UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

SYNAPTIC DEPRESSION AND ITS RELATION TO BEHAVIORAL HABITUATION WITHIN ANTERIOR PIRIFORM CORTEX

A Dissertation

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

Doctor of Philosophy

by

AARON ROBERT BEST

Norman, Oklahoma

2004

Downloaded from SHAREOK. Persistent Handle URL: https://hdl.handle.net/11244/779

UMI Number: 3143539



UMI Microform 3143539

Copyright 2004 by ProQuest Information and Learning Company.

All rights reserved. This microform edition is protected against unauthorized copying under Title 17, United States Code.

ProQuest Information and Learning Company
300 North Zeeb Road
PO Box 1346
Ann Arbor, MI 48106-1346

SYNAPTIC DEPRESSION AND ITS RELATION TO BEHAVIORAL HABITUATION WITHIN ANTERIOR PIRIFORM CORTEX

A Dissertation APPROVED FOR THE DEPARTMENT OF ZOOLOGY

BY

_____
Donald A. Wilson, Ph.D.

_____
Joseph A. Bastian, Ph.D.

_____
Ari Berkowitz, Ph.D.

_____
Douglas D. Gaffin, Ph.D.

_____
Robert L. Rennaker II, Ph.D.

© Copyright by AARON ROBERT BEST 2004
All Rights Reserved.

Acknowledgements

The ideas underlying this dissertation were developed at the University of Oklahoma within the Department of Zoology. I would like to thank the members of my doctoral committee Dr. Donald A. Wilson, Dr. Joseph A. Bastian, Dr. Ari Berkowitz, Dr. Douglas D. Gaffin and Dr. Robert L. Rennaker II for their assistance in the completion of the requirements of my doctoral training.

Table of Contents

Chapter 1

Introduction to Habituation and Short-Term Synaptic Depression……1

Chapter 2

Basics of olfaction: with a focus on the mammalian olfactory system……...12

Chapter 3

Coordinate Synaptic Mechanisms Contributing

to Olfactory Cortical Adaptation…….....55

Chapter 4

Further elaboration on the mechanisms of

LOT pre-synaptic depression…….....94

Chapter 5

Synaptic mechanism of habituation of a simple

olfactory behavior…….103

Chapter 6

Conclusions……...109

References……...114

Chapter 1

Introduction to Habituation

and

Short-Term Synaptic Depression

Early research in the study of habituation was limited by the idea of reflex invariability. In short, it was thought that the decrease in response to stimuli observed in both man and animal was the result of pathology and not neural plasticity (Christoffersen, 1997). Since this time, habituation has come to be recognized as a valid form of non-associative plasticity. Specifically, habituation is a form of learning defined by a decrement in behavioral response to repetitive stimuli that lack environmental relevance (Weinberger, 1995). Accordingly, because these stimuli are uninformative and, therefore, not associated with any relevant internal or external cue, habituation does not require associative plastic mechanisms like many other forms of learning. Thus, habituation can result from any mechanism that decreases the magnitude of communication between neurons and/or non-neural sensory cells over repeated presentation.

Habituation is present in most organisms across the Kingdom Animalia and is also found in the Kingdom Protista (Christoffersen, 1997), from ciliates to insects to mammals. In 1966, Thompson and Spencer developed a working definition for the character of habituation. They suggested that the term habituation should only be used for phenomena that meet nine specific criteria. One: Responses to stimuli that are repeated decrease. Two: This decrement is recovered spontaneously after the repeated stimuli cease. Three: If stimuli are repeated following recovery, habituation of responses occurs more rapidly. Four: Habituation rate increases as stimulus frequency increases. Five: Habituation rate is lower for stimuli of higher intensity. Six: Once habituated, the latency to recovery is lengthened by additional stimuli. Seven: Cross-habituation may

occur. Eight: A strong stimulus given following a weaker habituating stimulus can result in the reoccurrence of responses to the weaker stimuli (dishabituation). Nine: Dishabituation can also be habituated upon repetition.

Christoffersen (1997) recently made minor revisions to this list based on findings attained since 1966. One: Recovery from habituation has a dual time course in many instances; these two time course have been termed short-term habituation, the phenomena of most interest for this dissertation, and long-term habituation. It should be noted that long-term habituation has also been observed within the mammalian olfactory system (Dalton and Wysocki, 1996).

Long-term habituation may result in item three in the list by Spencer and Thompson regarding the increased rate of habituation on repeated trials. Importantly, because of long-term habituation most of the subsequent trials begin at a decreased level of response relative to the initial trial. Additionally, if no inter-trial recovery period is given, long-term habituation will not develop. This phenomenon is conceptually easy to deal with if it is presumed that different mechanisms are responsible for short- and long-term habituation. If a single long presentation is given in an attempt to induce long-term habituation following the induction of short-term habituation, it may be that the system is in a depressed state because of the short-term habituation such that the longer exposure can not act on the mechanism inducing long-term habituation.

Christoffersen (1997) also noted that the rule that increasing the rate of stimulation increases the rate of habituation does not take into account the normal stimulation parameters the neural circuit might receive. In at least one

situation, he noted that a neural circuit was found that habituated most to a specific frequency, and that this frequency is in line with what is environmentally useful. Thus, when studying habituation it is necessary to confirm that your stimulation parameters are within the natural range that evolution would have tuned the system to before making any conclusions.

The form that has been suggested for the mechanisms behind habituation varies widely across organisms and specific sensory systems (Horn, 1967). Two basic models for the mechanisms of habituation have been presented. In one theory, descending input from the cortex actively gates sensory inflow. For example, it has been proposed that habituation of an orienting response could result from cortical control of the reticular formation. Thus, when a novel stimulus is presented, the signal for that image is sent both to sensory cortex and to the reticular formation. Upon repeated presentation of this stimulus, sensory cortex may pass a signal to the reticular formation that prevents it from receiving the same signal. Thus, it was believed that cortex actively gates sensory input (reviewed but not supported by Horn, 1967).

This mechanism is likely untenable for many of the forms of habituation that have been studied, as highlighted by the fact that some forms of habituation can occur in decorticate animals (Horn, 1967). Horn suggested that a more reasonable hypothesis might be self-generated depression (SGD) of sensitivity. SGD could occur at one or more places anywhere along a sensory pathway. He proposed that a decrement in transfer integrity could be the result of any number of phenomena including after-hyperpolarization, conduction block due to build up

of $[K^+]_E$ in the domain of an active nerve fiber, vesicle depletion, recurrent inhibition, or pre-synaptic inhibition.

Synapses cannot be described completely by a single number that characterizes the post-synaptic voltage change in response to a single pre-synaptic action potential. The synaptic efficacy evolves over time in response to both the long-term firing activity at the synapse as well as more recent events in response to changes in both the pre- and post-synaptic elements. Here, I focus on those mechanisms that act over relatively short periods of time (ms to minutes) that do not alter the long-term efficacy of a synapse. These processes have the most relevance to the study of short-term habituation and are referred to as short-term synaptic plasticity. It should be noted, though, that short-term synaptic plasticity likely gates plastic mechanisms that act over longer time scales such as long term potentiation (LTP) and long-term depression (LTD) (Abbott and Nelson, 2000). Within the broad category of short-term synaptic plasticity, those mechanisms that act to decrease synaptic efficacy result in short-term synaptic depression.

Many factors can contribute to short-term synaptic depression and these mechanisms can either have a pre- or post-synaptic locus. Pre-synaptic mechanisms include vesicle depletion, depletion of a necessary substrate for vesicle release, and receptor-mediated decreases in transmitter release; post-synaptic effects include receptor desensitization or saturation of available post-synaptic receptors by transmitter (Zucker and Regehr, 2002). Additionally, many

other factors influence short-term synaptic plasticity, such as the dynamic concentration of extracellular ions (Rusakov and Fine, 2003).

There are a number of functions that are attributed to short-term synaptic plasticity and specifically short-term synaptic depression. Homosynaptic short-term synaptic depression may be used by neurons to help them code the rate of change of inputs that have vastly different constitutive firing frequencies; this process is referred to as cortical gain control(Abbott et al., 1997) .

Mechanisms similar to those described by Horn seem more reasonable for most forms of habituation in light of data collected since 1967. Much of what we know of mechanisms of habituation has come from the study of defensive behavior in invertebrates, such as the marine snail *Aplysia californica.* Specifically, Castellucci et al. (1970) provided evidence that there is a specific excitatory synapse at which the majority of the habituation to one type of sensory input occurs. The identified synapse was between sensory neurons and interneuron and motor neurons. Subsequently, Castellucci and Kandel (1974) found that the decrement in efficacy at the identified synapse resulted from a decrease in the number of transmitter quanta released per impulse. Concurrently, they showed that post-synaptic responsiveness was not altered by repeated stimulation. Thus, they described a pre-synaptic depression that was largely responsible for habituation of a response at the behavioral level.

Crayfish have provided another valuable preparation for the study of habituation. In response to mechanical stimulation of the tail in a crayfish, a single tail flip is elicited by a circuit that fires the lateral giant neuron. With

repeated stimulation, this behavior can be habituated (Zucker, 1972a). Zucker (1972b) was able to show that pre-synaptic depression of synapses from afferent fibers to interneurons was responsible for habituation of this response. Thus, as discussed above for *Aplysia*, habituation was mediated by a pre-synaptic depression.

Mechanistic study of habituation within spinal mammalian preparations has also been fruitful. Sherrington in 1898 observed that the flexion reflex habituates in response to closely spaced stimuli in spinal preparations (Spencer et al., 1966). Later, Posser and Hunter in 1936 were the first to describe the dishabituation of the flexion reflex in response to a strong “extrastimulus” in spinal preparations (Spencer et al., 1966). As stated previously, dishabituation is currently considered a required feature for a behavior to be defined as habituation.

Physiology and Habituation in the Olfactory System

Hasama in 1934 was the first to record voltage changes in olfactory-associated brain regions in the rabbit in response to odorants (Adrian and Ludwig, 1938). A short time later, Adrian and Ludwig (1938), using decayed animal tissue (earthworms and the head of a small alligator allowed to rot in 25 mls of water) as an olfactory stimulant, recorded the olfactory discharge of olfactory bulb (OB) output fibers in a number of fish species (primarily catfish). Although Hasama (1934) seems to have been the first to have record odorant-evoked activity from the mammalian forebrain, Adrian (1942)was likely the first

to record odor-evoked beta (15 – 40 Hz) frequency oscillations in the 15 - 20 Hz range in piriform cortex in the hedgehog and cat.

To the best of my knowledge, Adrian and Ludwig (1938) were the first to demonstrate adaptation to prolonged odorant stimulation electrophysiologically. Following odorant stimulation in fish preparations, both evoked and spontaneous OB activity was diminished. Adrian suggested that this adaptation of responses occurred as a result of olfactory receptor adaptation in the olfactory epithelia. Given what is known currently concerning receptor adaptation to continuous stimuli and possible incomplete wash of the fish's olfactory sac, he may have been correct in this interpretation. However, it is likely that more central bulbar effects may also have contributed.

In a later report, Adrian (1952) suggested that adaptation of bulbar responses in the mammal is likely not the result of receptor adaptation. He based this assumption on the fact that a continuous odorant stimulation produces adaptation over 2 to 3 minutes, but, under normal discontinuous stimulation, recovery would occur. Adrian (1952) went on to suggest that, because he did not observe failure of the receptors during discontinuous stimulation, habituation of odor responses in man (based on data collected in other organisms) is due to recovery of “intrinsic activity after its initial disorganization” following odorant stimulation. Although the recovery of intrinsic activity may be more of a character of odor adaptation than the cause, Adrian’s other interpretations are remarkably similar to what has been uncovered in the intervening fifty-two years.

Potter and Chorover (1976) observed that electroolfactogram (EOG) recordings reflecting olfactory receptor activity adapted appreciably to thirty-second continuous odor stimulation and recovered within thirty to ninety seconds. Ten of these 120-second trials resulted in no cumulative odor adaptation in the EOG. In contrast, mitral cell responses in the OB showed a steady accumulation of adaptation across the same trials that produced no decrement in the EOG. Importantly, the aforementioned work (Potter and Chorover, 1976) used a temporally lengthened artificial respiration pattern for stimulation that may have exaggerated the already brief odorant evoked receptor adaptation.

In a more recent report by Chaput (2000) the EOG was recorded in freely breathing anesthetized rats. Chaput observed that EOG responses synchronized with respiration, decreased during continuous odor presentation, but, importantly, showed significant sustained responses even during sustained sixty second presentations of odorant stimuli. It is likely that decreased odorant concentrations during exhalation allow partial recovery of olfactory receptor responses. At the mechanistic level, the adaptation of EOG responses that occurs during prolonged exposure of odorant receptors to odorants likely results from alteration of cAMP sensitivity of olfactory cyclic nucleotide-gated channels by Ca^{2+}/calmodulin (Kelliher et al., 2003).

Wilson (1998a) has shown that neurons in anterior piriform cortex (aPCX) adapt more quickly and with greater odor specificity than multiunit main OB activity. Additionally, Wilson (1998b) reported a synaptic depression of the mitral cell to aPCX synapse that is associated with adaptation of aPCX odor responses

as measured by shock stimulation of the lateral olfactory tract (LOT). Rapid adaptation of primary olfactory cortex responses in humans is also observed using magnetic resonance imaging (MRI) (Sobel et al., 2000; Poellinger et al., 2001) and can be observed using optical imaging in rats (Litaudon et al., 1997).

The rapid adaptation of aPCX responses relative to MOB responses is mirrored in some thalamocortical circuits (vision: Ohzawa et al., 1982; somatosensation: Chung et al., 2002) and may be a ubiquitous characteristic of sensory processing, although little is known of the underlying mechanisms of these cortical adaptation phenomena.

An additional question is whether centrifugal input can modulate cortical adaptation in the olfactory system. Potter and Chorover (1976) reported that removal of centrifugal input to the bulb causes a decrease in the latency to adaptation and an increase in the latency to recover from adaptation to odorants, although this effect may simply result from the exaggerated responses of mitral cells that occur under this condition.

Alternatively, reinforcement of an odorant with either stimulation of the medial forebrain bundle/lateral hypothalamus (Wilson and Sullivan, 1992) or mild cutaneous shock (Grajski and Freeman, 1989), results in decreased adaptation of bulb responses as measured by mitral/tufted cell firing rates or spatial electroencephalogram (EEG) patterns, respectively. Additionally, bulb responsiveness to non-reinforced odorants can also be maintained by infusion of norepinephrine into the bulb (Gray et al., 1986). These data suggest a role for

modulatory nuclei in the control of sensory adaptation. This may represent a neural correlate of attention state.

Conclusion

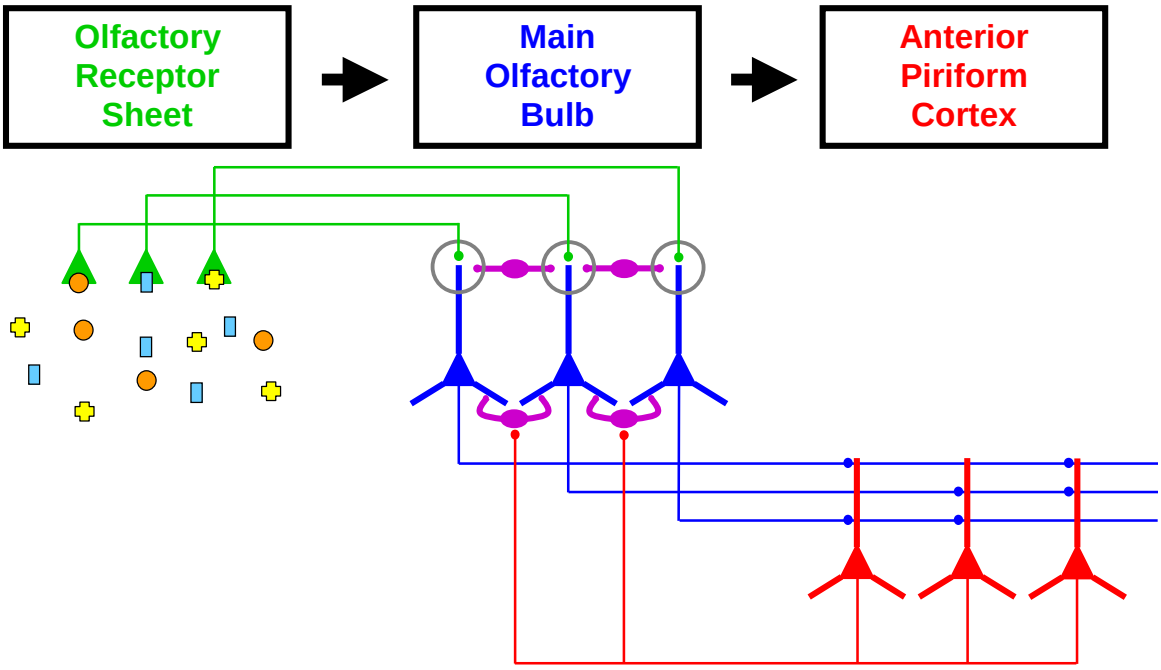
Ultimately, the goal of the research presented in this dissertation is to increase our understanding of habituation of odorant responses within the olfactory system. Specifically, I want to determine what role, if any, is played by the olfactory cortex, and specifically aPCX, in olfactory habituation. In general the goal of the last three chapters of this dissertation will be to present data that suggests that, not only is the synapse formed by mitral/tufted cells onto aPCX neurons the site of adaptation to repeated and prolonged odor responses that is seen under acute recording conditions. Additionally, I will show that this same phenomenon is likely to underlie habituation of responses to unreinforced odorants at the behavioral level in at least one behavioral task.

Chapter 2

Basics of olfaction: with a focus on the mammalian olfactory system.

Gross Anatomy of the Mammalian Olfactory System

Mammalian olfaction begins in the nose where odorant molecules bind to olfactory receptors on olfactory sensory neurons (OSN's). This causes the firing of OSN axons within the olfactory nerve leading to the main olfactory bulb. Mitral/tufted cells within the main olfactory bulb are activated which results in firing of synapses within aPCX. A circuit diagram is shown in figure 1.



The Stimulus

What defines a stimulus to the olfactory system? There is a necessity for odorants to be carried within the medium in which an organism exists, in air for terrestrial organisms and in water for aquatic organisms. Because of this, odorants must be volatile for terrestrial organisms to have access to them. Within terrestrial animals, most odorants are hydrophobic molecules that are

limited to around 300 daltons in size (Turin and Yoshii, 2003). This upper size limit is believed to result from upper limits to the molecular size that can bind to olfactory receptors and not decreased volatility with size (Turin and Yoshii, 2003). Terrestrial organisms are responsive to a great many molecules with vastly differing chemical structures (Amoore, 1970). Within fish, odorant molecules are primarily hydrophilic resulting from the aqueous environment in which fish exist (Hildebrand and Shepherd, 1997). For example, fish olfactory organs seem to be primarily responsive to hydrophilic compounds including amino acids and bile salts (Kang and Caprio, 1995).

At the periphery, the olfactory system must determine what molecular conformations make up each odorant molecule with which it comes into contact (Adrian, 1952; Polak, 1973; Malnic et al., 1999; Araneda et al., 2000). Simulataneously, the olfactory system must analyse feature information from the great many different molecules that are present in any single odor. It is believed that this feature information is then integrated into perceptual objects by more central olfactory-related structures (Wilson and Stevenson, 2003).

Olfactory Receptors

Chemical sensation begins at chemosensory receptors that can be located on a myriad of locations both within and on the external surface of an organism. In insects, OSN's are associated with several types of olfactory sensilla; which, although anatomically different from the olfactory structure of most vertebrates, share much with vertebrates in the biophysics of odorant-to-

OSN trafficking (Hildebrand and Shepherd, 1997). In both terrestrial invertebrates and vertebrates, many of the important odorants are hydrophobic. For this reason, before reaching the OSN's, the odorants must be adsorbed into either a waxy surface on the sensilla or a mucus layer covering the olfactory epithelia. Additionally, odorant binding proteins (OBP's) are presumed to help concentrate odorant near the OSN's in both invertebrates and vertebrates (Hildebrand and Shepherd, 1997).

Receptors on the surface of OSN's transduce odorant binding to a change in membrane potential in both invertebrates and vertebrates. However, although the morphology of the OSN's is evolutionarily conserved, the odorant binding receptors on the surface of OSN's diverge across phyla (Krieger and Breer, 1999). In fact, little sequence homology exists across different phyla for the seven-transmembrane G-protein coupled receptors that are believed to be the actual odorant receptors. Even the two families of odorant receptors that represent the input for the main olfactory system and the vomeronasal system of mammals seem to have diverged sharply (Krieger and Breer, 1999). The reason for this difference from the evolutionarily conserved nature of other G-protein coupled receptor families is not known.

The genes that code for putative mammalian odorant receptors were first discovered in 1991 (Buck and Axel, 1991). In mammals such as mice and rats, there are believed to be roughly 1000 different odorant receptor types (Young et al., 2002; Zhang and Firestein, 2002; Niimura and Nei, 2003). The genes that encode the odorant receptors are relatively small and are coded by a single

exon. Despite their relatively small size, odorant receptor genes comprise an estimated one to three percent of the mammalian genome (Reed, 2004).

Interestingly, comparison of the number of odorant receptor genes in invertebrates (*Drosophila melanogaster*: Clyne et al., 1999; Vosshall et al., 1999; *Caenorhabditis elegans*: Troemel et al., 1995; Sengupta et al., 1996; *Anopheles gambiae*: Fox et al., 2001 and vertebrates Young et al., 2002; Zhang and Firestein, 2002; Niimura and Nei, 2003) at least ~100 – 1000 in each despite the difference in overall genome size, suggests that large numbers of odorant receptor types are necessary for the coding of "odor space" (Reed, 2004). In contrast, fish have far fewer odororant receptor genes likely because of the limited number of odorants believed to be important for fish olfaction (Ngai et al., 1993).

Odorant receptors show a zonal pattern of expression within the mammalian olfactory epithelia in the nasal cavity (Ressler et al., 1993; Vassar et al., 1993); expression of any one odorant receptor is limited to one of four zones with intra-zone expression appearing random. It is not known if there is a purpose for this discontinuous pattern, although it has been suggested as early as 1952 that the spatial layout of the olfactory receptor sheet may lend itself to analysis based on molecular diffusion (Adrian, 1952). Work since this time has implicated a form of gas chromatographic separation of odorants across the olfactory epithelia in odor coding (Mozell, 1970; Mozell and Jagodowicz, 1973), although the most likely source of odor coding at the receptor level involves the

differential binding affinity of odorants to the ~1000 odorant receptor types (Adrian, 1952; Polak, 1973; Malnic et al., 1999; Araneda et al., 2000).

Although it has generally become accepted that there is one functional odorant receptor expressed per olfactory receptor neuron (Chess et al., 1994; Malnic et al., 1999; Serizawa et al., 2003), the data are not conclusive (Mombaerts, 2004). Additionally, although the pathway for odorant activation of OSN's has been thought to require cAMP formation, it is likely that more than one intracellular pathway exists for the transduction of odorant information in OSN's. Recordings from OSN's in culture suggest that transduction of odorant information from odorant receptors to a change in membrane potential relies on formation of cAMP in many cases (Schild and Restrepo, 1998). However, certain odorants produce increases in inositol-triphosphate (IP_3) that are not correlated with increases in cAMP. This suggests that there may be multiple signaling pathways (Schild and Restrepo, 1998).

Recordings from olfactory structures *in vivo* suggest at least two intracellular transduction pathways within OSN's. It is believed that increases in cAMP result in the opening of cyclic nucleotide-gated channels in the membrane of OSN's that leads to increases in intracellular calcium levels (Schild and Restrepo, 1998). In fact, mice that have had genetic deletion of their cyclic nucleotide-gated channels still display some odorant-induced responses in the olfactory receptor sheet, olfactory bulb (OB) and olfactory (piriform) cortex (Lin et al., 2004); suggesting other pathways.

One specific subtype of glomeruli, termed necklace glomeruli, receive projections from OSN's that lack the traditional cAMP-dependent intracellular signaling pathway. Necklace glomeruli are located along the border between the main and accessory OB. The OSN's that project to them use a cGMP increase to activate a phosphodiesterase for signal odorant binding (Zheng and Jourdan, 1988; Juilfs et al., 1997).

Calcium plays an important role as a third messenger in OSN signal transduction. Following the formation of cAMP and/or IP_3, levels of intracellular calcium rise. It is believed that calcium opens conductances in the OSN membrane. Although a number of conductances have been observed, in receptors that show increases in cAMP it is believed that one mechanism involves a Ca^{2+}-gated Cl- channel, activation of which results in the observed change in OSN potential that leads to transmitter release (Schild and Restrepo, 1998). It should be noted though, that the complete picture is far from clear.

OSN's are differentially responsive to odorants. It is believed that individual OSN's receive their selectivity via odorant receptor expression. It has been shown that certain OSN's are differentially responsive to odorants based on both odorant molecular character and odorant concentration (Duchamp-Viret and Duchamp, 1997; Touhara, 2002). Many odorant receptors show responsivity to multiple odorants. Early studies in both vertebrates and invertebrates demonstrated the existence of broadly tuned OSN's (Boeckh et al., 1965; Gesteland et al., 1965), although some insect OSN's are very narrowly tuned to certain pheromones. Interestingly, certain odorants can act as antagonists at

some odorant receptors, thus adding coding complexity to mixtures of odorants (Firestein, 2004).

While odorant receptor binding properties contribute to odorant coding, the defining of an odorant into a perceptual object relies on more central structures. For example, in C. *elegans*, it has been demonstrated that genetically targeting the expression of an odorant receptor to OSN's that usually do not express that receptor results in an altered behavioral response to the associated odorant from attraction to repulsion (Troemel et al., 1997). Thus, the circuit an OSN takes part in and not the receptor that it produces defines the odorant to an organism (Krieger and Breer, 1999).

From the mammalian olfactory epithelia, the axons of OSN's project through the cribriform plate dorsally and then caudally back to the superficial surface of the OB. OSN's expressing specific olfactory receptor proteins converge and synapse onto a pair of defined OB glomeruli, one on the lateral surface and one on the medial surface (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Wang et al., 1998). Genetic switching of the receptor expression of an OSN from one odorant receptor to another results in projection to the correct pair of glomeruli for the introduced odorant receptor (Wang et al., 1998; Bozza et al., 2002). If receptors that are normally in different zones of the olfactory epithelia are swapped, the transgenic OSN's then project their axons to defined but ectopic glomeruli within the bulb (Wang et al., 1998). Additional signaling systems also exist for control of OSN axon pathfinding (ephrins: Cutforth et al., 2003). Activity-dependent mechanisms for refinement of synaptic

targeting of axons from OSN's that express the same odorant receptor also are likely to contribute to overall OSN-derived synapse formation in the bulb (Zhao and Reed, 2001), this may vary, however, depending on the odorant receptor that a particular OSN type expresses (Zheng et al., 2000). Given the continual turnover of OSN's during the life of an organism, these systems must remain continuously viable (Gogos et al., 2000).

