Odor Specificity of Habituation in the Rat Anterior Piriform Cortex

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INTRODUCTION

Receptive fields of cortical sensory neurons are dynamic and can be shaped by experience. Plasticity of receptive fields can occur rapidly and may be expressed as temporary, reversible fluctuations in receptive field size or the strength of coupling of the neuron to an ensemble, or as long-term changes in connectivity (e.g., Edeline 1999; Kaas 1991; Recanzone et al. 1992; Weinberger 1998).

In the primary olfactory cortex (piriform cortex) of freely breathing rats, exposure to an unreinforced odor for < 1 min produces a rapid reduction in single-unit activity evoked by that odor (McCollum et al. 1991; Wilson 1998a). This reduction in odor-evoked activity in cortical neurons occurs despite the maintained odor-evoked input from the primary afferent to piriform neurons, the mitral cells of the main olfactory bulb (Wilson 1998a). In addition to a reduction in odor-evoked firing rate during odor habituation, the temporal pattern of cortical unit activity relative to mitral cell input also changes. Thus unreinforced odor exposure reduces piriform cortex responsiveness to mitral cell input, effectively isolating cortical units from the neural circuit processing that particular odor. In fact, odor habituation is associated with depression of mitral cell–piriform cortex neuron synaptic strength (Wilson 1998b). This synaptic depression recovers with a time course similar to the recovery of odor responses (~100–120 s) and thus could be a mechanism of reduced cortical responsiveness to afferent input during habituation.

An important aspect of understanding the nature of this form of rapidly induced cortical plasticity is to determine its specificity. That is, does unreinforced odor exposure produce a generalized depression of cortical unit responsiveness to mitral cell input, or does it modify the receptive field of the piriform unit to reduce responsiveness to some odors/inputs while sparing others? Modeling of rat piriform cortex odor responses suggests that a single odor may activate as few as 2–10% of synapses on a single piriform cortex layer II/III neuron (McCollum et al. 1991). Thus it seems possible for habituation to one odor to leave the large majority of afferent synapses on piriform cortex neurons unaffected.

Preliminary examination of habituation odor-specificity in anterior piriform cortex (aPCX) single units suggests that there is minimal cross-habituation between odors of structurally dissimilar molecules (Wilson 1998a). In this study, habituation odor specificity in aPCX single units was further examined by determining cross-habituation between markedly different odorants, between straight-chain alkane odorants varying in length by two carbons, and between binary mixtures and their components. The results suggest that habituation of aPCX odor responses is highly specific with minimal cross-habituation between either single odors or between mixtures and their components. These results have important implications not only for understanding odor habituation, but also for aPCX odor coding in general.

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. A hole was placed posterior to the olfactory bulb to allow access to the lateral olfactory tract (LOT) and a second hole was drilled over the aPCX, ~0–1 mm anterior to Bregma to allow a dorsal approach to the aPCX. The LOT was stimulated with constant current square wave pulses (50–1000 μA) with a tungsten monopolar electrode. In addition, respiratory activity was monitored via a piezoelectric device strapped to the animal’s chest.

For single-unit aPCX recordings, a tungsten recording electrode (5–12 Mohms) 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). In some
animals, histological confirmation of recording sites were also performed. Single units were isolated directly or were extracted through template matching (10 kHz sampling rate) using Spike2 software for the Macintosh (CED). The single-unit nature of the recordings were confirmed with autocorrelograms showing ≥2 ms refractory period. Additional details are provided in Wilson (1998a).

Odor stimulation

Animals were freely breathing at all times. 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 four-channel Picospritzer which forced air through odorant saturated syringe filters (2.7 μ glass microfiber, Whatman), creating odor concentrations of ~10⁻¹⁴ of saturated vapor. Odorants used included isomyl acetate, eugenol, anisole, limonene (all from Sigma), and peppermint (McCormick), and 1:1 mixtures of these odors (see below). These odors were chosen to allow comparison with our previous work on habituation (Wilson 1998a,b). In addition, a second group of cells were tested with a series of straight-chain alkanes, each differing in length by two carbons, pentane, heptane, and n-nonane (Sigma). These were also delivered at 10⁻¹⁴ concentration of saturated vapor. No behavioral and/or respiratory responses were observed to odor stimulation at the level of anesthesia used here.

