorated over time and remained poor until the end of the training (**Fig. 2A**). It may be because these two rats appeared to settle for escaping (jumping after foot shock was delivered) instead of actively avoiding foot shock. In contrast, among the poor learners in odor training, O-1 seemed to be able to pick up the skill after day 3, and O-3 was able to reach 50% avoidance on day 4 (**Fig. 2B**). On day 5, O-1 improved its daily best avoidance rate to 70% and had become one of the good learners. These differences between the two paradigms might be attributed to individual as well as paradigm differences.

In summary, there was a clear performance dichotomy between the rats with tone avoidance conditioning, while the same performance dichotomy was not as robust in odor training rats.

Rapid acquisition but poor behavioral retention

In the course of the five-day training, the rats appeared to acquire avoidance conditioning in each daily training session but forgot the newly learned behavior by the first training block of the next day (blocks 5, 9, 13, and 17) (**Fig. 2A&B**). In the tone training group, pair-wise comparisons indicated significant differences between blocks 4 and 5 ($p = 0.023$), blocks

8 and 9 (p = 0.021), blocks 12 and 13 (p = 0.018), and blocks 16 and 17 (p = 0.043) (**Fig. 2A**). In other words, mean avoidance rates of the rats declined after every 24-hour rest, suggesting poor behavioral retention for tone avoidance conditioning. However, the results with odor training rats revealed a different story. There was no significant difference between blocks 4 and 5 (p=0.051), blocks 8 and 9 (p=0.12), and blocks 12 and 13 (p = 0.07); the only significant difference was found between blocks 16 and 17 (p=0.006) (**Fig. 2B**). Together, the results suggested that behavioral retention was worse in tone training rats than in odor training ones, even though their behavioral patterns were similar.

Another way to investigate poor behavioral retention was to only look at mean avoidance rates in the first block of each day (blocks 1, 5, 9, 13, and 17) (**Fig. 3**). We first assessed whether there was a linear relationship between the avoidance rates and training days (**Fig. 3A**). Data of the tone training group showed a low linear correlation between the avoidance rate and training day (R^2 = 0.06), as only 6% of the variance in avoidance rates can be associated with the variance in training days. The slope of the trend line is -0.012, suggesting that the avoidance rates were slightly decreased as the training went on. Odor avoidance data indicated a

higher linear correlation between the avoidance rates and training days (R^2 = 0.4), as 40% of the variance in avoidance rates can be associated with the variance in training days. The slope of the trend line was 0.03, suggesting that the avoidance rates was slightly increased with the rats having more training.

In summary, avoidance conditioning in my design, on average, could be quickly learned by the rats in one training day. However, the rats appeared to have difficulty retaining the behaviors, as shown by significant differences between avoidance rates of the final blocks of one day and the first blocks of the next day.

DISCUSSION

My two-way avoidance data have confirmed previous findings that rats can use both auditory and olfactory signals to avoid upcoming dangerous events (Rohrbaugh et al., 1971; Hutton et al., 1974; Owens et al., 1996). Furthermore, our data are consistent with a study by Owens and colleagues that odor-cued animals had faster acquisition and better retention than tone-cued animals (Owens et al., 1996). The results also confirm several studies that have shown a dichotomy in active avoidance performance in subject populations (Torras-Garcia et al., 1997; Choi et al.,

2010) – i.e., some animals fail to learn the active avoidance behavior and inappropriately freeze instead.

Neural mechanisms underlying active avoidance have been investigated in two recent studies (Choi et al., 2010; Darvas et al., 2011) (**Fig. 4**). Choi and colleagues showed that the lateral and basal nuclei of the amygdala are essential for the performance of two-way active avoidance responses; lesions of these two areas impaired avoidance performance. Moreover, they showed that the central nucleus of the amygdala is not required for active avoidance, and may in fact constrain the instrumental avoidance response. In addition, Darvas and colleagues reported that dopamine (DA) signaling in the amygdala and the whole striatum is essential for acquiring two-way active avoidance. However, after prolonged overtraining, DA signaling in the striatum alone was sufficient to maintain two-way active avoidance, while DA signaling in the prefrontal cortex and the amygdala together was insufficient to maintain the behavior.

One of the goals of this study was to evaluate the possibility of using olfactory active avoidance conditioning for ensemble recordings from awake, behaving rats. As noted above, results have shown that the rats were able to use olfactory cues in two-way active avoidance conditioning.

The question is whether this paradigm is for ensemble recordings from awake, behaving rats? The advantages of this paradigm include: 1) simplicity of training, 2) speed of behavior acquisition, 3) ease for experimenters to observed odor-evoked behavioral responses, and 4) known underlying plasticity in multiple brain regions that mediate the learning. The disadtantages of this paradigm include: 1) requirement of animal movements, which may create electrical noise and movement artifacts in chronic recordings, 2) the foots hock, which itself can create electrical noise, 3) variability between animals in avoidance learning, 4) required multi-day training to induce reliable performance in some animals, and 5) variance of intensity and timing of odor stimuli, as locations of animal within the chamber cannot be controlled.

Together, these disadvantages outweighed the advantages and, I instead chose to use standard odor-shock conditioning, with freezing as the dependent behavioral variable. As described in Chapter 3, rats rapidly learn this task, movement artifacts are reduced, and the paradigm can be modified to examine both changes in learned fear and changes in odor acuity.

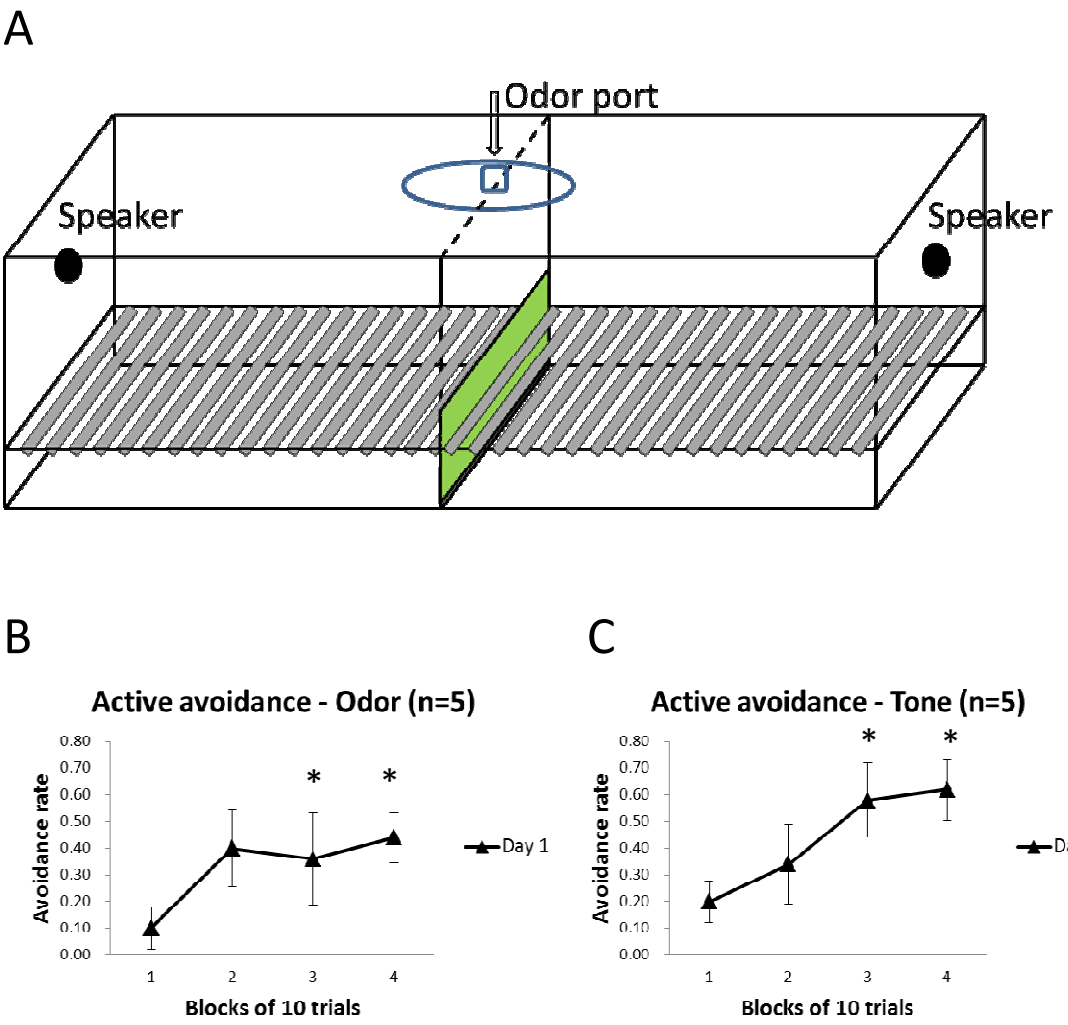
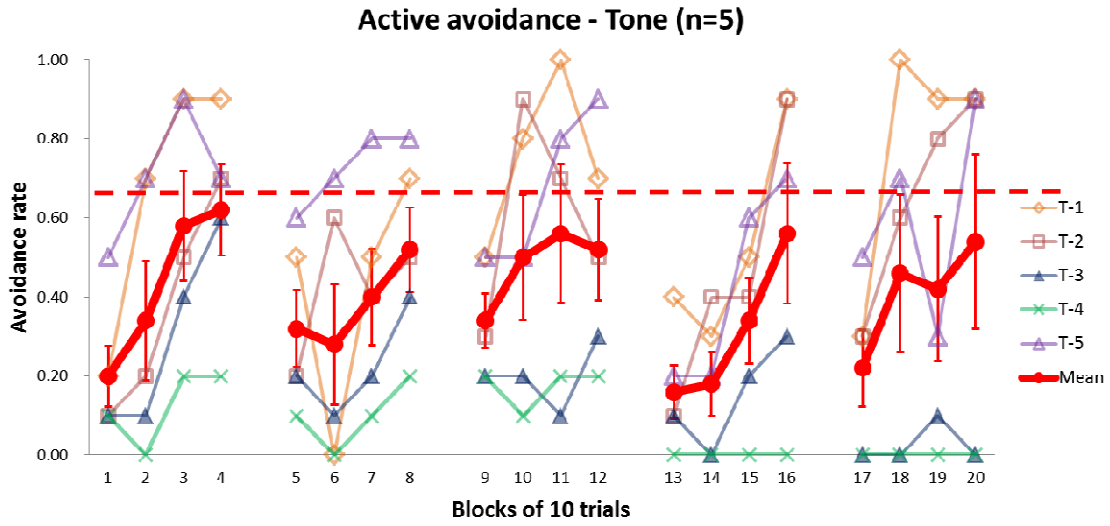


Figure 1. Mean avoidance rates in blocks of 10 trials in the rats. **A**, The custom modified shuttle box used in the behavioral training. **B**, Rats can be trained to perform active avoidance in response to a tone signal (75 dB, 27 Hz). Mean avoidance rates were significantly different in blocks 3 and 4 from in block 1. ***C***, Odors signal (propyl butyrate) can also evoke active avoidance behaviors in the rats. Mean avoidance rates were significantly different in blocks 2 and 4 from block 1. There were 4 blocks per day. Error bars represent s.e.m.. Asterisk: $p < 0.05$.

A



B

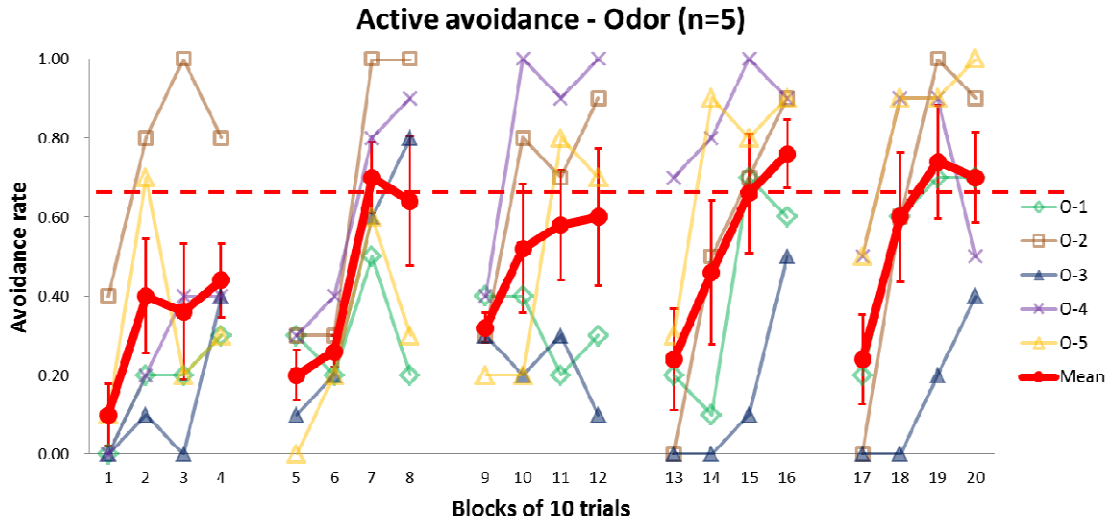


Figure 2. Performance curves for active avoidance training sessions over five days. **A**, Learning curves of the rats (T-1 to T-5) that were trained with tone cues. **B**, Learning curves of the rats (O-1 to O-5) that were trained with odor cues. Red dashed lines indicate 70% avoidance rate. Error bars represent s.e.m..