Very little has been found that is suggestive of synaptically based integrative processes at the level of the mammalian olfactory epithelia. Although there is some partitioning of odorant receptor expression to specific zones as stated previously (Ressler et al., 1993; Vassar et al., 1993), the overall pattern of expression seems to resemble random dispersion throughout each zone (Ressler et al., 1993; Levai et al., 2003). OSN's appear not to be synaptically connected, such as via dendrodendritic or recurrent synapses (Farbman, 1994; Hildebrand and Shepherd, 1997).

There exists the possibility of non-synaptic interactions between OSN's. Interestingly, there may be mechanisms of interaction over short distances between OSN's provided by membrane permeable gaseous messenger systems including CO and NO (Breer et al., 1992; Leinders-Zufall et al., 1995). Also, there exists the possibility of ephaptic interactions between the nonmyelinated axons of the OSN's that form the olfactory nerve (ON) (Bokil et al., 2001).

The actual pharmacology of the receptors may be a point of odorant information integration. It may be that certain odorants act as antagonists at receptors other than those that they excite in the classic manner of an agonist.

This antagonist activity may help shape the normal perception of mixtures of odorants (Firestein, 2004). The first major locus of integration in the mammalian olfactory system, however, is the OB. Integration is likely to begin earlier within invertebrate systems. In many invertebrate olfactory organs there is a stereotyped pattern of receptor expression within the two or more olfactory receptor cells within any one sensillum. Additional information on spatial odorant location is also available within many invertebrate chemosensory systems (Hildebrand and Shepherd, 1997).

Olfactory Bulb

OSN's in the mammalian olfactory system form glutamatergic synapses within the glomeruli of the OB (Sassoe-Pognetto et al., 1993; Berkowicz et al., 1994). The OB exists as an extension of the brain projecting rostrally. In many ways, it can be seen as the first point of integration of olfactory information. To this point, the olfactory system generally exists as a myriad of parallel processing pathways. After an odorant gains access to the olfactory epithelia, it activates a subset of the available OSN's depending upon the molecular functional groups that make up the odorant molecule (Adrian, 1952; Polak, 1973; Malnic et al., 1999; Araneda et al., 2000). These activated OSN's consist of groups of sensory neurons that express the same odorant receptor. The activity of each group can be considered one path in the parallel coding pathways from the olfactory epithelia to the OB. In fact, even the input stage of the OB is separated in this manner. Each group of OSN's, as defined by homotypic expression of odorant

receptors, projects to two specific glomeruli in the bulb and the pattern of expression across OSN types is nonoverlapping (Mombaerts et al., 1996).

There is a great deal of convergence from the level of the olfactory epithelium and its associated OSN's to the olfactory bulb and its associated glomeruli. Once the nonmyelinated axons of OSN's reach their appropriate glomeruli, they branch and form on average 8.1 terminals (Halasz and Greer, 1993). This expanded output of each OSN fiber contributes to the already massive convergence that occurs at this stage in the olfactory pathway. In mice, the number of OSN's is approximately 20×10^6 (Shepherd et al., 2004); the number of glomeruli is believed to be anywhere from 1800 to 3000 per OB in mice, rats and rabbits (Allison and Warwick, 1949; White, 1972; Meisami and Safari, 1981; Brunjes, 1983; Royet et al., 1988). Thus, approximately 11,000 OSN axons project to each glomerulus (Shepherd et al., 2004).

Glomerular activation has been studied in an attempt to visualize an olfactory sensory map within the OB. 2-deoxyglucose (2-DG) is a glucose analogue that is taken up by active cells, but neither excreted nor metabolized by the cell. This allows for autoradiographic analysis of active areas of the brain. This technique was first used within the olfactory system in the OB in 1975 in a preliminary report (Sharp et al., 1975). Using this technique, Sharp et al. (1977) were able to show that rudimentary activation patterns could be seen in a number of bulb lamina including the granule cell layer and the mitral cell layer, with the most intense labeling in the glomerular layer. They noted that individual glomeruli could be discriminated based on activity levels.

Early electrophysiological studies suggested that odorant stimulation results in differential activity across the OB (Adrian, 1953; Leveteau and MacLeod, 1966; Moulton, 1976). This observation was born out in subsequent studies using 2-DG methods (Stewart et al., 1979; Coopersmith and Leon, 1984). In short, different odors activate different subsets of glomeruli and the magnitude of the area of activation increases with increasing concentration (Stewart et al., 1979; Cinelli et al., 1995; Guthrie and Gall, 1995; Johnson et al., 1999; Rubin and Katz, 1999; Johnson and Leon, 2000). Additionally, these patterns of glomerular activation are consistent across individuals of the same species and this patterning is conserved across diverse phyla (Stewart et al., 1979; Jourdan et al., 1980; Friedrich and Korsching, 1997; Johnson et al., 1998; Galizia et al., 1999; Johnson et al., 1999; Rubin and Katz, 1999; Ng et al., 2002; Bozza et al., 2004).

More recent data have leant considerable support to the notion that activation of specific glomeruli contributes to the perception of an odor (Jourdan et al., 1980; Royet et al., 1987; Guthrie et al., 1993; Cinelli et al., 1995; Johnson et al., 1998; Johnson et al., 1999; Uchida et al., 2000). One recent study examined the effects of odor concentration on glomerular activation and compared the results with psychophysical reports of odor perception (Johnson and Leon, 2000). Two of the odorants tested recruited additional nonadjacent glomeruli as concentration increased, while three additional odorants did not. The former odorants are known from psychophysical studies to change in perceptual quality with increasing concentration in contrast to the latter odorants

which do not (Johnson and Leon, 2000). This finding strongly supports the notion that the differential activation of olfactory bulb glomeruli reflects coding of odor information.

Electrophysiological recordings and imaging data from the OB support the hypothesis that individual glomeruli can be considered a functional unit in the OB just as cortical columns exist as functional units within neocortical sensory systems (Guthrie et al., 1993; Kauer and Cinelli, 1993; Johnson and Leon, 2000; Johnson et al., 2002). These functional units consist of the activated glomerulus, the associated mitral/tufted cells fed by the activated glomerulus, and the interneurons in the glomerular and granule cell layers that take part in shaping the output of the glomerular "column".

The emerging view of bulbar odor coding consists of glomerular activation that is dependent on the chemical structure of the odor (Friedrich and Korsching, 1997; Johnson et al., 1998; Johnson et al., 1999; Rubin and Katz, 1999, 2001; Bozza et al., 2004). However, additional data may add complexity to this view. Currently, most experiments on odor coding in the OB rely on extended odorant exposure to build up an odor map in the bulb. Recent data suggest that the process may be more dynamic in nature (Luo and Katz, 2001; Spors and Grinvald, 2002). In these more recent studies, OB activation evolves over the course of odorant presentation. Over the course of hundreds of milliseconds, odor-specific sequences of glomerular activation have been observed. As odorant concentration is increased the pattern is changed only by having a

decreased latency to activation of glomeruli and by activation of additional glomeruli at longer latencies (Spors and Grinvald, 2002).

The axons of OSN's form contacts with both bulb projection neurons as well as interneurons. Specifically, terminals are formed with mitral and tufted projection neurons as well as periglomerular (PG) interneurons. Additionally, dendrodendritic synapses are found between mitral/tufted cells, which form Gray's type I synapses, and PG cells, which form Gray's type II synapses suggesting that these synapses are inhibitory. Additionally, Gray's type II synapses can be found on the dendrites of PG cells (Pinching and Powell, 1971a, b; Kasowski et al., 1999). PG cells usually arborize within only one glomerulus and PG cells that have axons tend to project them to neighboring glomeruli (Pinching and Powell, 1971a, b). It is believed that PG cells form a relatively tight network that may be important for coordination of rhythmicity within the olfactory system at frequencies similar to sniffing (Onoda and Mori, 1980; Wellis and Scott, 1990). Some PG cells likely form gamma aminobutyric acidergic (GABAergic) synapses, but they may still represent excitatory connections. Roughly twenty percent of rat PG cells contain the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) (Ribak et al., 1977).

One particular population of PG cells has recently been implicated in lateral inhibitory control of glomerular activation and, thus, a possible function in contrast enhancement or gain control. Short axon (SA) cells are a class of PG cells that, based on early Golgi reconstructions, were believed to have axons that spread no more than 2-3 glomeruli away from their soma (Pinching and Powell,

1971c). Newer techniques have resolved a network of glutamatergic excitatory connections between SA cells and inhibitory PG cells that extends over large areas of the OB. Thus, strong input to one glomerulus would excite SA cells and cause them to activate inhibitory PG cells that decrease the activity of other glomeruli across the surface of the OB (Aungst et al., 2003). Interestingly, given the ability of PG cells to undergo self-inhibition during intense firing, strong sensory stimulation might degrade this lateral inhibitory network (Smith and Jahr, 2002). An effect such as this could contribute to the previously mentioned increase in the number of glomeruli that show sensory-evoked responses to high odorant concentrations. This recruitment of glomeruli is usually attributed to additional ORN types being activated as odorant concentration rises; future research will be necessary to determine the relative contributions of the two mechanisms.

Additionally, PG cells can inhibit transmitter release from OSN axon terminals. PG cells contain the dopamine-synthesizing enzyme tyrosine hydroxylase (TH) (Halasz et al., 1977). Acting through D2 receptors on OSN terminals, dopamine can inhibit vesicle release (Ennis et al., 2001).

The primary pathway of information through the glomerular layer of the olfactory bulb consists of the OSN-to-mitral/tufted cell synapse. Mitral cells in mammals have large somata and give rise to a single, large apical dendrite that is targeted to a single glomerulus in which the dendrite branches profusely (Shepherd, 1972a; Orona et al., 1984). Mitral cells also possess secondary

dendrites that extend laterally and are situated primarily in the external plexiform layer (EPL), which lies deep to the glomerular layer (Orona et al., 1984).

The odor-evoked firing properties of mitral cells are complex. Responses can consist of excitation, inhibition or a combination of the two (Shipley and Ennis, 1996). The concentration of an odorant stimulus can significantly affect the firing properties of mitral cells to the extent that some mitral cells respond preferentially to odorants at specific concentrations (Kauer, 1974; Harrison and Scott, 1986; Meredith, 1986; Wellis et al., 1989).

Tufted cells are a second class of OB output neuron. The somata of tufted cells are located in the EPL while mitral cell somata lie deep to the EPL in the mitral cell layer. Tufted cell somata are generally smaller than mitral cell somata although they increase in size with depth (Pinching and Powell, 1971c). Interestingly, some tufted cells project apical dendrites to more than one glomerulus (Shipley and Ennis, 1996). There are approximately twice as many tufted cells as mitral cells (Allison, 1953).

There are three broad categories in which tufted cells are grouped based on the laminar placement of their somata in the EPL: internal, middle and external. Internal and middle tufted cells have similar projections to those of mitral cells. Thus, it is likely that they function in at least a manner rudimentarily similar to that of mitral cells (Schoenfeld and Macrides, 1984).

External tufted cells are particularly interesting given their axonal projection patterns. They form an association system within the bulb where in they link the lateral and medial circuits on opposite sides of the bulb that receive

input from OSN's expressing the same odorant receptor (Schoenfeld et al., 1985; Liu and Shipley, 1994; Belluscio et al., 2002). The synapses formed by this associational system are primarily formed on the dendrites of granule cells (Liu and Shipley, 1994). Interestingly, external tufted cell firing is tuned to the frequency of sniffing by intrinsic properties of the neurons (Hayar et al., 2004).

Internal and middle tufted cells also project locally, but their primary projection is to regions of the anterior olfactory cortex (AOC) and anterior portions of piriform cortex (Schoenfeld et al., 1985; Scott, 1986). It is believed that the majority of mitral and tufted cells release glutamate as their primary neurotransmitter (Liu et al., 1989).

Mitral and tufted cells differ in their stimulus response characteristics. Tufted cells have lower firing thresholds for olfactory nerve electrical stimulation (Schneider and Scott, 1983). This is mirrored in increased firing rates to odorant stimulation (Nagayama et al., 2004). Mitral cells show inhibitory or suppressive responses to stimulation of glomeruli neighboring those from which they receive strong excitation (Yokoi et al., 1995). Conversely, tufted cells do not seem to be under the control of the inhibitory surround system that can be seen to affect the firing of mitral cells (Nagayama et al., 2004). This is not completely surprising given that tufted cells have short lateral dendrites (see below) and, *in vitro*, electrically-induced lateral inhibition is smaller in tufted cells than in mitral cells (Mori et al., 1983; Christie et al., 2001). Additionally, there seem to be separate networks of granule cell-mediated inhibition for mitral and tufted cells (Ezeh et al., 1993; Nagayama et al., 2004).

A complex network exists that is derived from the mitral/tufted cell secondary dendrites and the large population of axon-less granule cells. Granule cells are GABAergic interneurons that are positioned in the inner-most lamina of the OB. The reciprocal synaptic connection between the mitral/tufted cells and the granule cells was the first dendrodendritic reciprocal synapse defined (Rall et al., 1966). There are approximately 50-100 granule cells to every one mitral cell in the olfactory bulb (Shepherd, 1972b).

Early studies in the rabbit suggested that there was inhibitory control of mitral cell output from the bulb. Specifically, antidromic activation of mitral cells by stimulation of the lateral olfactory tract results in a long-lasting inhibition of firing that is correlated with activation of granule cells (Yamamoto and Iwama, 1962; Phillips et al., 1963; Shepherd, 1963). Since these pioneering studies, the system has become well understood. Rall et al. (1966) first described the ultrastructural characteristics of the mitral/tufted to granule cell dendrodentritic reciprocal synapse. Specifically, they found that mitral cell lateral dendrites could be seen to form connections with spines on granule cells that consisted of neighboring synapses with one pre-synaptic element from each neuronal partner.

In 1982, the first physiological evidence to support the early EM findings became available (Jahr and Nicoll, 1982b). Jahr and Nicoll (1982b) used an *in vitro* turtle olfactory bulb preparation to deteremine whether mitral cell lateral dendrites could act as presynaptic elements. They found that when depolarizing current is passed into a mitral cell there is a TTX insensitive hyperpolarizing IPSP that follows the initial depolarization of the membrane; thus suggestive of a

mechanism that does not rely on axonal voltage gated sodium channel activation. They also discovered that the IPSP was mediated by $GABA_A$ receptor activation because of its sensitivity to bicuculline methiodide. Schoppa et al. (1998) and Isaacson and Strowbridge (1998)went on to show that this granule cell dendritic release of GABA requires the relatively long lasting Ca^{2+} currents mediated by NMDA receptor activation. Additionally, subsequent current through high voltage calcium channels of the N- and P/Q- types is also required for granule cell dendritic GABA release (Isaacson and Strowbridge, 1998).

The network of granule cells and their associated connections with mitral/tufted cells is believed to contribute significantly to bulbar feature detection. Mitral/tufted cell receptive ranges commonly consist of excitatory responses to one or more closely related molecules with inhibitory responses to odors that are slightly less similar (Yokoi et al., 1995). This inhibition was found to be mediated by GABAergic transmission within the EPL, suggesting that granule cells may underlie these inhibitory potentials (Yokoi et al., 1995). It is believed that this circuitry could contribute to help the olfactory system differentiate between odorants with similar functional groups.

Just as the PG cells are believed to contribute to the slow (~2 Hz) OB oscillations, the granule cell network is believed to contribute to fast bulbar oscillations in both the beta (~15-40 Hz) and gamma (~50-100 Hz) frequency range. Based on analysis of current source, currents within granule cells underlie the beta and gamma oscillations seen in local field potential (LFP) recordings (Freeman, 1972; Neville and Haberly, 2003).

There are significant subcortical and cortical projections to the OB. A disproportionate amount of this centrifugal input targets the granule cell population at the level of the the internal plexiform layer (IPL). The IPL is a thin layer that lies deep to the mitral cell body layer. The IPL contains the axons of mitral/tufted cells as well as the dendrites of granule cells and the axons of centrifugal fibers originating from various cortical and subcortical regions (Shipley and Ennis, 1996).

The subcortical input is primarily derived from modulatory nuclei. Part of this input is derived from cells in the horizontal limb of the diagonal band (HDB) (Carson, 1984a); many of these cells are either cholinergic or GABAergic (Macrides et al., 1981; Carson, 1984b; Zaborszky et al., 1986). Ten-hertz trains of electrical stimuli to the HDB result in current flow in granule cells and concurrent inhibition of mitral cells (Nickell and Shipley, 1988).

OB input from the locus coeruleus (LC) is also primarily directed to granule cells although the EPL and the mitral cell layer contain some LC fibers (McLean et al., 1989). Activation of the LC results in an increase in mitral cell responses to weak stimuli (Jiang et al., 1996). The development of OB projections from the LC correlates with development of this increase in mitral cell responsiveness to the effects of exogenous norepinephrine (Wilson and Leon, 1988). An additional interesting phenomenon is the reduction of habituation of responses to unconditioned stimuli following norepinephrine infusion (Gray et al., 1986). These circuit effects of norepinephrine likely contribute to the importance of this neuromodulator in OB forms of learning that are norepinephrine-

dependent (Bruce, 1960; Keverne and de la Riva, 1982; Pissonnier et al., 1985; Rosser and Keverne, 1985; Kaba et al., 1989; Sullivan et al., 1989). There are strong serotonergic projections from the raphe nuclei to the OB that have also been shown to be important for learning in a neonatal paradigm (McLean and Shipley, 1987).

One large source of input to the granule cell population is derived from axon collaterals that project back from olfactory cortex pyramidal cells. The cortical feedback is primarily from layer II/III pyramidal cells and is more intense from rostral than from caudal regions of olfactory cortex (Shipley and Adamek, 1984). It is believed that this input is excitatory upon granule cells and ultimately results in inhibition of mitral/tufted cells (Nakashima et al., 1978). The synapses formed are symmetrical with round vesicles suggestive of excitatory connections (Price and Powell, 1970). This projection is of specific interest because the exact function is not known. Given that this feedback projection is actually larger than the feedforward projection from the OB to olfactory cortex, it would seem that its function must be relatively important (Neville and Haberly, 2004).

Olfactory Cortex

As stated previously, the primary OB output to olfactory cortical areas is the LOT that is formed by the myelinated axons of mitral and tufted cells. Olfactory cortex consists of those areas that receive direct input from the OB (Price, 1973). Therefore, olfactory cortex subsumes the anterior olfactory cortex (AOC), medial olfactory cortex (MOC), piriform cortex (PCX), olfactory tubercle (OT), entorhinal cortex, agranular insula, and portions of the amygdala (Neville and Haberly, 2004).

Olfactory cortex is paleocortical tissue because of its phylogenetically older cytoarchitecture (Neville and Haberly, 2004); olfactory cortex generally has 3 or 4 layers rather than the usually 6 that are characteristic of neocortex. However, it should be noted that some portions of olfactory cortex, namely entorhinal cortex and agranular insula, are more highly laminated than paleocortical tissue and, thus, are in some ways more similar to neocortical tissue (Neville and Haberly, 2004).

Immediately caudal to the OB on the olfactory peduncle lies the AOC, previously considered a portion of the anterior olfactory nucleus (AON). The change in terminology occurred in part because the AOC is more appropriately cortical because of its cytoarchitectural characteristics than it is a nucleus of cells (Haberly, 2001). The AON also included the ventral tenia tecta (VTT) and the dorsal peduncular cortex (DPC). The VTT is located on the ventromedial wall of the olfactory peduncle while the DPC lies on the dorsomedial wall of the olfactory peduncle. Both structures are three-layered cortical tissue similar to piriform

cortex (Haberly and Price, 1978a; Haberly, 2001). Haberly (2001) termed these medial structures medial olfactory cortex (MOC) because of their different cytoarchitecture and connectivity compared to AOC.

AOC has reciprocal connections with anterior piriform cortex (aPCX) but not posterior piriform cortex (pPCX), while MOC connects with pPCX and entorhinal cortex and only has a unidirectional connection with AOC (Haberly and Price, 1978b; Luskin and Price, 1983b; Haberly, 2001). Addtionally, unlike the AOC, which has strong reciprocal OB connections, the MOC has little OB connectivity (Haberly and Price, 1978b; Luskin and Price, 1983b; Haberly, 2001). Haberly (2001) suggested that this connectivity results in a disynaptic pathway for information flow from posterior olfactory cortical regions to anterior olfactory cortical regions; a strong monosynaptic pathway from posterior to anterior is lacking. I will use the terminology of Haberly (2001) for this review as I agree with the alterations, although it should be noted that it is not a fully accepted change at this time.

The AOC is the first pyramidal cell-based cortical structure in the olfactory pathway. Little is known concerning the function of this laminated structure. It has a single, fairly homogenous layer of cells deep to the neuropil layer in which OB input projects along with associational fibers (Haberly and Price, 1978a). Association fibers arise from both the ipsilateral and contralateral AOC along with input from more caudal regions of olfactory cortex (Luskin and Price, 1983a, b; De Carlos et al., 1989), as well as the typical complement of subcortical input

from modulatory nuclei (Shipley and Ennis, 1996). The AOC also then projects back to OB and olfactory cortical areas (Haberly and Price, 1978a).

Beyond the ability to connect ipsilateral and contralateral olfactory systems specifically for memory retrieval, little is known of the function of the AOC (Kucharski and Hall, 1988). One phenomenon of interest is that the AOC, a small rostral part of the aPCX, and the OT are the only regions that receive tufted cell output (Haberly and Price, 1977; Scott, 1981; Schoenfeld and Macrides, 1984).

Tufted cells project to a limited number of regions and specifically to the AOC. There is a large population of tufted cells, twice the number of mitral cells, although not all project outside of the OB (Allison, 1953). Tufted cells have a number of characteristics that differentiate them from mitral cells. Many tufted cells project to more than one glomerulus (Shipley and Ennis, 1996). There are also differences in firing characteristics and inhibitory control (Nagayama et al., 2004).

Mitral and tufted cells may represent parallel pathways for processing of odorant information (Shepherd et al., 2004). Because of the lower threshold and decreased inhibition upon tufted cells, they may be more sensitive to lower concentrations of odorants than mitral cells (Nagayama et al., 2004). Additionally, paired-pulse facilitation of OB projections is significantly higher in both aPCX and pPCX than it is in AOC (McNamara et al., 2004). It is possible that this effect is caused by increased release probability during the initial pulse

of the LOT fibers within AOC. This would suggest that the OB to AOC synapse might be more reliable than the OB-to-aPCX/pPCX synapse.

Haberly (2001) suggests that the AOC may actually underlie the synthetic formation of odorant gestalts and proposes that the AOC functions like a primary sensory cortex. This is in contrast to the view that aPCX acts as the primary olfactory sensory cortex. Haberly (2001) suggests that aPCX may actually perform the functions of higher level sensory cortices. One piece of evidence used to support this hypothesis is that aPCX cortical neurons can alter their firing pattern in a manner that is temporally correlated with contextual information in a behavioral task (Schoenbaum and Eichenbaum, 1995). However, it remains possible that aPCX is in fact unimodal. As Haberly (2001) admits, these alterations in firing rate may simply reflect alterations in the attentional state of the animal. It has been observed that activation of the LC or HDB can alter neural activity within aPCX (Linster et al., 1999; Bouret and Sara, 2002). Analogous phenomena have been seen in primary visual cortex (Luck et al., 1997; Ress et al., 2000).

One possible alternative is that the olfactory system uses parallel pathways for detection and discrimination. This may mirror the situation within the visual parvocellular and magnocellular pathways. One pathway consisting of the mitral cell-to-aPCX fibers may be primarily involved in molecular recognition and discrimination. The more sensitive tufted/mitral cell-to-AOC pathway may instead be more involved in detection of low concentrations of odorants. In agreement with this hypothesis, recordings from mitral cells and AOC cells

suggest that the OB mitral cell population is better than the AOC at discrimination, which is in line with the idea that the AOC is specialized for detection and not discrimination of odorants (Boulet et al., 1978). Although it should be noted that detection and discrimination may not be mutually exclusive processes.

Mass release of norepinephrine and/or acetylcholine, which signals periods of alertness, causes a decrease in the efficacy of association fiber synapses within layer Ib (Hasselmo and Bower, 1992; Hasselmo et al., 1997; Linster et al., 1999; Linster and Hasselmo, 2001). In general, projections from more anterior olfactory structures synapse onto more distal segments of the pyramidal cell apical dendrites (Price, 1973; Luskin and Price, 1983a, b). For example, LOT fibers synapse on the most distal regions of the pyramidal cell apical dendrites in layer Ia. Deep to this, layer Ib contains intracortical and association fiber synapses that also show laminar selectivity. Fibers from more rostral regions of olfactory cortex synapse more distally in layer Ib. Thus, fibers from PPC would synapse closest to the pyramidal cell bodies and thus may have the greatest control over their firing; although active dendritic conductances could alter the effect of synaptic placement.

This laminar profile has one exception, though; axons projecting from AOC neurons synapse at the most proximal level of the pyramidal cell apical dendrites in aPCX, potentially giving AOC neurons more control over production of action potentials in these neurons than other anterior portions of the olfactory system would likely have. But, if AOC were to function as primary olfactory

cortex (and thus be responsible for the synthetic recombination of features detected at the periphery and the subsequent discrimination from other feature combinations), why would the input from the AOC to higher olfactory structures (aPCX) be diminished during times of heightened alertness (by neuromodulatory control of the association fibers in layer Ib)? It will be interesting to ascertain if AOC-based association fiber projections are attenuated in the same manner as the rest of the association fiber system. Up to this point, all studies have looked at the effects of neuromodulators during either direct stimulation of the association fibers (without knowledge of where the association fibers originated) or during stimulation within pPCX (Hasselmo and Bower, 1992; Hasselmo et al., 1997; Linster et al., 1999; Linster and Hasselmo, 2001).

I suggest that during normal states of decreased alertness, the tufted/mitral cell-to-AOC pathway is used for basic detection purposes. As an odorant is detected, modulatory nuclei increase the state of alertness of the animal and consequently decrease the relative importance of AOC input into aPCX. In this way, aPCX would be in a position to use its primarily mitral cell input to discriminate between odorants to ascertain whether the stimuli have behavioral significance.

The anterior demarcation for PCX is located at the point in the olfactory peduncle at which the dorsal surface of the peduncle fuses completely with the overlying brain tissue. The rhinal fissure is the demarcation between the caudal olfactory cortical structures and the perirhinal cortex located dorsally. The

posterior demarcation of PCX is the entorhinal cortex, which is laminated to a greater degree than PCX and the rest of the paleocortical brain regions.

PCX is divided into anterior piriform cortex (aPCX) and posterior piriform cortex (pPCX). The demarcation between aPCX and pPCX lies at the point where the myelinated LOT fibers end. Posterior to this point, mitral cell projections consist of nonmyelinated axon collaterals that spread out posteriorly. PCX consists of three to four laminae; four laminae exist only if you include the underlying endopiriform nucleus, which is usually not included (Haberly and Price, 1978b). There may be functional reasons for this division of the endopiriform nucleus and PCX as the endopiriform nucleus is responsive to gustatory as well as olfactory input (Fu et al., 2004).