The odor stimulation paradigm involved initial testing of odor response patterns, delivering 2-s odor pulses at 30–60 s intervals, with each odor repeated 1–3 times to allow baseline response magnitude measurements. Next a habituating stimulus was delivered for 50 s. Finally, posthabituation stimuli were delivered, beginning 20 s after the termination of the habituating stimulus. Both test (same odor as the habituating stimulus) and control (different odor) were delivered before and after the habituation exposure. The order of test stimuli presented after habituation (same odor or different) was varied in different cells to control for the differences in recovery time, and has previously been shown not to affect measures of habituation (Wilson 1998a).

Stimulus onset was triggered on the respiratory cycle at the inhalation/exhalation transition. Responses to 2–5 different odors were examined before a single, prolonged 50-s habituating stimulus was presented. The habituating stimulus consisted of 1) the same odor as one of the effective prehabitation test odors, 2) a 1:1 binary mixture of the test odor with a blank syringe filter (clean air), or 3) a 1:1 binary mixture of the test odor and a second odor. The flow rate through each individual syringe filter in a mixture was the same as during a single test stimulus; thus total odorant flowrate during a binary mixture was double that of a single odor presentation (the flow rate of the clean airstream carrier was constant throughout the experiment). The mixture of test odor with a blank syringe filter (condition 2 above) was included to control for potential changes in odor concentration that might influence habituation to mixtures (see RESULTS). Test stimuli (2 s) were resumed 10–30 s after the end of the habituation stimulus. Recovery from habituation was tested ≥100 s posthabituating stimulus (Wilson 1998b). In most cases, only one habituating series was used for a single odor in individual animals.

Odor response analysis

Spike counts during the 2-s stimuli were subtracted from counts during the immediate 2 s preceding the stimulus to determine response magnitude. In the cases of the few suppressive responses that were encountered, response magnitude was determined by expressing firing rate during the odor as a percent of baseline firing rate. In both cases, response magnitude to posthabituation stimuli were expressed relative to the mean response magnitude for that same odor prehabitation. Response magnitude to prolonged, 50-s odor stimuli were determined from the first 2 s of the stimulus. The extent of habituation during the 50-s stimulus was also determined for the alkane series to allow comparison with similar measurements performed previously with the other odors (Wilson 1998a). Habituation during the 50-s stimulus was determined as odor-evoked activity during the last 10 s of the habituating stimulus expressed as percent of activity during the first 10 s.

RESULTS

Single aPCX neurons varied in their responsiveness to odors. The large majority of responses observed here (>90%) were excitatory, although as previously reported (Wilson 1998a), suppressive responses also occurred. Single units within the same animal responded to between 0 and 5 different test odors (5 was the maximum number of odors tested for single cells). The within-cell habitation data reported below is from cells in which at least partial response recovery was observed within 2–5 min posthabituation, which served as a control for both response and preparation stability. No responses were observed to stimulation through a clean (blank) syringe filter.

Odor habituation in aPCX single units showed minimal cross-habituation to either different odors or to odor mixtures. Figure 1 shows representative PSTH’s from a single aPCX neuron habituated to peppermint and subsequently to a 1:1 binary mixture of peppermint and isomyl acetate. A comparison of Fig. 1A1 and 1B1 shows the stability of responsiveness