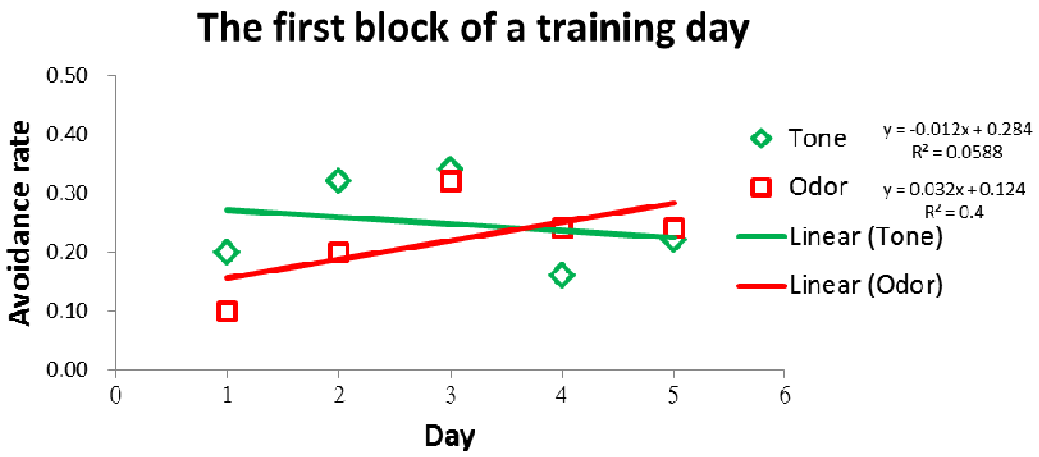


Figure 3. Relationships between mean first block avoidance rates and training days. Data represent average first block avoidance rate of tone (green diamonds) and odor (red squares) training rats. Green and red lines represented linear regressions for data of tone and odor training rats, respectively.

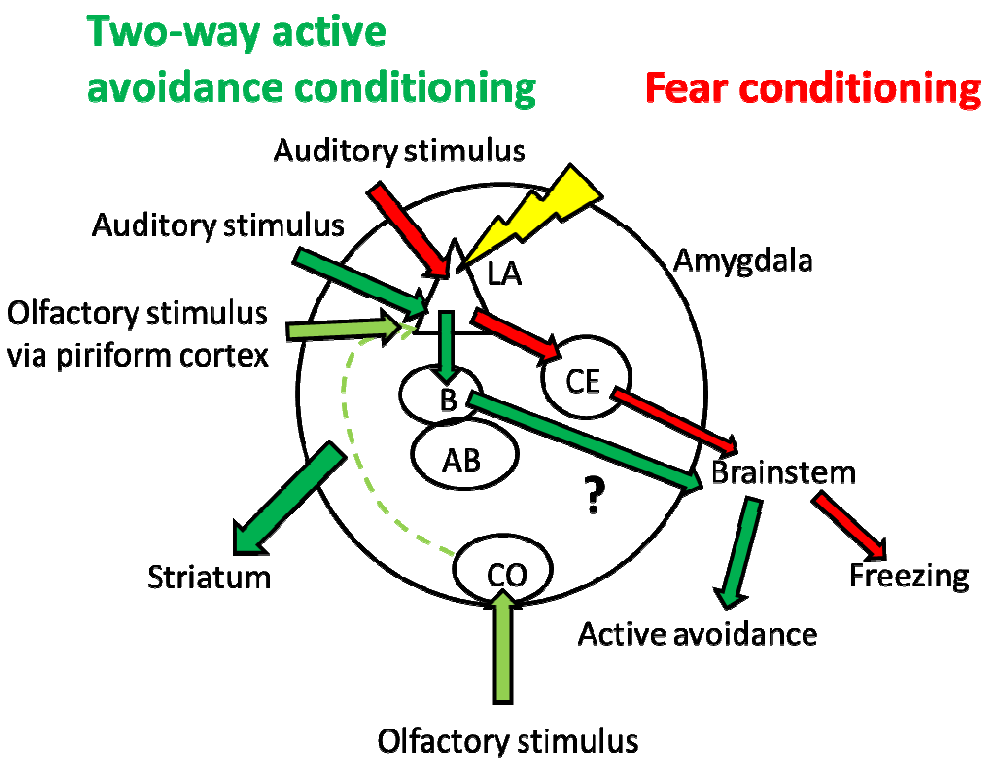


Figure 4. Hypothesized (Choi et al., 2010) neural pathways within the amygdala for two aversive conditioning paradigms. The red pathway represents a neural pathway for classical fear conditioning. The green pathways represent possible neural pathways for two-way active avoidance conditioning with tone and odor cues. **LA**, the lateral nucleus of the amygdala. **B**, the basal nucleus of the amygdala. **AB**, accessory basal nucleus of the amygdala. **CE**, the central nucleus of the amygdala. **CO**, the cortical nucleus of the amygdala.

CHAPTER 3

Generalized versus Stimulus-specific Learned Fear Differentially Modifies Stimulus Encoding in Primary Sensory Cortex in Awake Rats

ABSTRACT

Experience shapes both central olfactory system function and odor perception. In piriform cortex, odor experience appears critical for synthetic processing of odor mixtures. Synthetic odor processing contributes to perceptual learning and perceptual acuity, as well as contributing to memory for events and/or rewards associated with odors. Although reward-related odor learning has shown to induce plasticity in the piriform cortex, how aversive odor learning affects cortical odor processing is still unclear. Here, we examined the effect of odor fear conditioning on piriform cortical single-unit responses to the learned aversive odor, as well as its effects on responses to similar odors (overlapping mixtures) in freely moving rats. We found that odor-evoked fear responses were training paradigm-dependent. Simple association of a CS+ odor with foot shock (US) led to generalized fear (cue-evoked freezing) to similar odors. However, after differential conditioning, which included trials where a CS- odor (a mixture overlapping with the CS+) was not paired with shock, freezing responses were CS+ odor-specific and less generalized. Pseudo-conditioning led to no odor-evoked freezing. These differential levels of stimulus control over freezing were associated with different training-induced changes in single-unit odor responses in anterior piriform cortex (aPCX). Both simple and differential conditioning induced a significant decrease in aPCX single-unit spontaneous activity compared to pre-training levels while pseudo-conditioning did not. Simple conditioning enhanced mean receptive field size (breadth of tuning) of the aPCX units, while differential conditioning reduced mean receptive field size. These results suggest that generalized fear is associated with an impairment of olfactory cortical discrimination. Furthermore, changes in sensory processing are dependent on the nature of training, and can predict the behavioral outcome of the training.

INTRODUCTION

The olfactory system involves a memory-based odor processing function that allows its acuity to be constantly shaped by experience (Wilson and Stevenson, 2006). This function enables animals to not only identify myriad novel odors and odor combinations in the environment, but also associate odors with their ecological significance, which is critical for adaptive behavior. Experience-dependent perceptual changes (perceptual learning) and their underlying neural plasticity have been reported across phylogeny from the 1st- to 3rd- order neurons in the olfactory system (Davis, 2004).

In mammals, while robust experience-dependent plasticity is expressed in the olfactory bulb (Freeman and Schneider, 1982; Sullivan et al., 1989; Brennan et al., 1990; Fletcher and Wilson, 2003; Ravel et al., 2003; Mandairon et al., 2006; Doucette et al., 2011), the piriform cortex appears to play a special role in experience-dependent odorant perceptual synthesis and odor object formation (Haberly, 2001; Kadohisa and Wilson, 2006; Barnes et al., 2008; Li et al., 2008).

Work on olfactory perceptual learning has focused on enhanced acuity for learned or familiar odors (Cleland et al., 2002; Wilson, 2003; Li et

al., 2008; Moreno et al., 2009). Thus, molecularly similar odorant molecules that initially cannot be perceptually distinguished by naïve animals become discriminable with appropriate experience (Cleland et al., 2002; Fletcher and Wilson, 2002; Li et al., 2008). This improved acuity is associated with changes in the olfactory bulb (Fletcher and Wilson, 2003; Moreno et al., 2009; Doucette et al., 2011) and piriform cortex (Kadohisa and Wilson, 2006; Li et al., 2008; Chapuis and Wilson, 2010). Associative learning, for example, linking an odor with a specific biologically relevant meaning such as reward, can also modify olfactory bulb (Freeman and Schneider, 1982; Sullivan et al., 1989; Brennan et al., 1990; Ravel et al., 2003; Moreno et al., 2009; Doucette et al., 2011) and piriform cortical odor coding and physiology (Saar et al., 2002; Saar and Barkai, 2003; Kadohisa and Wilson, 2006; Calu et al., 2007; Roesch et al., 2007).

However, in some situations acuity appears to be reduced by experience. For example, standard fear conditioning involving pairing of a CS+ with a footshock can induce a generalized fear response to many stimuli in some way similar to the CS+. This has been demonstrated in a variety of sensory modalities and paradigms (Pavlov, 1927; Honig and Urcuioli, 1981; Shepard, 1987; Armony et al., 1997). Work in the auditory

system suggests that this fear generalization is in part mediated by changes within the sensory system itself, with an apparent loss of sensory acuity mediating the generalized response to stimuli the same as, or similar to, the CS+ (Armony et al., 1997; Ito et al., 2009). Differential conditioning, in contrast, where both a CS+ (e.g., predicting footshock) and a CS- (predicting no footshock) are used during conditioning, induces much less generalization and instead induces learned responses that are highly specific to the stimulus (Pavlov, 1927; Rescorla, 1976; Ito et al., 2009).

The present study had two primary goals. First, most work on changes in adult olfactory cortical single-unit responses to learned odors has relied on appetitive tasks (Saar et al., 2002; Kadohisa and Wilson, 2006; Calu et al., 2007; Roesch et al., 2007). Here, using chronic recording of anterior piriform cortical single units, we examined whether aversive conditioning could similarly modify olfactory cortical odor responses. Second, by using both standard (CS+ only) and differential (CS+ and CS-) fear conditioning, we tested the hypothesis that piriform cortical odor coding could be shifted in two opposing directions, depending on the nature of the conditioning and in concert with the learned behavioral response to odor (generalized or selective odor-evoked fear).

MATERIALS AND METHODS

Subjects. 27 male Long-Evans hooded rats (250-450g) were used as subjects (Charles River Laboratories, Wilmington, MA). Animals were housed individually in polypropylene cages on a 12-hr light/dark cycle, with food and water available *ad libitum*. Animal care protocols and all experiments were approved by the University of Oklahoma IACUC and the Nathan S. Kline Institute IACUC and in accordance with National Institutes of Health guidelines.

Odor Stimulation. Odors used were the monomolecular odorant limonene and a mixture (10C) that had 10 monomolecular odorants each at a concentration of 100 ppm based on vapor pressure and dilution in mineral oil. See **Fig. 1A** for a list of the components and mixtures. Other odors included 10C-1 (10C with isoamyl acetate removed), 10C-2 (10C with isoamyl acetate and nonane removed) and 10CR1 (10C with isoamyl acetate replaced by 2-methyl-2-buten-1-ol), identical to those used previously (Barnes et al., 2008). Odors were presented (5 sec) to the behavioral chamber via a flow-dilution olfactometer (1 LPM) that was controlled by a programmed script in Spike 2 (Cambridge Electronic Design,

UK). Odors were presented with an interval at least 60 seconds and at random distances from the animals' noses given their free mobility.

Electrodes. Extracellular recordings were obtained by using a drivable bundle of ten, 25-µm-diameter (38-µm-diameter with insulation) Formvar-insulated Nichrome wires (A-M Systems, Carlsborg, WA). A guide tube holding the wires was a 27-gauge thin wall cannula (Small Parts, Miami Lakes, FL). The electrode design was identical to those used previously (Roesch et al., 2007).

Surgical Procedures. Naïve animals were anesthetized and kept unconscious with an isoflurane anesthesia system (E-Z Systems, Palmer, PA) throughout the surgical process. The microwire bundle was chronically implanted in the left hemisphere and cemented on the rat's skull, with the tip slightly above or within aPCX (1.0 mm anterior to the bregma, 4 ~ 4.5 mm laterally, and 5 ~ 6 mm ventral to the surface of the brain). Immediately after the surgery, prior to recovery from anesthesia, an antibiotic and analgesic were subcutaneously injected in the rats (Enrofloxacin, 5 mg/kg; Buprenorphine, 0.01 mg/kg for analgesia, and 12 hours later). Animals were given two weeks for recovery from surgery before initial training sessions.

Fear-conditioning paradigm. Behavioral experiments were conducted in one of two custom chambers with a shock grid floor (Lafayette Instrument, Lafayette, IN or Coulbourn Instruments, Whitehall, PA). Rats were randomly divided into three groups: standard, differential, and pseudo-training groups. The standard training group received 10 trials of a 5-second 10C (CS+) odor followed by a 1-sec, 0.4-0.5 mA electric foot shock. The inter-trial interval for standard training was 120 seconds. The differential training group had a 5-sec 10C (CS+) odor paired with a foot shock and a 5-sec CS- odor (10C-2 or 10cR1 in different rats) with no foot shock. In differential conditioning, the rats received 10 CS+ and shock pairings as in standard conditioning, but in addition received 30 CS- trials randomly interspersed with the CS+ trials. Inter-trial interval in all cases was 120 seconds. The pseudo-training group received unpaired 5-sec 10C and foot shock, and each was randomly presented for 10 times. For this group, 10C presentations were kept at least 120 seconds apart to prevent olfactory habituation. Behavioral and neural testing was performed at least 24 hours after training.

Behavioral cue-evoked freezing tests. For behavioral analyses, 24 hours after training, a retention test was carried out in a transparent acrylic

testing chamber (10” W x 9.5” Lx 6” H) different from the training chambers. During testing, all rats were randomly presented with 10C, 10C-1, 10C-2, 10CR1 and limonene 3 times each and odor-evoked freezing (motionless except for breathing, slightly arched posture) was scored in response to each odor stimulus. All behaviors were also monitored by video. For behavior-only rats, the video camera was shooting from the side of the chamber, and this angle provided good visualization of freezing behavior of the rats. For chronically recorded rats, the camera was positioned above the chamber to record the freezing behavior from the top. This shooting angle caused problems of accurately visualizing freezing behavior in these rats. Thus, behavioral data and neural data are from different animals, although training and testing protocols were identical. The behavioral data were collected by a graduate student in the lab, Dylan Barnes and the behavioral tests were not blind.

Chronic single-unit recording. For animals used for single-unit recordings, behavioral training was conducted following several days of baseline recordings. The same three conditioning groups were used for behavioral testing: standard training, differential training, and pseudo-training. Post-training recordings sessions occurred over several days to

increase recording yield, thus before each daily post-training recording session, a reminder session (3 trials, paired or unpaired odor-shock) was given to the corresponding groups.