The most superficial layer of aPCX is layer Iα, which contains the myelinated axons of mitral and some tufted cells. However, the projection from tufted cells is limited to a small region in the anteroventral portion of aPCX (Haberly and Price, 1977). Depending on the rostrocaudal level, there are between 32 and 42 thousand myelinated axons in the LOT, with an average diameter of 1.3 µm (Price and Sprich, 1975).

Nonmyelinated axon collaterals project from the LOT and head deep to layer Ia, which is the exclusive site of synapse formation from LOT fibers into piriform cortex (Price, 1973). The synapses formed by these fibers are asymmetrical and have round vesicles, and thus are Gray's type I synapses (Haberly and Behan, 1983). This is in agreement with their excitatory action on

aPCX neurons as well as their likely use of glutamate as a neurotransmitter (Haberly, 1973a, b; Haberly and Shepherd, 1973; Liu et al., 1989).

Mitral cell synapses within aPCX have a relatively low vesicle packing density. Additionally, these synapses display paired-pulse facilitation at intervals between 10 and 250 ms (Bower and Haberly, 1986; McNamara et al., 2004). Train stimulation can induce depression of this synapse from 11% to > 60% depending on train parameters (Hasselmo and Bower, 1990; Best and Wilson, 2004).

The axonal arbors of OB projection fibers are distributed within olfactory cortex in an unusual manner relative to neocortical sensory regions. Within neocortical sensory areas most projections are point-to-point. Within anterior regions of olfactory cortex, projections are broadly distributed but not uniform; within regions of caudal olfactory cortex, projections are both broad and fairly uniform (Haberly and Price, 1977; Scott et al., 1980; Ojima et al., 1984; Buonviso et al., 1991; Zou et al., 2001).

Individually labeled mitral cells within the olfactory bulb usually project to both the AOC and the aPCX and send collaterals into each structure that form patches synapsing in layer Ia. Approximately one quarter of all mitral cells that were labeled in the rabbit also sent projections to the olfactory tubercle (OT) (Ojima et al., 1984). When retrograde tracers are introduced into olfactory cortical areas, widespread regions of the olfactory bulb are labeled, although there are regions of higher density staining (Haberly and Price, 1977; Scott et al., 1980). The fact that there are areas of the bulb that show a more dense labeling

of mitral and tufted cells following tracer injection into olfactory cortex corresponds well with what is known about projections of neighboring mitral cells in the bulb. Neighboring mitral cells have been demonstrated to project to olfactory cortex in overlapping patterns (Buonviso et al., 1991).

Transgenic expression of a transneuronal tracer in OSN neurons has been accomplished. Specifically, the tracer has been targeted to specific OSN's expressing only one of the full complement of odorant receptors (Zou et al., 2001). In this way, neurons within the olfactory system that are synaptically connected to receive input concerning the specific receptive range of just one type of odorant receptor can be visualized. This study corroborated previous studies showing projection patterns of mitral and tufted cells into olfactory cortex (Haberly and Price, 1977; Scott et al., 1980; Ojima et al., 1984; Buonviso et al., 1991).

As stated previously, all OSN's that express a specific odorant receptor gene project their axons to the same glomeruli (Ressler et al., 1994; Vassar et al., 1994; Mombaerts et al., 1996; Wang et al., 1998). Additionally, neighboring mitral/tufted cells often project their dendrites to the same glomeruli (Shepherd et al., 2004). Additionally, Buonviso et al. (1991) found that injection of tracer so that it was taken up by neighboring mitral cells in the OB resulted in the labeling of patches of fibers within olfactory cortex. Thus, not surprisingly, Zou et al. (2001) found that the axons of mitral/tufted cells that receive input from the same type of OSN converge in patches within olfactory cortex.

These synapses are sensitive to pre-synaptic modulation by a number of receptor mediated systems. Adenosine acting through A1 receptors can decrease electrically evoked responses at this synapse (see chapter 4). Additionally, activation of group III metabotropic glutamate receptors (mGluR's) also causes synaptic depression at this synapse (see chapters 3 & 4).

Post-synaptic responses at this afferent synapse are mediated by both NMDA and non-NMDA ionotropic glutamate receptors (Jung et al., 1990b). Additionally, there is evidence for post-synaptic actions by group I metabotropic receptors (group I mGluR). There is a membrane depolarization, increase in membrane excitabilty, and post-stimulus after-depolarization associated with pharmacological application of group I mGluR agonists (Libri et al., 1997). It is known that group I mGluR activation underlies a late response component following stimulation of layer Ib (Sugitani et al., 2002, 2004) and that activation of group I metabotropic receptors can enhance afferent evoked NMDA mediated responses (Collins, 1993).

The piriform cortex is highly connected internally as well as with other non-olfactory related brain areas via a dense association network that forms synapses in layers Ib, II, and III. Association fibers projecting from non-olfactory cortical areas synapse in layers Ib, II and III; in contrast, ipsilateral and contralateral olfactory cortex intrinsic connections are limited to layers Ib and III (Luskin and Price, 1983a, b).

There is a multitude of different cell types within PCX onto which these synapses are formed. Similar to neocortical regions there are large populations

of both pyramidal type output neurons as well as local GABAergic interneurons (Neville and Haberly, 2004). There are three basic types of pyramidal neurons within PCX. Most superficially located are the semilunar cells whose somata are arranged along layer IIa just deep to layer I (Haberly and Price, 1978b). These neurons have a number of interesting features. Although their apical spiny dendrites and their axonal morphology are similar other PCX pyramidal cells, semilunar cells lack basal dendrites, and, unlike other PCX pyramidal cells, feedback projections from these cells to the OB have not been observed (Haberly and Price, 1978b; Yang et al., 2004). Additionally, their apical dendritic morphology differs from that of other pyramidal cells. Rather than having the classic single superficially directed apical dendrite off of which thin dendrites branch, semilunar neurons can have multiple apical dendrites protruding from their soma (Haberly, 1983). They are also known to be sensitive to decreases in sensory experience as well as deafferentation (Heimer and Kalil, 1978; Wilson et al., 2000).

The next most superficial population of PCX pyramidal cells lie in layer IIb. These pyramidal cells, termed superficial pyramidal cells, have the traditional morphology consisting of a single apical dendrite that extends deep into layer I as well as a number of deeply directed basal dendrites (Haberly, 1983). This is the basic layout for the third class of pyramidal cells, deep pyramidal cells, as well. The basic morphological difference between the two groups is simply the location of the somata of deep pyramidal cells in layer III and the resulting increase in the length of the apical dendritic tree (Haberly, 1983).

The proportion of deep pyramidal cells decreases in deeper portions of layer III; concurrent with this is an increase in the prevalence of a type of glutamatergic spiny non-pyramidal multipolar neuron that is common in the depths of layer III and within the endopiriform nucleus (Tseng and Haberly, 1989a; Hoffman and Haberly, 1993). Multipolar glutamatergic neurons are not common within neocortical systems.

There are diverse populations of GABAergic interneurons within PCX. The most superficial GABAergic cell type is the horizontal cell within layer I. These neurons have very distinctive features. Horozontal cells are relatively large for interneurons, with somata measuring roughly 21 µm in diameter, and their dendrites stretch out across the surface of PCX, parallel to the surface. Their distribution within piriform cortex suggests certain physiological roles in that they are only located in anterior regions of PCX and they are highest in density near the LOT (Haberly and Feig, 1983; Haberly et al., 1987). It has been suggested that horizontal cells may contribute to feedforward inhibitory circuits within PCX. One additional group of interneurons that probably contributes as well are the small multipolar cells that also exist in layer I (Haberly et al., 1987). These small interneurons can be found at highest density deep in layer Ib in pPCX, although they are found throughout the rostrocaudal extent of PCX (Haberly et al., 1987).

Feedforward inhibition has been recorded in pyramidal cells within PCX (Tseng and Haberly, 1988). It is believed to result from a high density of

GABAergic synapses within layer I onto the distal dendrites of pyramidal cells (Haberly et al., 1987; Neville and Haberly, 2004).

A third important group of GABAergic interneurons are the large multipolar cells that are located throughout layer II and III. They are found at highest density in the superficial region of layer II (Haberly et al., 1987). Special attention has been paid to this population because of their similarity to basket cells in other regions of the brain. Their axons are myelinated, branch profusely, and form dense connections with the somata of pyramidal cells and interneurons within PCX (Neville and Haberly, 2004).

Basket synapses have been implicated in a number of physiological processes within PCX. They are believed to underlie the observed feedback inhibitory responses recorded from PCX neurons (Biedenbach and Stevens, 1969; Nemitz and Goldberg, 1983; Satou et al., 1983a; Tseng and Haberly, 1988; Gellman and Aghajanian, 1993). Recordings from interneuorons that may underlie these feedback inhibitory responses show response time courses following LOT stimulation that suggest that they are activated by pyramidal cell collaterals (Satou et al., 1983b). However it should be noted that analysis of the neurons that form these basket synapses revealed that there are a relative large number of overlapping groups based on molecular phenotype and morphology at both the light and electronmicroscopic level (Ekstrand et al., 2001).

The inhibitory synapses found on the distal portion of the apical dendrites of pyramidal cells have been found to have unique properties. It has been observed that they have a sufficiently long time course (IPSP time constant of

decay of 40 ms) to allow them to control NMDA-dependent forms of LTP at glutamatergic synapses targeting these dendritic regions (Kanter et al., 1996; Kapur et al., 1997a; Kapur et al., 1997b). Interestingly, inhibitory synapses with faster kinetics found more proximally on the apical dendrites and on the soma have been implicated in control of pyramidal cell spike frequency. Additionally, a $GABA_B$ receptor-dependent modulatory mechanism exists to alter the magnitude of inhibition upon the distal dendritic regions. This allows learning related plasticity to occur in the dendrites while overall firing output is kept under control (Kanter et al., 1996; Kapur et al., 1997a; Kapur et al., 1997b). This is similar to what is seen in some hippocampal circuits (Pearce, 1993; Pearce et al., 1995).

As stated previously, the PCX is highly interconnected via a complex association fiber system. A portion of this association system originates as non-myelinated collaterals that branch off from the myelinated axons of pyramidal cells. These non-myelinated collaterals form a large number of synapses within the region where they originate with a specifically high density of synapses within layer III (Haberly and Presto, 1986; Johnson et al., 2000). These synapses are Gray's type I synapses with round vesicles and asymmetrical synapses indicating that they are likely excitatory. These local synapses are formed on the basal dendrites of pyramidal cells and on the dendrites of local GABAergic interneurons. It has been suggested that this arrangement with GABAergic interneurons may play a role in feedback inhibitory processes (Haberly and Presto, 1986). Additionally, pyramidal cell axons can be seen to extend branches into a great many olfactory and non-olfactory cortical structures

(Johnson et al., 2000). Interestingly, pyramidal cell projections to distant regions of olfactory cortex make most of their synapses into layer Ib (Haberly and Presto, 1986).

Commissural projections contribute to these associational systems within the olfactory cortex but are not as heavy or widely distributed as ipsilaterally directed association fiber projections (Haberly and Price, 1978a, b). These connections have been shown to allow odorants presented unilaterally, to one nostril, to induce firing of cells in the contralateral aPCX (Wilson, 1997). More specifically, four classes of aPCX neuron spatial receptive fields have been observed that are relatively equally represented: cells that respond only to the ipsilateral stimulation, cells that only respond to contralateral stimulation, cells that respond to stimulation of either naris and cells that respond only to stimulation of both nares (Wilson, 1997, 2001b).

These association systems are organized in a manner very similar to the projections from the OB in that obvious topographic order is minimal or absent. Intracellular labeling of pyramidal cells in piriform cortex suggests that these cells extend their axons to broad areas of both olfactory cortex and neighboring brain regions (Johnson et al., 2000). A single pyramidal cell from pPCX is believed to make a small number of synapses on more than 1000 other neurons (Johnson et al., 2000). This pattern is also seen following extracellular dye labeling of all areas of olfactory cortex except OT; the OT does not seem to contribute to the association fiber system in olfactory cortex (Luskin and Price, 1983b).

Just as OB input synaptic responses can be decreased by a number of neuromodulatory substances (see chapter 4), the association fiber system is also under similar control, although it is responsive to additional substances. For instance, just as $GABA_B$ receptor activation can control transmitter release from inhibitory fibers onto the distal dendrites of pyramidal cells within layer Ia (Kapur et al., 1997a; Kapur et al., 1997b), $GABA_B$ receptor activation also presynaptically depresses excitatory association fiber synapses within layer Ib (Tang and Hasselmo, 1994). Additionally, both norepinephrine and acetylcholine have been observed to decrease association fiber-evoked responses within PCX (Hasselmo and Bower, 1992; Hasselmo et al., 1997; Linster and Hasselmo, 2001). Interestingly, acetylcholine also decreases the effect of inhibitory synapses within layer I. In contrast to activation of $GABA_B$ receptors, acetylcholine decreases inhibition to a greater extent within layer Ib (Patil and Hasselmo, 1999).

Olfactory cortex receives basal forebrain input that includes fibers projecting from cholinergic neurons (Haberly and Price, 1978b; Luskin and Price, 1982; Gaykema et al., 1990). In addition to the previously mentioned effects of acetylcholine within PCX, it also has a number of effects that effectively increase the excitability of pyramidal cell membranes (Constanti and Sim, 1987; Tseng and Haberly, 1989b; Linster and Hasselmo, 2001); much of these effects are believed to be mediated by muscarinic acetylcholine receptors. Interestingly, blockade of muscarinic cholinergic action in aPCX results in increased generalization between odorants. Specifically, scopolamine increases cross-

habituation between stimuli as measured by unit activity, without altering the neurons receptive range (Wilson, 2001a).

Noradrenergic input projects from the LC to the olfactory cortex (Mason and Corcoran, 1979; Fallon and Loughlin, 1982). The projection of norepinephrine fibers to PCX is fairly uniform. One interesting characteristic of this input is the parallel arrangement of norepinephrine-immunoreactive fibers within layer Ia (Shipley and Ennis, 1996). The effects of norepinephrine on PCX are varied. In addition to the aforementioned decrease in layer Ib excitatory synapse efficacy, norepinephrine is likely to play a role in the modulation of pre-synaptic depression of afferent synapses that has been implicated in short-term odor habituation (Best and Wilson, 2004). Additionally, it has been shown to excite inhibitory interneurons in PCX (Gellman and Aghajanian, 1993; Marek and Aghajanian, 1996). Concurrently, LC activation and the subsequent release of norepinephrine into PCX results in at least fifty percent of neurons becoming more responsive to odorant simuli (Bouret and Sara, 2002); this effect may partially result from decreased cortical adaptation (Best and Wilson, 2004).

The effects of $GABA_B$ agonists, acetylcholine and norepinephrine are all implicated in learning. All three modulatory mechanisms increase the relative efficacy of afferent fibers that carry odorant information from the periphery. This is believed to increase the chance of correctly forming NMDA-dependent forms of learning (Hasselmo, 1995; Hasselmo and Barkai, 1995; Hasselmo et al., 1997; Kapur et al., 1997a; Patil et al., 1998; Molyneaux and Hasselmo, 2002; Linster et al., 2003).

Relatively large projections exist from the dorsal raphe nucleus, a major source of serotonergic modulatory fibers, to the entire extent of PCX (De Olmos and Heimer, 1980; Vertes, 1991). Serotonin seems to be involved in excitation of both projection neurons and inhibitory interneurons that synapse onto projection neurons (Sheldon and Aghajanian, 1990, 1991; Gellman and Aghajanian, 1993). Inhibitory neurons may be under consititutive control via serotonin as at least one type of serotonin receptor antagonist can inhibit firing of putative inhibitory interneurons within PCX (Bloms-Funke et al., 1999).

Dopaminergic fibers are present over the entire rostrocaudal extent of PCX, but exist only in the deep portions of rostral PCX (Shipley and Ennis, 1996). Similar to norepinephrine and serotonin, dopamine causes an increase in spontaneous IPSP's in pyramidal cells that is correlated with an increase in the firing frequency of putative inhibitory interneurons in deep layers of PCX (Gellman and Aghajanian, 1993).

Odor Coding in Olfactory Cortex

Little can be inferred from our current anatomical and physiological knowledge of the mammalian olfactory cortex as far as how odorant information is coded. Based on immunohistochemical detection of immediate early genes and radiolabeling with 2-DG, it would seem that the code for any particular odor involves changes in activity within a relatively large number of cells within both aPCX and pPCX (Sharp et al., 1977; Cattarelli et al., 1988; Illig and Haberly, 2003). This is likely because application of odorants results in similar labeling regardless of the relative molecular similarity of the odorants compared; close or

dissimilar (Illig and Haberly, 2003). There was a slight shift in the pattern as concentration was increased but this pattern change was not dependent upon which odorant was tested (Illig and Haberly, 2003).

Within the locust olfactory system, some research has suggested that higher olfactory centers are selectively tuned to spikes from more peripheral olfactory centers that are phase locked to field oscillations (Laurent et al., 1996; Perez-Orive et al., 2002). Specifically, they suggest that rate coding is not particularly important because input that is not at a specific phase of the LFP will be filtered out by the neurons in the central olfactory structure.

Whether this will prove to be the case within mammalian olfactory cortex is unknown. Their proposed requirements for this sort of tuning include a feedforward inhibitory circuit, oscillatory changes in LFP's and afferent projections that are distributed. All of these characteristics exist within mammalian piriform cortex (Neville and Haberly, 2004). It will be interesting to find out if similar processes underlie mammalian cortical odor coding.

aPCX neuron receptive ranges tend to be complex. They generally can be activated by a number of different compounds (Haberly, 1969; Tanabe et al., 1975; Nemitz and Goldberg, 1983; Mccollum et al., 1991; Wilson, 1998a, 2000b, 2001b). Receptive ranges have been mapped across changes in carbon chain length and it appears, based on cross-habituation studies, that the neurons in aPCX have a higher degree of specificity than those in the OB that project to aPCX (Wilson, 2000b).

It appears to take more than ten seconds of odor exposure for aPCX cells to attain this higher level of molecular discrimination. The level of discriminability that aPCX neurons show to novel odors is fairly similar to that seen in olfactory bulb output neurons (Wilson, 2003). This increase in the level of discrimination ability by aPCX neurons to familiarized odors can be prevented by application of the muscarinic acetylcholine receptor antagonist scopolamine (Wilson, 2001a).

A form of perceptual learning may underlie this increase in discrimination ability within aPCX. Wilson and Stevenson (2003) have suggested that this may be mediated by a form of memory for the odorant that develops during familiarization. In essence, they suggest that the final goal of the olfactory system is not to detect each individual feature of an odorant, but instead to categorize groups of features into synthetic percepts against which future odorants can be tested for similarity. This theory of olfactory system function requires a high degree of neural plasticity to be available within the olfactory system. In fact this is the case. Plasticity can be found at all levels of the olfactory system.

LTP can be induced at a large number of synapses within the olfactory system. Synapses formed by ON fibers into the OB have been shown to undergo NMDA-dependent LTP (Ennis et al., 1998). Additionally, LTP can be induced at a number of synapses within the OB (Shepherd et al., 2004). Within piriform cortex, synapses of both the afferent and association system have been demonstrated to undergo NMDA-dependent LTP (Roman et al., 1987; Kanter and Haberly, 1990, 1993; Roman et al., 1993; Truchet et al., 2002). Additionally,

the projections from the olfactory cortex to the OB have also been shown to develop LTP (Patneau and Stripling, 1992; Stripling and Patneau, 1999). The function of much of this induced plasticity is unknown at this time.

Conclusion

Olfactory system functional architecture is defined by the need to make sense of stimuli that are in many ways very different from the sensory stimuli that are coded for by other sensory systems. Within the visual, auditory, and electrosensory systems there is a basic clear quantifiable variation in stimuli across a spectrum; in each case this spectrum is defined by frequency and/or spatial location. Within mechanosensation specifically, but other sensory systems as well, a basic dimension of a stimulus is defined by the somatic location of the receptor. Beneath these basic axes, additional subfeatures are used in each system to define a feature. But, what defines the gradients underlying odor coding? Is it possible that we simple don't yet comprehend the important odor derived dimensions that form maps within the olfactory system? We may come to find that coding in olfactory cortical structures is synthetic to the point that we may have difficulty discerning a map based on the molecular structure of odorants (Wilson and Stevenson, 2003).

Chapter 3

Coordinate Synaptic Mechanisms Contributing to Olfactory Cortical Adaptation

Aaron R. Best and Donald A. Wilson

Department of Zoology, University of Oklahoma, Norman, Oklahoma 73019

Reprinted with permission from the Journal of Neuroscience

Received Sep 15, 2003; revised October 28, 2003; accepted November 13, 2003.

This research was supported by grants from the National Institute on Deafness and Other Communication Disorders and the Oklahoma Center for the Advancement of Science and Technology to D.A.W. We thank Drs. J. Bastian and J. Larson for their help in the development of procedures used in this study and M. Fletcher for mitral/tufted cell data.

Copyright © 2004 Society for Neuroscience 0270-6474/04/240652-09$15.00/0

Abstract

Anterior piriform cortex (aPCX) neurons rapidly filter repetitive odor stimuli despite relatively maintained input from mitral cells. This cortical adaptation is correlated with short-term depression of afferent synapses, *in vivo*. The purpose of this study was to elucidate mechanisms underlying this nonassociative neural plasticity using *in vivo* and *in vitro* preparations and to determine its role in cortical odor adaptation. Lateral olfactory tract (LOT)-evoked responses were recorded in rat aPCX coronal slices. Extracellular and intracellular potentials were recorded before and after simulated odor stimulation of the LOT. Results were compared with *in vivo* intracellular recordings from aPCX layer II/III neurons and field recordings in urethane-anesthetized rats stimulated with odorants. The onset, time course, and extent of LOT synaptic depression during both *in vitro* electrical and *in vivo* odorant stimulation methods were similar. Similar to the odor specificity of cortical odor adaptation *in vivo*, there was no evidence of heterosynaptic depression between independent inputs *in vitro*. *In vitro* evidence suggests at least two mechanisms contribute to this activity-dependent synaptic depression: a rapidly recovering presynaptic depression during the initial 10-20 sec of the post-train recovery period and a longer lasting (~120 sec) depression that can be blocked by the metabotropic glutamate receptor (mGluR) II/III antagonist (*RS*)-α-cyclopropyl-4-phosphonophenylglycine (CPPG) and by the β-adrenergic receptor agonist isoproterenol. Importantly, in line with the *in vitro*

findings, both adaptation of odor responses in the 15-35 Hz spectral range and the associated synaptic depression can also be blocked by intracortical infusion of CPPG *in vivo*.

Introduction

Adaptation to repetitive, biologically non-meaningful stimulation is a critical function of sensory systems, and disorders of adaptation may contribute to some cognitive and psychiatric disorders (Geyer et al., 1990; Yeung-Courchesne and Courchesne, 1997). Although reductions in sensory system responses to stimuli may occur throughout the sensory pathway [e.g., in olfaction: olfactory receptors (Zufall et al., 1991); olfactory second-order neurons (Potter and Chorover, 1976; Mair, 1982); olfactory primary and higher order cortex (Mccollum et al., 1991; Wilson, 1998a)], behavioral adaptation (habituation) is believed to be mediated to a large extent by changes in cortical sensory responsiveness. This cortical adaptation could be mediated either by attenuation in synaptic excitation (i.e., synaptic depression) (Zucker, 1972b; Castellucci and Kandel, 1974; Chung et al., 2002) or enhancement in inhibition (Carandini and Ferster, 1997) of cortical neurons. Despite the importance of cortical adaptation for sensory and cognitive function, however, its mechanisms are not well understood.

In anterior piriform cortex (aPCX), glutamatergic mitral cell axons from within the lateral olfactory tract (LOT) project collaterals into layer Ia, where they synapse onto the distal apical dendrites of aPCX layer II/III neurons. Mitral cells conveying olfactory receptor-specific input terminate in clusters within the aPCX that overlap with input from different olfactory receptors, thus allowing convergence of odorant feature-specific activity on individual aPCX pyramidal neurons (Zou et al., 2001). Intracortical association fibers then significantly

enhance feature convergence and associative properties of the cortex (Johnson et al., 2000; Illig and Haberly, 2003). As in other sensory systems, olfactory cortical neurons undergo cortical adaptation in response to sensory stimulation in both awake (Mccollum et al., 1991) and anesthetized (Wilson, 1998a; Bouret and Sara, 2002) rats. This aPCX adaptation is highly odorant (i.e., input) specific (Wilson, 2000b) and occurs despite relatively maintained mitral cell activity (Wilson, 1998a). Intracellular recordings *in vivo* have shown a depression of LOT-evoked EPSPs coincident with adaptation of odor-evoked responses in aPCX layer II/III neurons (Wilson, 1998b). Potential mechanisms of this depression include modulation of presynaptic glutamate release by presynaptic metabotropic glutamate receptor (mGluR) II/III activation, which has been demonstrated at this synapse (Hasselmo and Bower, 1991), transmitter depletion, or postsynaptic changes comparable with long-term depression.

The present study examined the mechanisms of short-term synaptic depression that may underlie, or contribute to, cortical odor adaptation. Although plasticity at intracortical association synapses and changes upstream or downstream of the cortex may ultimately influence cortical sensory responsivity, the present work takes advantage of the unique, relatively simple cytoarchitecture of the piriform cortex to focus exclusively on changes at afferent synapses. Synaptic depression of cortical afferents induced by odorant stimulation *in vivo* was modeled using an *in vitro* aPCX slice preparation. Physiological and pharmacological characteristics of the novel train-evoked *in vitro* findings were subsequently compared with odor-evoked cortical adaptation *in vivo*. As a

measure of odor-induced cortical activity, fast cortical oscillations in the β range were analyzed (Boudreau and Freeman, 1963; Bressler and Freeman, 1980). β frequency oscillation was chosen because it is likely the result of a mechanism distinct from other fast aPCX oscillations (Kay and Freeman, 1998; Neville and Haberly, 2003) and has been shown to display adaptation under appropriate conditions (Doheny et al., 2000). The results suggest that *in vivo* cortical odor adaptation can be closely modeled with an *in vitro* stimulation paradigm inducing afferent synaptic depression and that both cortical afferent synaptic depression and cortical odor adaptation are subject to mGluR II/III modulation.

Methods

In vivo recording-

Male Long-Evans hooded rats (150-500 gm), obtained from Harlan (Indianapolis, IN), were anesthetized with urethane (1.5 gm/kg, i.p.) and placed in a stereotaxic apparatus. Additional urethane was administered as required. Respiration was monitored with a piezoelectric device (World Precision Instruments, Sarasota, FL) strapped to the chest. All experiments were done in accordance with the University of Oklahoma Animal Care and Use Committee.

In vivo *intracellular recording.* Intracellular recordings were made from layer II/III aPCX neurons with glass microelectrodes filled with 2 M potassium acetate (tip resistance 50-150 MΩ) as described previously (Wilson, 1998a, b). Layer II/III neurons were identified by the ability to evoke short-latency EPSPs with electrical stimulation of the LOT and by the characteristic shape of field potentials recorded extracellularly before or after cell penetration. Cells included for analyses had resting membrane potentials of at least -60 mV (mean = -73.7 ± 1.9 mV) and action potentials of at least 60 mV amplitude (mean = 78.1 ± 3.1 mV). Recordings were digitized at 5 kHz.

