Habituation of Odor Responses in the Rat Anterior Piriform Cortex

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Wilson, Donald A. Habituation of odor responses in the rat anterior piriform cortex. J. Neurophysiol. 79: 1425–1440, 1998. Simultaneous recordings of main olfactory bulb (MOB) and anterior piriform cortex (aPCX) neuron responses to repeated and prolonged odor pulses were examined in freely breathing, urethane-anesthetized rats. Comparisons of odor responses were made between multiunit recordings of MOB activity and single-unit extracellular and intracellular recordings of Layer II/III aPCX neurons. Odor stimuli consisted of either 2-s pulses repeated at 30-s intervals or a single, prolonged 50-s stimulus. Respiration rate was monitored throughout. MOB and aPCX neuron responses to odor were quantified both through firing frequency and through the temporal patterning of firing over the respiratory cycle. The results demonstrate that aPCX neurons habituate significantly more (faster) than MOB neurons with both repeated and prolonged stimulation paradigms. This enhanced habituation is expressed as both a decrease in aPCX firing despite maintained odor-evoked MOB input and as a decrease in aPCX respiratory cycle entrainment despite maintained MOB cyclic input. Intracellular aPCX recordings suggest that several mechanisms may be involved in this experience-induced change in aPCX function, including 1) decreased excitatory drive of aPCX neurons, 2) decreased excitability of aPCX neurons, and/or 3) enhancement in odor-evoked inhibition of aPCX neurons. These studies provide the initial basis for understanding the mechanisms of nonassociative plasticity in olfactory cortex.

INTRODUCTION

As with most sensory systems, olfactory system response characteristics are dependent on previous experience. In the olfactory bulb, the first central relay for olfactory information, associative learning (Freeman and Schneider 1982; Pagar 1974; Wilson and Leon 1988), prolonged odor exposure (Eckert and Schmidt 1985), and olfactory deprivation (Wilson and Sullivan 1995) can modify the spatiotemporal response patterns of olfactory bulb primary output neurons, the mitral/tufted cells.

Habituation, a simple form of nonassociative memory, is a common feature of sensory systems and is characterized as a decrease in responsiveness to repeated stimuli. Sensory habituation allows filtering of less significant, or predictable events, and is believed to involve higher-order processing, as opposed, for example, to receptor adaptation. Adaptation of olfactory receptor cells occurs relatively slowly (requiring several seconds of continuous stimulation) and appears to involve a stimulation-induced rise in intracellular Ca²⁺, which decreases the probability of adenosine 3',5'-cyclic monophosphate (cAMP)-gated ion-channel opening (Kramer and Siegelbaum 1992; Kurahashi and Menini 1997; Zufall et al. 1991). Interstimulus intervals of 30 s or more are sufficient to allow recovery of receptor adaptation to short odor pulses (Potter and Chorover 1976). Habituation in olfactory system second order neurons, mitral/tufted cells, however, has a slower onset and longer duration than receptor adaptation (Chaput and Panhuber 1982; Potter and Chorover 1976) and is modulated by centrifugal inputs to the olfactory bulb (Grajski and Freeman 1989; Potter and Chorover 1976; Scott 1977; Wilson and Sullivan 1992). The prolonged time course and role of central modulation suggests that habituation processes occur in the olfactory bulb in addition to the decrements in primary afferent activity.

This enhanced ability of more central structures to filter repetitive stimuli, compared with more peripheral structures, has been described in other sensory systems. For example, in audition, the auditory thalamic nucleus and cortex demonstrate rapid habituation to repeated short tones under conditions where the cochlear nucleus (the 1st central auditory nucleus) shows no habituation (Weinberger et al. 1975; Wickelgren 1968). Habituation, therefore, can be seen as a higher order process wherein, despite continued receptor and second-order neural activity, cortical neurons cease responding. If this is a general characteristic of sensory systems, then the primary olfactory cortex should demonstrate greater habituation than the olfactory bulb or receptor neurons. The present report compares olfactory bulb and olfactory cortical habituation to repeated and prolonged odor stimulation.

The primary olfactory cortex is the major target of olfactory bulb mitral/tufted cells, particularly the anterior piriform cortex (aPCX). Mitral/tufted cell axons form the lateral olfactory tract (LOT), which terminates on the distal half (layer Ia) of layer II/III aPCX pyramidal cell apical dendrites (Haberly 1985; Price 1987). The proximal half of the pyramidal apical dendrites (layer Ib) receives association fiber input from pyramidal cells in other regions of the olfactory cortex, as well as commissural input (Haberly 1985; Price 1987). Piriform cortex pyramidal cells also project heavily back to the ipsilateral olfactory bulb. At a gross level, the olfactory bulb projection to the piriform cortex appears distributed, with any one region of the bulb projecting throughout the piriform cortex and any one region of the piriform cortex receiving input from all regions of the bulb (Haberly 1985; Price 1987). The structure of the aPCX has led to the hypothesis that the piriform cortex functions as a distributed processing neural network and is critically involved in information processing and associative memory (Haberly 1985; Lynch and Granger 1991; Wilson and Bower 1988). Recent experimental evidence has demonstrated that synaptic strength within the piriform cortex can be modified by experience (Jung et al. 1990; Kanter and Haberly 1990; Litaudon et al. 1997; Roman et al. 1987; Stripling et al. 1992) and that the piriform cortex is active during multiple phases of olfactory learning behaviors (McCullom et al. 1991; Schoenbaum and Eichenbaum 1995).

Despite the fact that the aPCX is the primary sensory cortex in the olfactory system, relatively little is known about
basic sensory processing in this structure. Habituation is a relatively simple paradigm with which to examine both basic sensory processes and memory mechanisms in cortex. The present report describes an initial series of studies characterizing responses in the aPCX to both repeated and prolonged odor stimulation. Responses in the aPCX to odor stimuli were monitored with both extracellular and intracellular recordings and compared with simultaneous multunit responses of the ipsilateral main olfactory bulb. Responses were characterized in terms of firing rate and postsynaptic potential amplitudes and in terms of phase relationships to natural respiration cycles. The results demonstrate that habituation occurs more rapidly in the aPCX than in the olfactory bulb and that aPCX habituation reflects a decrease in excitatory drive and/or excitability of the aPCX and may also involve an enhancement in inhibition.