Data acquisition. Neural data were collected by a Multichannel Acquisition Processor system (Plexon, Dallas, TX). Two weeks following the surgery, animals were placed in a custom testing chamber (10" W x 10" L x 22" H) to record neural activity in piriform cortex. A DC fan was mounted on the wall and constantly drew air out of the chamber (**Fig. 3A**). In a recording session, neural data were continuously recorded while the animal was repeatedly presented with test odors. Test odors were 3 mixtures, 10C, 10C-1, and 10CR1 and 1 single odorant, limonene. At least 3 of the 4 tested odors were given to the animals for at least 3 times. Before each session, recordings were screened for quality of unit activity. If no isolatable spikes were found across all electrodes, recording was terminated for the day. The implanted wire bundles were lowered daily at the end of each session to a new recording site. The daily advancing distance was ~80 μm before reaching the depth of 6 mm, and it was then reduced to ~40 μm thereafter. After the advance, rats had at least a 24-hour rest before the next recording session (Schoenbaum et al., 2003). Recordings in aPCX (based on

histological analyses) before odor training were used as the pre-conditioning baseline. Data collected during the odor-shock training session were not analyzed due to excessive electrical noise generated by the shock system and movement artifacts from the shocked rats. Post-training recordings were conducted in the same way as baseline recordings, with electrode bundles advanced each day. The same recording procedures were used in all three conditioning groups.

Data analysis. Neural data recorded by the Plexon system were transformed into a format compatible with Spike2 and were analyzed off-line using Spike2's template sorting features. Before template sorting, most channels were bandpass-filtered at 300 to 3000 Hz using digital filters in Spike2. A threshold of spike amplitude was set to collect waveforms larger than 2.5:1 signal-to-noise ratio (Katz et al., 2002) (**Fig. 3D**). Isolations of single units were initially done using the template-matching function in Spike2. Each template was generated based on at least 60% match in shapes of the initial eight waveforms. Once a template was established, more similar waveforms are added into the template. Once the template matching was accomplished for all qualified waveforms in neural data of a recording session, non-action potential waveforms, such as electrical noise

and movement artifacts were manually removed from the templates using visual examination and the waveform-cutting function in Spike2. The identification of non-action potential waveforms was based on shapes, amplitudes, and whether they were simultaneously observed in multiple channels. Waveforms in different templates were finally examined using principle component analysis (PCA), in which clusters of action potentials were shown in principle component space. The cluster-cutting algorithm in Spike2 was used at this stage to further identified single units. Each single unit was required to have an inter-spike interval of at least 1-2 milliseconds. Typically, 1-3 units could be isolated on an active channel. Neural data collected 6 mm below the brain surface or deeper were considered to be from neurons in layer II/III of the piriform cortex and were confirmed by histological data (**Fig. 2**).

Odor-evoked activity was determined by spike counts from cumulative peri-stimulus time histograms (PSTHs) (from 5 seconds before and 10 seconds after odor onset, and with 100-ms bin width) based on 3 stimulus repetitions (**Fig. 3E**). A significant excitatory response of a single unit to a test odor was defined as odor-evoked activity that was at least 20% larger than the spontaneous activity (**Fig. 3E**). A significant suppression

was defined as a 20% or greater decrease in odor-evoked activity compared to spontaneous activity. The 20% value was chosen to provide a measure of reliable responses and corresponds to the magnitude of odor-evoked activity commonly observed in the olfactory cortex (Wilson, 1998). The critical factor in defining responses was selection of a standard measure that was applied to all recordings in all groups before and after training to assay learning-induced changes. The large bin widths were chosen because in this paradigm, odor stimuli were presented randomly to an animal that could be located anywhere within the conditioning chamber at stimulus onset. Thus precise timing of stimulus-response characteristics, as for example in odor-nose-poke paradigms (Calu et al., 2007; Roesch et al., 2007) was not possible. However, we were able to find reliable odor-evoked responses even in this more naturalistic paradigm.

Spike trains of units recorded from animals before and after training were analyzed for spontaneous activity rate, odor-evoked response probability, excitatory and suppressive response probability, and receptive field size (tuning breadth). Receptive field size of a single unit was calculated as the percentage of test odors to which a single unit showed a significant excitatory or suppressive response.

Histology. Following the final day of recording, implanted rats were given an overdose of urethane and then perfused transcardially with 0.9% saline followed by 10% formaldehyde. Brains removed from the skulls were stored in a 30% sucrose/10% formaldehyde solution for later sectioning. The brains were sectioned coronally at 40 μm, mounted on the slides, and stained with cresyl violet or nuclear fast red. Electrode tracks and recording locations were verified under a light microscope and images were acquired using a digital camera (**Fig. 2**).

RESULTS

Three odor-fear conditioning paradigms were used to train 12 rats (3 groups, 4 in each group) for behavioral analysis. Animals used for chronic recording received the same kind of training, but due to difficulties in accurately visualizing freezing behavior while recording in this system, behavioral and neural data are from different rats. Neural activity was recorded in aPCX from 15 freely moving rats (standard training, n = 6; differential training, n = 4; pseudo-training, n = 5). A total of 528 aPCX single units were isolated and analyzed.

Olfactory fear generalization is dependent on nature of the training

Rats were trained in standard, differential or pseudo odor-shock conditioning and odor-evoked freezing was measured in a different context 24 hours after training (**Fig. 1B**). A mixed ANOVA was performed, with group (standard, differential and pseudo) and odor (10C, 10C-1, 10C-2, 10CR1 and limonene). The 3 training groups showed distinct odor-evoked fear responses (**Fig. 1C**), with significant main effects of odor, $F(4, 50) = 4.00$, $p < 0.01$, and training groups, $F(2, 50) = 60.60$, $p < 0.0001$). Pseudo-trained rats did not show significant freezing in response to any test odor (**Fig. 1C, pseudo**). The standard training group, which had 10C as the CS+, showed significant freezing responses to 10C and the overlapping mixtures, 10C-1, 10C-2 and 10CR1, as well as to limonene (Fisher's PLSD, $p < 0.05$) (**Fig. 1C, standard**). There was no significant difference in freezing across all test odors, suggesting that the acquired fear of 10C was generalized to the other odors. In contrast, the differential training group, which was trained to associate 10C (CS+) with foot-shock and 10C-2 or 10CR1 (CS-) with safety (no shocks), showed significant freezing to 10C only. Furthermore, 10C evoked significantly stronger freezing than 10C-1, 10CR1 or limonene (Fisher's PLSD, $p < 0.05$). The results suggest that differential training

induced highly odor-specific fear, while standard training induced highly generalized odor fear. In separate animals we examined whether these disparate behavioral outcomes were associated with changes in cortical odor processing.

Neural responses

A total of 528 single units were isolated from 15 animals. In the standard training group, we recorded from 95 units before training and 121 units after training. In the pseudo-trained group, we recorded from 54 units before and 55 units after training. In the differential group, we recorded from 122 units before and 87 units after training. Both spontaneous and odor-evoked activity were compared within each group before and after training. Mean number of single-units isolated from individual animals > 30.

Non-specific decreases in aPCX spontaneous activity associated with olfactory aversive learning

We first examined spontaneous aPCX single-unit activity, and there was a main effect of training groups (one-way ANOVA, $F(5, 528) = 5.39$, $p < 0.0001$). There was no significant difference in spontaneous activity across

the three conditioning groups pre-training. However, both standard and differential training induced a significant decrease (**Fig. 4**) in average spontaneous firing rate in aPCX neurons (Fisher's PLSD, standard group, $p < 0.0001$; differential group, $p < 0.005$). No significant change was observed in pseudo-trained rats. Thus, regardless of the specific paradigm, odor fear conditioning induced a decrease in aPCX spontaneous activity that was not observed after pseudo-conditioning.

Training paradigm-dependent changes in aPCX odor-evoked activity

We first looked at the proportion of single units that showed an excitatory response to the test odors before (pre) and after (post) training. We found a trend toward a non-odor-specific decrease in the proportion of single units that showed excitatory (>20% increase from baseline) responses to the test odors in the standard and differential training groups (**Fig. 5 B1 & C1**). In the standard training group, however, only the proportion of limonene-responsive single units was significantly decreased ($x^2 = 4.19$, $p = 0.04$) (**Fig. 5 B1**). In the differential training group, a decrease was found in the proportion of single units responding to 10CR1 ($x^2 = 4.70$, $p = 0.03$) and 10C-1 ($x^2 = 10.35$, $p = 0.001$) (**Fig. 5 C1**). Both standard and

differential training induced a significant decrease in the proportion of units showing excitatory responses to odor (any of the 4 test odors) compared to pre-training (**Fig. 5 A1) (**mixed ANOVA, a significant main effect of training, $F(1, 9) = 5.98$, $p = 0.037$; paired t-test (t = 5.66, df = 3), $p = 0.011$; paired t-test (t = 4.135, df = 3), $p = 0.026$). Finally, there was no change in the proportion of excitatory responses after pseudo-conditioning (**Fig. 5 A1**).

In contrast to excitatory responses, the proportion of units showing suppressive responses was significantly enhanced after standard training (**Fig. 5 A2**; mixed ANOVA, a significant main of training, $F(1, 9) = 19.680$, $p < 0.01$; paired t-test (t = -6.4, df = 3), $p = 0.008$), with no change after differential or pseudo-conditioning. The enhancement in suppressive responses was not stimulus-dependent, and was expressed significantly by limonene ($x^2 = 11.87$, $p < 0.001$), 10C ($x^2 = 5.71$, $p = 0.017$), and 10CR1 ($x^2 = 9.35$, $p = 0.002$) (**Fig. 5 B2**). Other than a significant increase in suppressive responses to 10C-1 ($x^2 = 5.10$, $p = 0.02$), no clear training-induced pattern was found in the differential group (**Fig. 5 C2**).

Training paradigm-dependent changes in aPCX single-unit receptive field breadth

Receptive field (RF) size of each aPCX single unit before (pre) and after (post) training was calculated as the proportion of the test odors that evoked either a significant excitatory response or a significant inhibitory response.

There was no difference across groups in receptive field size pre-conditioning. Furthermore, receptive field size was not modified post-pseudo-conditioning. However, standard training (which induces generalized odor fear) resulted in a significant increase in mean single-unit total receptive field size in aPCX compared to pre-training levels (repeated-measures ANOVA, a significant main effect of paradigm x training, $F(2, 528) = 3.96$, $p = 0.02$; t-test ($t = -2.44$, $df = 189.33$), $p = 0.015$) (**Fig. 6 A, Standard**). This increase was predominantly due to an increase in receptive fields for suppressive responses (**Fig. 6 B**; repeated-measures ANOVA, a significant main effect of training, $F(1, 528) = 13.9$, $p < 0.001$; t-test ($t = -4.84$, $df = 214$), $p < 0.001$), even though standard training did induce a significant decrease in excitatory receptive field size (**Fig. 6 B**; repeated-measures ANOVA, a significant main effect of training, $F(1, 528) = 8.03$, $p = 0.005$; t-test ($t = 2.1$,

df = 189), p = 0.034). In contrast, differential training, which led to odor-specific fear, induced a decrease in mean total receptive field size, although this was not quite significant (t-test (t = 1.57, df = 207), p = 0.118) (**Fig.6 A Differential**). Differential conditioning did, however, induce a significant decrease in mean excitatory RF size (repeated-measures ANOVA, a significant main effect of training, $F(1, 528) = 8.03$, p = 0.005; t-test (t = 3.35, df = 204), p = 0.001) (**Fig. 6 C**). Thus, standard conditioning which induced generalized, non-odor-specific fear also resulted in a decrease in odor selectivity of aPCX single units, while differential conditioning, which induced relatively odor-specific fear, resulted in an increase in odor selectivity of aPCX single-units. Pseudo-conditioning induced no detectable change in receptive fields.

Learned changes in cortical single-unit virtual ensembles

Virtual ensembles of piriform cortical single units were created from merged recordings across animals within each training condition (Kadohisa and Wilson, 2006; Barnes et al., 2008; Chapuis and Wilson, 2010). Given the relatively high proportion of units that did not show reliable responses to odors, only those cells that were excited (as defined above) by at least one

of the test odor mixtures were included here (Standard conditioning, Pre, n = 47, Post, n = 58; Differential conditioning, Pre, n = 67; Post, n = 31; Pseudo-conditioning, Pre, n = 37, Post, n = 28). Bivariate correlations between odor-evoked firing rates (spike count during 5-sec odor stimulation – pre-stimulus spike count) within each ensemble for the 10C-1 and 10CR1 odors relative to the 10C CS+ odor were examined in both the pre-training and post-training sessions.

In these freely moving animals with extensive initial exposure to the odors during the pre-training sessions, both 10C-1 and 10R1 responses were strongly decorrelated from the 10C CS+ responses within all three conditioning groups prior to conditioning and there were no differences between groups during pre-conditioning (10c vs 10C-1, mean (± SEM) ensemble correlation across the 3 groups, r = 0.01 ± 0.04; 10C vs 10CR1, r = -0.03 ± 0.11). The ensemble response decorrelation of odors, however, was modified in a task-specific manner post-conditioning (**Fig. 7**). Standard conditioning produced a small increase in cortical ensemble response correlation between the CS+ odor and similar mixtures. This should have the effect of enhancing the similarity of these odors. Differential conditioning, in contrast, induced enhanced response decorrelation of the

CS+ odor from similar mixtures, increasing the difference in encoding of these odors. Finally, pseudo-conditioning produced a dramatic increase in correlation ($p < 0.05$) between the odor responses, again reflecting enhanced similarity of their encoding within the piriform cortex. Though the standard and differential conditioning-induced changes in cortical ensemble response correlation were small, they correspond to the distinct changes in odor selectivity of the learned odor-evoked fear responses in these two groups.

DISCUSSION

The present results demonstrate that increases and decreases in apparent odor perceptual acuity are associated with specific, opposing changes in anterior piriform single-unit receptive fields and virtual ensembles recorded in freely moving rats. Thus, standard odor-shock conditioning, which results in generalized freezing responses to odorant mixtures overlapping with the CS+, was associated with an increase in piriform cortical single-unit odor receptive field width and an increase in cortical ensemble response correlation of odor mixtures with the CS+. In contrast, differential odor-shock conditioning, using both a CS+ and CS-, induced freezing responses relatively specific to the CS+ odor, a decrease in

single-unit receptive field width and enhanced ensemble response decorrelation of the mixtures from the CS+. Pseudo-conditioning induced no freezing and no receptive field changes though it did significantly enhance correlation between overlapping mixtures. The increased response correlation in the pseudo-trained rats may reflect a decrease in acuity for similar odors with no significant meaning (Chapuis and Wilson, 2010). In addition to these paradigm-specific changes, both standard and differential conditioning, but not pseudo-conditioning reduced spontaneous firing rates, as recorded in the conditioning chamber. This reduction could enhance signal-to-noise ratios of odor-evoked activity, although given the observed decrease in odor-evoked excitatory responses, the effect on signal-to-noise ratios is unclear. Together, the results suggest that cortical and behavioral olfactory acuity can be either increased or decreased, based on experience, and that generalized fear may reflect not only changes in emotion circuits such as in the amygdala, but also changes within early sensory pathways.