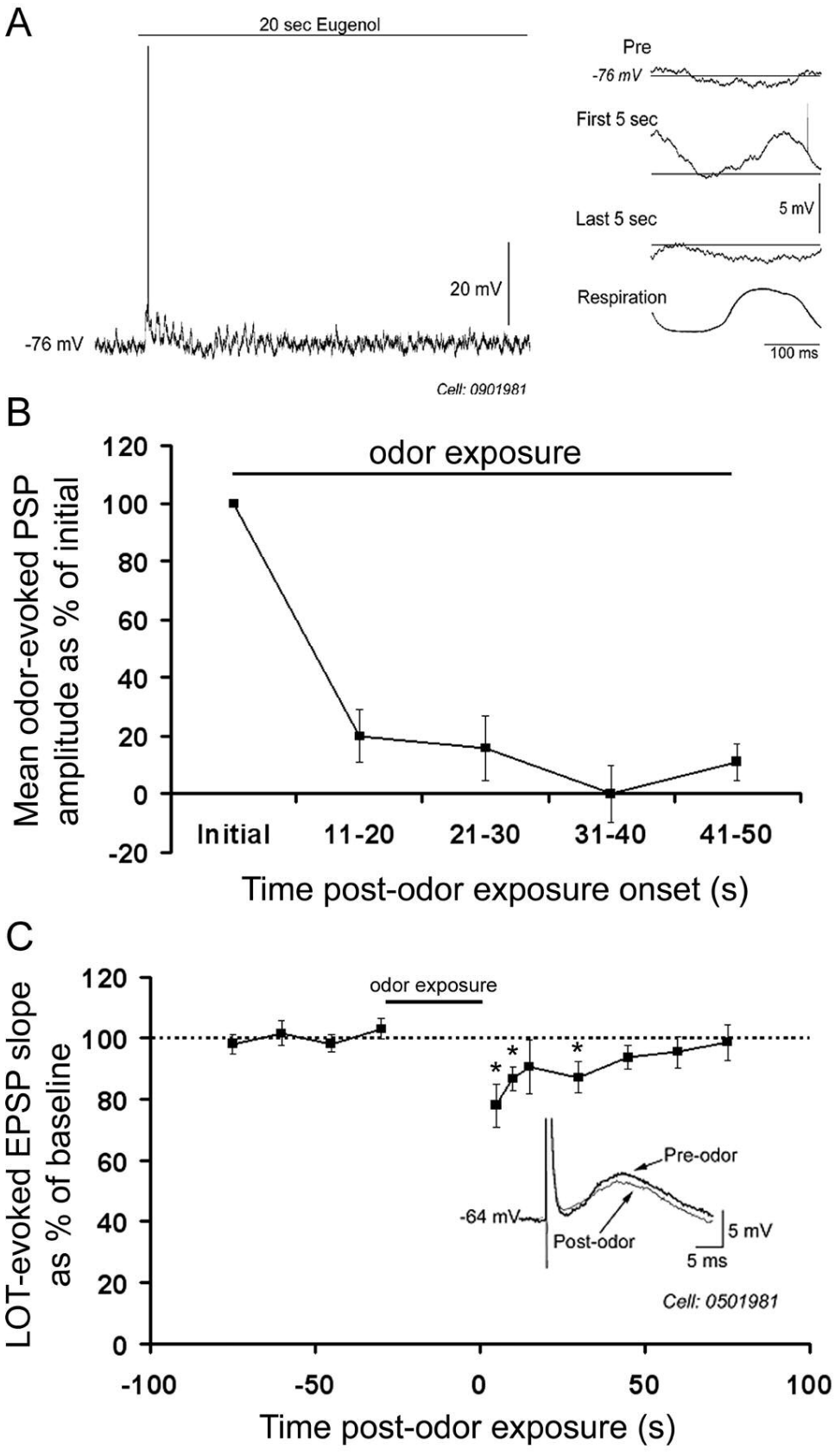
Test odor stimulation consisted of 2 sec stimuli from a flow-dilution olfactometer at a flow rate of 1 l/min and stimulus concentrations of ~1:10 of saturated vapor. Odorants included isoamyl acetate, anisole, eugenol (all from

Sigma, St. Louis, MO), and peppermint (McCormick, Hunt Valley, MD). Stimulus onset was triggered by the transition from inhalation to exhalation. Additional details of stimulus control are provided in Wilson (1998a).

To determine the effect of prolonged odor exposure on LOT-evoked EPSPs, paired-pulse stimuli [30 msec interpulse interval (IPI), 5 sec interpair interval] were applied for at least 60 sec before and after a 50 sec odor exposure. Only one 50 sec odor presentation was given per animal. Initial EPSP slope (millivolts per second) was determined from averaged responses to both the conditioning and the test pulses. Responses to the conditioning pulse were used to confirm an odor adaptation-induced synaptic depression, whereas the ratio of test response to conditioning response was used to determine the effect of odor adaptation on paired-pulse facilitation (PPF). The odor-evoked response was quantified by examining the amplitude of respiration-entrained postsynaptic potentials (PSPs) (Wilson, 1998a). Adaptation of the odor-evoked response was quantified by comparing the amplitude of these respiration-entrained odor-evoked PSPs at 10 sec intervals during the 50 sec stimulus with the initial, peak response (see Fig. 2).

Figure 2. Piriform cortex adaptation to 50 sec odor exposure, *in vivo*. *A*, Example of an *in vivo*, intracellular recording from a layer II/III aPCX neuron during a 50 sec exposure to the odorant eugenol. The first 20 sec of the odorant stimulus are shown on the left. In this cell, eugenol evoked a single spike followed by several

seconds of respiration entrained subthreshold oscillations. On the right, membrane potential averages triggered off the respiratory cycle are shown (inhalation is up in the respiration trace). Note the large respiration-linked odor-evoked depolarization during the first 5 sec of odor stimulation, which is mostly absent by the end of the 50 sec stimulus. The horizontal line in these traces corresponds to the -76 mV resting membrane potential. *B*, Mean respiration-entrained PSP peak-to-peak amplitude before and during 50 sec odorant exposure (n = 15 cells). Note the rapid decrease in response magnitude over the first 20 sec. *C*, LOT-evoked, intracellularly recorded EPSPs were monitored before and after 50 sec of odorant stimulation (*A, B*). EPSP initial slope was significantly depressed immediately after odor offset (asterisk signifies significantly different from baseline, $p < 0.05$) and recovered within 100 sec (n = 15 cells). Inset shows a representative example of *in vivo*, intracellularly recorded, LOT-evoked EPSPs before and after 50 sec of odor stimulation. Bars indicate duration of odor exposure.


A
20 sec Eugenol
20 mV
-76 mV
Cell: 0901981
Pre
-76 mV
First 5 sec
5 mV
Last 5 sec
Respiration
100 ms
B
odor exposure
Mean odor-evoked PSP amplitude as % of initial
120
100
80
60
40
20
0
-20
Initial
11-20
21-30
31-40
41-50
Time post-odor exposure onset (s)
C
odor exposure
LOT-evoked EPSP slope as % of baseline
120
100
80
60
40
20
0
-100
-50
0
50
100
Pre-odor
-64 mV
Post-odor
5 mV
5 ms
Cell: 0501981
Time post-odor exposure (s)

In vivo *field recording and drug infusion.* For electrode/cannula placement, burr holes were drilled in the skull dorsal to the intended site of placement. Burr hole position was guided by known cranial landmarks. Field EPSPs (fEPSPs) were recorded with tungsten microelectrodes (A-M Systems, Carlsborg, WA) directed to layer Ia in aPCX via physiological markers. A similar tungsten microelectrode was placed in the LOT anterior to the aPCX to activate the mitral cell synapses within aPCX. Electrode placement was confirmed histologically in 40 µ cresyl violet-stained sections. For drug infusion, a 26 gauge blunt-tip syringe needle (Hamilton) was attached to the recording electrode using cyanoacrylate (ND Industries, Troy, MI). The cannula was staggered back ~250 µ to prevent damage to the recording site. Infusion was done at 0.15 µl/min for 20 min. The tissue was allowed to rest for 5 min after infusion before recording. The infusate consisted of a 0.01% solution of fast green dye (for visual confirmation of infusion), artificial CSF as prepared for the *in vitro* studies, and dissolved (*RS*)-α-cyclopropyl-4-phosphonophenylglycine (CPPG) (2.5 mM) where appropriate. A 2.5 mM concentration of CPPG was chosen to ensure that mGluR II/IIIs were blocked in as large a portion of aPCX as possible.

LOT stimulation current was adjusted to produce a 300 µV negative deflection in the A1 wave as recorded in aPCX. The A1 wave is associated with activation of synapses in layer Ia wherein LOT fibers synapse (Haberly, 1998). Although other sources of adaptation are within the olfactory system, we specifically focused on this initial EPSP in aPCX associated with activation of

layer Ia synapses. Odor stimuli were from a flow-dilution olfactometer at a flow rate of 1 liter/min and stimulus concentrations of ~1:2 of saturated vapor. An odorant mixture was used to maximize the number of active LOT fibers synapsing within aPCX. The odorant mixture consisted of isoamyl acetate, ethyl butyrate (both from Sigma), lemon, and peppermint (both from McCormick, Hunt Valley, MD). The high odor concentration, odor mixture, and low LOT stimulation level were used to maximize the possibility of observing changes in the LOT shock-evoked field potential after stimulation with 50 sec of odor.

Tests for the effects of 50 sec of odor stimulation on subsequent odor responses were accomplished with a 2 sec odor test pulse given 50 sec after the cessation of a 50 sec conditioning odor presentation. Only one 50 sec odor presentation was given per animal. Oscillatory local field potential activity (sampled at 10 KHz) was quantified for β band activity (15-35 Hz) using fast Fourier transforms (FFTs) with a hanning window with 2048 bins (Spike2; Cambridge Electronic Design, Cambridge, UK). For baseline odor responsiveness, a 2 sec window was taken at the beginning of the 50 sec habituating odor presentation. The window was begun at the point within 0.8 sec after odor initiation at which the cortex began responding. A 2 sec window of non-odor background activity taken before the 50 sec odor onset was then processed and subtracted from the FFT for the odor response. This was done to normalize the level of odor-evoked oscillation against the background activity. The 2 sec test odor given 50 sec after the cessation of the habituating odor was then analyzed in the same way. To calculate the level of adaptation of odor responses,

the normalized odor-evoked oscillatory power from the habituating odor was subtracted from the test stimulus value.

In these same animals, to test for changes in the LOT shock-evoked response after the 50 sec habituating odor presentation, LOT shock-evoked responses measured immediately before the 2 sec test odor were compared with 90 sec of LOT shock-evoked responses given at 0.1 Hz before the 50 sec conditioning odor presentation. Quantification of the LOT shock-evoked waveform consisted of measuring the initial slope of the A1 component.

***In vitro* recording-**

Male Long-Evans hooded rats were maintained with food and water *ad libitum* on a 12 hr light/dark cycle. After anesthetization with isoflurane (Abbott Laboratories, North Chicago, IL), rats were decapitated, and the brains were dissected and blocked in ice-cold, oxygenated ACSF containing (in mM): 124 NaCl, 5 KCl, 1.24 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 26 $NaHCO_3$, and 10 glucose, and 400 µm coronal sections were taken through the aPCX using a Pelco 1000 Plus Vibratome (Redding, CA). Slices were allowed to incubate for at least 1 hr in an interface chamber before recording. All recordings were done in an interface chamber except for the pharmacological experiments in which the tissue was submerged. All recordings were done at room temperature in ACSF bubbled with 95% O_2/5% CO_2. In all pharmacological experiments, superfusion occurred at a rate of 0.75 ml/min.

Bipolar stainless steel (0.28 mm Teflon coated; A-M Systems) stimulating electrodes were placed on the LOT. fEPSP's were recorded with tungsten microelectrodes (A-M Systems) from the superficial portion of layer Ia in aPCX. For intracellular recordings, microelectrode pipettes were filled with 2 M potassium acetate. EPSPs were recorded from layer II/III aPCX neurons. Intracellular recordings were used only if the resting membrane potential of the cell was at least -60 mV (mean = -68.6 ± 2.6 mV). In one cell, membrane potential was held with 0.1 nA of hyperpolarizing current. Additionally, cells were not used if overshooting action potentials could not be elicited. Slices were allowed to rest at least 5 min between test runs.

Test runs consisted of a baseline period of 1.5-5 min of test shock stimuli (0.1 Hz) followed by 10-50 sec of train stimuli. Within any test run, test and train stimuli were given at the same stimulus intensity. Stimulus intensity where not stated explicitly was set at 4x fEPSP threshold. Threshold was determined from averages of five test stimuli. Test/train stimulus amplitude ranged from 15 to 48 µA. Parametric tests done consecutively within slices were in random order.

In vitro pharmacology. After initial baseline testing of train-induced cortical adaptation, a second run was given after application of drug that was followed by washout (>10 min) and a final recovery test run. This was the protocol used for the *in vitro* pharmacological experiments throughout this study.

The mGluR III agonist L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) and the mGluR II/III antagonist CPPG were obtained from Tocris Cookson (Ellisville,

MO). Isoproterenol and all other chemicals were obtained from Sigma (St. Louis, MO). Isoproterenol solutions included equimolor ascorbic acid. Concentrations for all compounds were selected on the basis of published reports [L-AP4 (Pekhletski et al., 1996); CPPG (Harrison and Jahr, 2003); isoproterenol (Kawaguchi and Shindou, 1998)].

Because there was no immediately obvious change in baseline responses after bath application of CPPG, wash-in time and concentration were determined in separate experiments in which the antagonist was used to block the effects of bath-applied L-AP4 (100 µM) (data not shown).

In vitro *data analysis.* Amplified signals (DAM50 and Intra 767; World Precision Instruments) were collected at 10 KHz, digitized and analyzed with Spike2 software (Cambridge Electronic Design). Slope measurements were taken from the falling (fEPSP) or the rising (EPSP) phase of the shock-evoked responses that correspond to monosynaptic currents (Ketchum and Haberly, 1993). *In vitro* recordings were digitally low-pass filtered (extracellular recordings at 500 Hz; intracellular recordings at 50 Hz).

Comparison of control baseline versus drug baseline consisted of paired *t* tests. All other analyses consisted of one-way or two-way ANOVAs followed by Fisher's *post hoc* tests where appropriate. Normalization of data was used for comparison between treatments *in vitro*. Normalization consisted of averaging of the data points before the train, dividing all responses to test stimuli data by this number, and multiplying each by 100.

Results

In vivo cortical adaptation to odors-

Data were obtained from 15 cells responsive to at least one odorant recorded from 12 animals. Figure 2 shows a typical odorant-evoked response and its rapid adaptation during a prolonged stimulus. Odorant-evoked subthreshold PSPs displayed adaptation, with mean respiration-entrained odorant-evoked PSPs substantially reduced within 10-20 sec and nearly completely eliminated by the end of the 50 sec stimulus (Fig. 1). Associated with this cortical adaptation to the odorant stimulus was a depression of LOT-evoked monosynaptic EPSPs (Fig. 1*C*) (50 sec of odor stimulation resulted in a significant decrease of LOT-evoked EPSPs (n = 15; $F_{(10,140)}$ = 2.24; p < 0.05). *Post hoc* tests revealed significant decreases post-odor. As reported previously (Wilson, 1998b), this odorant stimulation-induced synaptic depression recovered within ~80 sec after the end of the habituating stimulus.

No significant effect of odor-cortical adaptation was detected on LOT PPF, *in vivo* (PPF pre-odor exposure = 129.4 ± 8.4% vs PPF 10-15 sec post-odor exposure = 125.6 ± 15.8%; NS; data not shown). This may reflect a lack of sensitivity in this *in vivo* measurement, however, because odor stimulation (and thus cortical adaptation) affects only a small subset of the LOT synapses activated by LOT

electrical stimulation. Thus, these experiments were repeated *in vitro*, using electrical stimulation of the LOT as the stimulus to induce cortical adaptation.

LOT stimulation *in vitro* can produce cortical adaptation-

We used an *in vitro* preparation combined with electrical stimulation of the LOT to simulate olfactory cortical adaptation at the mitral cell to aPCX neuron synapse. Electrical stimulation of the LOT consisted of 80 msec, 100 Hz trains repeated at 2 Hz, which roughly although conservatively mimics mitral cell odor-evoked activity. These parameters were determined from analyses of a subset of mitral cell single-unit data collected from urethane-anesthetized rats by (Fletcher and Wilson, 2003). In these mitral cells, odor-evoked spiking occurred in phase with respiration, which occurred at 2 Hz. Within each respiration-entrained burst, 70% of spiking occurred with interspike intervals of between 10 and 30 msec (i.e., 33-100 Hz). The total mean number of spikes occurring during a 50 sec odor stimulus in this data set was 1246 ± 173 (SEM) (n = 6 mitral cells), whereas the total number of shocks delivered during our electrical LOT stimulation paradigm was 800 stimuli. As shown in Figure 3, *A* and *B*, 50 sec of electrical LOT stimulation, simulating odor-evoked afferent input, resulted in a significant decrease of LOT-evoked fEPSPs (n = 27; $F_{(17,468)}$ = 37.71; p < 0.0001). Within-train responses recorded intracellularly from layer II/III aPCX neurons show early onset of the depression, similar to odor-evoked cortical adaptation. The magnitude of the depression of both the *in vivo* odor-evoked PSPs and the *in vitro* shock-evoked EPSPs recorded intracellularly from layer II/III aPCX neurons

was similar (Figs. 1B,2C). As noted above, the magnitude of the odor exposure-induced depression of LOT-evoked EPSPs is less than the magnitude of depression in the other paradigms, but most likely reflects the small subset of LOT axons activated by a particular odor (see below). Additionally, the time course for recovery of both the *in vitro* cortical adaptation and the *in vivo* cortical adaptation appeared similar, with depression recovering in all three paradigms within 60-100 sec, although again *in vivo* LOT-evoked EPSPs appeared to recover faster than the other two paradigms (Figs. 1C,2C,3) (Wilson, 1998b). This slower recovery rate of the *in vitro* depression may be attributable in part to the difference in temperatures of the *in vivo* and *in vitro* (room temperature, 25°C) preparations.

Figure 3. *In vitro*, 50 sec of simulated odor experience (train stimulation as described in Materials and Methods) results in aPCX synaptic depression. *A*, Representative example of an individual *in vitro* test run with extracellular fEPSPs before and after train stimulation. *B*, Average fEPSP data for 27 slices showing aPCX cortical adaptation. Asterisk signifies significant difference from baseline ($p < 0.05$). *C*, Within-train development of cortical adaptation recorded intracellularly in layer II/III aPCX neurons *in vitro* ($n = 7$ cells). Waveforms are examples of intracellular responses to the first LOT train stimulus and after 10 and 50 sec of trains repeated at 0.5 Hz. Bars indicate duration of trains stimulation.

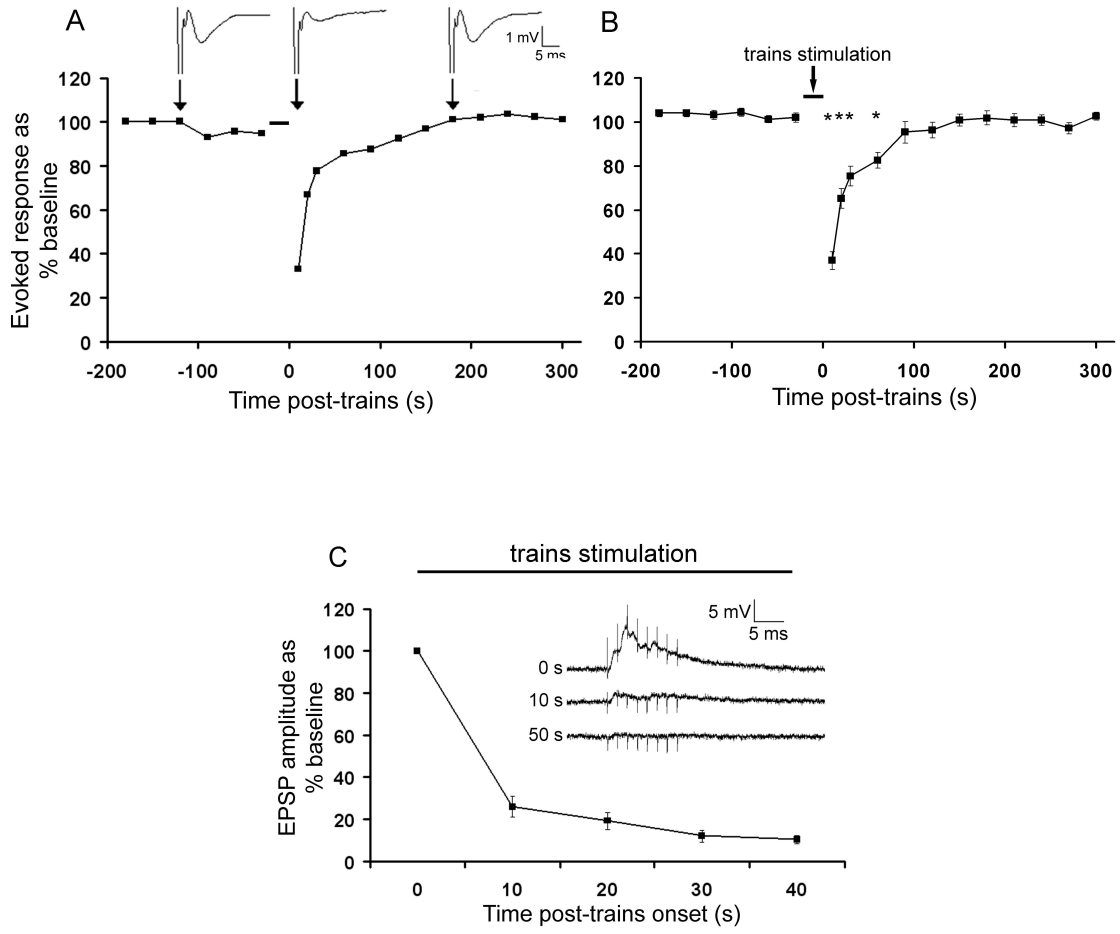
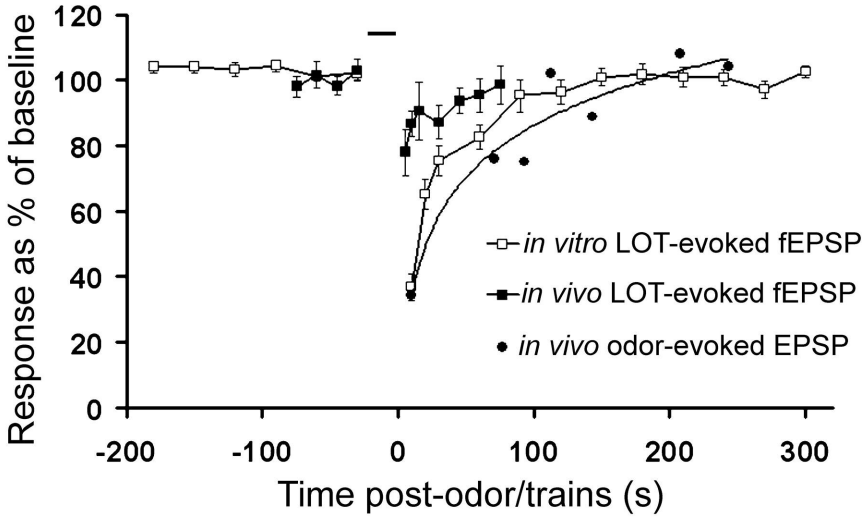


Figure 4. Overlay of *in vivo* odor exposure-induced LOT synaptic depression (connected filled squares; data from Fig. 1*C*), *in vitro* train-induced LOT synaptic depression (connected open squares; data from Fig. 2*B*), and *in vivo* odor exposure-induced odor-evoked PSP depression (filled circles and trend line; adapted from Wilson, 1998b) showing similarity of recovery time course for each paradigm. Both the time course and magnitude of *in vitro* train-induced depression and *in vivo* odor-evoked depression were similar (see text). The *in vivo* LOT-evoked EPSPs did not depress to the same extent as in the other two paradigms, presumably because of the fact that only a small subset of electrically

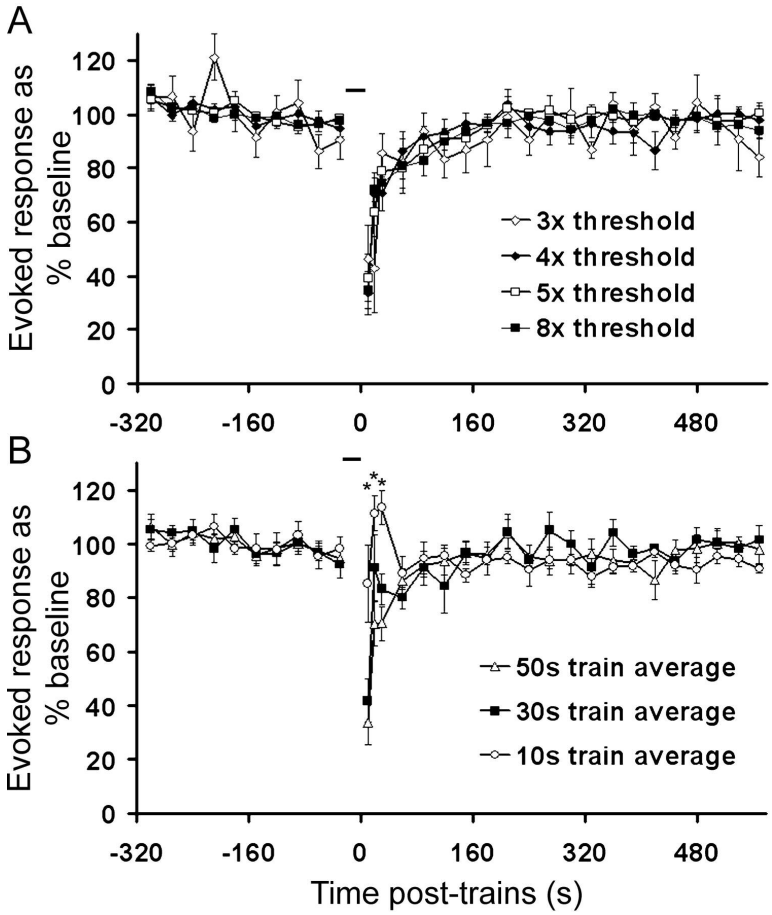
activated LOT synapses would have been activated by the habituating odor exposure. Bar indicates duration of odor exposure or trains stimulation.