**METHODS**

**Subjects**

Male Long-Evans hooded rats (150–450 g) obtained from Charles River Labs were used as subjects. Animals were housed in polypropylene cages lined with wood chips. Food and water were available ad libitum. Lights were maintained on a 12:12 light:dark cycle with testing occurring during the light portion of the cycle.

**Electrophysiology**

Animals were anesthetized with urethan (1.5 g/kg) and placed in a stereotaxic apparatus. The main olfactory bulb (MOB) was exposed through a hole drilled in the dorsal surface of the skull, a second hole placed posterior to the MOB to allow access to the lateral olfactory tract (LOT), and a third hole was drilled over the aPCX, ~0–1 mm anterior to Bregma (incisor bar at ~3 mm). MOB recordings were made with tungsten microelectrodes (5–12 MΩ, A-M Systems). The LOT was stimulated with constant current square wave pulses (50–1,000 μA) with a tungsten monopolar electrode.

Olfactory bulb activity was monitored with multunit recordings from the mitral cell body layer, approximately in the middle of the anterior-posterior extent of the bulb. Multunit activity was bandpass filtered (300 Hz to 3 kHz) and passed through a window discriminator. Multunit odor responses were analyzed with peri-stimulus time histograms (PSTHs).

Extracellular single-unit activity and intracellular potentials were recorded in the aPCX. For single-unit recordings, a tungsten recording electrode (5–12 MΩ) was lowered from the dorsal skull surface. Physiological confirmation of recording electrode placement in Layer II/III of the piriform was done with LOT electrical stimulation. Recordings were done at, or slightly dorsal to, the reversal point of the LOT-evoked population potential (Haberly and Shepherd 1973). Single-units were isolated directly or were extracted through template matching (10-kHz sampling rate), using Spike2 software for the Macintosh (Cambridge Electronic Design). The single-unit nature of the recordings were confirmed with autocorrelograms showing at least a 3-ms refractory period. After the recording session, the location of the electrode tip was generally marked by electrolytic lesions, and placements in the aPCX confirmed histologically.

A dorsal approach was also used for intracellular recordings with glass microelectrodes filled with 2 M potassium acetate (tip resistance 60–150 MΩ). For intracellular recordings additional surgical procedures were performed to stabilize the preparation, including draining of the cisterna magna and stabilizing the vertebral column. Resting membrane potentials of analyzed cells were at least −60 mV and spike amplitudes were >50 mV. Extracellular DC potential was subtracted from the intracellular potential to determine resting membrane potential. Stable recordings (membrane potential and action-potential amplitude) were maintained for between 10 min and >1 h (generally 15–30 min). Cells were identified as aPCX neurons by their response to LOT stimulation. Intracellular recordings were digitized at either 5 or 20 kHz and analyzed with Spike2 software.

In addition to MOB and aPCX neural activity, respiratory activity was monitored with a piezoelectric device monitoring chest wall movements. The output of the piezoelectric device was amplified, filtered, and fed through a window discriminator that provided pulses synchronous with the transition from exhalation to inhalation.

**Odor stimulation**

Animals were freely breathing through both nares at all times and stimulated with a flow dilution olfactometer. A continuous stream (500 ml/min) of air, passed through an activated charcoal filter and humidified, was blown across the nares of the animal. Odor vapor was added to the airstream with a computer controlled Hamilton syringe pump, which forced air through odorant saturated filter paper at 50 ml/min, creating odor concentrations of 10⁻¹ of saturated vapor. Odorants used included isoamyl acetate, eugenol, anisole, and camphor (all from Sigma). No behavioral and/or respiratory responses were observed from odor stimulation at the level of anesthesia used here. In addition to respiration rate, which was monitored in all animals, respiration volume (amplitude of chest wall movements) was monitored in a small subset of animals. Neither rate nor volume was detectably changed by odor stimulation.

Habituation training consisted of either nine repetitions of 2-s stimuli with a 30-s interstimulus interval or a single, prolonged 50-s stimulus. In most cases, only one of these paradigms was used for a single odor in individual animals.

In the extracellular recording experiments, stimulus onset occurred randomly with respect to respiratory cycle, as has been the procedure in previous studies (McCollum et al. 1991; Wilson 1997). For intracellular recordings, the stimulus delivery system was modified such that stimulus onset was triggered on the respiratory cycle (at the exhalation/inhalation transition). This allowed averaging of response waveforms across repeated trials.

**Odor response analysis**

MOB and aPCX spike train responses to odors were analyzed in two ways. First, response magnitude was determined with spike counts. Spike counts (both aPCX and MOB) during the 2-s stimulus with a 30-s interstimulus interval or a single, prolonged 50-s stimulus were compared with counts during the immediate 2-s prestimulus. Response magnitude to subsequent stimuli were expressed relative to response magnitude to the first stimulus. For prolonged odor stimuli, spike counts during consecutive 10-s intervals during the stimulus were expressed relative to spike counts during the initial 10-s of stimulation.

The second method of response analysis involved relating spike activity to respiratory cycle. This method was only used with the prolonged stimulation paradigm, where sufficient aPCX activity permitted the production of accurate phase histograms. Phase histograms (6° binwidth) of MOB and aPCX spike activity relative to respiration were constructed for the following 25-s time periods: prestimulation, 0–25 s, 26–50 s of odor stimulation, immediately postodor, and after recovery from stimulation. Mean vectors (angle: 0°–360°; length: 0–1) describing neural activity and respiratory cycle phase relationships were determined for both the MOB and aPCX during each of these time periods. Minimal phase differences (i.e., 0°–180°) between the MOB and aPCX were then calculated (see Fig. 6). An example of a minimal phase difference
calculation is that the minimal difference between 340 and 20° is 40° and not 320°.

Finally, analyses of intracellularly recorded odor evoked post-synaptic potentials (PSPs) were performed on averaged waveforms (2–3 stimuli) pre- and posthabitation (repeated stimulus training). Analysis of averaged waveforms rather than single responses was preferred because it reduced minor, random fluctuations in PSP amplitude. PSPs to initial stimulation and posthabitation were analyzed by calculating the response amplitude and width (ms) at half-amplitude (half-width). In cells with multiple respiration-entrained PSPs during the 2-s stimulus, measurements were taken from a single respiration entrained PSP only. PSP amplitude was measured from baseline to peak, away from evoked action potentials (a procedure that may have resulted in slight underestimation of maximal PSP amplitude, particularly on trials early in the habituation training).