The difference in neural and behavioral outcomes between the standard and differential conditioning paradigms has been suggested to reflect the fact that comparative judgments between stimulus pairs are not

required in the single-CS (standard training) paradigms, whereas they are an essential ingredient for the discriminative (differential training) paradigms (Guttman and Kalish, 1956; Ganz, 1962; Honig and Urcuioli, 1981). The comparative nature of differential conditioning presumably involves higher centers that utilize olfactory information for associative memory and decision making, such as the amygdala (Sullivan et al., 2000; Rosenkranz and Grace, 2002; Roesch et al., 2010), orbitofrontal cortex (Schoenbaum et al., 2003), entorhinal cortex (Kay and Freeman, 1998) and hippocampal formation (Wiebe and Staubli, 2001; Knafo et al., 2005). For example, odor-shock conditioning has been demonstrated to modify CS+ odor-evoked responses in the basolateral amygdala (Sullivan et al., 2000; Rosenkranz and Grace, 2002; Hegoburu et al., 2009). These networks presumably interact to help shape odor processing in the piriform cortex via feedback connections (see below).

We have recently observed similar task-dependent enhancement and reduction of behavioral and piriform cortical sensory acuity using appetitive tasks (Chapuis and Wilson, 2010). Although not directly comparable, both this study and theirs showed strong behavioral generalization and increased cortical response correlation after specific training protocols.

Whether the mechanisms of these changes induced by appetitive and aversive conditioning are the same is unclear. Work in humans suggests stimulus generalization is greater after aversive conditioning than after appetitive conditioning (Schechtman et al., 2010). Further work will be required to explore differences between these paradigms.

In addition to changes in sensory-evoked activity, single-unit spontaneous activity was also significantly decreased after either standard or differential conditioning, but not after pseudo-conditioning. The suppression of spontaneous firing could in part be mediated by norepinephrine, which is released by the locus coeruleus during novel or arousing events (Foote et al., 1980; Sara et al., 1994). This possibility is supported by: 1) norepinephrine release in aPCX is enhanced by foot shock in rats; this effect can last at least 20 min. (Smith et al., 2009); 2) norepinephrine has been reported to excite inhibitory interneurons and enhance both frequencies and amplitudes of IPSPs in pyramidal cells of the PCX (Gellman and Aghajanian, 1993); 3) norepinephrine has been shown to suppress excitatory afferent and intrinsic fiber synaptic transmission in the piriform cortex (Hasselmo et al., 1997). A computational modeling study utilizing these data showed clear norepinephrine-mediated suppression of

spontaneous activity of pyramidal cells in PCX (Hasselmo et al., 1997). This suppression of spontaneous firing may enhance the signal-to-noise ratio in the piriform cortex, as also suggested in the inferior colliculus (Voytenko and Galazyuk, 2010). This effect could enhance detection of odor cues in a dangerous environment and perhaps enhance separation of cue odors from background odors. However, much of the observed changes in odor-evoked activity were in suppressive type responses. Thus, it is unclear whether this model contributes to the observed, learned neurobehavioral effects of aversive conditioning. Furthermore, the effects of norepinephrine on olfactory responses are not just suppressive, as norepinephrine also enhances entrainment of piriform cortical single units to respiration (Bouret and Sara, 2002) and enhances mitral cell responses to olfactory sensory neuron input (Jiang et al., 1996). Thus, again a contribution from either changes in local inhibitory circuitry (Zhang et al., 2006; Suzuki and Bekkers, 2007; Poo and Isaacson, 2009) or higher order centers such as the amygdala (Luna, 2011), orbitofrontal cortex (Cohen et al., 2008) or entorhinal cortex (Ferry et al., 1996) may contribute to this observed spontaneous activity decrease.

In fact, in support of a potential change in local inhibitory circuitry, as noted above, much of the overall increase in receptive field breadth in the standard conditioning rats was due to an increase in inhibitory receptive field size. Standard conditioning actually reduced excitatory receptive field size, as did differential conditioning. In naïve animals, inhibitory interneurons in piriform cortex are much more broadly odor-responsive than pyramidal cells (Poo and Isaacson, 2009). Whether the enhancement in suppressive response tuning after standard conditioning was due to further broadening of inhibitory interneuron receptive fields or increased effectiveness of this inhibition on an already suppressed firing rate is not clear and is under investigation.

Although previous recordings in awake rats using a differential association appetitive task (go/no-go test) have shown odor-specific changes in aPCX-evoked activity (Roesch et al., 2007), differences in aPCX responses we recorded before and after training appear to be non-odor-specific (**Fig. 5 B1-2 & C1-2**). This difference may result from different aPCX plasticity requirements to reach behavioral criteria for the paradigms. Rapidly acquired aversive conditioning can quickly modify odor-evoked post-synaptic potentials in neurons of the lateral nucleus of the amygdala

(Rosenkranz and Grace, 2002), which may contribute to odor-evoked freezing behaviors. When fast responses are needed, precise CS-US association may not be required in the piriform cortex. In contrast, the slowly acquired olfactory discrimination tasks often involve complex rule learning and spatial learning (Saar et al., 1998, 1999; Cleland et al., 2002) and require more specific odor and perhaps multimodal associative encoding in the piriform cortex to reach the behavioral criteria (Schoenbaum and Eichenbaum, 1995; Calu et al., 2007; Roesch et al., 2007).

Summary

The present results showed that standard and differential fear conditioning induced different levels of odor-fear generalization in rats. There were corresponding training paradigm-dependent differences in aPCX single unit activity after training, suggesting that plasticity of piriform cortical networks may contribute to odor generalization and discrimination induced by aversive conditioning. Both aversive learning paradigms induced a significant reduction in spontaneous activity that was not observed in pseudo-conditioned rats. This reduction may increase signal-to-noise ratio in the aPCX and presumably enhance cue odor detection in a dangerous

environment. Together, the results suggest that changes within the primary sensory system may contribute to differing outcomes of fear conditioning. Generalized fear responses may in part be due to reduced selectivity of piriform cortical odor responses.

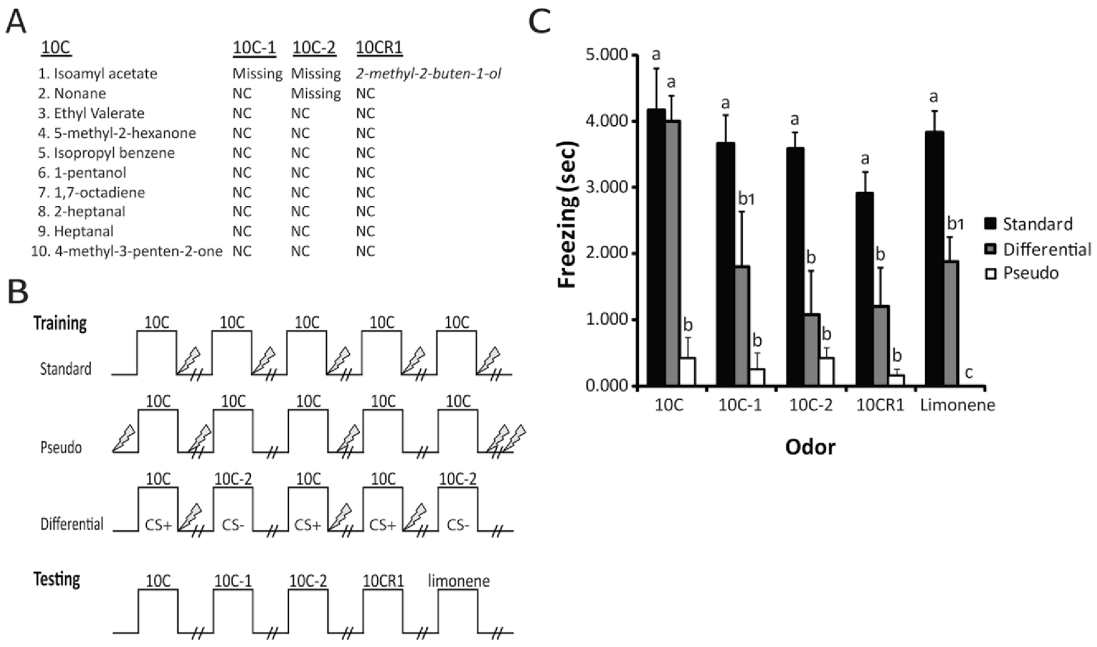


Figure 1. Olfactory fear conditioning with different paradigms. **A,** Odor mixtures used in the experiments. 10C, an odor mixture that consists of 10 different odorants. 10C-1, 10C with 1 odorant missing. 10C-2, 10C with 2 odorants missing. 10CR1, 10C with 1 odorant replaced by a new odorant. NC, no change. **B,** Odor-fear training paradigms. Standard training had 10 trials of 10C paired with an electric foot shock. Pseudo-training had 10 unpaired presentations of 10C and foot shock. Differential training had 10 trials of 10C (CS+) paired with a foot shock and 10C-2 (CS-) without a following foot shock. A retention test was carried out 24 hrs after the training session. All rats were randomly presented with 10C, 10C-1, 10C-2, 10CR1 and limonene 3 times. Odor-evoked freezing behavior of the rats was scored and recorded. **C,** Average freezing of the non-implanted rats in response to odor stimuli after training. Standard, differential and pseudo represent different training paradigms. Bars that are marked with a are significantly different from bars that are marked with b1, b and c (Fisher's PLSD, $p < 0.05$). There is no significant difference between bars that have the same mark. b1 and b are not significantly different from each another. b1 and c are significantly different (Fisher's PLSD, $p < 0.05$). Error bars represent s.e.m.

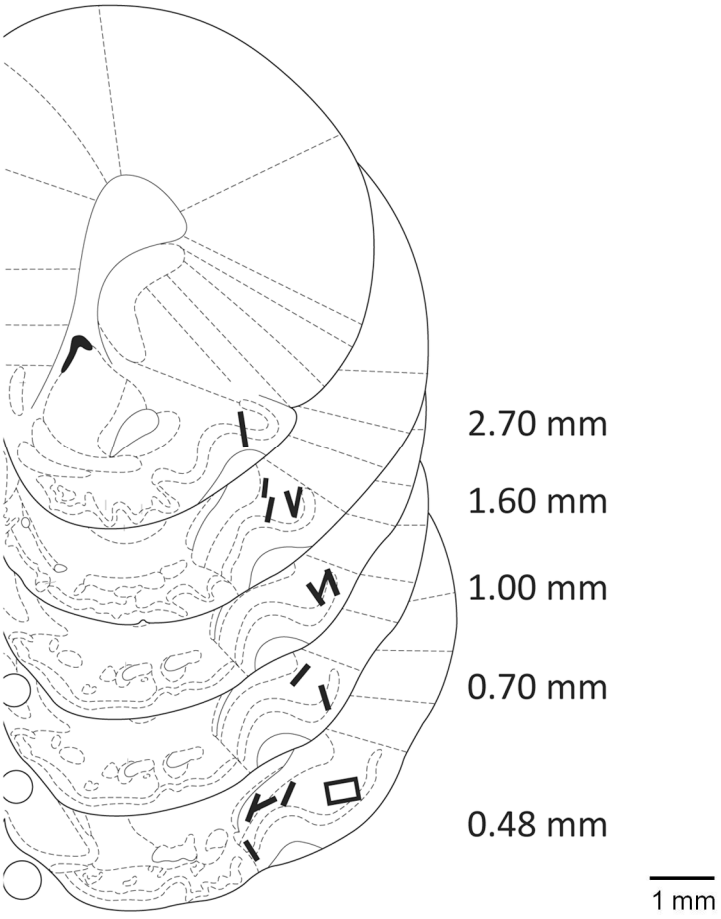


Figure 2. Representation of electrode tracks of 15 rats implanted with a movable microwire bundle. Black bars represent electrode tracks reconstructed from the brain sections. Recording sites were along the tracks. The black open rectangle represents possible recording sites across layer III of piriform cortex where track reconstruction was not available. The data suggested recordings were localized to layer II/III of anterior piriform cortex. Outlines are reproduced from Paxinos & Watson (2009) and represent sections ranging from 2.70 to 0.48 mm anterior to Bregma.