Post-train depression is stimulus intensity independent *in vitro*-

Increasing LOT electrical stimulus intensity increases the number of synchronously activated afferent fibers. To determine whether cortical adaptation was modulated by the number of active afferent synapses for induction, thus potentially involving an associative mechanism, test pulse and train stimulus intensity were varied. Stimulus intensity for test runs was manipulated by multiplying threshold values (as determined in Materials and Methods) by a factor of 3, 4, 5, or 8. Test pulse and train intensity were equal. Altering the stimulus intensity (and thus the number of active afferent fibers) did not alter the magnitude or time course of recovery over the first minutes post-train (n = 6; $F_{(3,60)}$ = 0.01; NS) (Fig.5 *A*).

Figure 5. *In vitro* synaptic depression is not dependent on stimulus intensity but is dependent on stimulus duration. *A*, Magnitude and time course of recovery of *in vitro* cortical adaptation are independent of afferent stimulus intensity. Synaptic depression during the first minutes post-train does not differ between groups after 50 sec of train stimulation at varying stimulus intensities (n = 6 slices per intensity). *B*, Varying the duration of train stimulation significantly altered the magnitude of synaptic depression during the first minutes post-train between groups, with 10 sec insufficient to induce depression (n = 6 slices per duration). Asterisks signify significant differences between the 10 sec duration group and all other groups ($p < 0.05$). Bars indicate duration of trains stimulation.



Post-train depression is dependent on the duration of the conditioning train *in vitro*-

To examine the time course of development of the post-train depression, the duration of the conditioning train was varied. Train durations of 10, 30, and 50 sec revealed that post-train depression was dependent on train duration. Trains of 10 sec failed to produce significant depression, whereas 30 and 50 sec trains evoked a similar magnitude and duration of post-train depression (n = 6; main effect of train duration, $F_{(2,45)}$ = 19.96; p < 0.0001) (Fig. 5*B*). *Post hoc* tests revealed significant differences between post-train depression after a 10 sec train versus that for 30 and 50 sec trains during the first 30 sec of the recovery period.

Post-train depression is homosynaptic *in vitro*-

Adaptation to odors is highly odorant specific, with minimal cross-adaptation between odors (Wilson, 2000b), and exposure to an odor to which a cortical neuron does not respond produces no depression of the LOT-evoked EPSP in that cell (Wilson, 1998b). To further test the synaptic specificity of the *in vitro* adaptation, intracellular recording from aPCX layer II/III neurons was combined with stimulation with two separate bipolar electrodes on non-overlapping portions of the LOT. The fEPSP recorded in layer Ia corresponds to the activation of the apical dendrites of layer II/III aPCX neurons via mitral cell synapses (Haberly, 1998). Because this synaptic depression was found to be stimulus intensity independent, current was varied to produce EPSPs that were

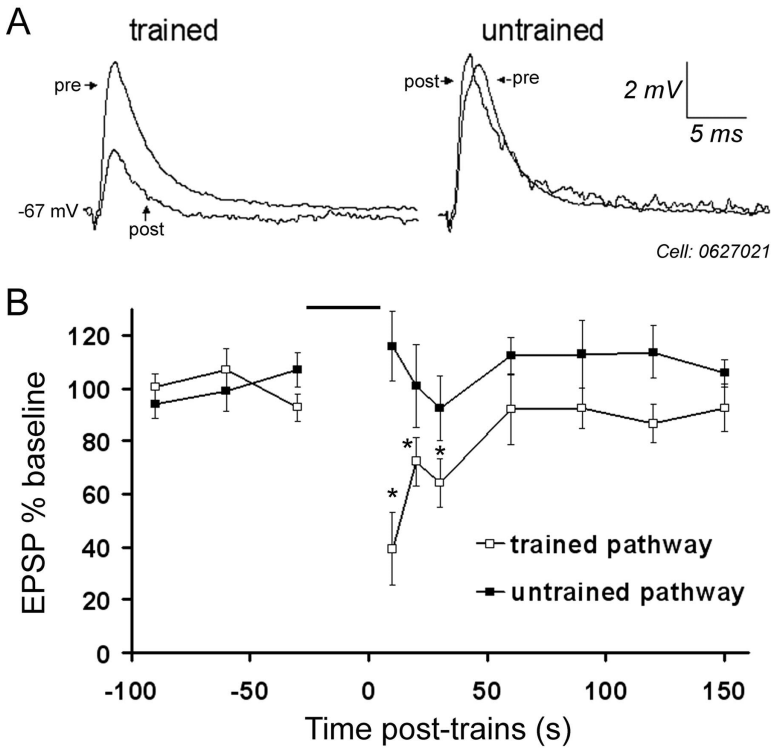
subthreshold for spiking. It was confirmed that each electrode was firing a separate population of synapses with analysis of paired stimuli. In short, it was first verified that both pathways expressed PPF at a 30 msec IPI. It was then confirmed that a conditioning stimulus given to one pathway did not result in facilitation of responses evoked by the alternate pathway (data not shown).

Once pathway separation was verified, test stimuli were alternated between pathways (0.1 Hz). A conditioning train was then given to one pathway followed by continued alternating test stimuli. The alternating stimuli were begun after the train in the conditioned and unconditioned pathway in a counterbalanced manner across slices.

Trains produced homosynaptic post-train depression in intracellularly recorded aPCX layer II/III neurons similar to that recorded in extracellular fEPSPs (Fig. 5). Conditioning trains given to LOT fibers exciting a layer II/III aPCX neuron did not affect unconditioned synapses onto the same neuron (n = 8; main effect of pathway, $F_{(1,70)}$ = 20.319; p < 0.0001) (Fig. 6). *Post hoc* tests revealed a significant difference between the baseline and post-train shock-evoked field responses in the trained pathway, with no change in the untrained pathway.

Figure 6. *In vitro* synaptic depression is homosynaptic. LOT-evoked, intracellularly recorded EPSPs were monitored before and after a 50 sec train stimulation to the trained pathway, whereas the untrained pathway was left

unstimulated. *A*, Intracellular EPSPs recorded after test stimuli given to the trained and untrained pathways in a single cell before and within the first 30 sec after the train protocol. *B*, Train-induced depression was selective to the stimulated pathway (*n* = 8 cells). An asterisk signifies significant difference from baseline ($p < 0.05$). Bar indicates duration of trains stimulation.

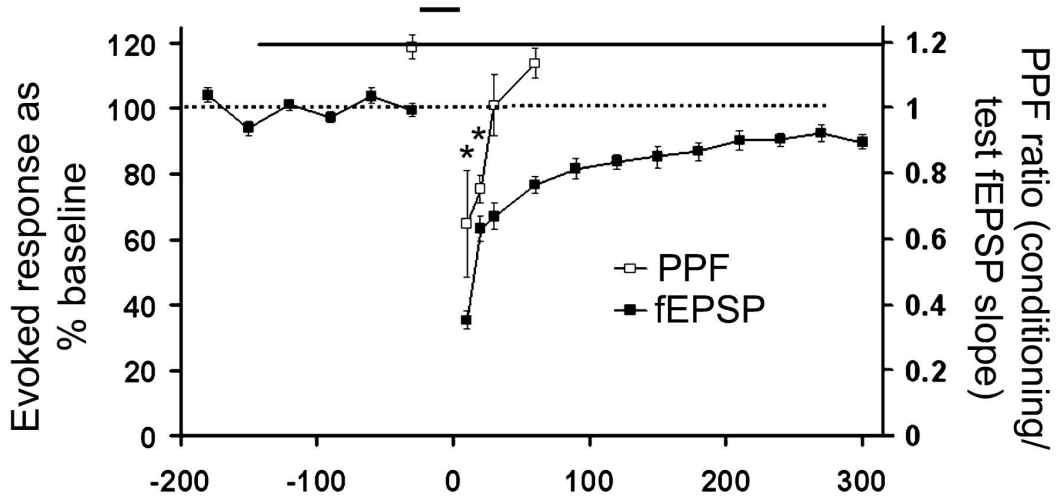


Paired-pulse analysis reveals the possibility of at least two components to the synaptic depression-

It is commonly interpreted that changes in PPF can result from vesicle depletion (Zucker and Regehr, 2002). To test whether post-train depression is

associated with changes in PPF and thus possibly vesicle depletion, five paired test stimuli (30 msec IPI) delivered before the conditioning train were averaged and compared with six paired test stimuli (0.1 Hz) given post-train. PPF was observed during the pre-train baseline period. PPF was reversed to paired-pulse depression for up to 20 sec post-train (n = 10; main effect of train, $F_{(4,36)}$ = 9.385; p < 0.0001) (Fig. 7). *Post hoc* tests revealed a significant decrease in the paired-pulse ratio immediately after the train. PPF returned to pre-train levels within the first 30 sec post-train. Importantly, this time course of recovery is much faster than the latency to full recovery from the train-induced depression and thus suggests a two-part mechanism.

Figure 7. *In vitro* PPF was significantly decreased immediately post-train but returned to baseline before recovery of cortical adaptation (n = 9 slices). Asterisks signify significant differences in PPF from baseline (p < 0.05). Horizontal lines represent baseline levels for PPF (top line) and fEPSP (bottom line). Bar indicates duration of trains stimulation.

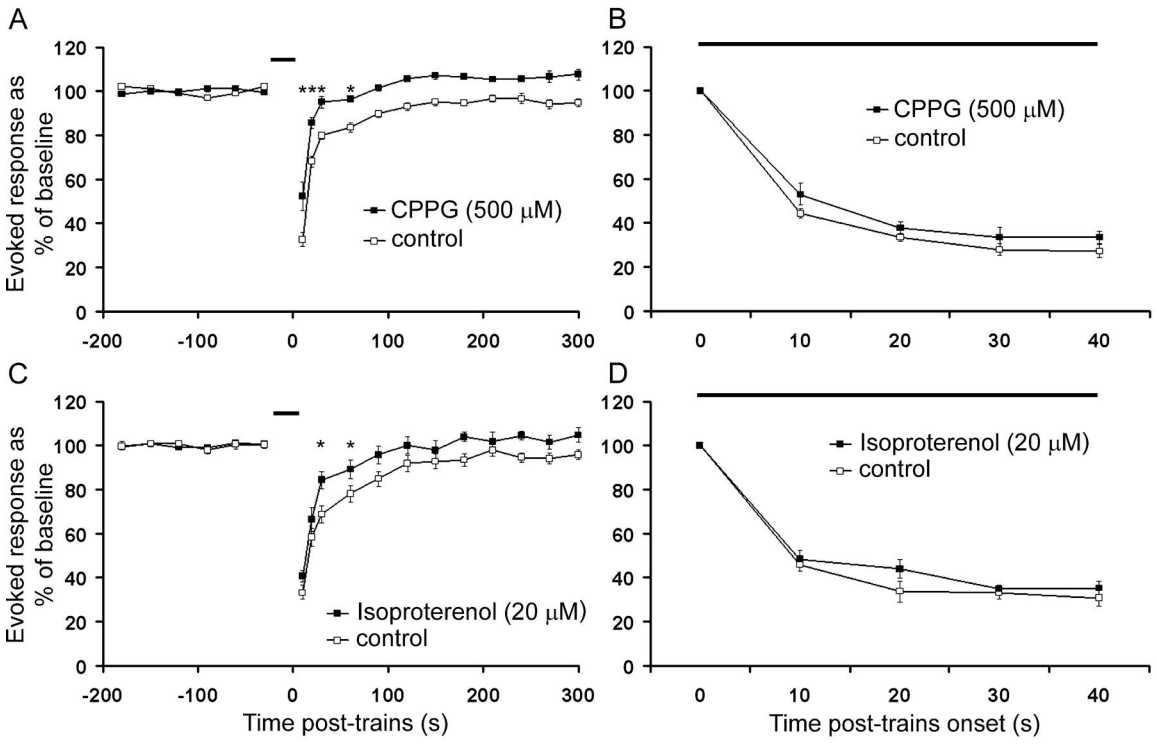


mGluR II/III activation mediates a late component of olfactory cortical adaptation *in vitro*-

mGluR IIIs are known to be located on the presynaptic boutons of mitral cell axons within aPCX layer Ia (Kinzie et al., 1995; Wada et al., 1998) and are known to decrease excitatory transmission at this synapse within aPCX slices (Hasselmo and Bower, 1991) and at mitral/tufted cell synapses in culture (Trombley and Westbrook, 1992), presumably by reducing transmitter release. mGluR IIs are also located at the mitral cell to aPCX synapse (Wada et al., 1998) and have been shown to function in a manner similar to mGluR IIIs at mitral cell synapses in culture (Schoppa and Westbrook, 1997). Given their location and the functional consequences of their activation, it is possible that mGluR II/IIIs may modulate or mediate a portion of olfactory cortical adaptation.

In line with previous literature, the mGluR III agonist L-AP4 (100 µM) decreased mean LOT-evoked fEPSP slope (n = 9; paired t tests, $t_{(8)}$ = -8.03; p < 0.0001). Bath application of the mGluR II/III antagonist CPPG (500 µM) significantly accelerated recovery from synaptic depression during the first min post-train (500 µM; n = 8; $F_{(1,42)}$ = 27.27; p < 0.001) (Fig. 8*A*). *Post hoc* tests revealed significant differences from control during the first min post-train. Application of CPPG at this concentration blocks both mGluR II and III receptors. Thus, the mGluR II/III antagonist CPPG selectively disrupts the late phase of synaptic depression.

Figure 8. Blockade of mGluR II/III receptors with CPPG or activation of noradrenergic β-receptors reduces train-induced depression. *A*, CPPG application (*n* = 9 slices) had no effect on baseline response slope but resulted in a significant reduction in post-train depression, with fEPSPs returning to baseline within 30 sec post-train. Asterisks signify significant difference between drug conditions ($p < 0.05$). *B*, CPPG had no effect on within-train response magnitude. *C*, Activation of noradrenergic β-receptors with isoproterenol *in vitro* had no effect on baseline fEPSP response slope but significantly reduced post-train synaptic depression (*n* = 9 slices). Asterisks signify significant difference between drug conditions ($p < 0.05$). *D*, Isoproterenol had no effect on within-train response magnitude. These data suggest that the onset and early phase of cortical adaptation rely on a different mechanism than the later phases, which are blocked by both CPPG and isoproterenol. Bars indicate duration of trains stimulation.



Although CPPG (500 μM) was able to block a large portion of the post-train depression, it had no significant effect on within-train depression (Fig. 8*B*), similar to its small but statistically significant effect on the early phase of post-train depression (Fig. 8*A*). Additionally, when PPF stimulation was combined with CPPG (500 μM) application, there was no alteration in PPF recovery time course from control, suggesting that the alteration in PPF and the group II/III mGluR-mediated effects were separate phenomena (n = 4 slices; repeated measures ANOVA; NS). CPPG (500 μM) application did not affect baseline PPF (n = 4 slices; paired t test, $t_{(3)}$ = -2.75; NS). These data suggest that the onset and early phases of recovery of *in vitro* cortical adaptation are mediated by a different mechanism than the late phase, which is blocked by CPPG.

β-adrenergic activation modulates a late component of olfactory cortical adaptation *in vitro*-

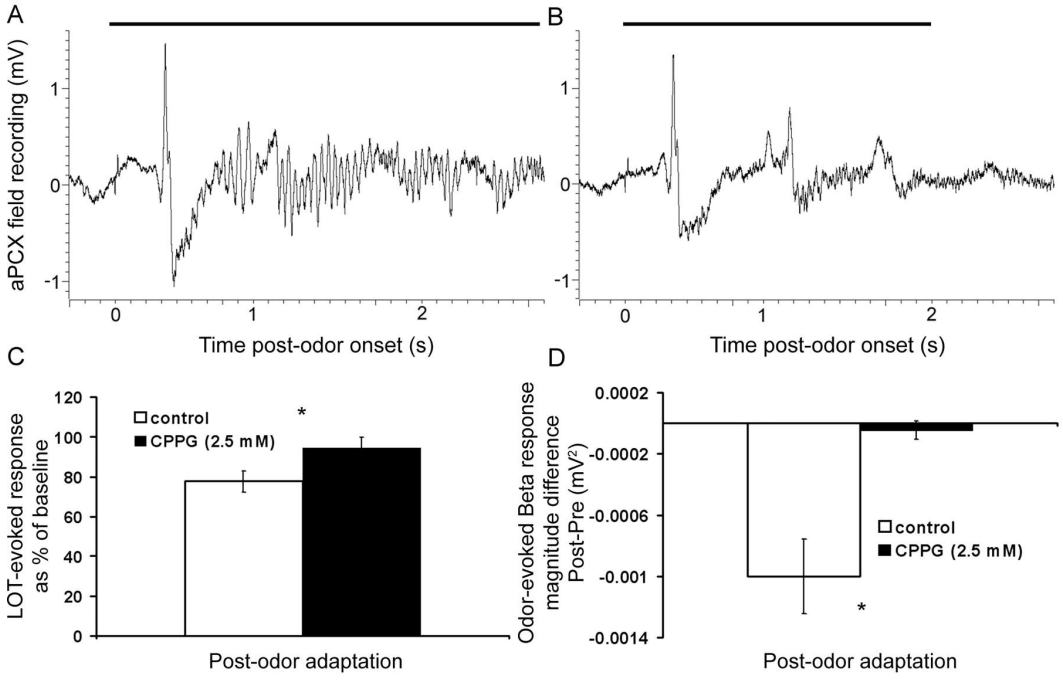
Noradrenergic inputs to the piriform cortex originate in the locus coeruleus (LC); activity in this structure is regulated in response to novelty and arousal (Foote et al., 1980). β-adrenergic receptors have been demonstrated in layer I and II within aPCX (Shipley and Ennis, 1996), and norepinephrine (NE) can modulate afferent input to the piriform cortex *in vitro*, although to a lesser extent than the effect of norepinephrine on association fiber synapses (Hasselmo et al., 1997). NE release and LC activity are known to increase during arousal, vigilance, and exposure to novelty (Vankov et al., 1995) and also increase both mitral/tufted cell responses to olfactory nerve input and aPCX responses to odors (Jiang et al., 1996). We thus examined whether NE modulators alter cortical adaptation. No significant effect of the β-receptor agonist isoproterenol (20 μM) was observed on baseline responses; however, as shown in Figure 8*C*, isoproterenol significantly reduced post-train depression, most notably during the later phase (n = 9; $F_{(1,48)}$ = 5.74; p < 0.05) (Fig. 8*B*). *Post hoc* tests revealed significant differences between control and isoproterenol treatments post-train.

Similar to the effects of CPPG, isoproterenol, which significantly facilitated recovery from synaptic depression, had no significant effect on depression onset during the train stimulation (Fig. 8*D*), again suggesting distinct mechanisms of early and late phases of this synaptic depression.

mGluR II/III modulation of both aPCX odor response adaptation and the associated synaptic depression, *in vivo*-

If the mGluR II/III-mediated depression of afferent synapses described *in vitro* is involved in cortical adaptation to odors, then adaptation of cortical odor responses should be reduced by CPPG *in vivo*. To test whether *in vivo* odor-evoked cortical adaptation and the associated synaptic depression can be affected by blockade of mGluR II/IIIs, ACSF or ACSF plus CPPG (2.5 mM) was infused into aPCX. Cortical odor responses were monitored during drug infusion using local field potential recordings of odor-evoked β (15-35 Hz) oscillations subtracted from pre-odor β activity (Boudreau and Freeman, 1963; Bressler and Freeman, 1980). The odor used was a high concentration mixture to maximize the number of afferent synapses involved, as described in Materials and Methods. In control ACSF-only animals, 2 sec odor stimulation evoked a strong increase in β oscillation, which was reduced by >70% in responses to 2 sec test pulses after a prolonged 50 sec odor exposure (Fig. 9). This depression of odor-evoked β responses was associated with a significant depression of LOT-evoked fEPSPs measured 50 sec after the end of the odor exposure (Fig. 9*C*). The magnitude of the LOT-evoked fEPSP depression was not as large as that of the odor-evoked β activity, presumably because of the population of LOT synapses not activated by the odor, as described above.

Figure 9. Blockade of mGluR II/III receptors with CPPG *in vivo* decreases the extent of post-odor synaptic depression and adaptation of odor responses. *A*, Example of an odor response recorded in aPCX layer during the initial portion of the 50 sec conditioning odor. *B*, Response to the 2 sec test odor given 50 sec after the cessation of the conditioning odor in the control animal shown in *A*. *C*, LOT shock-evoked response slopes in aPCX were depressed after 50 sec of odor exposure. CPPG infusion significantly decreased the extent of synaptic depression. Asterisks signify significant difference between drug conditions ($p < 0.05$). *D*, Fifty seconds of odor presentation resulted in adaptation to a 2 sec odor given 50 sec after the conditioning odor. CPPG infusion significantly decreased the extent of odor adaptation. Asterisks signify significant difference between drug conditions ($p < 0.05$). Bars indicate duration of odor exposure.



Infusion of CPPG (2.5 mM) did not produce a detectable change in baseline (non-odor evoked) β frequency power within a 2 sec window before odor stimulation (n = 7; unpaired t test, $t_{(12)}$ = 0.614; NS) or in initial odor-evoked β frequency power (n = 7; unpaired t test, $t_{(12)}$ = -2.06; NS); however, similar to the effect of CPPG on train-induced synaptic depression *in vitro*, CPPG (2.5 mM) infusion *in vivo* significantly decreased the extent of odor-evoked depression of LOT-evoked fEPSPs induced by the prolonged 50 sec odor exposure (n = 7; unpaired t test, $t_{(12)}$ = 2.23; $p < 0.05$) (Fig. 9*C*). Importantly, CPPG (2.5 mM) infusion also significantly decreased the extent of odor exposure-induced adaptation of odor-evoked β frequency responses (n = 7; unpaired t test, $t_{(12)}$ = 2.20; $p < 0.05$) (Fig. 9*D*).

Discussion

In vitro synaptic depression resembles *in vivo* cortical adaptation-

Although behavioral responsiveness to odors is ultimately dependent on a myriad of factors including receptor adaptation and synaptic state in the OB, piriform cortex, and other regions, we hypothesize that the synaptic depression described here may contribute significantly to both olfactory cortical adaptation and behavioral odor habituation. The ability to disrupt *in vitro* and *in vivo* synaptic depression and cortical odor response adaptation with CPPG strongly suggests a similar underlying mechanism for these phenomena. In addition, the homosynaptic nature of synaptic depression described here may contribute to the odor specificity of cortical olfactory adaptation and behavioral olfactory habituation (Wilson, 2000b; Fletcher and Wilson, 2002).

Mechanisms of *in vitro* synaptic depression-

Our results suggest that at least two mechanisms act in tandem to produce depression of afferent synapses in aPCX. PPF occurs under control conditions with interpulse intervals of 30 msec. PPF shifts to paired-pulse depression immediately post-train but recovers to facilitation more quickly (~20 sec) than the post-train synaptic depression (~100 sec). One possible interpretation of the shift to paired-pulse depression is transmitter vesicle depletion (Zucker and Regehr, 2002), and the observed recovery time is similar

to that reported for replenishment of a readily releasable pool of synaptic vesicles at central synapses in other systems (Stevens and Tsujimoto, 1995; Dobrunz and Stevens, 1997). Thus, it seems possible that the early phase of cortical adaptation may result from a rapidly recovering vesicle depletion that emerges during the prolonged train stimulation, although additional analyses are required to test this possibility.

It is likely that activation of mGluR II/IIIs located on the presynaptic boutons of mitral cells contributes to a separate, longer lasting component of olfactory cortical adaptation. Presynaptic mGluRs can function as autoreceptors that reduce presynaptic Ca^{2+} influx and thus glutamate release when activated by glutamate (Anwyl, 1999). The mGluR II/III antagonist CPPG was able to block primarily that portion of the synaptic depression that cannot be attributed to vesicle depletion on the basis of the recovery time course of PPF (Figs. 7,8A). Furthermore, CPPG did not alter the time course of PPF recovery after train stimulation (data not shown). Finally, CPPG was not able to block the depression observed within trains (Fig. 8*B*). Together, these data suggest that at least two mechanisms contribute to olfactory cortical adaptation: a rapidly recovering synaptic vesicle depletion and a more slowly recovering activation of presynaptic group II/III metabotropic glutamate receptors. Both mechanisms appear to require at least 10 sec of stimulation to emerge, similar to the emergence of cortical adaptation to odorants.

It should be noted that mGluR receptors also modulate intracortical association fiber synapses in the piriform cortex (Hasselmo and Bower, 1990), which could also influence cortical oscillations beyond the effects of afferent synaptic depression described here. Association fiber synapses are capable of synaptic plasticity (Kanter and Haberly, 1990), and future work will be needed to explore the role of these synapses in cortical adaptation.

Protein kinase activation is able to inhibit mGluR II/III activity in a number of systems (Schaffhauser et al., 2000; Cai et al., 2001). Activation of the stimulatory G-protein (G_s) coupled β-adrenergic receptor has been found to mimic this effect, and activation is believed to work through similar mechanisms (Cai et al., 2001). Interestingly, β-adrenergic receptor activation was also found to decrease the extent of cortical adaptation. It is possible that this represents a mechanism for control of cortical adaptation and for behavioral state regulation of cortical responsiveness (Bouret and Sara, 2002). One putative mechanism for this action could be direct inhibition of mGluR II/III activity via phosphorylation of the receptor (Cai et al., 2001), although further studies will be required to identify specific molecular mechanisms.

Relationship to other forms of olfactory cortical plasticity-

Brief, tetanic stimulation of the LOT can produce, under some circumstances, long-term potentiation (LTP) of afferent synapses in aPCX (Roman et al., 1987; Jung et al., 1990a; Kanter and Haberly, 1990), although LTP of these cortical afferents is more difficult to induce than LTP of association

fiber synapses within the aPCX. In the present *in vitro* paradigm, there was no evidence for LTP of LOT-evoked potentials induced by the prolonged afferent stimulation protocol. Furthermore, depression did not emerge until after at least 10 sec of the train stimulation protocol, similar to the emergence of odor-evoked adaptation, and thus more commonly used brief stimulation paradigms should not have observed the depression that we describe here. Although what we observed could be the result of classically described NMDA-dependent long-term depression (Bear and Malenka, 1994) given the presence of NMDA receptors at these synapses (Shipley and Ennis, 1996), it seems unlikely given the rapid recovery time of this synaptic depression. In fact, the extent of *in vitro* synaptic depression was not stimulus intensity dependent, suggesting that the number of coactive fibers did not contribute to the depression in some associative or potentially NMDA receptor-dependent manner, although NMDA dependence has not been examined directly.

At present, it is not clear what the effects of this stimulation protocol would have on association fiber synapses, although it is predicted, given past *in vitro* findings, that LTP or potentiation in general may be a more likely outcome than adaptation. Work in both thalamocortical systems (Crair and Malenka, 1995; Beierlein and Connors, 2002) and piriform cortex (Hasselmo and Bower, 1990; Stripling and Patneau, 1999; Haberly, 2001; Best and Wilson, 2003) suggests differential rules and developmental periods for plasticity between afferent and association fiber systems. We are currently investigating this question.