Statistical comparisons were made with ANOVAs and/or repeated measures ANOVAs with post hoc tests where appropriate, or with paired t-tests.

RESULTS

Extracellular recordings

REPEATED, SHORT-ODOR STIMULI. Responses to 9 repetitions of 2-s odor stimuli were measured in a total of 25 layer II/III aPCX single units in 9 animals. No clear differences were noted in habituation between odorants tested (isoamyl acetate, eugenol, anisole, camphor); thus, responses to all stimuli were combined for statistical analyses.

Odor stimulation produced an increase in MOB multiunit activity throughout the duration of the stimulus, often strongly entrained to the respiratory cycle. In contrast, as previously reported (Wilson 1997), simultaneously recorded aPCX responses were frequently very brief, often consisting of a single initial burst of activity at odor onset (Fig. 1).

This brief response evoked by a single stimulus was mirrored by a rapid habituation to repeated stimuli. As shown in the examples in Fig. 2A, despite a relatively well-maintained MOB multiunit response to repeated stimuli (30-s interstimulus interval), aPCX single-unit responses habituated rapidly and completely within 5–10 stimuli. This rapid aPCX habituation appeared to occur with both excitatory and suppressive responses (Fig. 2A), although the present report will focus on excitatory responses unless otherwise noted. The relatively maintained MOB response suggests that aPCX habituation cannot be accounted for by a decrease in intensity of the stimulus itself.

A quantitative analysis demonstrated that response magnitude in both the MOB and aPCX decreased with repeated stimulation, although significantly more so in the aPCX (Fig. 2B). After nine repetitions of 2-s odor stimuli, MOB response magnitude was ~75% of initial values. This amount of habituation in multiunit responses corresponds well with a previous report from our lab on mitral/tufted cell single-unit habituation by using similar stimulation parameters (Wilson and Sullivan 1992). In contrast, aPCX response magnitude was reduced to nonsignificant levels within 6–9 stimuli. A two-way, repeated measures analysis of variance (ANOVA) (brain region X trial number) showed a significant difference between brain regions [F(8,256) = 11.30, P < 0.01]. Posthoc Fisher comparisons revealed that aPCX relative response magnitude was significantly less than MOB relative response magnitude by the fourth stimulus (P < 0.05).

PROLONGED ODOR STIMULATION. Responses to prolonged, 50-s odor stimulation were examined in 30 layer II/III aPCX neurons in 10 animals. As above, no clear differences were noted in habituation between odorants tested; thus, responses to all stimuli were combined for statistical analyses.

As with repeated, short stimuli, the aPCX single-unit response magnitude to prolonged stimulation declined more rapidly than MOB response magnitude (Fig. 3). Examples of two aPCX units recorded simultaneously with MOB multiunit responses are shown in Fig. 3A. In this example, both the MOB and aPCX units show an initial rapid increase...
In a small subset of cells, habituation selectivity was tested. Habituation selectivity was tested in four cells that gave reliable responses to at least two different odors. Response magnitude to one odor (test odor) was examined with a single 2-s stimulus. The cell was then habituated to a second odor with a prolonged, 50-s stimulus. At variable intervals after the end of the habituating stimulus, the test odor and the

![Fig. 2](image-url)

**Fig. 2.** Rapid habituation of aPCX single-unit responses to repeated 2-s odor stimuli. A: raster plots of representative aPCX unit responses and a MOB multiunit response to repeated 2-s stimuli [interstimulus interval (ISI) averaged 30 s]. Each horizontal line represents a stimulus repeat. Note that both excitatory (aPCX #1 and #2) and suppressive (aPCX #3) aPCX responses habituated nearly completely within 5–10 stimuli. MOB response to odor was reduced but did not completely habituate. (Examples were not from simultaneous recordings.) B: mean response magnitude (as a percent of response magnitude to 1st stimulus) to repeated stimuli in MOB and aPCX (n = 25 aPCX neurons). aPCX single-unit habituated significantly more rapidly than MOB (*significant difference between aPCX and MOB, P < 0.05).

![Fig. 3](image-url)

**Fig. 3.** Rapid habituation of aPCX single-units to prolonged odor stimulation. A: representative PSTHs of 2 aPCX single-units and MOB multiunit activity recorded simultaneously in response to a 50-s odor stimulus. B: simultaneously recorded multiunit aPCX activity and multiunit MOB activity in response to a 50-s odor stimulus. C: mean relative response magnitude (as percent of activity evoked during 1st 10 s of stimulation) to prolonged odor stimulation in MOB and aPCX (n = 30 aPCX neurons). aPCX activity habituated significantly more than MOB activity by last half of prolonged stimulus (*significant difference between aPCX and MOB, P < 0.05).
habituated odor were repeated as 2-s stimuli. Figure 4 shows examples of two different aPCX neurons stimulated with this paradigm. For the cell in Fig. 4A, prolonged eugenol stimulation reduced the response to a subsequent 2-s eugenol stimulus, but had no clear effect on the response to isoamyl acetate (35 spikes evoked during the initial 2-s ISO stimulus, 32 spikes evoked by the 2nd). For the cell in Fig. 4B, habituation to anisole had no obvious effect on the subsequent response to isoamyl acetate (5 spikes during the initial ISO stimulus, 7 spikes during the 2nd), although the anisole response remained diminished (note the difference in time scale in Fig 4, A and B). In the four cells tested no evidence of cross-habituation was found [mean response magnitude to the test stimulus was 119 ± 12% (mean ± SE) of prehabituation magnitude]. Thus, habituation in the aPCX appears to be odor specific, although this issue must be addressed with a more thorough set of odors.

A second method of analyzing odor response patterns is to express spontaneous and evoked activity as a function of respiratory cycle (Chaput et al. 1992; Macrides and Chorover 1972). The prolonged odor stimulation paradigm allowed sufficient sampling of activity to perform such an analysis even on slow firing aPCX neurons. An example is shown in Fig. 5. Activity of an aPCX single-unit in response to isoamyl acetate stimulation (2 s) is shown in Fig. 5A. The events marked “inhalation trigger” correspond to the transition from exhalation to inhalation as determined by chest wall movement, shown as the cyclic waveform at top. As can be seen, this aPCX unit fired in bursts at a regular relationship to respiration during odor stimulation. The cyclic nature of odor-evoked unit activity can be expressed in a phase histogram (Fig. 5B) and be quantified as a mean vector (Fig. 5C), where the angle (0–360°) reflects the mean phase relationship of unit activity with respect to the respiratory cycle and the length of the vector (0–1) reflects the relative spread or variability of the phase distribution (Chaput et al. 1992).