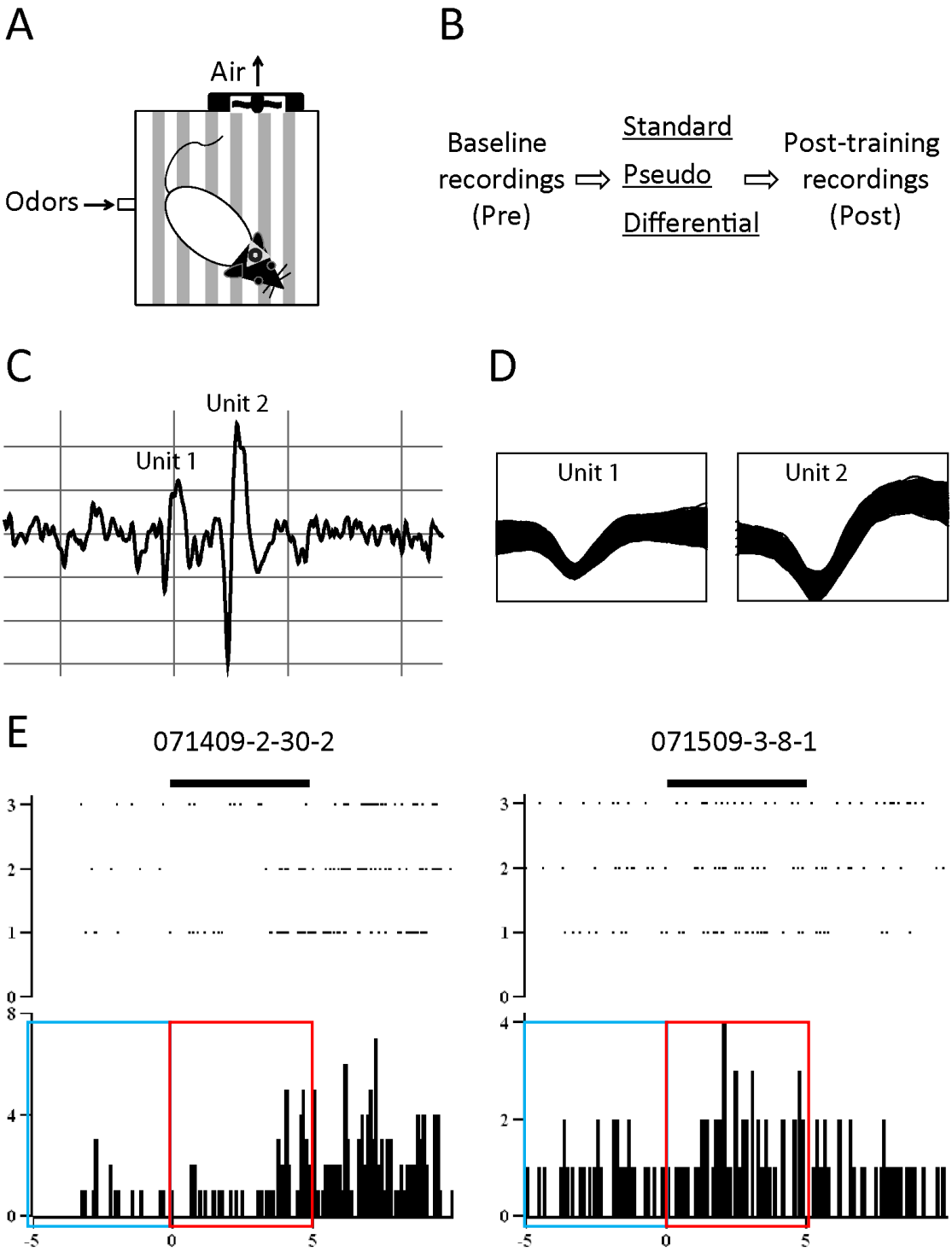


Figure 3. Anterior piriform cortex (aPCX) single-unit recordings from awake, freely moving rats. **A,** A rat implanted with a movable wire bundle was in the testing chamber for chronic unit recording and odor training. The gray bars in the chamber represent the metal grid floor that was part of the electric shocking system. **B,** Procedures of chronic unit recordings. Note

that each rat only was only trained once on a single day, while recording sessions were conducted for multiple days before and after the training day. **C,** A digital-filtered (bandpass, 300-3000 Hz) trace showing spikes from a single microwire placed in aPCX. One larger and one smaller spike can be seen. The trace has a time base of 5 ms per division. **D,** Extracted waveforms of units 1 and 2 from the trace in C. The signal-to-noise ratio of unit 1 (left) was 2.5:1; that of unit 2 (right) was 6:1. **E,** Examples of peri-stimulus time histograms (PSTHs) with raster plots of two single-units (left and right) in response to 10CR1. Histograms showed cumulative spike count of three trials, with a 100-ms bin width. Blue boxes (from -5 to 0 sec) represent spontaneous activity and red boxes (from 0 to 5 sec) indicated odor-evoked activity. Both units showed a significant excitatory response to 10CR1. Horizontal black bars indicate odor delivery (5 sec).

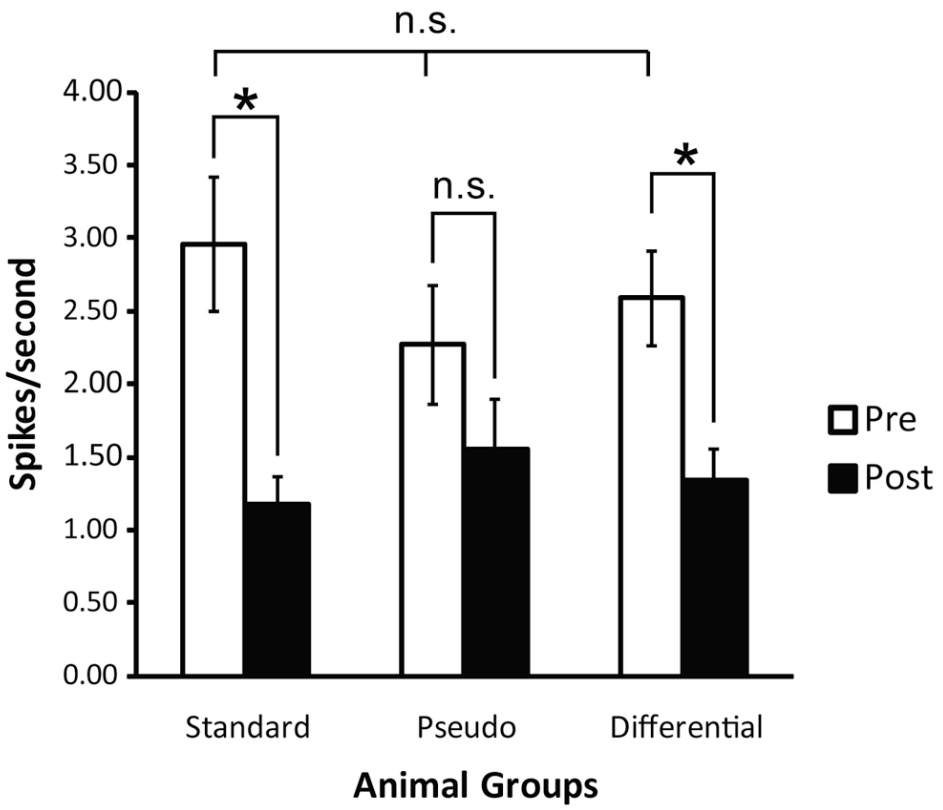


Figure 4. Effects of odor-fear conditioning on spontaneous activity of aPCX neurons. Standard and differential training induced a significant decrease in spontaneous firing rate of neurons in the aPCX. This decrease was not significant in the pseudo-trained group. There was no significant difference in spontaneous activity of the animals before training. Error bars represent s.e.m. * $P < 0.01$.

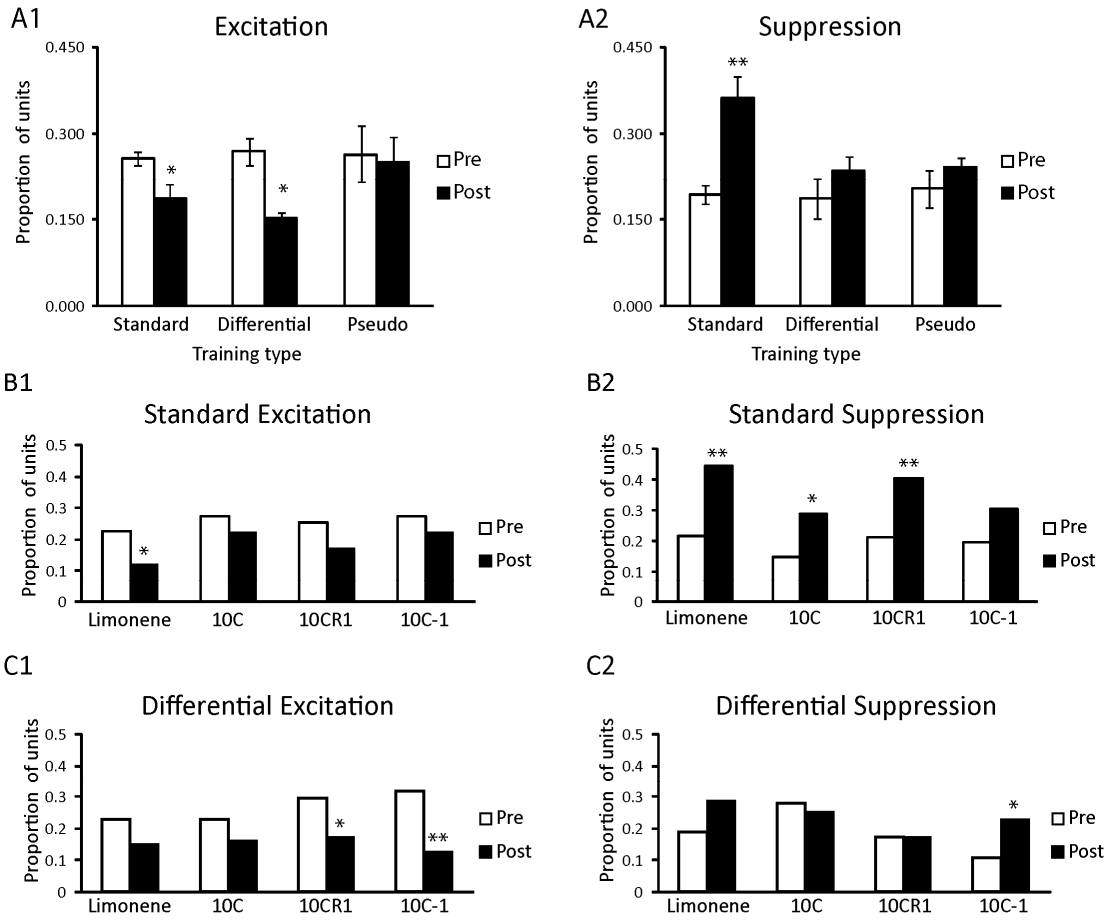


Figure 5. Training effects on aPCX single-unit odor responses. **A1,** Standard and differential training induced proportionally less excitatory single-unit odor responses to the test odors. **A2,** Standard training induced proportionally more suppressive odor responses. **B1,** A smaller proportion of the single units showed excitatory responses to limonene after standard training. **B2,** Proportionally more odor suppression was induced by standard training. **C1,** A non-odor-specific effect on excitatory odor responses was induced by the differential training. **C2,** Effects on suppressive odor responses induced by the differential training were more odor-specific. Note that data of excitation and suppression in A1 were the average of unit response data to each test odor shown in B1 and B2. Odors used were 10C, 10C-1, 10CR1 and limonene. Error bars represent s.e.m. * $p < 0.05$. ** $p < 0.01$. For calculation of single-unit odor responses, see **Methods**.

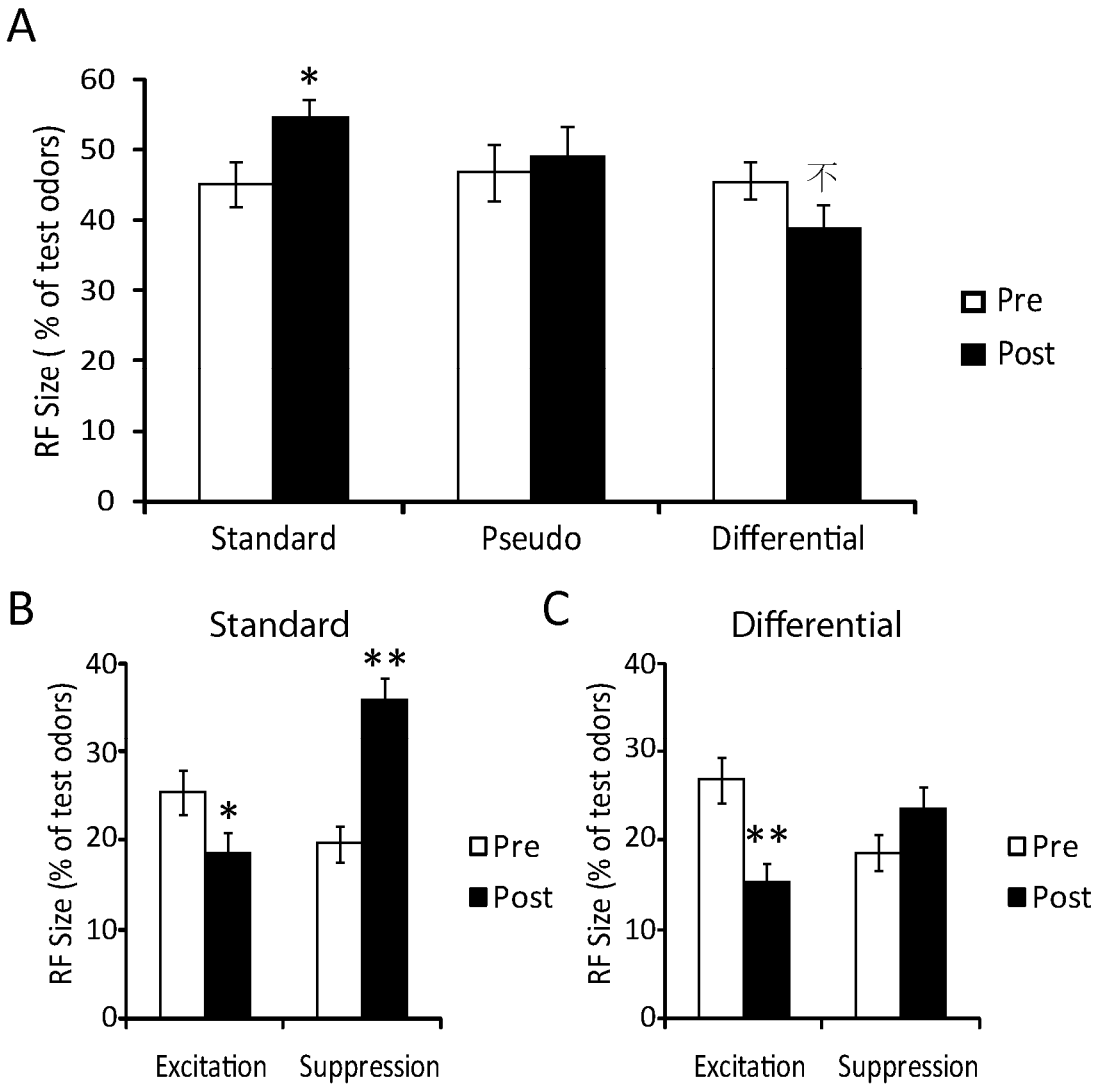


Figure 6. Training effects on aPCX single-unit receptive fields. **A,** Standard training induced a significant increase in mean RF size of aPCX neurons. In contrast, differential training induced a decrease (although not significant) in mean RF size. No change was induced by the pseudo-training. **B,** Standard training induced a significant decrease in excitatory RF size and an increase in suppressive RF size. This suggested not only that the neurons fired to a smaller percentage of the test odors, but also that they were suppressed by the test odors. **C,** A significant decrease in excitatory receptive field size was found in aPCX neurons after differential training. This suggested that the neurons were more narrowly tuned after the training. Error bars represent s.e.m. * $P < 0.05$, ** $P < 0.01$, 不 $P = 0.12$. For receptive field calculation, see **Methods**.