Noradrenergic modulation of cortical adaptation-

Norepinephrine from the nucleus locus coeruleus has been shown to regulate both sensory responses (Foote et al., 1980; Waterhouse et al., 1998) and synaptic plasticity (Pettigrew and Kasamatsu, 1978) in many brain regions. In the olfactory system, norepinephrine modulates behavioral odor responsiveness (Gray et al., 1986), OB lateral inhibition (Jahr and Nicoll, 1982a; Trombley and Shepherd, 1992), mitral cell responsiveness to olfactory nerve input (Jiang et al., 1996) and odor stimuli (Gervais and Pager, 1983), and piriform cortex single-unit responses to odors (Bouret and Sara, 2002), as well as behavioral and neural olfactory system plasticity (Gray et al., 1986; Sullivan et al., 1989; Brennan and Keverne, 1997). The present results suggest that norepinephrine may also modulate aPCX cortical adaptation via β-adrenergic receptors. Maintenance of cortical responses in the presence of norepinephrine (i.e., during arousal or in response to novel stimuli) by reducing adaptation may facilitate synaptic plasticity believed to be required for cortical odor memory, as discussed above (Wilson and Stevenson, 2003).

Summary

We present a form of short-term synaptic depression of an identified cortical afferent synapse that may contribute to olfactory cortical adaptation. Furthermore, we present evidence of a multicomponent mechanism of that synaptic depression involving potential transmitter depletion and presynaptic autoreceptor activation. This synaptic depression is homosynaptic and thus could

contribute to the odor specificity of aPCX cortical adaptation. Furthermore, the synaptic depression can be modulated by norepinephrine, suggesting a potential synaptic basis of state-dependent sensory processing in the olfactory system. Finally, blockade of this synaptic depression prevents adaptation of odor-evoked cortical responses. Future work will attempt to directly test the role of these mechanisms in behavioral habituation to odors. Understanding cortical adaptation at the mitral cell-piriform cortex synapse may elucidate mechanisms that underlie cortical sensory adaptation generally. Additional insight may also be gained into subcortical sensory relays, because mGluR activation and vesicle depletion have been associated with adaptation within these systems as well.

Chapter 4

Further elaboration on the mechanisms of LOT pre-synaptic depression

Introduction

The following chapter contains additional data that were deemed extraneous by the reviewers at Journal of Neuroscience or collected subsequently and therefore I will present it here. The data verify that the LOT-to-aPCX synapse undergoes synaptic depression in the presence of A1 adenosine receptor agonists. Subsequently, I tested the ability of DPCPX, an antagonist at A1 adenosine receptors, to block train-induced synaptic depression. I also present data that shows that activation of group III, but not by group II, mGluR's by L-AP4 at this synapse also causes synaptic depression.

Methods

The methods for this work are the same as for chapter 3. All data are shown in both normalized and non-normalized forms including CPPG data that were previously shown in chapter 3.

Results

A1 adenosine receptor activation decreases transmission at the LOT-aPCX neuron synapse

A1 adenosine receptors are known to be located on the pre-synaptic boutons of mitral cell axons within layer Ia (Scholfield and Steel, 1988) and are known to decrease excitatory transmission at this synapse (Kuroda et al., 1976; Scholfield, 1978; Motley and Collins, 1983).

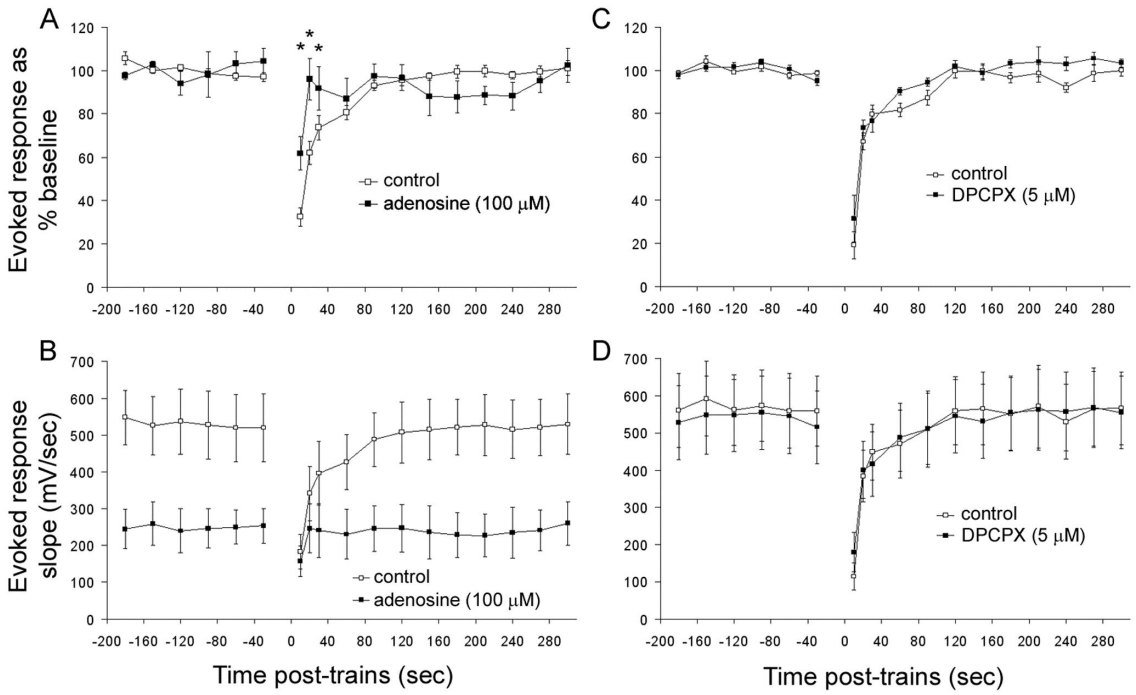
In order to determine a role for A1 adenosine receptor activation during olfactory cortical adaptation, adenosine (100 µM) was bath applied to slices using a within slice, repeated measures design. Following initial baseline testing of train-induced cortical adaptation, a second run was given during activation of the adenosine receptors. This was followed by washout (> 10 min) and a final recovery test run. This is the protocol used for the pharmacological experiments within this chapter.

Adenosine (100 µM) bath application ($n = 6$, $t(5) = -6.87$, $p = 0.001$) decreased baseline response values. Additionally, application of adenosine ($n = 6$, $F(1,30) = 8.18$, $p < 0.05$; Fig. 10A is normalized & 10B is non-normalized) resulted in shorter recovery time as measured by comparing normalized data. Post-hoc tests revealed significant differences within the first 30 sec of recovery. Thus, activation of adenosine receptors is not additive with the *in vitro* cortical adaptation mechanism. Adenosine receptor activation may, therefore, mask this cortical adaptation, which could suggest a possible common mechanism.

***In vitro* olfactory cortical adaptation does not result from accumulation of adenosine**

In order to test whether A1 adenosine receptors mediate or modulate this cortical adaptation, the A1 adenosine receptor antagonist DPCPX (5 µM) was bath applied. Application of DPCPX (5 µM) resulted in no significant change in recovery during the first minute post-train ($n = 7$, $F(1,36) = 4.76$, $p < 0.05$, post-hoc analysis revealed no significant pair wise differences between means; Fig. 10C is normalized & 10D is non-normalized).

Figure 10



Group III metabotropic glutamate receptor (mGluR) activation decreases transmission at the LOT-aPCX neuron synapse

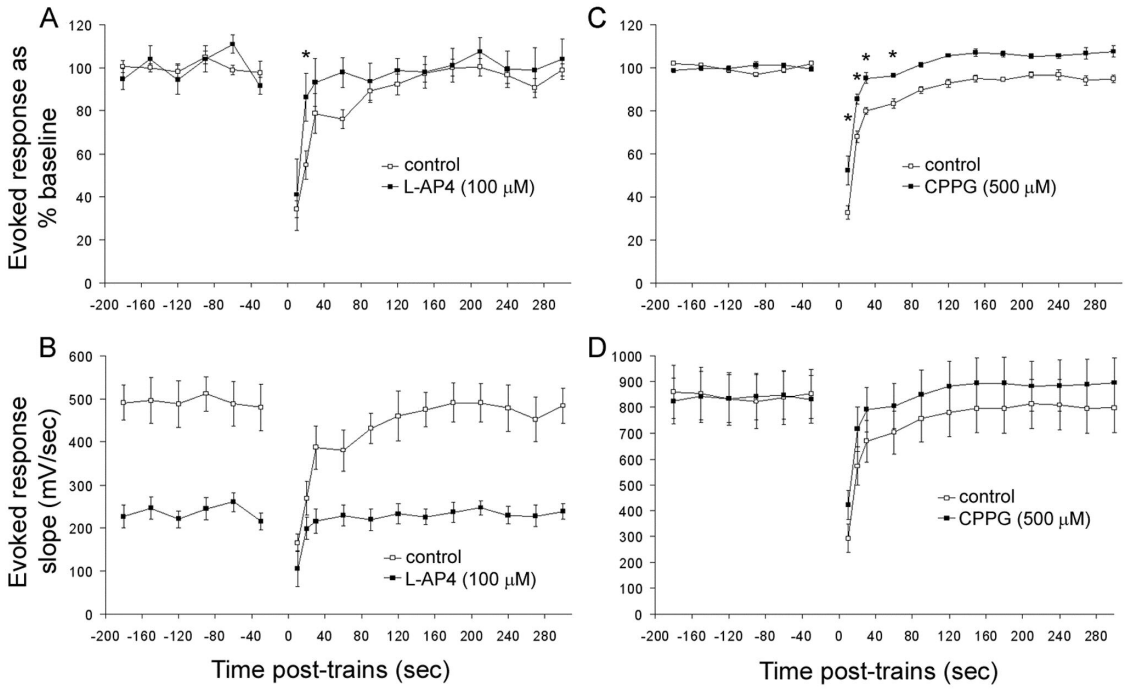
Group III mGluR's are known to be located on the pre-synaptic boutons of mitral cell axons within aPCX layer Ia (Kinzie et al., 1995; Kinzie et al., 1997; Saugstad et al., 1997; Wada et al., 1998) and are known to decrease excitatory transmission at this synapse within slice preparations of aPCX (Hasselmo and Bower, 1991), and at mitral/tufted cell synapses in culture (Trombley and Westbrook, 1992). Given their location and the functional consequences of their

activation, it is possible that group III mGluR's may modulate or mediate one part of olfactory cortical adaptation.

The group III mGluR agonist L-AP4 decreased mean LOT-evoked fEPSP slope ($n = 9$, paired t-tests, $t(8) = -8.03$, $p < 0.0001$). Additionally, application of L-AP4 during the train ($n = 9$, $F(1,48) = 5.76$, $p < 0.05$; Fig. 11A is normalized & 11B is non-normalized) resulted in a shorter recovery time as measured by comparing normalized data. Post-hoc tests revealed significant differences within the first 30 sec of recovery.

The effect of group III mGluR activation is not additive with that of the cortical adaptation mechanism. Thus, it is possible that group III mGluR activation results in masking of the cortical adaptation, which could suggest a possible common mechanism. As previously stated, blockade of group II/III mGluR activation results in blockade of a significant portion of the train-induced synaptic depression (see chapter 3 for statistical analysis); the present data are given for comparison of normalized and non-normalized results (Fig. 11C is normalized & 11D is non-normalized).

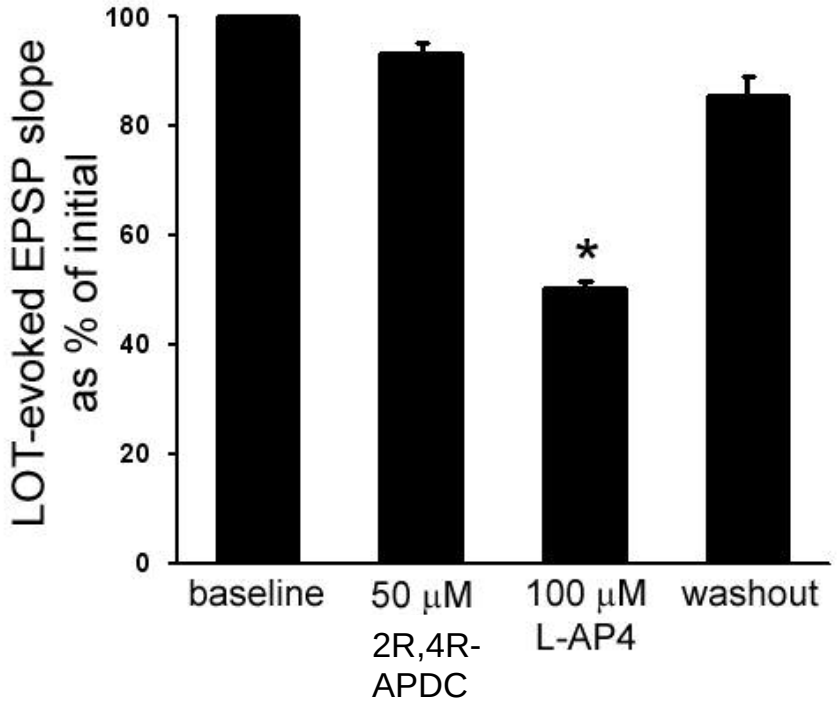
Figure11



Group III mGluR activation, but not Group II mGluR activation, depresses the the LOT to aPCX synapse in this strain of *Rattus norvegicus*

Blockade of mGluR II/III receptors with CPPG reduces train-induced depression. But it is not clear if group II mGluR receptors are active at this synapse. ACPD application (n = 8 slices) had no effect on baseline fEPSP response slope (Fig. 12). As has been shown previously, L-AP4 application significantly reduced fEPSP response slope. Confirmation of L-AP4 receptor (group III mGluR) activity was tested in 6 of the 8 slices tested for 2R,4R-APDC activity. Asterisks signify a significant difference from baseline condition ($p < 0.05$) (Fig. 12).

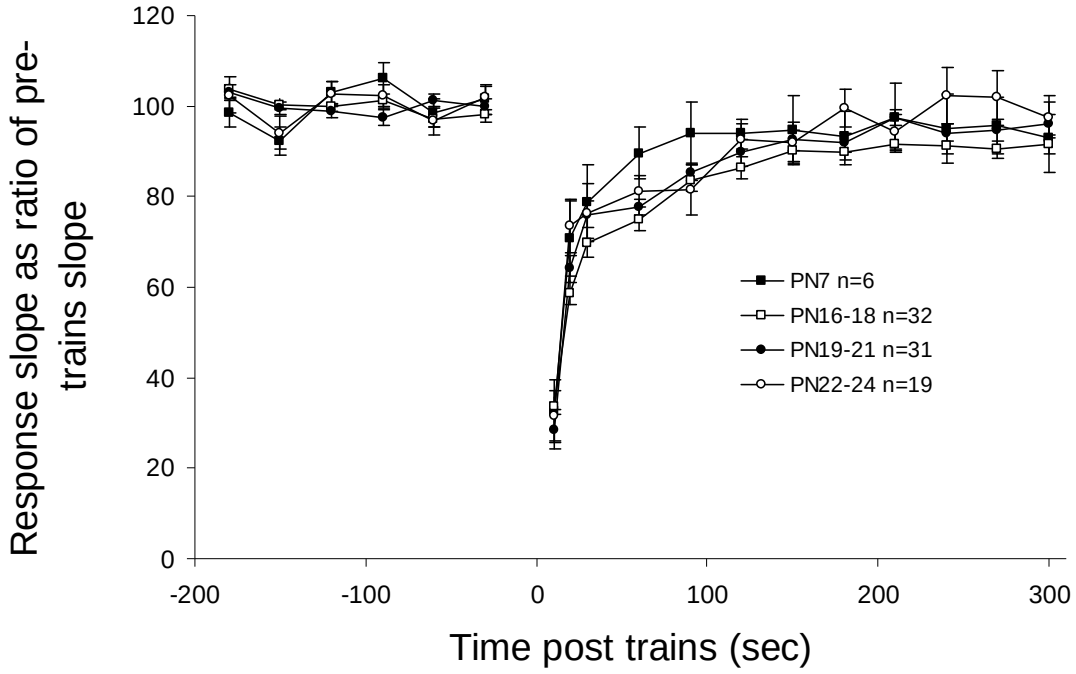
Figure 12



Train-induced depression of the LOT to aPCX synapse may be developmentally stable by post-natal day 7

Slices were taken from rats on post-natal day 7 and were stimulated with the same train paradigm as used in chapter 3. Data collected were compared to data taken previously at three different ages. Statistics were done as in chapter 3. No statistical difference was found between the four age groups over the first minute of recovery (n= 6, 32, 31, 19 in ascending order by age; repeated measures ANOVA; F(3,252) = 2.06; N.S.; Fig. 13).

Figure 13



Discussion

Based on these data, it would seem that, in line with the literature, both L-AP4 and adenosine acting on group III mGluR's and adenosine A1 receptors respectively can produce synaptic depression at this synapse. Although one report has observed group II mGluR-mediated synaptic depression in guinea pigs at this synapse, we did not observe this using 2R,4R-APDC (Libri et al., 1997); this is in line with the weak immunolabeling of group II mGluR's in rats at this synapse (Wada et al., 1998).

Based on data presented here and in chapter 3 it appears that the train-induced synaptic depression that I have been studying is likely mediated in large

part by the activation of group III mGluR's. In contrast, blockade of activation of A1 adenosine receptors has no effect on this train-induced depression. Given that adenosine is produced as a metabolic byproduct, it will be interesting to see what functions this mechanism of synaptic depression performs (Zucker and Regehr, 2002).

Based on previous behavioral data using odor-evoked heart rate orienting response and a cross habituation paradigm, it is likely that whatever mechanism is responsible for habituation of the odor-evoked heart rate orienting response is mature early in post-natal development (Fletcher and Wilson, 2001). In fact, as seen in figure 13, train-induced depression of the LOT-aPCX synapse is developmentally stable by at least PN7.

Chapter 5

Synaptic mechanism of habituation of a simple olfactory behavior

Introduction

Defining the circuits that are involved in production of specific behaviors is an ultimate goal of neuroscience. The knowledge of what processes must occur for an organism to sense and respond to stimuli within its environment allows us to better understand ourselves and better equip ourselves to treat neuropathology. The path to this goal has led to the development of a number of very fruitful invertebrate behavioral models (Kandel and Schwartz, 1982; Christoffersen, 1997). But, this goal has been more challenging in the mammalian central nervous system. Here we present data indicating that the likely site for mediation of habituation of a simple olfactory mediated behavior can be traced to synaptic depression of a defined synapse with a defined pharmacological basis.

Olfactory sensation in mammals begins within the nose at olfactory sensory neurons (OSN's) in the olfactory epithelia. Binding of an odorant to an OSN results in release of glutamate by the receptor neuron onto the dendrites of mitral/tufted cells in the olfactory bulb. The axons of mitral/tufted cells form the lateral olfactory tract (LOT) which projects caudally to olfactory cortical structures including the anterior piriform cortex (aPCX). When activated, mitral/tufted cells release glutamate at the LOT-aPCX synapse.

We have previously shown that repeated activation of this synapse *in vitro* in a manner conservatively similar to odor-elicited firing results in a synaptic depression very similar in recovery time course and magnitude to odor-induced adaptation at this synapse (Best and Wilson, 2004); subsequently we found that

this synaptic depression requires group II/III mGluR activation for full expression. Group II/III mGluR's are very specifically expressed in a manner consistent with a role as presynaptic autoreceptors at this synapse; although group II expression seems to be limited if present in the rat (Wada et al., 1998). Consistent with this, although we have observed L-AP4-mediated synaptic depression as a result of mGluR III activation at this synapse, as others have (Hasselmo and Bower, 1991; Trombley and Westbrook, 1992); we do not observe (2R,4R)-APDC-mediated synaptic depression as a result of mGluR II activation at this synapse, as has been observed in guinea-pig (unpublished observations; Libri et al., 1997). This may reflect species differences. Given that there is no known functional difference between these two types of receptors at this synapse, I will refer to them simply as mGluR autoreceptors.

Previously, we tested the effects of blockade of mGluR autoreceptors in aPCX *in vivo* during prolonged (50 sec) odor presentation. Prolonged (50 sec) odor exposure results in decreased responsiveness to the odor presented and an associated synaptic depression measured by shock stimulation of the LOT (Wilson, 1998a, b, 2000a). This adaptation to odor stimuli in aPCX occurs more quickly and with greater odor specificity than odor adaptation in more peripheral olfactory structures (Potter and Chorover, 1976; Wilson, 2000b). mGluR autoreceptor blockade significantly decreased both odor-evoked adaptation and the associated synaptic depression (Best and Wilson, 2004). Given that more central olfactory structures adapt to odors more quickly and recover more slowly

than more peripheral structures, adaptation within aPCX may play a significant role in habituation of odor responses at the behavioral level.

Initial presentation of an odor to a rat results in bradycardia, a behavior termed the odor-induced heart-rate orienting response. The heart-rate orienting response can be habituated during repeated presentation of an odor. It is believed to arise as follows. Odor information travels from the olfactory bulb and/or olfactory cortex to the central nucleus of the amygdala (Davis, 2000). The central nucleus is connected to the dorsal motor nucleus of the vagus which has the ability to induce bradykadia (Davis, 2000). It is believed that activation of this pathway induces the heart-rate orienting response.

Habituation of the heart-rate orienting response is relatively odor specific, suggesting that the aPCX and its increased discrimination abilities relative to the olfactory periphery are required for the production of this behavior (Fletcher and Wilson, 2002). Thus, we tested the ability of mGluR autoreceptor blockade in aPCX to diminish habituation of the heart rate orienting response as measured by Fletcher and Wilson (2002) Infusion of CPPG (2.5 mM) bilaterally into aPCX as in Best and Wilson (2004) dramatically and significantly decreased the extent of habituation of the heart-rate orienting response ($n = 11$; one-tailed paired t-test $t(10) = -2.77$; $p < 0.001$; Fig. 13). Histological location of canulae is shown in figure 14. Thus, we suggest that not only does the circuit for the heart-rate orienting response require aPCX, but that habituation of this response is mediated in large part by activation of presynaptic mGluR autoreceptors at the input stage of aPCX.

Figure 13

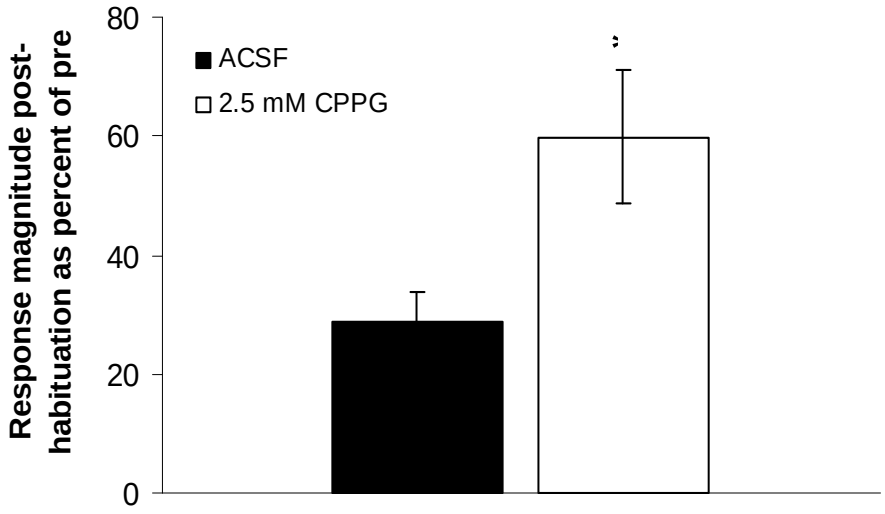
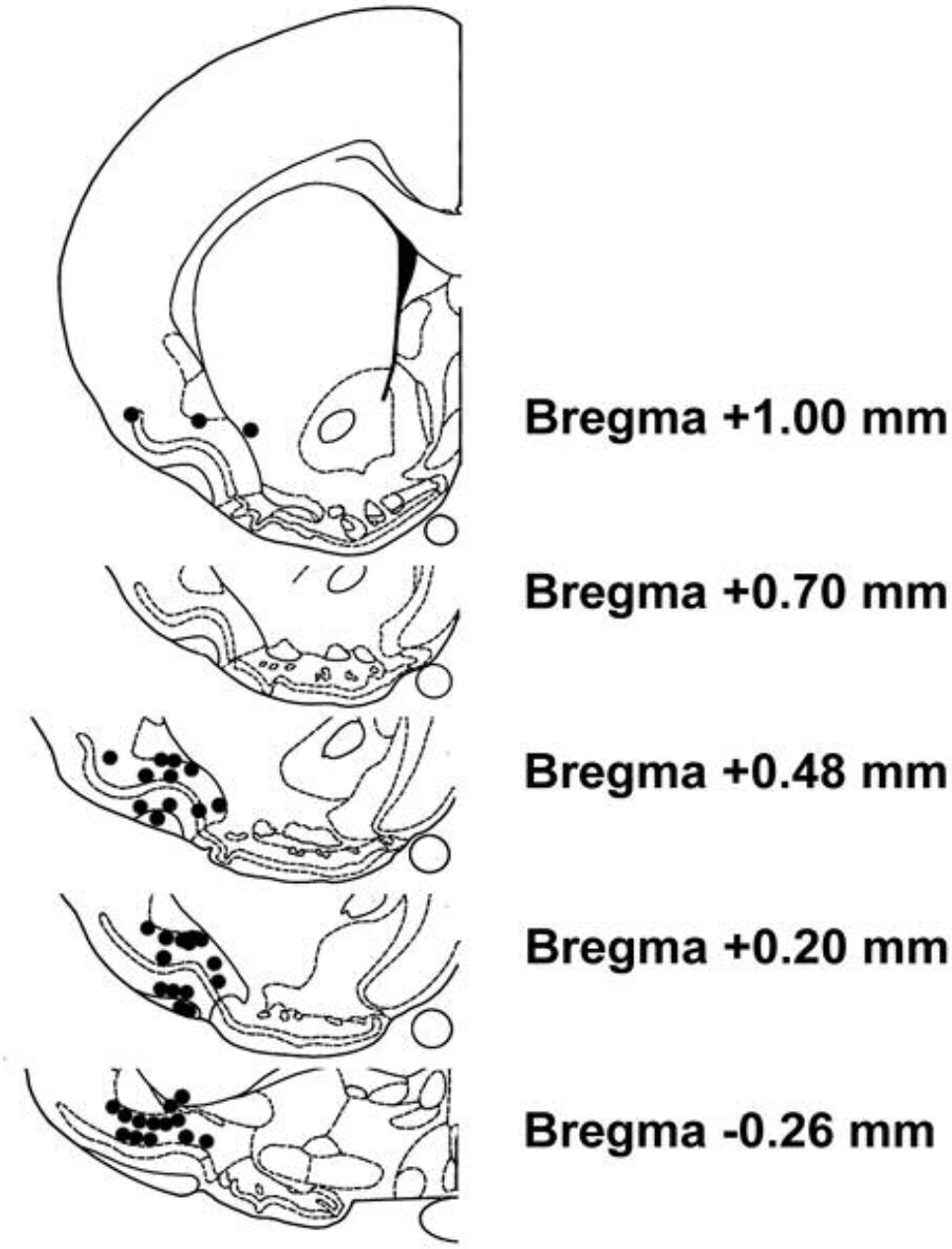

Response magnitude post-habituation as percent of pre
80
60
40
20
0
ACSF
2.5 mM CPPG
*

Figure 14


Bregma +1.00 mm
Bregma +0.70 mm
Bregma +0.48 mm
Bregma +0.20 mm
Bregma -0.26 mm

Chapter 6

Conclusions

In summary, my dissertation research suggests that synaptic depression can be induced by repeated stimulation at the LOT-aPCX synapse. Further, *in vitro* this depression is likely mediated by an early vesicle depletion-like mechanism followed by a slowly developing but longer lasting presynaptic depression caused by activation of group III metabotropic glutamate receptors.