By using mean vector analysis, both MOB and aPCX activity can be expressed relative to the respiratory cycle over the course of prolonged odor stimulation. Figure 6A shows rasterplots of MOB multiunit and simultaneously recorded aPCX single-unit activity before, during, and after 50-s odor stimulation. Each horizontal line in the raster plot represents one respiratory cycle, initiated at the exhalation/inhalation transition. Respiration rate remained stable throughout the recording session, centering on a 420-ms
The respiratory cycle. Mean vectors for the MOB moved to $\sim 245^\circ$ and the aPCX mean vector moved to $\sim 190^\circ$. This relative phase shift disappeared by the last half of the stimulus (stimulus 26–50 s), with both MOB and aPCX vectors $\sim 245^\circ$. Poststimulus activity rapidly returned to baseline patterns.

This example demonstrates that the relationship between the MOB and aPCX, relative to the respiratory cycle, is dynamic and that similar to response magnitude, aPCX odor-induced phase shifts habituate rapidly. Although the MOB multiunit phase relationship to respiration varied slightly in individual animals (Chaput et al. 1992), grouped data show that the MOB mean vector was relatively stable across the prolonged odor stimulus (Fig. 7A). The minimum phase difference between MOB and aPCX activity, before, during and after odor stimulation was quantified for each aPCX neuron. On the basis of this preliminary analysis, it appeared that aPCX neurons could be separated by their initial response to odor. A histogram of the minimum phase difference at odor onset (1st 25 s of odor stimulation) between aPCX unit activity and simultaneously recorded MOB multiunit activity is shown in Fig. 7B. At odor onset, half of the recorded aPCX neurons fired out of phase with MOB activity (large phase difference), while the other half fired relatively in phase with the MOB (small phase difference). On the basis of this histogram, the aPCX neurons were dichotomized for analysis purposes (2 groups separated by the arrow in Fig. 7B).

As shown in Fig. 7C, on average, aPCX and MOB prestimulation activity occurs $\sim 90^\circ$ out of phase relative to the respiratory cycle. At odor onset (1–25 s) however, one subgroup of aPCX neurons (out phase; $n = 15$ out of 30) shifted to a nearly $150^\circ$ phase difference from the MOB, relative to the respiratory cycle. (Phase shifts can occur in either direction—leading or lagging. The present analysis only addresses absolute value of the shift). The second subgroup (in phase; $n = 15$ of 30) began to fire at odor onset in phase with the MOB relative to respiration, with a mean phase difference of $30^\circ$. An example of an in phase aPCX neuron is shown in Fig. 13. There was no significant difference in preodor spontaneous activity in the two groups of neurons (in phase spontaneous activity $= 0.77 \pm 0.24$ Hz; out phase $= 1.07 \pm 0.24$ Hz; not significant).

Although MOB activity remained relatively locked to a particular phase of respiration throughout the prolonged stimulation (Fig. 7A), aPCX activity did not. The phase shifts in aPCX activity observed at odor onset diminished by the second 25 s of stimulation (Figs. 6 and 7), at a time when aPCX response magnitude had also diminished (Fig. 3). At odor offset (post and recovery time points), aPCX and MOB phase relationships relative to respiration returned to prestimulus values, occasionally with an initial ‘‘rebound’’ effect as shown in Fig. 7C and as discussed below. Statistical analysis of minimal phase differences between MOB and aPCX activity over the course of prolonged odor stimulation (Fig. 7C) revealed a significant difference between in-phase and out-phase neurons [repeated measures ANOVA, cell type X time interaction, $F(4,112) = 15.15, P < 0.01$]. Post hoc Fisher comparisons revealed significant differences between in-phase and out-phase neurons during odor stimulation and during the immediate poststimulation period ($P < 0.05$). Furthermore, although both In phase and Out phase neurons showed an initial significant change (Fisher, $P <$
FIG. 6. Representative simultaneous aPCX and MOB recording showing aPCX phase shift at onset of prolonged odor stimulation. A: raster plots of MOB multiunit and aPCX single-unit activity. Consecutive horizontal lines are consecutive respiratory cycles, with cycle trigger at time 0. Odor was presented for 50 s during vertical line. B: interval histogram (5-ms binwidth) of respiratory activity (exhalation to inhalation transition events), showing stability of respiratory cycle over course of this experiment. C: mean vector plots of MOB and aPCX unit activity as a function of respiratory cycle. Prestimulus activity in both MOB and aPCX occurs at nearly same phase of respiration. At odor onset (stimulus 1–25 s) aPCX activity changes its phase relationship to respiration, while MOB shows a much smaller deviation. This results in a separation of activity in MOB and aPCX relative to respiration. During last half of odor stimulus (stimulus 25–50 s), phase mismatch between MOB and aPCX is no longer apparent and at odor offset (post-stimulus) activity in both structures returns to near prestimulus values.

0.05) in minimal phase difference with the MOB at odor onset, these shifts were not significantly different from preodor values during the second half of the stimulus.

POSSIBLE MECHANISMS. A decrease in excitatory responses to odor stimulation may be due to decreases in excitatory drive or excitability and/or increases in inhibition. Several observations of extracellular unit activity suggest that the reduction in aPCX responses to repeated or prolonged odor stimulation may, in part, reflect active processes such as enhanced inhibition. For example, in three cells (of 25), repeated short odor stimulation resulted in a switch from net excitatory responses to net suppressive responses, despite continued excitatory responses in the MOB (e.g., Fig. 8A). Another example of a switch from net excitation to net suppression was observed in the prolonged odor stimulation paradigm (Fig. 8B). The cell shown in Fig. 8B displayed an increase in activity at odor onset which shifted to a decrease in activity to below spontaneous levels by the end of the stimulus. At odor offset, there appeared to be an excitatory rebound.