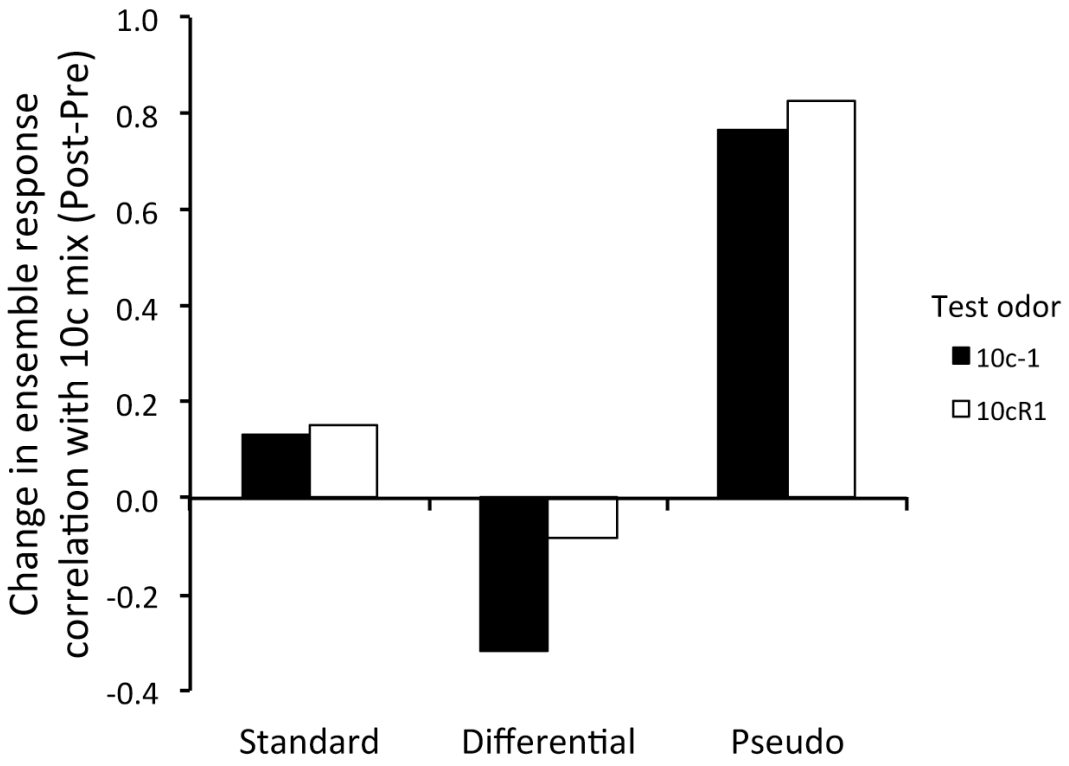


Figure 7. Training effects on aPCX single-unit virtual ensemble response decorrelation of odors. All single units showing an excitatory response to at least one of the test odors were merged into virtual ensembles for analysis of changes in ensemble correlation/decorrelation in response to overlapping odor mixtures (see text). Relative to pre-training levels, standard training produced a slight enhancement in ensemble response correlation between test odors, while differential training produced an enhancement in response decorrelation between test odors. Pseudo-conditioning produced a significant enhancement in ensemble response correlation relative to pre-training.

CHAPTER 4

Conclusions and Future Directions

In summary, my experiments showed that the rats can use odor cues to acquire two-way active avoidance conditioning. Furthermore, data from awake single-unit recording showed that classical fear conditioning can induce paradigm-specific plasticity in anterior piriform cortex, which may explain different odor-evoked fear responses induced by the three fear conditioning paradigms.

Results of my two-way active avoidance experiments indicate that both odor and tone cues can support avoidance conditioning, but that both training groups have individuals that failed to reach the behavioral criterion or had difficulty improving or maintaining their performance. One hypothesis for these low performers is that their cue-evoked freezing behaviors outweighed their active avoidance responses (Choi et al., 2010).

Among these low performers, tone-trained rats (T-3 & T-4) appeared to give up on improving avoidance rates in the last two training sessions, whereas the odor-trained rat (O-3) was still capable of improving its performance until the end of training. Given that higher cue intensity may evoke stronger freezing behavior, the group difference might result from stronger relative intensity of tone signals compared to odor cues. This hypothesis is hard to test because it is hard to compare relative intensity

between two sensory modalities in rodents. However, it would be interesting to see if lower tone or odor intensities could reduce the percentage of low performers in the population.

Tone signals have relatively constant stimulus intensity during delivery. In contrast, odor intensity is hard to control, particularly in a large enclosure as used here. It may take a certain amount of time for the odor to fully disperse throughout the chamber. Even if odor intensity in the environment could reach a constant level, it may still fluctuate for each animal in different phases of a respiratory cycle. Thus, the animal might actively adjust odor intensity via differential sampling (e.g., different sniff volumes) which may not apply to tone signals (Johnson et al., 2003). To monitor odor intensity, I would need a better odor delivery design and a way to measure respiration of the animal.

A similar design of odor stimulation was used in our awake recording experiment in which the rats were freely moving and sampling odor cues that were delivered into air in the shocking chamber. This passive odor sampling design prevents anticipation that is often involved in an active odor sampling design. However, passive odor sampling does not allow good control over odor intensity and precise timing when the rats first sampled

the odor cue. The more naturalistic odor delivery we used may correspond better to the situations rats encounter in the wild, but also adds variability to the data and impairs quantitative analysis.

In future experiments, odor delivery could be better controlled by adding a tubing system onto the recording cable, which would be connected to the headstage during awake recording, so that the distance between the odor source and the rat's nose would be constant. It would also be helpful to monitor the rat's respiration cycle, which could be achieved by using a thermocouple sensor implanted in a rat's nasal cavity (Cury and Uchida, 2010).

The identification of the OR gene family enables genetic engineering techniques to be applied to investigating the olfactory pathway (Buck, 1996). Researchers are now able to tag homogeneous OSNs and glomeruli with fluorescent markers to identify OR/glomerulus-specific projection patterns in the olfactory cortex (Sosulski et al., 2011). Until now, data have indicated that odor representation in the piriform cortex is spatially distributed and without modular odor processing units (Illig and Haberly, 2003; Rennaker et al., 2007; Stettler and Axel, 2009; Sosulski et al., 2011). However, as direct OB projections to the cortical amygdala are patchy and

stereotypical (Sosulski et al., 2011), the amygdala might perform modular odor processing specific to certain odor stimuli. More investigations are needed to clarify the functional roles of the cortical amygdala in central odor processing.

The largest part of the olfactory cortex, the piriform cortex, has features of an associative cortex (Schoenbaum and Eichenbaum, 1995; Haberly, 2001; Calu et al., 2007; Roesch et al., 2007) and its cells have narrower molecular receptive ranges (receptive fields) than OB neurons (Wilson, 2000b, a; Barnes et al., 2008). This may be analogous to functions of inferotemporal cortex in the ventral stream of the visual system (Nassi and Callaway, 2009). Cortical nucleus of the amygdala may be analogous to the modular face processing area - fusiform face area in the inferotemporal cortex. Thus, studies in inferotemporal cortex might provide directions for future investigations in the olfactory cortex.

LITERATURE CITED

Allison AC (1953) The structure of the olfactory bulb and its relationship to the olfactory pathways in the rabbit and the rat. J Comp Neurol 98:309-353.

Apicella A, Yuan Q, Scanziani M, Isaacson JS (2010) Pyramidal cells in piriform cortex receive convergent input from distinct olfactory bulb glomeruli. J Neurosci 30:14255-14260.

Araneda RC, Kini AD, Firestein S (2000) The molecular receptive range of an odorant receptor. Nat Neurosci 3:1248-1255.

Armony JL, Servan-Schreiber D, Romanski LM, Cohen JD, LeDoux JE (1997) Stimulus generalization of fear responses: effects of auditory cortex lesions in a computational model and in rats. Cereb Cortex 7:157-165.

Barkai E, Hasselmo ME (1994) Modulation of the input/output function of rat piriform cortex pyramidal cells. J Neurophysiol 72:644-658.

Barkai E, Saar D (2001) Cellular correlates of olfactory learning in the rat piriform cortex. Rev Neurosci 12:111-120.

Barnes DC, Hofacer RD, Zaman AR, Rennaker RL, Wilson DA (2008) Olfactory perceptual stability and discrimination. Nat Neurosci 11:1378-1380.

Bayer SA (1983) 3H-thymidine-radiographic studies of neurogenesis in the rat olfactory bulb. Exp Brain Res 50:329-340.

Berkowicz DA, Trombley PQ, Shepherd GM (1994) Evidence for glutamate as the olfactory receptor cell neurotransmitter. J Neurophysiol 71:2557-2561.

Bignetti E, Cavaggioni A, Pelosi P, Persaud KC, Sorbi RT, Tirindelli R (1985) Purification and characterisation of an odorant-binding protein from cow nasal tissue. Eur J Biochem 149:227-231.

Boekhoff I, Tareilus E, Strotmann J, Breer H (1990) Rapid activation of alternative second messenger pathways in olfactory cilia from rats by different odorants. Embo J 9:2453-2458.

Bouret S, Sara SJ (2002) Locus coeruleus activation modulates firing rate and temporal organization of odour-induced single-cell responses in rat piriform cortex. Eur J Neurosci 16:2371-2382.

Bozza T, Feinstein P, Zheng C, Mombaerts P (2002) Odorant receptor expression defines functional units in the mouse olfactory system. J Neurosci 22:3033-3043.

Brennan P, Kaba H, Keverne EB (1990) Olfactory recognition: a simple memory system. Science 250:1223-1226.

Broadwell RD, Jacobowitz DM (1976) Olfactory relationships of the telencephalon and diencephalon in the rabbit. III. The ipsilateral centrifugal fibers to the olfactory bulbar and retrobulbar formations. J Comp Neurol 170:321-345.

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

Buck LB (1996) Information coding in the vertebrate olfactory system. Annual Review of Neuroscience 19:517-544.

Buonviso N, Chaput MA (1990) Response similarity to odors in olfactory bulb output cells presumed to be connected to the same glomerulus: electrophysiological study using simultaneous single-unit recordings. J Neurophysiol 63:447-454.

Caggiano M, Kauer JS, Hunter DD (1994) Globose basal cells are neuronal progenitors in the olfactory epithelium: a lineage analysis using a replication-incompetent retrovirus. Neuron 13:339-352.

Calu DJ, Roesch MR, Stalnaker TA, Schoenbaum G (2007) Associative Encoding in Posterior Piriform Cortex during Odor Discrimination and Reversal Learning. Cereb Cortex 17:1342-1349.

Cang J, Isaacson JS (2003) In vivo whole-cell recording of odor-evoked synaptic transmission in the rat olfactory bulb. J Neurosci 23:4108-4116.

Carr WE, Gleeson RA, Trapido-Rosenthal HG (1990) The role of perireceptor events in chemosensory processes. Trends in Neurosciences 13:212-215.

Chapuis J, Wilson DA (2010) Effect of olfactory learning on pattern separation and completion processes in the piriform cortex. In: Society for Neuroscience Annual Meeting Abstracts. San Diego, CA.

Chen Z, Lancet D (1984) Membrane proteins unique to vertebrate olfactory cilia: candidates for sensory receptor molecules. Proc Natl Acad Sci U S A 81:1859-1863.

Choi JS, Cain CK, LeDoux JE (2010) The role of amygdala nuclei in the expression of auditory signaled two-way active avoidance in rats. Learn Mem 17:139-147.

Cleland TA, Morse A, Yue EL, Linster C (2002) Behavioral models of odor similarity. Behav Neurosci 116:222-231.

Cohen Y, Reuveni I, Barkai E, Maroun M (2008) Olfactory learning-induced long-lasting enhancement of descending and ascending synaptic transmission to the piriform cortex. J Neurosci 28:6664-6669.

Coskun V, Luskin MB (2002) Intrinsic and extrinsic regulation of the proliferation and differentiation of cells in the rodent rostral migratory stream. J Neurosci Res 69:795-802.

Cury KM, Uchida N (2010) Robust odor coding via inhalation-coupled transient activity in the mammalian olfactory bulb. Neuron 68:570-585.

Darvas M, Fadok JP, Palmiter RD (2011) Requirement of dopamine signaling in the amygdala and striatum for learning and maintenance of a conditioned avoidance response. Learn Mem 18:136-143.

Davis BJ, Macrides F (1981) The organization of centrifugal projections from the anterior olfactory nucleus, ventral hippocampal rudiment, and piriform cortex to the main olfactory bulb in the hamster: an autoradiographic study. J Comp Neurol 203:475-493.

Davis RL (2004) Olfactory learning. Neuron 44:31-48.

Davison IG, Ehlers MD (2011) Neural circuit mechanisms for pattern detection and feature combination in olfactory cortex. Neuron 70:82-94.

De Rosa E, Hasselmo ME (2000) Muscarinic cholinergic neuromodulation reduces proactive interference between stored odor memories during associative learning in rats. Behav Neurosci 114:32-41.

Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 17:5046-5061.

Doucette W, Gire DH, Whitesell J, Carmean V, Lucero MT, Restrepo D (2011) Associative cortex features in the first olfactory brain relay station. Neuron 69:1176-1187.

Ferry B, Oberling P, Jarrard LE, Di Scala G (1996) Facilitation of conditioned odor aversion by entorhinal cortex lesions in the rat. Behavioral neuroscience 110:443-450.

Firestein S (1992) Electrical signals in olfactory transduction. Current Opinion in Neurobiology 2:444-448.