In vivo odor adaptation and the associated synaptic depression seem to also be mediated in large part by activation of group III metabotropic glutamate receptors. I have not confirmed whether the early vesicle depletion-like mechanism associated with *in vitro* train-induced synaptic depression is present during *in vivo* odor adaptation. Additionally, odor -evoked heart-rate orienting behavior seems to be gated by activation of group III metabotropic glutamate receptors in awake rats.

Thus, as in a number of invertebrate models of habituation, it appears that habituation of the odorant-evoked heart-rate orienting response is mediated in large part by pre-synaptic depression at a defined synapse (Castellucci et al., 1970; Zucker, 1972a, b; Castellucci and Kandel, 1974; Best and Wilson, 2004). This finding also holds an additional implication, specifically, that aPCX is necessary for the production of the odor-evoked heart-rate orienting response.

These data suggest that habituation can be mediated by synaptic depression in both invertebrates and mammals (Kandel and Schwartz, 1982; Christoffersen, 1997). Additionally, Horn's (1967) hypothesis of self-generated depression (SGD) of sensitivity involving a decrement in transfer integrity may

reasonably fit the data presented here concerning this particular mechanism of odor habituation.

Synaptic depression makes a very significant contribution to odor habituation at this synapse in aPCX, although it should be noted that the locus of odor habituation cannot be placed exclusively at this synapse in aPCX. Other possibly more basic odor processing does not actually travel through aPCX. For example, many OB outputs go directly to other non-aPCX forebrain areas such as the olfactory tubercle, but these projections also contain pre-synaptic mGluR receptors and thus may utilize a mechanism of habituation similar to the one I have proposed for aPCX (Wada et al., 1998).

The synaptic mechanism proposed here also presents an opportunity for the study of dishabituation. As stated previously, dishabituation is the accelerated recovery of responses following a habituating stimulus, caused by an extrastimulus; dishabituation is considered a classical characteristic of habituation (Thompson and Spencer, 1966). The modulatory mechanism involving activation of β-adrenergic receptors that I described in chapter 3 may be a candidate mechanism for dishabituation of odor habituation. Specifically, an extrastimulus may be able to activate the locus coeruleus resulting in norepinephrine release. This norepinephrine could then act to inhibit synaptic depression at the LOT-aPCX synapse, possibly resulting in dishabituation of odor responses.

There are a number of questions that have been left open by the data presented in this dissertation. One study that would be informative involves

recording from aPCX while presenting a habituating series of odor stimuli as used for the behavioral data in awake animals presented in chapter 5. In this way, it would be possible to tell whether or not aPCX activity is in fact attenuated in an awake, behaving animal as it is following sustained odor presentation in a urethane-anesthetized preparation (Best and Wilson, 2004).

Additional information could be gained by spreading the stimulus presentations out temporally during the habituating series of odor stimuli. Currently, our paradigm does not allow the analysis of heart rate during the habituating series of stimuli. If the stimuli were spaced further apart, it would be possible to correlate the level of LFP activity with the extent of odor-induced bradykardia.

An additional research direction available for future study involves the effect of norepinephrine on *in vivo* odor adaptation and behavioral odor habituation. I found that *in vitro* train-induced synaptic depression could be diminished by activation of β-adrenergic receptors (Best and Wilson, 2004). Whether this represents a possible mechanism for modulation of odor adaptation *in vivo* has not been confirmed.

I have preliminary evidence that this may be the case. Train stimulation as used *in vitro* does not produce depression in an *in vivo* urethane-anesthetized preparation. There are reasons that I have not considered this to be evidence against my *in vitro*-derived paradigm though. Shocking the LOT *in vivo* produces a synchronous volley within the cortex that activates a great many more synapses than would ever be activated during odor presentation. It is known that

activation of aPCX results in firing of the HDB (Linster and Hasselmo, 2000), which raises the possibility that other neuromodulatory nuclei are also activation by strong aPCX stimulation.

This phenomenon was part of the impetus to test the effects of β-adrenergic receptor activation on synaptic depression *in vitro,* combined with a study I had recently read (Cai et al., 2001). So, in a single animal, I injected propranolol, a β-adrenergic receptor antagonist, systemically and gave train stimulations like those I used *in vitro*. Before the drug was applied there was no synaptic depression following the trains, but after the drug took effect, I observed synaptic depression following the trains. This suggests that, during train stimulation *in vivo,* norepinephrine is released and blocks the effects of the pre-synaptic mGluR autoreceptors, inhibiting synaptic depression. However, this study should be done more precisely using canulation and propranolol delivery directly to aPCX.

References

Abbott LF, Nelson SB (2000) Synaptic plasticity: taming the beast. Nat Neurosci 3 Suppl:1178-1183.

Abbott LF, Varela JA, Sen K, Nelson SB (1997) Synaptic depression and cortical gain control. Science 275:220-224.

Adrian ED (1942) Olfactory Reactions in the Brain of the Hedgehog. J Physiol 100:459-473.

Adrian ED (1952) The electrical activity of the mammalian olfactory bulb.

Adrian ED (1953) Sensory messages and sensation; the response of the olfactory organ to different smells. Acta Physiol Scand 29:5-14.

Adrian ED, Ludwig C (1938) Nevous Discharges from the Olfactory Organs of Fish. J Physiol 94:441-460.

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.

Allison AC, Warwick RTT (1949) Quantitative observations on the olfactory system of the rabbit. Brain 72:186-187.

Amoore JE (1970) Molecular Basis of Odor. Springfield, IL: Charles C. Thomas.

Anwyl R (1999) Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. Brain Res Brain Res Rev 29:83-120.

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

Aungst JL, Heyward PM, Puche AC, Karnup SV, Hayar A, Szabo G, Shipley MT (2003) Centre-surround inhibition among olfactory bulb glomeruli. Nature 426:623-629.

Bear MF, Malenka RC (1994) Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 4:389-399.

Beierlein M, Connors BW (2002) Short-term dynamics of thalamocortical and intracortical synapses onto layer 6 neurons in neocortex. J Neurophysiol 88:1924-1932.

Belluscio L, Lodovichi C, Feinstein P, Mombaerts P, Katz LC (2002) Odorant receptors instruct functional circuitry in the mouse olfactory bulb. Nature 419:296-300.

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

Best AR, Wilson DA (2003) A postnatal sensitive period for plasticity of cortical afferents but not cortical association fibers in rat piriform cortex. Brain Res 961:81-87.

Best AR, Wilson DA (2004) Coordinate synaptic mechanisms contributing to olfactory cortical adaptation. J Neurosci 24:652-660.

Biedenbach MA, Stevens CF (1969) Synaptic organization of cat olfactory cortex as revealed by intracellular recording. J Neurophysiol 32:204-214.

Bloms-Funke P, Gernert M, Ebert U, Loscher W (1999) Extracellular single-unit recordings of piriform cortex neurons in rats: influence of different types of

anesthesia and characterization of neurons by pharmacological manipulation of serotonin receptors. J Neurosci Res 55:608-619.
Boeckh J, Kaissling KE, Schneider D (1965) Insect olfactory receptors. Cold Spring Harb Symp Quant Biol 30:263-280.
Bokil H, Laaris N, Blinder K, Ennis M, Keller A (2001) Ephaptic interactions in the mammalian olfactory system. J Neurosci 21:RC173.
Boudreau JC, Freeman WJ (1963) Spectral analysis of electrical activity in the prepyriform cortex of cat. Exp Neurol 8:423-430.
Boulet M, Daval G, Leveteau J (1978) Qualitative and quantitative odour discrimation by mitral cells as compared to anterior olfactory nucleus cells. Brain Res 142:123-134.
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.
Bower JM, Haberly LB (1986) Facilitating and nonfacilitating synapses on pyramidal cells: a correlation between physiology and morphology. Proc Natl Acad Sci U S A 83:1115-1119.
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.
Bozza T, McGann JP, Mombaerts P, Wachowiak M (2004) In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. Neuron 42:9-21.
Breer H, Klemm T, Boekhoff I (1992) Nitric oxide mediated formation of cyclic GMP in the olfactory system. Neuroreport 3:1030-1032.
Brennan PA, Keverne EB (1997) Neural mechanisms of mammalian olfactory learning. Prog Neurobiol 51:457-481.
Bressler SL, Freeman WJ (1980) Frequency analysis of olfactory system EEG in cat, rabbit, and rat. Electroencephalogr Clin Neurophysiol 50:19-24.
Bruce HM (1960) A block to pregnancy in the mouse caused by proximity of strange males. J Reprod Fertil 1:96-103.
Brunjes PC (1983) Olfactory bulb maturation in Acomys cahirinus: is neural growth similar in precocial and altricial murids? Brain Res 284:335-341.
Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65:175-187.
Buonviso N, Revial MF, Jourdan F (1991) The Projections of Mitral Cells from Small Local Regions of the Olfactory Bulb: An Anterograde Tracing Study Using PHA-L (Phaseolus vulgaris Leucoagglutinin). Eur J Neurosci 3:493-500.
Cai Z, Saugstad JA, Sorensen SD, Ciombor KJ, Zhang C, Schaffhauser H, Hubalek F, Pohl J, Duvoisin RM, Conn PJ (2001) Cyclic AMP-dependent protein kinase phosphorylates group III metabotropic glutamate receptors and inhibits their function as presynaptic receptors. J Neurochem 78:756-766.
Carandini M, Ferster D (1997) A tonic hyperpolarization underlying contrast adaptation in cat visual cortex. Science 276:949-952.

Carson KA (1984a) Quantitative localization of neurons projecting to the mouse main olfactory bulb. Brain Res Bull 12:629-634.
Carson KA (1984b) Localization of acetylcholinesterase-positive neurons projecting to the mouse main olfactory bulb. Brain Res Bull 12:635-639.
Castellucci V, Pinsker H, Kupfermann I, Kandel ER (1970) Neuronal mechanisms of habituation and dishabituation of the gill-withdrawal reflex in Aplysia. Science 167:1745-1748.
Castellucci VF, Kandel ER (1974) A quantal analysis of the synaptic depression underlying habituation of the gill-withdrawal reflex in Aplysia. Proc Natl Acad Sci U S A 71:5004-5008.
Cattarelli M, Astic L, Kauer JS (1988) Metabolic mapping of 2-deoxyglucose uptake in the rat piriform cortex using computerized image processing. Brain Res 442:180-184.
Chaput MA (2000) EOG responses in anesthetized freely breathing rats. Chem Senses 25:695-701.
Chess A, Simon I, Cedar H, Axel R (1994) Allelic inactivation regulates olfactory receptor gene expression. Cell 78:823-834.
Christie JM, Schoppa NE, Westbrook GL (2001) Tufted cell dendrodendritic inhibition in the olfactory bulb is dependent on NMDA receptor activity. J Neurophysiol 85:169-173.
Christoffersen GR (1997) Habituation: events in the history of its characterization and linkage to synaptic depression. A new proposed kinetic criterion for its identification. Prog Neurobiol 53:45-66.
Chung S, Li X, Nelson SB (2002) Short-term depression at thalamocortical synapses contributes to rapid adaptation of cortical sensory responses in vivo. Neuron 34:437-446.
Cinelli AR, Hamilton KA, Kauer JS (1995) Salamander olfactory bulb neuronal activity observed by video rate, voltage-sensitive dye imaging. III. Spatial and temporal properties of responses evoked by odorant stimulation. J Neurophysiol 73:2053-2071.
Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J, Carlson JR (1999) A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in Drosophila. Neuron 22:327-338.
Collins GG (1993) Actions of agonists of metabotropic glutamate receptors on synaptic transmission and transmitter release in the olfactory cortex. Br J Pharmacol 108:422-430.
Constanti A, Sim JA (1987) Muscarinic receptors mediating suppression of the M-current in guinea-pig olfactory cortex neurones may be of the M2-subtype. Br J Pharmacol 90:3-5.
Coopersmith R, Leon M (1984) Enhanced neural response to familiar olfactory cues. Science 225:849-851.
Crair MC, Malenka RC (1995) A critical period for long-term potentiation at thalamocortical synapses. Nature 375:325-328.
Cutforth T, Moring L, Mendelsohn M, Nemes A, Shah NM, Kim MM, Frisen J, Axel R (2003) Axonal ephrin-As and odorant receptors: coordinate determination of the olfactory sensory map. Cell 114:311-322.

Dalton P, Wysocki CJ (1996) The nature and duration of adaptation following long-term odor exposure. Percept Psychophys 58:781-792.
Davis M (2000) The role of the amygdala in conditioned and unconditioned fear and anxiety. In: The Amygdala
a functional analysis, 2nd Edition (Aggleton JP, ed), pp 213-288. New York: Oxford University Press.
De Carlos JA, Lopez-Mascaraque L, Valverde F (1989) Connections of the olfactory bulb and nucleus olfactorius anterior in the hedgehog (Erinaceus europaeus): fluorescent tracers and HRP study. J Comp Neurol 279:601-618.
De Olmos J, Heimer L (1980) Double and triple-labeling of neurons with fluorescent substances; the study of collateral pathways in the ascending raphe system. Neurosci Lett 19:7-12.
Dobrunz LE, Stevens CF (1997) Heterogeneity of release probability, facilitation, and depletion at central synapses. Neuron 18:995-1008.
Doheny HC, Faulkner HJ, Gruzelier JH, Baldeweg T, Whittington MA (2000) Pathway-specific habituation of induced gamma oscillations in the hippocampal slice. Neuroreport 11:2629-2633.
Duchamp-Viret P, Duchamp A (1997) Odor processing in the frog olfactory system. Prog Neurobiol 53:561-602.
Ekstrand JJ, Domroese ME, Feig SL, Illig KR, Haberly LB (2001) Immunocytochemical analysis of basket cells in rat piriform cortex. J Comp Neurol 434:308-328.
Ennis M, Linster C, Aroniadou-Anderjaska V, Ciombor K, Shipley MT (1998) Glutamate and synaptic plasticity at mammalian primary olfactory synapses. Ann N Y Acad Sci 855:457-466.
Ennis M, Zhou FM, Ciombor KJ, Aroniadou-Anderjaska V, Hayar A, Borrelli E, Zimmer LA, Margolis F, Shipley MT (2001) Dopamine D2 receptor-mediated presynaptic inhibition of olfactory nerve terminals. J Neurophysiol 86:2986-2997.
Ezeh PI, Wellis DP, Scott JW (1993) Organization of inhibition in the rat olfactory bulb external plexiform layer. J Neurophysiol 70:263-274.
Fallon JH, Loughlin SE (1982) Monoamine innervation of the forebrain: collateralization. Brain Res Bull 9:295-307.
Farbman AI (1994) Developmental biology of olfactory sensory neurons. Semin Cell Biol 5:3-10.
Firestein S (2004) A code in the nose. Sci STKE 2004:pe15.
Fletcher M, Wilson DA (2001) Ontogeny of odor discrimination: a method to assess novel odor discrimination in neonatal rats. Physiol Behav 74:589-593.
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. Proc Natl Acad Sci U S A 77:3033-3037.
Fox AN, Pitts RJ, Robertson HM, Carlson JR, Zwiebel LJ (2001) Candidate odorant receptors from the malaria vector mosquito Anopheles gambiae and evidence of down-regulation in response to blood feeding. Proc Natl Acad Sci U S A 98:14693-14697.
Freeman WJ (1972) Depth recording of averaged evoked potential of olfactory bulb. J Neurophysiol 35:780-796.
Friedrich RW, Korsching SI (1997) Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging. Neuron 18:737-752.
Fu W, Sugai T, Yoshimura H, Onoda N (2004) Convergence of olfactory and gustatory connections onto the endopiriform nucleus in the rat. Neuroscience 126:1033-1041.
Galizia CG, Sachse S, Rappert A, Menzel R (1999) The glomerular code for odor representation is species specific in the honeybee Apis mellifera. Nat Neurosci 2:473-478.
Gaykema RP, Luiten PG, Nyakas C, Traber J (1990) Cortical projection patterns of the medial septum-diagonal band complex. J Comp Neurol 293:103-124.
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.
Gervais R, Pager J (1983) Olfactory bulb excitability selectively modified in behaving rats after local 6-hydroxydopamine treatment. Behav Brain Res 9:165-179.
Gesteland RC, Lettvin JY, Pitts WH (1965) Chemical transmission in the nose of the frog. J Physiol 181:525-559.
Geyer MA, Swerdlow NR, Mansbach RS, Braff DL (1990) Startle response models of sensorimotor gating and habituation deficits in schizophrenia. Brain Res Bull 25:485-498.
Gogos JA, Osborne J, Nemes A, Mendelsohn M, Axel R (2000) Genetic ablation and restoration of the olfactory topographic map. Cell 103:609-620.
Grajski KA, Freeman WJ (1989) Spatial EEG correlates of nonassociative and associative olfactory learning in rabbits. Behav Neurosci 103:790-804.
Gray CM, Freeman WJ, Skinner JE (1986) Chemical dependencies of learning in the rabbit olfactory bulb: acquisition of the transient spatial pattern change depends on norepinephrine. Behav Neurosci 100:585-596.
Guthrie KM, Gall CM (1995) Functional mapping of odor-activated neurons in the olfactory bulb. Chem Senses 20:271-282.
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.

Haberly L, Behan M (1983) Structure of the piriform cortex of the opossum. III. Ultrastructural characterization of synaptic terminals of association and olfactory bulb afferent fibers. J Comp Neurol 219:448-460.
Haberly LB (1969) Single unit responses to odor in the prepyriform cortex of the rat. Brain Res 12:481-484.
Haberly LB (1973a) Summed potentials evoked in opossum prepyriform cortex. J Neurophysiol 36:775-788.
Haberly LB (1973b) Unitary analysis of opossum prepyriform cortex. J Neurophysiol 36:762-774.
Haberly LB (1983) Structure of the piriform cortex of the opossum. I. Description of neuron types with Golgi methods. J Comp Neurol 213:163-187.
Haberly LB (1998) Olfactory Cortex. In: The synaptic organization of the brain., 4 Edition (Shepherd GM, ed), pp 377-416. New York: Oxford Universty Press.
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, Shepherd GM (1973) Current-density analysis of summed evoked potentials in opossum prepyriform cortex. J Neurophysiol 36:789-802.
Haberly LB, Price JL (1977) The axonal projection patterns of the mitral and tufted cells of the olfactory bulb in the rat. Brain Res 129:152-157.
Haberly LB, Price JL (1978a) Association and commissural fiber systems of the olfactory cortex of the rat. II. Systems originating in the olfactory peduncle. J Comp Neurol 181:781-807.
Haberly LB, Price JL (1978b) Association and commissural fiber systems of the olfactory cortex of the rat. J Comp Neurol 178:711-740.
Haberly LB, Feig SL (1983) Structure of the piriform cortex of the opossum. II. Fine structure of cell bodies and neuropil. J Comp Neurol 216:69-88.
Haberly LB, Presto S (1986) Ultrastructural analysis of synaptic relationships of intracellularly stained pyramidal cell axons in piriform cortex. J Comp Neurol 248:464-474.
Haberly LB, Hansen DJ, Feig SL, Presto S (1987) Distribution and ultrastructure of neurons in opossum piriform cortex displaying immunoreactivity to GABA and GAD and high-affinity tritiated GABA uptake. J Comp Neurol 266:269-290.
Halasz N, Greer CA (1993) Terminal arborizations of olfactory nerve fibers in the glomeruli of the olfactory bulb. J Comp Neurol 337:307-316.
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.
Harrison J, Jahr CE (2003) Receptor occupancy limits synaptic depression at climbing fiber synapses. J Neurosci 23:377-383.

Harrison TA, Scott JW (1986) Olfactory bulb responses to odor stimulation: analysis of response pattern and intensity relationships. J Neurophysiol 56:1571-1589.
Hasselmo ME (1995) Neuromodulation and cortical function: modeling the physiological basis of behavior. Behav Brain Res 67:1-27.
Hasselmo ME, Bower JM (1990) Afferent and association fiber differences in short-term potentiation in piriform (olfactory) cortex of the rat. J Neurophysiol 64:179-190.
Hasselmo ME, Bower JM (1991) Selective suppression of afferent but not intrinsic fiber synaptic transmission by 2-amino-4-phosphonobutyric acid (AP4) in piriform cortex. Brain Res 548:248-255.
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.
Hayar A, Karnup S, Shipley MT, Ennis M (2004) Olfactory bulb glomeruli: external tufted cells intrinsically burst at theta frequency and are entrained by patterned olfactory input. J Neurosci 24:1190-1199.
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.
Hoffman WH, Haberly LB (1993) Role of synaptic excitation in the generation of bursting-induced epileptiform potentials in the endopiriform nucleus and piriform cortex. J Neurophysiol 70:2550-2561.
Horn G (1967) Neuronal mechanisms of habituation. Nature 215:707-711.
Illig KR, Haberly LB (2003) Odor-evoked activity is spatially distributed in piriform cortex. J Comp Neurol 457:361-373.
Isaacson JS, Strowbridge BW (1998) Olfactory reciprocal synapses: dendritic signaling in the CNS. Neuron 20:749-761.
Jahr CE, Nicoll RA (1982a) Noradrenergic modulation of dendrodendritic inhibition in the olfactory bulb. Nature 297:227-229.
Jahr CE, Nicoll RA (1982b) An intracellular analysis of dendrodendritic inhibition in the turtle in vitro olfactory bulb. J Physiol 326:213-234.
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 BA, Leon M (2000) Modular representations of odorants in the glomerular layer of the rat olfactory bulb and the effects of stimulus concentration. J Comp Neurol 422:496-509.
Johnson BA, Woo CC, Leon M (1998) Spatial coding of odorant features in the glomerular layer of the rat olfactory bulb. J Comp Neurol 393:457-471.
Johnson BA, Woo CC, Hingco EE, Pham KL, Leon M (1999) Multidimensional chemotopic responses to n-aliphatic acid odorants in the rat olfactory bulb. J Comp Neurol 409:529-548.
Johnson BA, Ho SL, Xu Z, Yihan JS, Yip S, Hingco EE, Leon M (2002) Functional mapping of the rat olfactory bulb using diverse odorants reveals modular responses to functional groups and hydrocarbon structural features. J Comp Neurol 449:180-194.
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.
Jourdan F, Duveau A, Astic L, Holley A (1980) Spatial distribution of [14C]2-deoxyglucose uptake in the olfactory bulbs of rats stimulated with two different odours. Brain Res 188:139-154.
Juilfs DM, Fulle HJ, Zhao AZ, Houslay MD, Garbers DL, Beavo JA (1997) A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. Proc Natl Acad Sci U S A 94:3388-3395.
Jung MW, Larson J, Lynch G (1990a) Long-term potentiation of monosynaptic EPSPs in rat piriform cortex in vitro. Synapse 6:279-283.
Jung MW, Larson J, Lynch G (1990b) Role of NMDA and non-NMDA receptors in synaptic transmission in rat piriform cortex. Exp Brain Res 82:451-455.
Kaba H, Rosser A, Keverne B (1989) Neural basis of olfactory memory in the context of pregnancy block. Neuroscience 32:657-662.
Kandel ER, Schwartz JH (1982) Molecular biology of learning: modulation of transmitter release. Science 218:433-443.
Kang J, Caprio J (1995) In vivo responses of single olfactory receptor neurons in the channel catfish, Ictalurus punctatus. J Neurophysiol 73:172-177.
Kanter ED, Haberly LB (1990) NMDA-dependent induction of long-term potentiation in afferent and association fiber systems of piriform cortex in vitro. Brain Res 525:175-179.
Kanter ED, Haberly LB (1993) Associative long-term potentiation in piriform cortex slices requires GABAA blockade. J Neurosci 13:2477-2482.
Kanter ED, Kapur A, Haberly LB (1996) A dendritic GABAA-mediated IPSP regulates facilitation of NMDA-mediated responses to burst stimulation of afferent fibers in piriform cortex. J Neurosci 16:307-312.
Kapur A, Lytton WW, Ketchum KL, Haberly LB (1997a) Regulation of the NMDA component of EPSPs by different components of postsynaptic GABAergic inhibition: computer simulation analysis in piriform cortex. J Neurophysiol 78:2546-2559.

Kapur A, Pearce RA, Lytton WW, Haberly LB (1997b) GABAA mediated IPSCs in piriform cortex have fast and slow components with different properties and locations on pyramidal cells. J Neurophysiol 78:2531-2545.
Kasowski HJ, Kim H, Greer CA (1999) Compartmental organization of the olfactory bulb glomerulus. J Comp Neurol 407:261-274.
Kauer JS (1974) Response patterns of amphibian olfactory bulb neurones to odour stimulation. J Physiol 243:695-715.
Kauer JS, Cinelli AR (1993) Are there structural and functional modules in the vertebrate olfactory bulb? Microsc Res Tech 24:157-167.
Kay LM, Freeman WJ (1998) Bidirectional processing in the olfactory-limbic axis during olfactory behavior. Behav Neurosci 112:541-553.
Kelliher KR, Ziesmann J, Munger SD, Reed RR, Zufall F (2003) Importance of the CNGA4 channel gene for odor discrimination and adaptation in behaving mice. Proc Natl Acad Sci U S A 100:4299-4304.
Ketchum KL, Haberly LB (1993) Membrane currents evoked by afferent fiber stimulation in rat piriform cortex. I. Current source-density analysis. J Neurophysiol 69:248-260.
Keverne EB, de la Riva C (1982) Pheromones in mice: reciprocal interaction between the nose and brain. Nature 296:148-150.
Kinzie JM, Saugstad JA, Westbrook GL, Segerson TP (1995) Distribution of metabotropic glutamate receptor 7 messenger RNA in the developing and adult rat brain. Neuroscience 69:167-176.
Kinzie JM, Shinohara MM, van den Pol AN, Westbrook GL, Segerson TP (1997) Immunolocalization of metabotropic glutamate receptor 7 in the rat olfactory bulb. J Comp Neurol 385:372-384.
Krieger J, Breer H (1999) Olfactory reception in invertebrates. Science 286:720-723.
Kucharski D, Hall WG (1988) Developmental change in the access to olfactory memories. Behav Neurosci 102:340-348.
Kuroda Y, Saito M, Kobayashi K (1976) Concomitant changes in cyclic AMP level and postsynaptic potentials of olfactory cortex slices induced by adenosine derivatives. Brain Res 109:196-201.
Laurent G, Wehr M, Davidowitz H (1996) Temporal representations of odors in an olfactory network. J Neurosci 16:3837-3847.
Leinders-Zufall T, Shepherd GM, Zufall F (1995) Regulation of cyclic nucleotide-gated channels and membrane excitability in olfactory receptor cells by carbon monoxide. J Neurophysiol 74:1498-1508.
Levai O, Breer H, Strotmann J (2003) Subzonal organization of olfactory sensory neurons projecting to distinct glomeruli within the mouse olfactory bulb. J Comp Neurol 458:209-220.
Leveteau J, MacLeod P (1966) Olfactory discrimination in the rabbit olfactory glomerulus. Science 153:175-176.
Libri V, Constanti A, Zibetti M, Postlethwaite M (1997) Metabotropic glutamate receptor subtypes mediating slow inward tail current (IADP) induction and inhibition of synaptic transmission in olfactory cortical neurones. Br J Pharmacol 120:1083-1095.