The rebound phenomenon at odor offset was also apparent in the respiratory cycling of some cells. For example, the cell shown in Fig. 9 fired in phase with the MOB relative to respiration during prestimulus activity. At odor onset, the
As can be seen in the rasterplot, aPCX activity immediately post odor is nearly the mirror image of that at odor onset. At recovery, the aPCX mean vector angle returned to a near prestimulus value. MOB mean vector angle did not shift more than 40° throughout this recording.

Intracellular recordings

A total of 23 intracellular recordings was made from aPCX neurons in 15 animals. Mean resting membrane potential was $-72.5 \pm 1.8$ mV (11 cells with $V_m$ between $-60$ and $-70$ mV and 12 cells with a $V_m$ of at least $-70$ mV). aPCX neurons were generally identified by their synaptic response to LOT stimulation (Fig. 10A). This response consisted of a short latency excitatory postsynaptic potential (EPSP), often resulting in an action potential and followed by a prolonged inhibitory postsynaptic potential (IPSP) (Scholfield 1978). Figure 10 also shows examples of excitatory and inhibitory aPCX responses to odor. Figure 10, B and C, shows simultaneous recordings of MOB multiunit activity and an excitatory aPCX response. The increase in aPCX activity was associated with a membrane depolarization of 5–10 mV. The shape and time course of membrane depolarizations varied in different cells. The cell in Fig.

aPCX neuron shifted out of phase with the MOB and the strength of this phase shift decreased by the second half of the stimulus (decrease in vector length). At odor offset, the mean vector angle of the aPCX neuron shifted nearly 180°.
10CI showed a prolonged depolarization lasting the duration of the 2-s stimulus. Most neurons, however, showed more cyclic and/or brief depolarization’s. For example, the cell depicted in Fig. 11 in response to aniseole showed a brief depolarization to the initial respiratory cycle, with much reduced or absent depolarization’s on subsequent inhalations. The neurons in Fig. 13 responding to eugenol and isooamyl acetate, on the other hand, showed cyclic depolarizing-hyperpolarizing sequences over several respiratory cycles. Predominantly inhibitory responses could also be observed, as shown in Fig. 10C2. The cessation of spontaneous activity was associated with a measurable membrane hyperpolarization. However, as with the extracellular data above, only initially excitatory responses were analyzed here.

REPEATED, SHORT ODOR STIMULI. Intracellular responses to repeated, 2-s odor stimuli were measured in a total of 11 layer II/III aPCX neurons. As with the extracellular recordings, aPCX responses habituated rapidly to repeated
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Aged response waveform to 3 stimuli. After repeated odor stimulation, the magnitude of the aPCX odor evoked depolarization was reduced, such that no odor-evoked spikes were observed (Fig. 11A; averaged response to 3 stimuli). Note that both the amplitude and the duration of this evoked PSP decreased with habituation. Resting membrane potential did not substantially change over the course of the habituation training in this example.

Odor-evoked PSP amplitude and half-width duration were quantified in averaged responses (Fig. 11B). In cells demonstrating multiple PSPs to single stimuli, only one odor-evoked PSP was measured within the response. Paired t-tests showed a significant reduction in both odor-evoked aPCX PSP amplitude (66% of initial amplitude; Fig. 11B, paired t-test of mV data, t(10) = 3.65, P < 0.01) and half-width with repeated odor stimulation (73% of initial duration, Fig. 11B, paired t-test of ms data, t(10) = 3.81, P < 0.01). No consistent change in resting membrane potential was observed over the course of training (mean within cell change in Vm = 2.5 ± 1.3 mV; 2 cells hyperpolarized, 5 cells depolarized, and 4 cells showed no change). Thus while MOB multiunit input to the aPCX was reduced to 80% of initial values by repeated odor stimulation, aPCX PSP amplitude was reduced to 66% and duration to 73% of initial values.

PROLONGED ODOR STIMULATION. Intracellular responses to prolonged, 50-s odor stimulation were examined in 12 layer II/III aPCX neurons. Particular attention was paid to odor evoked changes in respiration entrained membrane potential oscillations. As with 2-s duration stimuli, responses evoked by prolonged stimulation were generally depolarizing/hyperpolarizing oscillations linked to the respiratory cycle. A typical example is shown in Fig. 12. In this example, before odor onset, both MOB activity and aPCX spiking activity were loosely locked to respiration, with a 140° phase difference between the aPCX and MOB relative to the respiratory cycle. aPCX membrane potential (average of 10 respiratory cycles, triggered on the exhalation/inhalation transition) mirrored the aPCX spiking temporal pattern, with a weak depolarizing/hyperpolarizing sequence over a single respiratory cycle. At odor onset, MOB activity become more strongly entrained to respiration, with a slight phase shift. aPCX spiking activity, however, showed a very strong phase shift, with a mean vector angle nearly perfectly matching the MOB (example of an in-phase neuron). This aPCX phase shift was associated with a pronounced increase in the amplitude of the depolarizing/hyperpolarizing PSP sequence. By midstimulus (15-s postodor onset), while MOB activity remained strongly entrained to respiration at a similar mean vector angle, aPCX activity demonstrated another phase shift back toward the resting phase angle. aPCX membrane potential similarly returned to the preodor pattern, with a weak depolarizing/hyperpolarizing sequence. Importantly, this return to prestimulation PSP patterns occurred despite the maintained MOB odor-evoked activity. As shown in Fig. 12, no clear change in resting membrane potential was noted. After a brief recovery period, the cell was restimulated with a 2-s pulse of isoamyl acetate. As shown in Fig. 12, this repeat stimulus again produced a shift in the aPCX activity vector and a similar depolarizing/hyperpolarizing sequence of membrane oscillation.

FIG. 10. Examples of intracellular recorded aPCX neuron responses to stimulation. A: typical aPCX neuron response to lateral olfactory tract (LOT) electrical stimulation. B: PSTH of MOB response to isoamyl acetate, recorded simultaneously with aPCX neuron shown in C1. C1: aPCX intracellular response to odor. Note prolonged membrane depolarization and associated increase in spiking. Depolarizing responses were frequently much shorter. C2: a different aPCX neuron showing a predominant hyperpolarization to isoamyl acetate stimulation. Odor stimulus duration in B and C is 2 s, voltage calibration is 25 mV for A and C, and time calibration for A is 50 ms. Resting membrane potential is shown to right of trace in this and subsequent figures.