Firestein S, Werblin F (1989) Odor-induced membrane currents in vertebrate-olfactory receptor neurons. Science 244:79-82.

Firestein S, Darrow B, Shepherd GM (1991a) Activation of the sensory current in salamander olfactory receptor neurons depends on a G protein-mediated cAMP second messenger system. Neuron 6:825-835.

Firestein S, Zufall F, Shepherd GM (1991b) Single odor-sensitive channels in olfactory receptor neurons are also gated by cyclic nucleotides. J Neurosci 11:3565-3572.

Fletcher ML, Wilson DA (2002) Experience modifies olfactory acuity: acetylcholine-dependent learning decreases behavioral generalization between similar odorants. J Neurosci 22:RC201.

Fletcher ML, Wilson DA (2003) Olfactory bulb mitral-tufted cell plasticity: odorant-specific tuning reflects previous odorant exposure. J Neurosci 23:6946-6955.

Foote SL, Aston-Jones G, Bloom FE (1980) Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. Proceedings of the National Academy of Sciences of the United States of America 77:3033-3037.

Freeman WJ, Schneider W (1982) Changes in spatial patterns of rabbit olfactory EEG with conditioning to odors. Psychophysiology 19:44-56.

Fried HU, Fuss SH, Korsching SI (2002) Selective imaging of presynaptic activity in the mouse olfactory bulb shows concentration and structure dependence of odor responses in identified glomeruli. Proc Natl Acad Sci U S A 99:3222-3227.

Friedrich RW, Korsching SI (1997) Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging. Neuron 18:737-752.

Friedrich RW, Laurent G (2001) Dynamic optimization of odor representations by slow temporal patterning of mitral cell activity. Science 291:889-894.

Ganz L (1962) Hue generalization and hue discriminability in Macaca mulatta. Journal of Experimental Psychology 64:142-150.

Gellman RL, Aghajanian GK (1993) Pyramidal cells in piriform cortex receive a convergence of inputs from monoamine activated GABAergic interneurons. Brain Res 600:63-73.

Getchell TV, Margolis FL, Getchell ML (1984) Perireceptor and receptor events in vertebrate olfaction. Progress in Neurobiology 23:317-345.

Gottfried JA, Winston JS, Dolan RJ (2006) Dissociable codes of odor quality and odorant structure in human piriform cortex. Neuron 49:467-479.

Graziadei PP, Graziadei GA (1979) Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. Journal of neurocytology 8:1-18.

Guthrie KM, Anderson AJ, Leon M, Gall C (1993) Odor-induced increases in c-fos mRNA expression reveal an anatomical "unit" for odor processing in olfactory bulb. Proc Natl Acad Sci U S A 90:3329-3333.

Guttman N, Kalish HI (1956) Discriminability and stimulus generalization. Journal of Experimental Psychology 51:79-88.

Haberly LB (2001) Parallel-distributed processing in olfactory cortex: new insights from morphological and physiological analysis of neuronal circuitry. Chem Senses 26:551-576.

Haberly LB, Price JL (1978) Association and commissural fiber systems of the olfactory cortex of the rat. J Comp Neurol 178:711-740.

Halasz N, Ljungdahl A, Hokfelt T (1979) Transmitter histochemistry of the rat olfactory bulb III. Autoradiographic localization of [3H]GABA. Brain Res 167:221-240.

Halasz N, Ljungdahl A, Hokfelt T, Johansson O, Goldstein M, Park D, Biberfeld P (1977) Transmitter histochemistry of the rat olfactory bulb. I. Immunohistochemical localization of monoamine synthesizing enzymes. Support for intrabulbar, periglomerular dopamine neurons. Brain Res 126:455-474.

Hamilton KA, Kauer JS (1985) Intracellular potentials of salamander mitral/tufted neurons in response to odor stimulation. Brain Res 338:181-185.

Hamilton KA, Kauer JS (1989) Patterns of intracellular potentials in salamander mitral/tufted cells in response to odor stimulation. J Neurophysiol 62:609-625.

Hasselmo ME, Bower JM (1992) Cholinergic suppression specific to intrinsic not afferent fiber synapses in rat piriform (olfactory) cortex. J Neurophysiol 67:1222-1229.

Hasselmo ME, Barkai E (1995) Cholinergic modulation of activity-dependent synaptic plasticity in the piriform cortex and associative memory function in a network biophysical simulation. J Neurosci 15:6592-6604.

Hasselmo ME, Linster C, Patil M, Ma D, Cekic M (1997) Noradrenergic suppression of synaptic transmission may influence cortical signal-to-noise ratio. J Neurophysiol 77:3326-3339.

Hegoburu C, Sevelinges Y, Thevenet M, Gervais R, Parrot S, Mouly AM (2009) Differential dynamics of amino acid release in the amygdala and olfactory cortex during odor fear acquisition as revealed with simultaneous high temporal resolution microdialysis. Learn Mem 16:687-697.

Heimer L, Kalil R (1978) Rapid transneuronal degeneration and death of cortical neurons following removal of the olfactory bulb in adult rats. J Comp Neurol 178:559-609.

Hildebrand JG, Shepherd GM (1997) Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. Annu Rev Neurosci 20:595-631.

Honig WK, Urcuioli PJ (1981) The legacy of Guttman and Kalish (1956): Twenty-five years of research on stimulus generalization. J Exp Anal Behav 36:405-445.

Huque T, Bruch RC (1986) Odorant- and guanine nucleotide-stimulated phosphoinositide turnover in olfactory cilia. Biochem Biophys Res Commun 137:36-42.

Hutton RS, Wenzel BM, Baker T, Homuth M (1974) Two-way avoidance learning in pigeons after olfactory nerve section. Physiol Behav 13:57-62.

Illig KR (2005) Projections from orbitofrontal cortex to anterior piriform cortex in the rat suggest a role in olfactory information processing. J Comp Neurol 488:224-231.

Illig KR, Haberly LB (2003) Odor-evoked activity is spatially distributed in piriform cortex. J Comp Neurol 457:361-373.

Imamura K, Mataga N, Mori K (1992) Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. I. Aliphatic compounds. J Neurophysiol 68:1986-2002.

Isaacson JS (2001) Mechanisms governing dendritic gamma-aminobutyric acid (GABA) release in the rat olfactory bulb. Proc Natl Acad Sci U S A 98:337-342.

Isaacson JS, Strowbridge BW (1998) Olfactory reciprocal synapses: dendritic signaling in the CNS. Neuron 20:749-761.

Ito W, Pan BX, Yang C, Thakur S, Morozov A (2009) Enhanced generalization of auditory conditioned fear in juvenile mice. Learn Mem 16:187-192.
Jiang M, Griff ER, Ennis M, Zimmer LA, Shipley MT (1996) Activation of locus coeruleus enhances the responses of olfactory bulb mitral cells to weak olfactory nerve input. J Neurosci 16:6319-6329.
Johnson BN, Mainland JD, Sobel N (2003) Rapid olfactory processing implicates subcortical control of an olfactomotor system. J Neurophysiol 90:1084-1094.
Johnson DM, Illig KR, Behan M, Haberly LB (2000) New features of connectivity in piriform cortex visualized by intracellular injection of pyramidal cells suggest that "primary" olfactory cortex functions like "association" cortex in other sensory systems. J Neurosci 20:6974-6982.
Jones DT, Reed RR (1989) Golf: an olfactory neuron specific-G protein involved in odorant signal transduction. Science 244:790-795.
Kadohisa M, Wilson DA (2006) Separate encoding of identity and similarity of complex familiar odors in piriform cortex. Proc Natl Acad Sci U S A 103:15206-15211.
Kajiya K, Inaki K, Tanaka M, Haga T, Kataoka H, Touhara K (2001) Molecular bases of odor discrimination: Reconstitution of olfactory receptors that recognize overlapping sets of odorants. J Neurosci 21:6018-6025.
Kanter ED, Haberly LB (1993) Associative long-term potentiation in piriform cortex slices requires GABAA blockade. J Neurosci 13:2477-2482.
Katoh K, Koshimoto H, Tani A, Mori K (1993) Coding of odor molecules by mitral/tufted cells in rabbit olfactory bulb. II. Aromatic compounds. J Neurophysiol 70:2161-2175.
Katz DB, Simon SA, Nicolelis MA (2002) Taste-specific neuronal ensembles in the gustatory cortex of awake rats. J Neurosci 22:1850-1857.
Kauer JS, Senseman DM, Cohen LB (1987) Odor-elicited activity monitored simultaneously from 124 regions of the salamander olfactory bulb using a voltage-sensitive dye. Brain Res 418:255-261.
Kay LM, Freeman WJ (1998) Bidirectional processing in the olfactory-limbic axis during olfactory behavior. Behavioral neuroscience 112:541-553.
Kendrick KM, Levy F, Keverne EB (1992) Changes in the sensory processing of olfactory signals induced by birth in sleep. Science 256:833-836.
Knafo S, Barkai E, Herrero AI, Libersat F, Sandi C, Venero C (2005) Olfactory learning-related NCAM expression is state, time, and location specific

and is correlated with individual learning capabilities. Hippocampus 15:316-325.
Kobayakawa K, Kobayakawa R, Matsumoto H, Oka Y, Imai T, Ikawa M, Okabe M, Ikeda T, Itohara S, Kikusui T, Mori K, Sakano H (2007) Innate versus learned odour processing in the mouse olfactory bulb. Nature 450:503-508.
LeDoux JE (2000) Emotion circuits in the brain. Annu Rev Neurosci 23:155-184.
Lee KH, Wells RG, Reed RR (1987) Isolation of an olfactory cDNA: similarity to retinol-binding protein suggests a role in olfaction. Science 235:1053-1056.
Leung CH, Wilson DA (2003) Trans-neuronal regulation of cortical apoptosis in the adult rat olfactory system. Brain Res 984:182-188.
Leveteau J, MacLeod P (1966) Olfactory discrimination in the rabbit olfactory glomerulus. Science 153:175-176.
Li W, Howard JD, Parrish TB, Gottfried JA (2008) Aversive learning enhances perceptual and cortical discrimination of indiscriminable odor cues. Science 319:1842-1845.
Linster C, Hasselmo ME (2001) Neuromodulation and the functional dynamics of piriform cortex. Chem Senses 26:585-594.
Luna VM (2011) The piriform cortex utilizes different microcircuits to process cortical and amygdaloid synaptic inputs. In: Association for Chemoreception Sciences. St Petersburg, Florida.
Luskin MB, Price JL (1983) The laminar distribution of intracortical fibers originating in the olfactory cortex of the rat. J Comp Neurol 216:292-302.
Majak K, Ronkko S, Kemppainen S, Pitkanen A (2004) Projections from the amygdaloid complex to the piriform cortex: A PHA-L study in the rat. J Comp Neurol 476:414-428.
Malnic B, Hirono J, Sato T, Buck LB (1999) Combinatorial receptor codes for odors. Cell 96:713-723.
Mandairon N, Stack C, Kiselycznyk C, Linster C (2006) Broad activation of the olfactory bulb produces long-lasting changes in odor perception. Proc Natl Acad Sci U S A 103:13543-13548.
Manson MD, Boos W, Bassford PJ, Jr., Rasmussen BA (1985) Dependence of maltose transport and chemotaxis on the amount of maltose-binding protein. The Journal of biological chemistry 260:9727-9733.

Martin C, Gervais R, Hugues E, Messaoudi B, Ravel N (2004) Learning modulation of odor-induced oscillatory responses in the rat olfactory bulb: a correlate of odor recognition? J Neurosci 24:389-397.

Meisami E (1989) A proposed relationship between increases in the number of olfactory receptor neurons, convergence ratio and sensitivity in the developing rat. Brain Res Dev Brain Res 46:9-19.

Meredith M (1986) Patterned response to odor in mammalian olfactory bulb: the influence of intensity. J Neurophysiol 56:572-597.

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

Mombaerts P, Wang F, Dulac C, Chao SK, Nemes A, Mendelsohn M, Edmondson J, Axel R (1996) Visualizing an olfactory sensory map. Cell 87:675-686.

Moreno MM, Linster C, Escanilla O, Sacquet J, Didier A, Mandairon N (2009) Olfactory perceptual learning requires adult neurogenesis. Proc Natl Acad Sci U S A 106:17980-17985.

Mori K (1987) Membrane and synaptic properties of identified neurons in the olfactory bulb. Prog Neurobiol 29:275-320.

Mori K, Takagi SF (1978) An intracellular study of dendrodendritic inhibitory synapses on mitral cells in the rabbit olfactory bulb. J Physiol 279:569-588.

Mori K, Yoshihara Y (1995) Molecular recognition and olfactory processing in the mammalian olfactory system. Prog Neurobiol 45:585-619.

Mori K, Mataga N, Imamura K (1992) Differential specificities of single mitral cells in rabbit olfactory bulb for a homologous series of fatty acid odor molecules. J Neurophysiol 67:786-789.

Mori K, Nagao H, Yoshihara Y (1999) The olfactory bulb: coding and processing of odor molecule information. Science 286:711-715.

Moriceau S, Roth TL, Sullivan RM (2010) Rodent model of infant attachment learning and stress. Dev Psychobiol 52:651-660.

Morrison EE, Costanzo RM (1990) Morphology of the human olfactory epithelium. J Comp Neurol 297:1-13.

Mouly AM, Gervais R (2002) Polysynaptic potentiation at different levels of rat olfactory pathways following learning. Learn Mem 9:66-75.

Mouly AM, Fort A, Ben-Boutayab N, Gervais R (2001) Olfactory learning induces differential long-lasting changes in rat central olfactory pathways. Neuroscience 102:11-21.

Nakamura T, Gold GH (1987) A cyclic nucleotide-gated conductance in olfactory receptor cilia. Nature 325:442-444.

Nassi JJ, Callaway EM (2009) Parallel processing strategies of the primate visual system. Nat Rev Neurosci 10:360-372.

Neville KR, Haberly L (2004) Olfactory cortex. In: The synaptic organization of the brain, 5th Edition (Shepherd GM, ed), pp 415-454. New York: Oxford University Press.

Nickell WT, Shipley MT (1993) Evidence for presynaptic inhibition of the olfactory commissural pathway by cholinergic agonists and stimulation of the nucleus of the diagonal band. J Neurosci 13:650-659.