![Figure 1](http://jn.physiology.org/DownloadedfromHttp%3A%2F%2Fjn.physiology.org%2FDownloadedfrom)
to 2-s peppermint test pulses. Figure 1A2 shows the response to the 50-s peppermint habituation stimulus. After an initial strong response, the firing activity returned to prestimulation levels, i.e., complete habituation, by the end of the 50-s stimulus. Figure 1A3 shows the response was still significantly reduced to a subsequent (30-s delay) peppermint test pulse. The response of this same cell to a mixture of peppermint and isoamyl acetate is shown in Fig. 1A2. The response to this mixture was also initially strong but completely habituated by the end of the 50-s presentation. As shown in Fig. 1B3, there was minimal effect of habituation to the mixture on subsequent response to a peppermint test stimulus (30-s delay), despite the fact that the habituating mixture contained the same volume of peppermint odor as shown in Fig. 1A2. Similar results were obtained when the habituating stimulus was a single odor completely different from the test stimulus.

Figure 2 displays the mean response magnitude for each habituation condition. Response magnitude for the 2-s posttest stimuli and the first 2 s of the habituation stimuli are expressed as a percent of prehabituation magnitude. Cells tested in the self-habituation paradigm (n = 17) included cells habituated to either 50 s of the odor alone (test odor A, habituate odor A, and test odor A) or to 50 s of the odor in a 1:1 mixture with air from a clean syringe filter (test odor A, habituate odor A + blank, and test odor A). There was no difference between these groups, thus their data are combined for statistical analyses. Response magnitude to the test odor was significantly reduced by self-habituation [mean posthabituation response magnitude = 16.9 ± 4.7% (SE) of baseline response]. The habituated response recovered within 2–5 min as previously reported (Wilson 1998b).

There was no evidence of cross-habituation to different, single odors (n = 24; test odor C, habituate odor A, and test odor C; Fig. 2). Response magnitude to test stimuli was statistically unchanged after habituation to a different odor (mean posthabituation response magnitude = 94.5 ± 14.3% of baseline response). Finally, habituation to odor mixtures (n = 24; test odor A, habituate odor A + B, and test odor A) produced significantly less habituation to the components of the mixture than self-habituation [mean posthabituation response magnitude = 75.2 ± 10.9% of baseline response; one-way analysis of variance (ANOVA), F(2,62) = 10.57, P < 0.001]. Post hoc Scheffe tests revealed that response magnitude after self-habituation was significantly less than after either cross-habituation or habituation to mixtures (P < 0.01), whereas response magnitudes after cross- or mixture-habituation were not significantly different from each other. Similar results were found with all combinations of odors and odor mixtures tested.

The reduced habituation to mixture components after odor mixture stimulation could be because of the fact that responses to odor mixtures were reduced compared with responses to a single stimulus (mixture suppression). Mean response magnitude during the first 2 s of the mixture habituating stimulus was significantly suppressed relative to the response magnitude to the prehabituation component alone (mean = 70.1 ± 11.7%; t(24) = 2.56, P < 0.02), whereas mean response magnitude to the self-habituation stimulus was not significantly different from prehabituation magnitude [mean = 86.8 ± 18.4%; t(16) = 0.72, not significant (NS)]. Perhaps the mechanisms of odor habituation require a threshold intensity of activity that was not obtained during stimulation with an odor mixture.

Two approaches were used to test this hypothesis. First, the magnitude of the response to the mixture was plotted as a function of the amount of habituation to a component of that mixture (Fig. 3). If the threshold hypothesis is correct, then cells with a vigorous response to the mixture should tend to show greater habituation to the components. This relationship was not observed (r = 0.03, P > 0.1, NS). Cells showing very weak relative responses to the mixture were just as likely to show no habituation as cells that had strong responses to the mixture (Fig. 3).