Odor stimulation, despite relatively minor MOB multiunit habituation (MOB multiunit response magnitude at end of habituation training, mean = 80 ± 19% of initial magnitude). An example is shown in Fig. 11. The initial aPCX response to anisole stimulation was a brief burst of action potentials at odor onset lying on top of a pronounced 5–7 mV depolarization, with smaller depolarizations not evoking spikes occurring on subsequent inhalations (Fig. 11A, averaged response waveform to 3 stimuli).
FIG. 11. **A**: example of habituation of aPCX postsynaptic potential (PSP) responses to repeated anisole. This neuron displayed a single depolarization at odor onset that decreased in amplitude and duration during habituation. Response waveforms are averages of three consecutive responses. Action potential amplitude decreased because of averaging. Calibration for both records is 5 mV.

**B**: mean amplitude (left) and half-width (right) of odor-evoked PSP before and after habituation. Both amplitude and half-width were significantly reduced by habituation training (* difference from initial value, P < 0.05).

PSP morphology, while generally including both depolarizing and hyperpolarizing components over the respiratory cycle, varied between cells. Examples are shown in Fig. 13 from two different cells responding to two different odors. The eugenol odor-evoked response of the cell in Fig. 13 consisted of an increase in the amplitude of a relatively simple depolarization, bounded by hyperpolarizing waves. The amplitude of the hyperpolarization decreased over the course of prolonged stimulation. The cell responding to isoamyl acetate showed a much more complex odor-evoked PSP. At odor onset, two to three depolarizing waves were apparent, again bounded by hyperpolarization. The amplitude of these depolarizing PSPs decreased with extended stimulation, such that by the end of the prolonged stimulus, the middle of the respiratory cycle was again dominated by hyperpolarization. The cell tested with isoamyl acetate was also tested for recovery of odor response after a period of nonstimulation. Restimulation with isoamyl acetate evoked a similar depolarizing response after recovery (data not shown). These shifts in the time and magnitude of different PSP components may explain the phase shifts in activity relative to respiration observed above.

The amplitude of odor-evoked PSPs was measured in respiration triggered, average waveforms taken over 10-s intervals (~20 respiratory cycles) of the 50-s stimulus. Because PSPs appeared to include both depolarizations and hyperpolarizations, PSP amplitude was measured as the maximal peak to peak amplitude. Mean PSP amplitude measured in this way for the initial 10-s stimulus period was 4.4 ± 0.53 mV (n = 12). PSP amplitude in subsequent 10-s intervals was expressed as a percentage of the initial 10-s value and is shown in Fig. 13, along with MOB multiunit odor response magnitude from simultaneous recordings. Respiration entrained, odor-evoked PSP magnitude in the aPCX declined over the course of prolonged odor stimulation significantly greater than the MOB multiunit response [repeated measures ANOVA, area X time interaction: F(4,88) = 3.09, P < 0.05]. aPCX response magnitude declined to ~46% of initial levels by the end of the 50-s stimulus, while MOB PSP amplitude declined to ~90% of initial levels (post hoc Fisher tests revealed a significant difference between the MOB and aPCX at the last 3 time points, P < 0.05).

**DISCUSSION**

The present results describe a form of nonassociative plasticity in the aPCX. The response of aPCX layer II/III neurons to odors is decreased by repeated or prolonged odor stimulation. The depression of aPCX single-unit spiking responses is greater than the depression of activity of the primary aPCX afferent, the ipsilateral MOB. That is, relatively small decreases in MOB input to the aPCX during odor stimulation are associated with larger decreases in aPCX output. The decrease in aPCX spiking activity is associated with a decrease in both the magnitude and duration of intracellularly recorded, odor-induced PSPs. These changes do not appear to be the result of generalized changes in aPCX or CNS excitability given that the habituation is odor specific. Furthermore, the effects are repeatable within cells, suggesting that deterioration of recording conditions cannot account for the results.

Anatomically, the MOB projection to the piriform is divergent and nontopographic, with any one region of the MOB projecting to wide areas of the piriform and any one
FIG. 12. Representative example of intracellular recorded aPCX neuron response to prolonged odor stimulation. Top: baseline and onset of extended (50 s) isoamyl acetate stimulus. PSTH (100 ms bins) of MOB activity shows increased peak activity and cyclic, respiration entrainment throughout initial 10 s of stimulation displayed. aPCX activity is initially also entrained to respiration (emphasized by vertical lines) but rapidly returns to prestimulus patterns (▲). Bottom: phase histograms, vector plots and mean aPCX membrane potential responses during 10 respiratory cycles of preodor, odor onset, mid odor (15 s into stimulus), and recovery. During preodor period, MOB, and aPCX activity are weakly out of phase to each other with respect to respiration. aPCX membrane potential remains relatively flat over respiratory cycle. At odor onset, MOB and aPCX activity occur in phase with each other with respect to respiration. Respiration entrained peaks in MOB and aPCX activity are associated with a strong depolarization in aPCX membrane potential. However, by mid-stimulus, despite a continued strong respiratory phase relationship in MOB, aPCX activity, and membrane potential return to preodor patterns. After a 2-min recovery period, repeat stimulation with isoamyl acetate produced a similar odor evoked response in this cell, demonstrating that habituation of odor response was not the result of a deterioration of recording. Vectors are plotted in a circle with radius 0.6. Calibration for intracellular records is 5 mV and 200 ms.
region of the piriform receiving input from wide regions of the MOB (Haberly 1985; Price 1987). Thus each aPCX principal neuron receives input from many MOB mitral/tufted cells in scattered areas of the bulb. Given the nature of this projection, the present experiments monitored MOB activity with multiunit recordings to allow sampling of the population of inputs to the aPCX, rather than single-unit recording from mitral/tufted cells that may or may not have contacted the recorded aPCX neuron. Although multiunit recordings can mask changes in individual neuron firing patterns, the present estimates of MOB habituation to repeated odors corresponds well with previous work in our lab on single-unit mitral/tufted cell habituation using similar odor presentation protocols (Wilson and Sullivan 1992).