Lin W, Arellano J, Slotnick B, Restrepo D (2004) Odors detected by mice deficient in cyclic nucleotide-gated channel subunit A2 stimulate the main olfactory system. J Neurosci 24:3703-3710.
Linster C, Hasselmo ME (2000) Neural activity in the horizontal limb of the diagonal band of broca can be modulated by electrical stimulation of the olfactory bulb and cortex in rats. Neurosci Lett 282:157-160.
Linster C, Hasselmo ME (2001) Neuromodulation and the functional dynamics of piriform cortex. Chem Senses 26:585-594.
Linster C, Wyble BP, Hasselmo ME (1999) Electrical stimulation of the horizontal limb of the diagonal band of broca modulates population EPSPs in piriform cortex. J Neurophysiol 81:2737-2742.
Linster C, Maloney M, Patil M, Hasselmo ME (2003) Enhanced cholinergic suppression of previously strengthened synapses enables the formation of self-organized representations in olfactory cortex. Neurobiol Learn Mem 80:302-314.
Litaudon P, Datiche F, Cattarelli M (1997) Optical recording of the rat piriform cortex activity. Prog Neurobiol 52:485-510.
Liu CJ, Grandes P, Matute C, Cuenod M, Streit P (1989) Glutamate-like immunoreactivity revealed in rat olfactory bulb, hippocampus and cerebellum by monoclonal antibody and sensitive staining method. Histochemistry 90:427-445.
Liu WL, Shipley MT (1994) Intrabulbar associational system in the rat olfactory bulb comprises cholecystokinin-containing tufted cells that synapse onto the dendrites of GABAergic granule cells. J Comp Neurol 346:541-558.
Luck SJ, Chelazzi L, Hillyard SA, Desimone R (1997) Neural mechanisms of spatial selective attention in areas V1, V2, and V4 of macaque visual cortex. J Neurophysiol 77:24-42.
Luo M, Katz LC (2001) Response correlation maps of neurons in the mammalian olfactory bulb. Neuron 32:1165-1179.
Luskin MB, Price JL (1982) The distribution of axon collaterals from the olfactory bulb and the nucleus of the horizontal limb of the diagonal band to the olfactory cortex, demonstrated by double retrograde labeling techniques. J Comp Neurol 209:249-263.
Luskin MB, Price JL (1983a) The laminar distribution of intracortical fibers originating in the olfactory cortex of the rat. J Comp Neurol 216:292-302.
Luskin MB, Price JL (1983b) The topographic organization of associational fibers of the olfactory system in the rat, including centrifugal fibers to the olfactory bulb. J Comp Neurol 216:264-291.
Macrides F, Davis BJ, Youngs WM, Nadi NS, Margolis FL (1981) Cholinergic and catecholaminergic afferents to the olfactory bulb in the hamster: a neuroanatomical, biochemical, and histochemical investigation. J Comp Neurol 203:495-514.
Mair RG (1982) Adaptation of rat olfactory bulb neurones. J Physiol 326:361-369.
Malnic B, Hirono J, Sato T, Buck LB (1999) Combinatorial receptor codes for odors. Cell 96:713-723.

Marek GJ, Aghajanian GK (1996) Alpha 1B-adrenoceptor-mediated excitation of piriform cortical interneurons. Eur J Pharmacol 305:95-100.
Mason ST, Corcoran ME (1979) Catecholamines and convulsions. Brain Res 170:497-507.
Mccollum J, Larson J, Otto T, Schottler F, Granger R, Lynch G (1991) Short-Latency Single Unit Processing in Olfactory Cortex. Journal of Cognitive Neuroscience 3:293-299.
McLean JH, Shipley MT (1987) Serotonergic afferents to the rat olfactory bulb: I. Origins and laminar specificity of serotonergic inputs in the adult rat. J Neurosci 7:3016-3028.
McLean JH, Shipley MT, Nickell WT, Aston-Jones G, Reyher CK (1989) Chemoanatomical organization of the noradrenergic input from locus coeruleus to the olfactory bulb of the adult rat. J Comp Neurol 285:339-349.
McNamara AM, Cleland TA, Linster C (2004) Characterization of the synaptic properties of olfactory bulb projections. Chem Senses 29:225-233.
Meisami E, Safari L (1981) A quantitative study of the effects of early unilateral olfactory deprivation on the number and distribution of mitral and tufted cells and of glomeruli in the rat olfactory bulb. Brain Res 221:81-107.
Meredith M (1986) Patterned response to odor in mammalian olfactory bulb: the influence of intensity. J Neurophysiol 56:572-597.
Molyneaux BJ, Hasselmo ME (2002) GABA(B) presynaptic inhibition has an in vivo time constant sufficiently rapid to allow modulation at theta frequency. J Neurophysiol 87:1196-1205.
Mombaerts P (2004) Odorant receptor gene choice in olfactory sensory neurons: the one receptor-one neuron hypothesis revisited. Curr Opin Neurobiol 14:31-36.
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.
Mori K, Kishi K, Ojima H (1983) Distribution of dendrites of mitral, displaced mitral, tufted, and granule cells in the rabbit olfactory bulb. J Comp Neurol 219:339-355.
Motley SJ, Collins GG (1983) Endogenous adenosine inhibits excitatory transmission in the rat olfactory cortex slice. Neuropharmacology 22:1081-1086.
Moulton DG (1976) Spatial patterning of response to odors in the peripheral olfactory system. Physiol Rev 56:578-593.
Mozell MM (1970) Evidence for a chromatographic model of olfaction. J Gen Physiol 56:46-63.
Mozell MM, Jagodowicz M (1973) Chromatographic separation of odorants by the nose: retention times measured across in vivo olfactory mucosa. Science 181:1247-1249.
Nagayama S, Takahashi YK, Yoshihara Y, Mori K (2004) Mitral and tufted cells differ in the decoding manner of odor maps in the rat olfactory bulb. J Neurophysiol 91:2532-2540.

Nakashima M, Mori K, Takagi SF (1978) Centrifugal influence on olfactory bulb activity in the rabbit. Brain Res 154:301-306.
Nemitz JW, Goldberg SJ (1983) Neuronal responses of rat pyriform cortex to odor stimulation: an extracellular and intracellular study. J Neurophysiol 49:188-203.
Neville KR, Haberly LB (2003) Beta and gamma oscillations in the olfactory system of the urethane-anesthetized rat. J Neurophysiol 90:3921-3930.
Neville KR, Haberly LB (2004) Olfactory Cortex. In: The synaptic organization of the brain., 5 Edition (Shepherd GM, ed), pp 415-454. New York: Oxford University Press.
Ng M, Roorda RD, Lima SQ, Zemelman BV, Morcillo P, Miesenbock G (2002) Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. Neuron 36:463-474.
Ngai J, Dowling MM, Buck L, Axel R, Chess A (1993) The family of genes encoding odorant receptors in the channel catfish. Cell 72:657-666.
Nickell WT, Shipley MT (1988) Two anatomically specific classes of candidate cholinoceptive neurons in the rat olfactory bulb. J Neurosci 8:4482-4491.
Niimura Y, Nei M (2003) Evolution of olfactory receptor genes in the human genome. Proc Natl Acad Sci U S A 100:12235-12240.
Ohzawa I, Sclar G, Freeman RD (1982) Contrast gain control in the cat visual cortex. Nature 298:266-268.
Ojima H, Mori K, Kishi K (1984) The trajectory of mitral cell axons in the rabbit olfactory cortex revealed by intracellular HRP injection. J Comp Neurol 230:77 87.
Onoda N, Mori K (1980) Depth distribution of temporal firing patterns in olfactory bulb related to air-intake cycles. J Neurophysiol 44:29-39.
Orona E, Rainer EC, Scott JW (1984) Dendritic and axonal organization of mitral and tufted cells in the rat olfactory bulb. J Comp Neurol 226:346-356.
Patil MM, Hasselmo ME (1999) Modulation of inhibitory synaptic potentials in the piriform cortex. J Neurophysiol 81:2103-2118.
Patil MM, Linster C, Lubenov E, Hasselmo ME (1998) Cholinergic agonist carbachol enables associative long-term potentiation in piriform cortex slices. J Neurophysiol 80:2467-2474.
Patneau DK, Stripling JS (1992) Functional correlates of selective long-term potentiation in the olfactory cortex and olfactory bulb. Brain Res 585:219-228.
Pearce RA (1993) Physiological evidence for two distinct GABAA responses in rat hippocampus. Neuron 10:189-200.
Pearce RA, Grunder SD, Faucher LD (1995) Different mechanisms for use-dependent depression of two GABAA-mediated IPSCs in rat hippocampus. J Physiol 484 (Pt 2):425-435.
Pekhletski R, Gerlai R, Overstreet LS, Huang XP, Agopyan N, Slater NT, Abramow-Newerly W, Roder JC, Hampson DR (1996) Impaired cerebellar synaptic plasticity and motor performance in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. J Neurosci 16:6364-6373.

Perez-Orive J, Mazor O, Turner GC, Cassenaer S, Wilson RI, Laurent G (2002) Oscillations and sparsening of odor representations in the mushroom body. Science 297:359-365.
Pettigrew JD, Kasamatsu T (1978) Local perfusion of noradrenaline maintains visual cortical plasticity. Nature 271:761-763.
Phillips CG, Powell TP, Shepherd GM (1963) Responses of Mitral Cells to Stimulation of the Lateral Olfactory Tract in the Rabbit. J Physiol 168:65-88.
Pinching AJ, Powell TP (1971a) The neuropil of the glomeruli of the olfactory bulb. J Cell Sci 9:347-377.
Pinching AJ, Powell TP (1971b) The neuropil of the periglomerular region of the olfactory bulb. J Cell Sci 9:379-409.
Pinching AJ, Powell TP (1971c) The neuron types of the glomerular layer of the olfactory bulb. J Cell Sci 9:305-345.
Pissonnier D, Thiery JC, Fabre-Nys C, Poindron P, Keverne EB (1985) The importance of olfactory bulb noradrenalin for maternal recognition in sheep. Physiol Behav 35:361-363.
Poellinger A, Thomas R, Lio P, Lee A, Makris N, Rosen BR, Kwong KK (2001) Activation and habituation in olfaction--an fMRI study. Neuroimage 13:547-560.
Polak EH (1973) Mutiple profile-multiple receptor site model for vertebrate olfaction. J Theor Biol 40:469-484.
Potter H, Chorover SL (1976) Response plasticity in hamster olfactory bulb: peripheral and central processes. Brain Res 116:417-429.
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 (1970) An experimental study of the origin and the course of the centrifugal fibres to the olfactory bulb in the rat. J Anat 107:215-237.
Price JL, Sprich WW (1975) Observations on the lateral olfactory tract of the rat. J Comp Neurol 162:321-336.
Rall W, Shepherd GM, Reese TS, Brightman MW (1966) Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. Exp Neurol 14:44 56.
Reed RR (2004) After the holy grail: establishing a molecular basis for Mammalian olfaction. Cell 116:329-336.
Ress D, Backus BT, Heeger DJ (2000) Activity in primary visual cortex predicts performance in a visual detection task. Nat Neurosci 3:940-945.
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 (1994) Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. Cell 79:1245-1255.
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.

Roman F, Staubli U, Lynch G (1987) Evidence for synaptic potentiation in a cortical network during learning. Brain Res 418:221-226.
Roman FS, Chaillan FA, Soumireu-Mourat B (1993) Long-term potentiation in rat piriform cortex following discrimination learning. Brain Res 601:265-272.
Rosser AE, Keverne EB (1985) The importance of central noradrenergic neurones in the formation of an olfactory memory in the prevention of pregnancy block. Neuroscience 15:1141-1147.
Royet JP, Sicard G, Souchier C, Jourdan F (1987) Specificity of spatial patterns of glomerular activation in the mouse olfactory bulb: computer-assisted image analysis of 2-deoxyglucose autoradiograms. Brain Res 417:1-11.
Royet JP, Souchier C, Jourdan F, Ploye H (1988) Morphometric study of the glomerular population in the mouse olfactory bulb: numerical density and size distribution along the rostrocaudal axis. J Comp Neurol 270:559-568.
Rubin BD, Katz LC (1999) Optical imaging of odorant representations in the mammalian olfactory bulb. Neuron 23:499-511.
Rubin BD, Katz LC (2001) Spatial coding of enantiomers in the rat olfactory bulb. Nat Neurosci 4:355-356.
Rusakov DA, Fine A (2003) Extracellular Ca2+ depletion contributes to fast activity-dependent modulation of synaptic transmission in the brain. Neuron 37:287-297.
Sassoe-Pognetto M, Cantino D, Panzanelli P, Verdun di Cantogno L, Giustetto M, Margolis FL, De Biasi S, Fasolo A (1993) Presynaptic co-localization of carnosine and glutamate in olfactory neurones. Neuroreport 5:7-10.
Satou M, Mori K, Tazawa Y, Takagi SF (1983a) Neuronal pathways for activation of inhibitory interneurons in pyriform cortex of the rabbit. J Neurophysiol 50:74-88.
Satou M, Mori K, Tazawa Y, Takagi SF (1983b) Interneurons mediating fast postsynaptic inhibition in pyriform cortex of the rabbit. J Neurophysiol 50:89-101.
Saugstad JA, Kinzie JM, Shinohara MM, Segerson TP, Westbrook GL (1997) Cloning and expression of rat metabotropic glutamate receptor 8 reveals a distinct pharmacological profile. Mol Pharmacol 51:119-125.
Schaffhauser H, Cai Z, Hubalek F, Macek TA, Pohl J, Murphy TJ, Conn PJ (2000) cAMP-dependent protein kinase inhibits mGluR2 coupling to G-proteins by direct receptor phosphorylation. J Neurosci 20:5663-5670.
Schild D, Restrepo D (1998) Transduction mechanisms in vertebrate olfactory receptor cells. Physiol Rev 78:429-466.
Schneider SP, Scott JW (1983) Orthodromic response properties of rat olfactory bulb mitral and tufted cells correlate with their projection patterns. J Neurophysiol 50:358-378.
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.
Schoenfeld TA, Macrides F (1984) Topographic organization of connections between the main olfactory bulb and pars externa of the anterior olfactory nucleus in the hamster. J Comp Neurol 227:121-135.

Schoenfeld TA, Marchand JE, Macrides F (1985) Topographic organization of tufted cell axonal projections in the hamster main olfactory bulb: an intrabulbar associational system. J Comp Neurol 235:503-518.
Scholfield CN (1978) Depression of evoked potentials in brain slices by adenosine compounds. Br J Pharmacol 63:239-244.
Scholfield CN, Steel L (1988) Presynaptic K-channel blockade counteracts the depressant effect of adenosine in olfactory cortex. Neuroscience 24:81-91.
Schoppa NE, Westbrook GL (1997) Modulation of mEPSCs in olfactory bulb mitral cells by metabotropic glutamate receptors. J Neurophysiol 78:1468-1475.
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.
Scott JW (1981) Electrophysiological identification of mitral and tufted cells and distributions of their axons in olfactory system of the rat. J Neurophysiol 46:918-931.
Scott JW (1986) The olfactory bulb and central pathways. Experientia 42:223-232.
Scott JW, McBride RL, Schneider SP (1980) The organization of projections from the olfactory bulb to the piriform cortex and olfactory tubercle in the rat. J Comp Neurol 194:519-534.
Sengupta P, Chou JH, Bargmann CI (1996) odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. Cell 84:899-909.
Serizawa S, Miyamichi K, Nakatani H, Suzuki M, Saito M, Yoshihara Y, Sakano H (2003) Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. Science 302:2088-2094.
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.
Sharp FR, Kauer JS, Shepherd GM (1977) Laminar analysis of 2-deoxyglucose uptake in olfactory bulb and olfactory cortex of rabbit and rat. J Neurophysiol 40:800-813.
Sheldon PW, Aghajanian GK (1990) Serotonin (5-HT) induces IPSPs in pyramidal layer cells of rat piriform cortex: evidence for the involvement of a 5-HT2-activated interneuron. Brain Res 506:62-69.
Sheldon PW, Aghajanian GK (1991) Excitatory responses to serotonin (5-HT) in neurons of the rat piriform cortex: evidence for mediation by 5-HT1C receptors in pyramidal cells and 5-HT2 receptors in interneurons. Synapse 9:208-218.
Shepherd GM (1963) Neuronal Systems Controlling Mitral Cell Excitability. J Physiol 168:101-117.
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., 5 Edition (Shepherd GM, ed), pp 165-216. New York: Oxford University Press.
Shipley MT, Adamek GD (1984) The connections of the mouse olfactory bulb: a study using orthograde and retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase. Brain Res Bull 12:669-688.
Shipley MT, Ennis M (1996) Functional organization of olfactory system. J Neurobiol 30:123-176.
Smith TC, Jahr CE (2002) Self-inhibition of olfactory bulb neurons. Nat Neurosci 5:760-766.
Sobel N, Prabhakaran V, Zhao Z, Desmond JE, Glover GH, Sullivan EV, Gabrieli JD (2000) Time course of odorant-induced activation in the human primary olfactory cortex. J Neurophysiol 83:537-551.
Spencer WA, Thompson RF, Neilson DR, Jr. (1966) Response decrement of the flexion reflex in the acute spinal cat and transient restoration by strong stimuli. J Neurophysiol 29:221-239.
Spors H, Grinvald A (2002) Spatio-temporal dynamics of odor representations in the mammalian olfactory bulb. Neuron 34:301-315.
Stevens CF, Tsujimoto T (1995) Estimates for the pool size of releasable quanta at a single central synapse and for the time required to refill the pool. Proc Natl Acad Sci U S A 92:846-849.
Stewart WB, Kauer JS, Shepherd GM (1979) Functional organization of rat olfactory bulb analysed by the 2-deoxyglucose method. J Comp Neurol 185:715-734.
Stripling JS, Patneau DK (1999) Potentiation of late components in olfactory bulb and piriform cortex requires activation of cortical association fibers. Brain Res 841:27-42.
Sugitani M, Sugai T, Onoda N (2002) Postsynaptic activity of metabotropic glutamate receptors in the piriform cortex. Neuroreport 13:1473-1476.
Sugitani M, Sugai T, Onoda N (2004) Postsynaptic metabotropic glutamate receptor mGluR1 mediates the late component of signal propagation in the guinea pig piriform cortex: optical imaging study. Neurosci Lett 356:29-32.
Sullivan RM, Wilson DA, Leon M (1989) Norepinephrine and learning-induced plasticity in infant rat olfactory system. J Neurosci 9:3998-4006.
Tanabe T, Iino M, Takagi SF (1975) Discrimination of odors in olfactory bulb, pyriform-amygdaloid areas, and orbitofrontal cortex of the monkey. J Neurophysiol 38:1284-1296.
Tang AC, Hasselmo ME (1994) Selective suppression of intrinsic but not afferent fiber synaptic transmission by baclofen in the piriform (olfactory) cortex. Brain Res 659:75-81.
Thompson RF, Spencer WA (1966) Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychol Rev 73:16-43.
Touhara K (2002) Odor discrimination by G protein-coupled olfactory receptors. Microsc Res Tech 58:135-141.

Troemel ER, Kimmel BE, Bargmann CI (1997) Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in C. elegans. Cell 91:161-169.
Troemel ER, Chou JH, Dwyer ND, Colbert HA, Bargmann CI (1995) Divergent seven transmembrane receptors are candidate chemosensory receptors in C. elegans. Cell 83:207-218.
Trombley PQ, Westbrook GL (1992) L-AP4 inhibits calcium currents and synaptic transmission via a G-protein-coupled glutamate receptor. J Neurosci 12:2043-2050.
Trombley PQ, Shepherd GM (1992) Noradrenergic inhibition of synaptic transmission between mitral and granule cells in mammalian olfactory bulb cultures. J Neurosci 12:3985-3991.
Truchet B, Chaillan FA, Soumireu-Mourat B, Roman FS (2002) Learning and memory of cue-reward association meaning by modifications of synaptic efficacy in dentate gyrus and piriform cortex. Hippocampus 12:600-608.
Tseng GF, Haberly LB (1988) Characterization of synaptically mediated fast and slow inhibitory processes in piriform cortex in an in vitro slice preparation. J Neurophysiol 59:1352-1376.
Tseng GF, Haberly LB (1989a) Deep neurons in piriform cortex. I. Morphology and synaptically evoked responses including a unique high-amplitude paired shock facilitation. J Neurophysiol 62:369-385.
Tseng GF, Haberly LB (1989b) Deep neurons in piriform cortex. II. Membrane properties that underlie unusual synaptic responses. J Neurophysiol 62:386-400.
Turin L, Yoshii F (2003) Structure-Odor Relationships: A Modern Perspective. In: Handbook of Olfaction and Gustation, 2nd Edition (Doty RL, ed), pp 275-294. New York: Basel.
Uchida N, Takahashi YK, Tanifuji M, Mori K (2000) Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features. Nat Neurosci 3:1035-1043.
Vankov A, Herve-Minvielle A, Sara SJ (1995) Response to novelty and its rapid habituation in locus coeruleus neurons of the freely exploring rat. Eur J Neurosci 7:1180-1187.
Vassar R, Ngai 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.
Vertes RP (1991) A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. J Comp Neurol 313:643-668.
Vosshall LB, Amrein H, Morozov PS, Rzhetsky A, Axel R (1999) A spatial map of olfactory receptor expression in the Drosophila antenna. Cell 96:725-736.
Wada E, Shigemoto R, Kinoshita A, Ohishi H, Mizuno N (1998) Metabotropic glutamate receptor subtypes in axon terminals of projection fibers from the main and accessory olfactory bulbs: a light and electron microscopic immunohistochemical study in the rat. J Comp Neurol 393:493-504.

Wang F, Nemes A, Mendelsohn M, Axel R (1998) Odorant receptors govern the formation of a precise topographic map. Cell 93:47-60.
Waterhouse BD, Moises HC, Woodward DJ (1998) Phasic activation of the locus coeruleus enhances responses of primary sensory cortical neurons to peripheral receptive field stimulation. Brain Res 790:33-44.
Weinberger NM (1995) Dynamic regulation of receptive fields and maps in the adult sensory cortex. Annu Rev Neurosci 18:129-158.
Wellis DP, Scott JW (1990) Intracellular responses of identified rat olfactory bulb interneurons to electrical and odor stimulation. J Neurophysiol 64:932-947.
Wellis DP, Scott JW, Harrison TA (1989) Discrimination among odorants by single neurons of the rat olfactory bulb. J Neurophysiol 61:1161-1177.
White EL (1972) Synaptic organization in the olfactory glomerulus of the mouse. Brain Res 37:69-80.
Wilson DA (1997) Binaral interactions in the rat piriform cortex. J Neurophysiol 78:160-169.
Wilson DA (1998a) Habituation of odor responses in the rat anterior piriform cortex. J Neurophysiol 79:1425-1440.
Wilson DA (1998b) Synaptic correlates of odor habituation in the rat anterior piriform cortex. J Neurophysiol 80:998-1001.
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 (2001a) Scopolamine enhances generalization between odor representations in rat olfactory cortex. Learn Mem 8:279-285.
Wilson DA (2001b) Receptive fields in the rat piriform cortex. Chem Senses 26:577-584.
Wilson DA (2003) Rapid, experience-induced enhancement in odorant discrimination by anterior piriform cortex neurons. J Neurophysiol 90:65-72.
Wilson DA, Leon M (1988) Noradrenergic modulation of olfactory bulb excitability in the postnatal rat. Brain Res 470:69-75.
Wilson DA, Sullivan RM (1992) Blockade of mitral/tufted cell habituation to odors by association with reward: a preliminary note. Brain Res 594:143-145.
Wilson DA, Stevenson RJ (2003) The fundamental role of memory in olfactory perception. Trends Neurosci 26:243-247.
Wilson DA, Best AR, Brunjes PC (2000) Trans-neuronal modification of anterior piriform cortical circuitry in the rat. Brain Res 853:317-322.
Yamamoto C, Iwama K (1962) Intracellular Potential Recording from Olfactory Bulb Neurones of Rabbit. Proceedings of the Japan Academy 38:63-&.
Yang J, Ul Quraish A, Murakami K, Ishikawa Y, Takayanagi M, Kakuta S, Kishi K (2004) Quantitative analysis of axon collaterals of single neurons in layer IIa of the piriform cortex of the guinea pig. J Comp Neurol 473:30-42.
Yeung-Courchesne R, Courchesne E (1997) From impasse to insight in autism research: from behavioral symptoms to biological explanations. Dev Psychopathol 9:389-419.

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.
Young JM, Friedman C, Williams EM, Ross JA, Tonnes-Priddy L, Trask BJ (2002) Different evolutionary processes shaped the mouse and human olfactory receptor gene families. Hum Mol Genet 11:535-546.
Zaborszky L, Carlsen J, Brashear HR, Heimer L (1986) Cholinergic and GABAergic afferents to the olfactory bulb in the rat with special emphasis on the projection neurons in the nucleus of the horizontal limb of the diagonal band. J Comp Neurol 243:488-509.
Zhang X, Firestein S (2002) The olfactory receptor gene superfamily of the mouse. Nat Neurosci 5:124-133.
Zhao H, Reed RR (2001) X inactivation of the OCNC1 channel gene reveals a role for activity-dependent competition in the olfactory system. Cell 104:651-660.
Zheng C, Feinstein P, Bozza T, Rodriguez I, Mombaerts P (2000) Peripheral olfactory projections are differentially affected in mice deficient in a cyclic nucleotide-gated channel subunit. Neuron 26:81-91.
Zheng LM, Jourdan F (1988) Atypical olfactory glomeruli contain original olfactory axon terminals: an ultrastructural horseradish peroxidase study in the rat. Neuroscience 26:367-378.
Zou Z, Horowitz LF, Montmayeur JP, Snapper S, Buck LB (2001) Genetic tracing reveals a stereotyped sensory map in the olfactory cortex. Nature 414:173-179.
Zucker RS (1972a) Crayfish escape behavior and central synapses. I. Neural circuit exciting lateral giant fiber. J Neurophysiol 35:599-620.
Zucker RS (1972b) Crayfish escape behavior and central synapses. II. Physiological mechanisms underlying behavioral habituation. J Neurophysiol 35:621-637.
Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Annu Rev Physiol 64:355-405.
Zufall F, Shepherd GM, Firestein S (1991) Inhibition of the olfactory cyclic nucleotide gated ion channel by intracellular calcium. Proc R Soc Lond B Biol Sci 246:225-230.