The second test of the threshold hypothesis was a direct examination of self-habituation to mixtures (n = 11; test odor A + B, habituate odor A + B, and test odor A + B). If mixture suppression reduces activity below that required for habituation mechanisms, then minimal self-habituation to mixtures should be observed. On the contrary, self-habituation to mixtures was as pronounced as self-habituation to single odors (mean response magnitude post self-habituation to mixtures =

**FIG. 3.** Relationship between initial response magnitude to odor mixtures and degree of habituation to the components. No significant relationship was observed (r = 0.03).
The minimal cross-habituation between a binary mixture and its components suggests a very high degree of odor specificity. Another test for the degree of odor specificity is to use odor molecules that vary along a single dimension, such as has been used very effectively for understanding coding in the olfactory bulb and in human psychophysics (e.g., Cometto-Muniz et al. 1998; Mori and Yoshihara 1995). We chose to use straight chain alkanes varying in length by two carbons; pentane, heptane, and n-nonane. A set of 12 aPCX cells were tested with these odorants. Single cells responded to 0–3 of the stimuli. Eight of the cells responsive to $\geq 2$ of the alkane odors were tested for habituation and cross-habituation. Habituation to these odors was similar to that previously reported for the odors used above (Wilson 1998a). Responses to the prolonged stimulus habituated rapidly, with odor-evoked activity during the last 10 s of the 50-s stimulus at 34.2 ± 12.5% of odor-evoked activity during the first 10 s of stimulation. As shown in Fig. 5, this habituation was odor specific. Response magnitude to a 2-s odor pulse 20–50 s after the termination of the habituating stimulus revealed significant self-habituation (mean 30.4 ± 10.1% of prehabitation response magnitude) and no cross-habituation to a neighboring alkane odor (mean 105.4 ± 12.7%; unpaired $t$-test, $t(14) = 4.62, P < 0.01$).

**DISCUSSION**

The present results demonstrate that odor habituation in the piriform cortex is odor specific. That is, exposure to one odor does not produce cross-habituation to other, markedly different odors nor to structurally similar odors differing in length by only two carbons. Furthermore, the results suggest that exposure to a 1:1 binary odor mixture produces minimal cross-habituation to components of that mixture. This latter finding suggests a form of synthetic/configural processing of novel odors in the olfactory system, wherein mixtures are processed relatively independently of their components in the aPCX.

The demonstration of self-habituation to mixtures suggests that the lack of habituation to components of mixtures is not...
caused by mixture suppression-induced lowering of afferent activity to the point that mechanisms of habituation are not activated. Rather, these results suggest that mixtures of novel odors are processed as unique stimuli. Mixtures which include familiar or learned odor components may be processed differently (Livermore et al. 1997; Staubli et al. 1987).

The locus of this mixture synthesis is unknown, but presumably involves events at the receptor sheet, olfactory bulb and cortex. Odor mixture interactions/synthesis have been described in olfactory receptors (Ache 1989; Caprio et al. 1989; Cromarty and Derby 1998; Derby et al. 1991; Gentilcore and Derby 1998; Johnson et al. 1985), as have mixture interactions in both the invertebrate olfactory lobe (Joerges et al. 1997; Linster and Smith 1997; Vickers et al. 1998) and rat olfactory bulb (Bell et al. 1987). For example, in the lobster Panulirus argus, interactions between odorants can occur either at odorant receptor binding sites or through interactions between odorant activated ionic conductances and second messenger cascades within individual receptor neurons (Ache and Zhinarov 1995; Cromarty and Derby 1998). These interactions can result in either suppression or enhancement of receptor cell responses to mixtures compared with responses to the components of those mixtures. In both the invertebrate olfactory lobe and rat olfactory bulb, the mixture evoked pattern of receptor activity can be further differentiated from individual component response patterns through glomerular layer processing, resulting in mixture specific spatial patterns of glomerular activation (Bell et al. 1987; Joerges et al. 1997) and mixture specific output neuron responses (Vickers et al. 1998). If these kinds of interactions occur in the present preparation, they could begin to account for both the mixture suppression of response magnitude observed here (Fig. 3) and the minimal cross habituation between mixtures and their components (Fig. 2).