It should be noted, however, that recent work suggests that the MOB projection specifically to the aPCX may not be entirely random, with instead very small, localized populations of mitral cells projecting to discrete regions of the aPCX (Buonviso et al. 1991; Ojima et al. 1984). Depending on the extent of this topography in microcircuitry, future correlations of MOB and aPCX activity may require a more thorough sampling of MOB activity to ensure that the MOB

![Image of a figure showing respiration-triggered PSPs before and during odor stimulation in 2 cells from 2 different animals.](http://jn.physiology.org/)

**FIG. 13.** A: respiration-triggered PSPs before and during odor stimulation in 2 cells from 2 different animals. PSP averages taken over 10-s periods (averages of ~20 traces, spike amplitudes clipped by averaging). At top is average respiratory waveform and cumulative PSTHs of MOB multiunit activity (triggered on respiratory cycle) taken over course of 50-s stimulus. Horizontal lines on aPCX PSPs represent approximate resting membrane potential for each cell, i.e., prominent odor-evoked waveforms that show habituation. Note hyperpolarizing PSP in cell stimulated with eugenol and depolarizing PSP in cell stimulated with isoamyl acetate. Over course of extended odor stimulus, PSP amplitudes in both cells decreased. **B**: mean relative intracellular PSP amplitude (as percent of amplitude evoked during 1st 10 s of stimulation) to prolonged odor stimulation in aPCX and simultaneously recorded MOB multiunit activity. Habituation of aPCX PSP amplitude was significantly greater than habituation of MOB multiunit activity over course of prolonged stimulation. * Significant difference between MOB and aPCX, P < 0.05.
region presynaptic to the aPCX neuron of interest is accurately monitored. Nonetheless, in the present report, similar results were obtained despite variation between and within animals in precise MOB and aPCX recording placement, suggesting that the enhanced habituation in aPCX relative to MOB is robust and not dependent on mismatch between recording sites in the two structures.

The single-unit aPCX spike train responses to odor stimulation reported here were similar to previous reports (Duchamp-Viret et al. 1996; Giachetti and MacLeod 1975; Haberly 1969; McCollum et al. 1991; Nemitz and Goldberg 1983; Schoenbaum and Eichenbaum 1995; Tanabe et al. 1975; Wilson 1997). aPCX responses were frequently brief, despite maintained odor driven MOB input. Odor-induced firing was often oscillatory, occurring at set phase relationships to the respiratory cycle. This oscillatory activity in the aPCX could occur in phase with MOB input or out of phase and changed during prolonged stimulation. Within the respiratory cycle oscillations, odor driven instantaneous firing frequencies were generally 50–100 Hz, although values as high as 300 Hz were observed (based on intracellular recordings only). These frequencies include the range of previously described odor-evoked gamma frequency oscillations (40–60 Hz) in piriform and olfactory bulb local field potential recordings (Eeckman and Freeman 1990; Ketchum and Haberly 1993).

Intracellular PSP responses to odors included both depolarization and hyperpolarization. These responses are similar to those reported in the only other intracellular study of piriform responses to odors (Nemitz and Goldberg 1983). The present experiments, however, allowed odor-induced PSPs to be more accurately monitored because of the synchronization of odor stimulation and respiratory cycle. In the present study, odor-induced PSPs were often oscillatory over the respiration cycle, similar to spike trains, with depolarization occurring either in phase, or out of phase with MOB multiunit activity. In some cells, an odor-induced PSP was observed on the first respiratory cycle of the stimulus and not on subsequent cycles, despite maintained MOB multiunit activity. It should be noted, however, that a similar phenomenon of responding only to the first inhalation of an odor has been reported in a small subset of intracellularly recorded MOB mitral (Wells et al. 1989) in studies using artificial sniffs. Furthermore, a subset of neurons in the frog primary olfactory cortex respond only very briefly (often with only a single spike) at odor onset (Duchamp-Viret et al. 1996).

The differential role of LOT and association afferents in driving depolarizing/hyperpolarizing odor-induced PSPs is not known. Electrical stimulation of either the LOT or association fibers in piriform cortical slices induces one or more EPSP-IPSP sequences in pyramidal cell neurons (Ketchum and Haberly 1993; Scholfield 1978). Similarly, in the present in vivo experiments, both electrical stimulation of the LOT and odor stimulation evoked apparent EPSP-IPSP sequences (Fig. 10). The duration of the depolarizing component, in particular, was substantially longer in odor-evoked responses than in electrical stimulation evoked responses, although this would be expected given the relatively prolonged, nonsynchronous activation of mitral cells by odorants during natural respiration.

**Respiration and olfactory system activity**

Odor information enters the mammalian olfactory system in relatively discrete temporal units, in synchrony with the respiratory cycle (Macrides and Chorover 1972). The respiratory entrainment of olfactory system activity has been studied in most detail in the olfactory bulb (e.g., Chaput et al. 1992; Macrides and Chorover 1972). Individual mitral/tufted cells demonstrate one of a variety of respiration related firing patterns during odor stimulation, although the majority have peak periods of activity near the inspiration/expiration transition (Chaput et al. 1992). The present result of MOB multiunit activity mean vector angle of 200–250° (Fig. 7) corresponds very well with Chaput et al. (1992) single-unit data (mean vector angle ~220°), despite the difference in techniques in the two studies.

The results reported here demonstrate that activity in the aPCX is also entrained to the respiratory cycle, although aPCX temporal patterns may be more dynamic than observed in the MOB. During prestimulus activity, aPCX membrane potential was characterized by small depolarizing/hyperpolarizing sequences over the respiratory cycle. At odor onset, at least two odor-induced respiratory phase relationships were observed, with half of the aPCX neurons firing at a different phase of respiration than the MOB and half firing at a similar phase of respiration as the MOB. The phasic firing patterns appear to be due to odor-induced increases in the hyperpolarizing and depolarizing PSPs over the respiratory cycle. Although these PSP can be very complex, in general, cells display either a hyperpolarizing-depolarizing sequence or a depolarizing-hyperpolarizing sequence over a single respiratory cycle (e.g., Fig. 13). Intracellular recording and labeling will allow correlation of firing pattern and morphology to begin to determine the significance of these two different response classes.