Nolte J (2002) The Chemical Senses of Taste and Smell. In: The Human Brain, 5th Edition, pp 319-336. St. Louis: Mosby, Inc.

Nowycky MC, Mori K, Shepherd GM (1981) GABAergic mechanisms of dendrodendritic synapses in isolated turtle olfactory bulb. J Neurophysiol 46:639-648.

Olender T, Lancet D, Nebert DW (2008) Update on the olfactory receptor (OR) gene superfamily. Hum Genomics 3:87-97.

Owens JG, James RA, Moss OR, Morgan KT, Bowman JR, Struve MF, Dorman DC (1996) Design and evaluation of an olfactometer for the assessment of 3-methylindole-induced hyposmia. Fundam Appl Toxicol 33:60-70.

Pace U, Hanski E, Salomon Y, Lancet D (1985) Odorant-sensitive adenylate cyclase may mediate olfactory reception. Nature 316:255-258.

Patil MM, Hasselmo ME (1999) Modulation of inhibitory synaptic potentials in the piriform cortex. J Neurophysiol 81:2103-2118.

Pavlov IP (1927) Conditioned Reflexes. New York: Dover Publications.

Pevsner J, Snyder SH (1990) Odorant-binding protein: odorant transport function in the nasal epithelium. Chemical Senses 15:217-222.

Pevsner J, Sklar PB, Snyder SH (1986) Odorant-binding protein: localization to nasal glands and secretions. Proceedings of the National Academy of Sciences of the United States of America 83:4942-4946.

Pevsner J, Reed RR, Feinstein PG, Snyder SH (1988) Molecular cloning of odorant-binding protein: member of a ligand carrier family. Science 241:336-339.

Pinching AJ, Powell TP (1971) The neuron types of the glomerular layer of the olfactory bulb. J Cell Sci 9:305-345.

Poo C, Isaacson JS (2009) Odor representations in olfactory cortex: "sparse" coding, global inhibition, and oscillations. Neuron 62:850-861.

Price JL (1973) An autoradiographic study of complementary laminar patterns of termination of afferent fibers to the olfactory cortex. J Comp Neurol 150:87-108.

Price JL, Powell TP (1970a) The synaptology of the granule cells of the olfactory bulb. J Cell Sci 7:125-155.

Price JL, Powell TP (1970b) The morphology of the granule cells of the olfactory bulb. J Cell Sci 7:91-123.

Ravel N, Chabaud P, Martin C, Gaveau V, Hugues E, Tallon-Baudry C, Bertrand O, Gervais R (2003) Olfactory learning modifies the expression of odour-induced oscillatory responses in the gamma (60-90 Hz) and beta (15-40 Hz) bands in the rat olfactory bulb. Eur J Neurosci 17:350-358.

Reed RR (1992) Signaling pathways in odorant detection. Neuron 8:205-209.

Rennaker RL, Chen CF, Ruyle AM, Sloan AM, Wilson DA (2007) Spatial and temporal distribution of odorant-evoked activity in the piriform cortex. J Neurosci 27:1534-1542.

Rescorla RA (1976) Stimulus generalization: some predictions from a model of Pavlovian conditioning. J Exp Psychol Anim Behav Process 2:88-96.

Ressler KJ, Sullivan SL, Buck LB (1993) A zonal organization of odorant receptor gene expression in the olfactory epithelium. Cell 73:597-609.

Ressler KJ, Sullivan SL, Buck LB (1994a) A molecular dissection of spatial patterning in the olfactory system. Curr Opin Neurobiol 4:588-596.

Ressler KJ, Sullivan SL, Buck LB (1994b) Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. Cell 79:1245-1255.

Restrepo D, Miyamoto T, Bryant BP, Teeter JH (1990) Odor stimuli trigger influx of calcium into olfactory neurons of the channel catfish. Science 249:1166-1168.

Ribak CE, Vaughn JE, Saito K, Barber R, Roberts E (1977) Glutamate decarboxylase localization in neurons of the olfactory bulb. Brain Res 126:1-18.

Rochefort C, Gheusi G, Vincent JD, Lledo PM (2002) Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. J Neurosci 22:2679-2689.

Roesch MR, Stalnaker TA, Schoenbaum G (2007) Associative encoding in anterior piriform cortex versus orbitofrontal cortex during odor discrimination and reversal learning. Cereb Cortex 17:643-652.

Roesch MR, Calu DJ, Esber GR, Schoenbaum G (2010) Neural correlates of variations in event processing during learning in basolateral amygdala. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:2464-2471.

Rohrbaugh M, Brennan JF, Riccio DC (1971) Control of two-way shuttle avoidance in rats by auditory frequency and intensity. J Comp Physiol Psychol 75:324-330.

Rosenkranz JA, Grace AA (2002) Dopamine-mediated modulation of odour-evoked amygdala potentials during pavlovian conditioning. Nature 417:282-287.

Royet JP, Distel H, Hudson R, Gervais R (1998) A re-estimation of the number of glomeruli and mitral cells in the olfactory bulb of rabbit. Brain Res 788:35-42.

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

Saar D, Barkai E (2003) Long-term modifications in intrinsic neuronal properties and rule learning in rats. Eur J Neurosci 17:2727-2734.

Saar D, Grossman Y, Barkai E (1998) Reduced after-hyperpolarization in rat piriform cortex pyramidal neurons is associated with increased learning capability during operant conditioning. Eur J Neurosci 10:1518-1523.

Saar D, Grossman Y, Barkai E (1999) Reduced synaptic facilitation between pyramidal neurons in the piriform cortex after odor learning. J Neurosci 19:8616-8622.

Saar D, Grossman Y, Barkai E (2001) Long-lasting cholinergic modulation underlies rule learning in rats. J Neurosci 21:1385-1392.

Saar D, Grossman Y, Barkai E (2002) Learning-induced enhancement of postsynaptic potentials in pyramidal neurons. J Neurophysiol 87:2358-2363.

Sara SJ, Vankov A, Herve A (1994) Locus coeruleus-evoked responses in behaving rats: a clue to the role of noradrenaline in memory. Brain Res Bull 35:457-465.

Schechtman E, Laufer O, Paz R (2010) Negative valence widens generalization of learning. J Neurosci 30:10460-10464.

Schoenbaum G, Eichenbaum H (1995) Information coding in the rodent prefrontal cortex. I. Single-neuron activity in orbitofrontal cortex compared with that in pyriform cortex. J Neurophysiol 74:733-750.

Schoenbaum G, Setlow B, Saddoris MP, Gallagher M (2003) Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. Neuron 39:855-867.

Schoppa NE, Kinzie JM, Sahara Y, Segerson TP, Westbrook GL (1998) Dendrodendritic inhibition in the olfactory bulb is driven by NMDA receptors. J Neurosci 18:6790-6802.

Servan-Schreiber D, Printz H, Cohen JD (1990) A network model of catecholamine effects: gain, signal-to-noise ratio, and behavior. Science 249:892-895.

Sevelinges Y, Gervais R, Messaoudi B, Granjon L, Mouly AM (2004) Olfactory fear conditioning induces field potential potentiation in rat olfactory cortex and amygdala. Learn Mem 11:761-769.

Sharp FR, Kauer JS, Shepherd GM (1975) Local sites of activity-related glucose metabolism in rat olfactory bulb during olfactory stimulation. Brain Res 98:596-600.

Shepard RN (1987) Toward a universal law of generalization for psychological science. Science 237:1317-1323.

Shepherd GM (1972a) Synaptic organization of the mammalian olfactory bulb. Physiol Rev 52:864-917.

Shepherd GM (1972b) The neuron doctrine: a revision of functional concepts. Yale J Biol Med 45:584-599.

Shepherd GM, Chen WR, Greer CA (2004) Olfactory bulb. In: The Synaptic Organization of the Brain (Shepherd GM, ed), pp 165-216. New York: Oxford University Press.

Sicard G, Holley A (1984) Receptor cell responses to odorants: similarities and differences among odorants. Brain Res 292:283-296.

Sklar PB, Anholt RR, Snyder SH (1986) The odorant-sensitive adenylate cyclase of olfactory receptor cells. Differential stimulation by distinct classes of odorants. The Journal of biological chemistry 261:15538-15543.

Smith JJ, Shionoya K, Sullivan RM, Wilson DA (2009) Auditory stimulation dishabituates olfactory responses via noradrenergic cortical modulation. Neural Plast 2009:754014.

Sosulski DL, Lissitsyna Bloom M, Cutforth T, Axel R, Datta SR (2011) Distinct representations of olfactory information in different cortical centres. Nature 472:213-216.

Stettler DD, Axel R (2009) Representations of odor in the piriform cortex. Neuron 63:854-864.

Stopfer M, Laurent G (1999) Short-term memory in olfactory network dynamics. Nature 402:664-668.

Strotmann J, Conzelmann S, Beck A, Feinstein P, Breer H, Mombaerts P (2000) Local permutations in the glomerular array of the mouse olfactory bulb. J Neurosci 20:6927-6938.

Sullivan RM, Wilson DA, Leon M (1989) Norepinephrine and learning-induced plasticity in infant rat olfactory system. J Neurosci 9:3998-4006.

Sullivan RM, Landers M, Yeaman B, Wilson DA (2000) Good memories of bad events in infancy. Nature 407:38-39.

Suzuki N, Bekkers JM (2006) Neural coding by two classes of principal cells in the mouse piriform cortex. J Neurosci 26:11938-11947.

Suzuki N, Bekkers JM (2007) Inhibitory interneurons in the piriform cortex. Clin Exp Pharmacol Physiol 34:1064-1069.

Torras-Garcia M, Portell-Cortes I, Costa-Miserachs D, Morgado-Bernal I (1997) Long-term memory modulation by posttraining epinephrine in rats: differential effects depending on the basic learning capacity. Behav Neurosci 111:301-308.

Touhara K, Sengoku S, Inaki K, Tsuboi A, Hirono J, Sato T, Sakano H, Haga T (1999) Functional identification and reconstitution of an odorant receptor in single olfactory neurons. Proc Natl Acad Sci U S A 96:4040-4045.

Treloar HB, Feinstein P, Mombaerts P, Greer CA (2002) Specificity of glomerular targeting by olfactory sensory axons. J Neurosci 22:2469-2477.

Trombley PQ, Shepherd GM (1997) The olfactory bulb. In: Encyclopedia of Neuroscience (Edelman GM, Smith B, eds). Amsterdam: Elservier.

Tseng GF, Haberly LB (1989) Deep neurons in piriform cortex. II. Membrane properties that underlie unusual synaptic responses. J Neurophysiol 62:386-400.

Tsuboi A, Yoshihara S, Yamazaki N, Kasai H, Asai-Tsuboi H, Komatsu M, Serizawa S, Ishii T, Matsuda Y, Nagawa F, Sakano H (1999) Olfactory neurons expressing closely linked and homologous odorant receptor genes tend to project their axons to neighboring glomeruli on the olfactory bulb. J Neurosci 19:8409-8418.

Vassar R, Ngal J, Axel R (1993) Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. Cell 74:309-318.

Vassar R, Chao SK, Sitcheran R, Nunez JM, Vosshall LB, Axel R (1994) Topographic organization of sensory projections to the olfactory bulb. Cell 79:981-991.

Vogt RG, Prestwich GD, Lerner MR (1991) Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. Journal of Neurobiology 22:74-84.

Voytenko SV, Galazyuk AV (2010) Suppression of spontaneous firing in inferior colliculus neurons during sound processing. Neuroscience 165:1490-1500.

Wachowiak M, Denk W, Friedrich RW (2004) Functional organization of sensory input to the olfactory bulb glomerulus analyzed by two-photon calcium imaging. Proc Natl Acad Sci U S A 101:9097-9102.

Wang F, Nemes A, Mendelsohn M, Axel R (1998) Odorant receptors govern the formation of a precise topographic map. Cell 93:47-60.

Wellis DP, Kauer JS (1994) GABAergic and glutamatergic synaptic input to identified granule cells in salamander olfactory bulb. J Physiol 475:419-430.

Wiebe SP, Staubli UV (2001) Recognition memory correlates of hippocampal theta cells. J Neurosci 21:3955-3967.

Wilson DA (1998) Habituation of odor responses in the rat anterior piriform cortex. J Neurophysiol 79:1425-1440.

Wilson DA (2000a) Odor specificity of habituation in the rat anterior piriform cortex. J Neurophysiol 83:139-145.

Wilson DA (2000b) Comparison of odor receptive field plasticity in the rat olfactory bulb and anterior piriform cortex. J Neurophysiol 84:3036-3042.

Wilson DA (2001) Scopolamine enhances generalization between odor representations in rat olfactory cortex. Learn Mem 8:279-285.

Wilson DA (2003) Rapid, experience-induced enhancement in odorant discrimination by anterior piriform cortex neurons. J Neurophysiol 90:65-72.

Wilson DA, Leon M (1987) Evidence of lateral synaptic interactions in olfactory bulb output cell responses to odors. Brain Res 417:175-180.

Wilson DA, Stevenson RJ (2003) Olfactory perceptual learning: the critical role of memory in odor discrimination. Neurosci Biobehav Rev 27:307-328.

Wilson DA, Stevenson RJ (2006) Learning to smell : olfactory perception from neurobiology to behavior. Baltimore: Johns Hopkins University Press.

Yang X, Renken R, Hyder F, Siddeek M, Greer CA, Shepherd GM, Shulman RG (1998) Dynamic mapping at the laminar level of odor-elicited responses in rat olfactory bulb by functional MRI. PNAS 95:7715-7720.

Yokoi M, Mori K, Nakanishi S (1995) Refinement of odor molecule tuning by dendrodendritic synaptic inhibition in the olfactory bulb. Proc Natl Acad Sci U S A 92:3371-3375.

Zhang C, Szabo G, Erdelyi F, Rose JD, Sun QQ (2006) Novel interneuronal network in the mouse posterior piriform cortex. J Comp Neurol 499:1000-1015.

Zhang X, Firestein S (2002) The olfactory receptor gene superfamily of the mouse. Nat Neurosci 5:124-133.

Zimmer LA, Ennis M, Shipley MT (1999) Diagonal band stimulation increases piriform cortex neuronal excitability in vivo. Neuroreport 10:2101-2105.