Odor habituation induced by the present paradigm (50 s stimulation) is associated with depression of lateral olfactory tract (LOT) synapses within the aPCX (Wilson 1998b). This synaptic depression is odor specific, not a generalized systemic depression, in that cells exposed to odors to which they do not respond show no synaptic change. Furthermore, the synaptic depression recovers with a time course similar to odor response recovery, and has been hypothesized to contribute to habituation-induced reduction of aPCX odor responses. If LOT synaptic depression is a major component of habituation in aPCX neurons, then the present finding of odor specificity suggests that different odors activate different populations of synapses on individual aPCX neurons, with only minimal overlap. In fact, even with mixture stimulation, the population of synapses activated by the mixture must only minimally overlap with the population of synapses activated by individual components. If there was substantial overlap between groups of synapses activated by different odors, then depression of those synapses by habituation to one odor should produce cross-habituation to other odors using the same synapses. No cross-habituation
between odors, even odors differing in length by only two carbon atoms, was observed. Thus either habituation-associated LOT synaptic depression is not involved in expression of reduced odor response magnitude, or different odors activate different, nonoverlapping sets of synapses on aPCX neurons.

The odor specificity of habituation in the aPCX described here clearly demonstrates that odor habituation does not reflect a decreased excitability of aPCX neurons to all synaptic inputs, but rather, perhaps reflects a depression of odor specific synapses. Several mechanisms of synaptic depression have been described in other systems, including neurotransmitter depletion and presynaptic receptor modulation of neurotransmitter release (Castellucci et al. 1970; Hasselmo and Bower 1991; Trombley and Westbrook 1992; Zucker 1972) and NMDA-dependent depression of postsynaptic sensitivity (Linden and Connor 1995). The role of these mechanisms in aPCX odor habituation are currently being examined (Wilson 1998c).

Odor receptive fields and odor coding

These results demonstrate that individual components of an aPCX neuron’s odor receptive field can be independently modified by experience. The breadth of aPCX odor receptive fields may not be caused by a loose coding of molecular features resulting in responses to several odors. Rather, aPCX odor receptive fields may represent a collection of relatively independent responses to several different odors or odor features. Experience can then modify these receptive fields by strengthening or weakening independent components within the field. For example, in the present experiment, repeated, nonreinforced presentation of an odor resulted in temporary reduction in the representation of that odor in receptive fields of single aPCX neurons (and presumably through the cortex as a whole), leaving responsiveness to other odors intact. It is predicted that different kinds of experience (e.g., associative learning) may produce the opposite effect by selectively enhancing responsiveness to reinforced stimuli, as has been described in other sensory systems (Edeline 1999; Weinberger 1998).

In fact, it should be noted that the odors used here and in previous work are novel odors to these animals. Theories of piriform cortex function (Ambros-Ingeron et al. 1990; Haberly 1985; Haberly and Bower 1989) suggest that as odors and odor combinations are repeatedly experienced, associative changes in synaptic strength of cortical connections occur, modifying subsequent response patterns to those odors. Thus odor receptive fields, interactions between odors, and cross-habituation may vary as a function of odorant familiarity (Livermore et al. 1997; Staubli et al. 1987).

Although not specifically designed to address the nature of odor coding in the olfactory system, the present results suggest a form of synthetic odor processing. At a behavioral level, olfactory system processing of odor mixtures appears to involve both component/analytic processes as well as configural/synthetic processes. For example, humans and other animals exposed to an odor mixture can, under some conditions, identify a subset of the components within that mixture (analytic processing) but also appear to treat the mixture as a unique stimulus in itself (synthetic processing, Blakstein and Engen 1987; Chandra and Smith 1998; Derby et al. 1996; Laing 1995; Laska and Hudson 1993; Linster and Smith 1999; Livermore and Laing 1998; Livermore et al. 1997; Staubli et al. 1987).

The minimal cross-habituation between a binary mixture and its components observed in the present study suggests that the mixture is processed by individual aPCX neurons as a unique stimulus, different from either of its constituent parts.

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