The odor-induced enhancement in respiration-locked PSPs and the changes in synchrony of aPCX and MOB oscillations relative to the respiratory cycle may be critically involved in coding olfactory information (Laurent 1996). With extended odor stimulation, the magnitude of these PSPs decrease and phasic aPCX firing patterns return to baseline, despite continued phasic MOB input (e.g., Figs. 7 and 12). This shift in temporal patterning of aPCX activity relative to the MOB and respiration may be as important an indicant of aPCX habituation as changes in spike frequency (Laurent 1996; Ravel et al. 1997).

The use of natural respiration as opposed to artificial sniffs complicates analysis of synaptic events underlying odor-evoked PSPs in the aPCX. However, the aPCX and olfactory system function under conditions of natural respiration (which involves both inhalation and exhalation), not artificial, square wave inhalations. The present results demonstrate, that under natural (although anesthetized) stimulation conditions, aPCX neurons demonstrate strong oscillations in membrane potential, frequently showing odor evoked depolarization bounded by hyperpolarization within single respiratory cycles. Repeated or extended odor stimulation can result in a decrease in the amplitude (and duration) of both of these components.

**Mechanisms of habituation**

Repeated or prolonged odor stimulation significantly reduced aPCX responsiveness to subsequent stimulation, as
measured both in spike-train frequency and PSP amplitude and duration. Importantly, a similar rapid decrease in responsiveness to odors was observed in the majority of Layer II piriform cortex neurons recorded in awake, freely moving rats (McCollum et al. 1991), suggesting that the results reported here are not specific to the anesthetized preparation. Habituation of aPCX spike trains was more rapid and/or more complete than MOB multiunit habituation. Three potential mechanisms can be posited for this apparent enhanced habituation in the aPCX compared with its primary sensory afferent. Repeated or prolonged odor stimulation may 1) decrease excitatory drive of aPCX neurons (via afferent and/or association fibers), 2) decrease postsynaptic excitability of aPCX neurons, and 3) increase inhibition of aPCX neurons. These mechanisms are not mutually exclusive and may all contribute to the observed results. Each of these mechanisms is discussed below.

First, the excitatory drive of aPCX neurons is unquestionably decreased by habituation training. As demonstrated by the multiunit MOB recordings, mitral/tufted cells have reduced firing rates to repeated odors (Fig. 2). This decrease in excitatory drive reduces aPCX odor-evoked PSP amplitude (Fig. 11), which in turn reduces odor-induced aPCX spiking (Fig. 2). The reduction in primary afferent evoked spiking may reduce recurrent excitation mediated by intracortical association fibers, thus further reducing aPCX excitation (Ketchum and Haberly 1993). The result would be an amplified, nonlinear decrease in aPCX output for a small reduction in input firing frequency, as is demonstrated in the present report. Thus the aPCX, using a very simple threshold mechanism, could filter out repetitive, nonsignificant inputs from the MOB.

A second potential mechanism that may be involved in habituation of aPCX neuron odor responses is a decrease in excitability of aPCX neurons. A decrease in excitability could occur through a mechanism such as long-term depression (LTD) (Linden and Connor 1995) or changes in activity of modulatory inputs. The present data do not specifically address this possibility, although intracellularly recorded PSP amplitude decreased in both habituation paradigms significantly greater than the decrease in MOB firing frequency. Any change in excitability would need to be input/pattern specific given that habituation as induced here was odor specific (Fig. 4). An examination of response threshold to electrical and odor stimulation may help identify excitability changes. Current models of plasticity and memory formation in the piriform include LTD as a critical component of the piriform synaptic network (Hasselmo and Barkai 1995) and the firing patterns of aPCX neurons to odor stimulation described here could support either LTD or LTP. The time course of habituation effects was not quantitatively assessed in the present study. Qualitatively, large decrements in response magnitude generally lasted several minutes (e.g., Fig. 4), although rarely >10 min. A more detailed examination of the time course of habituation will help isolate potential mechanisms.

Centrifugal modulatory inputs can also affect postsynaptic excitability. Previous work in our laboratory and other laboratories (Grajski and Freeman 1989; Potter and Chorover 1976; Scott 1977; Wilson and Sullivan 1992) has demonstrated that habituation and plasticity in the MOB is influenced by centrifugal modulatory inputs, such as norepinephrine (Gray et al. 1986; Sullivan et al. 1989). Similarly, acetylcholine has been shown to modulate piriform excitability and olfactory system plasticity (Hasselmo and Barkai 1995; Ravel et al. 1994).

Finally, habituation of aPCX odor response magnitude and duration could be due to an enhancement in inhibition. Several observations support this hypothesis. First, as shown in the intracellular records, inhibition is a critical component of aPCX neuron odor responses. Inhibition is generally evoked after strong, odor-induced depolarization (e.g., Fig. 12) and is also routinely observed shaping aPCX firing patterns over the respiratory cycle (e.g., Fig. 13). Second, in the extracellular recording experiments, several cells were observed that changed their net odor response from excitation to inhibition over the course of habituation training (e.g., Fig. 8). Third, in the intracellular records, odor-evoked PSP half-width was reduced by habituation, which could be due to either a reduction in association fiber mediated recurrent excitation (see above) or to an enhancement in inhibition. The odor-evoked PSP in Fig. 11 in response to anisole, for example, shows a reduction in excitatory PSP half-width coincident with an apparent increase in amplitude of a late hyperpolarizing PSP. However, no evidence of a sustained, tonic hyperpolarization, as has been recently observed during adaptation in the visual cortex (Carandini and Ferster 1997), was detected. Finally, experience-dependent enhancement of inhibition has been previously demonstrated in the olfactory system as an effective means of storing information (Wilson et al. 1987). To completely account for the observed results, as with the other mechanisms, enhanced inhibition must be odor specific to account for the lack of cross habituation. Pharmacological manipulations will be required to further test this hypothesis.

In comparing MOB and aPCX habituation rates, it should also be noted that the aPCX projects heavily back to the MOB, terminating primarily on inhibitory granule cells (Price and Powell 1970). Thus a reduction in aPCX odor-evoked activity could result in disinhibition of MOB mitral/tufted cells. Enhanced aPCX habituation, therefore, could actually help maintain MOB responsiveness. Clearly, more detailed analysis, perhaps using a combination of local electrical stimulation and odor stimulation, unilateral stimulation of aPCX neurons with binaral receptive fields (Wilson 1997) and pharmacological manipulations will be necessary to localize and identify specific mechanisms of this simple form of olfactory memory.

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