Comparison of Odor Receptive Field Plasticity in the Rat Olfactory Bulb and Anterior Piriform Cortex

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INTRODUCTION

Current theories of odor discrimination by mammalian olfactory systems begin with molecular feature recognition by a large set of odor receptors (Buck 1996; Mombaerts 1999). A single receptor neuron, expressing perhaps a single receptor protein, may respond to many odors (Duchamp-Viret et al. 1999; Kaluza and Breer 2000; Malnic et al. 1999) if each of those odors includes the appropriate feature. This feature recognition is refined through precise axonal projection patterns to glomeruli in the main olfactory bulb (MOB) and through lateral interactions between those glomeruli and between their output neurons (mitral/tufted cells; Bozza and Kauer 1998; Vassar et al. 1994; Yokoi et al. 1995). The individual features of a particular odorant may then be combined through precise temporal synchrony in mitral/tufted cell spike trains (Kashiwadani et al. 1999) and/or through convergence within the primary olfactory (piriform) cortex (Haberly 1985; Lynch 1986; Mori et al. 1999).

Similar to olfactory receptor neurons, MOB mitral/tufted cells (Katoh 1993; Meredith 1986; Wellis et al. 1989) and anterior piriform cortex (aPCX) neurons (Haberly 1969; McCollum et al. 1991; Tanabe et al. 1975; Wilson 1998a) have relatively broad receptive fields, responding to multiple odorants. Within a particular class of odorants (e.g., aliphatic acids or aldehydes), receptive fields can be mapped along a dimension of carbon chain length (Katoh et al. 1993). Using this paradigm, molecular or odor receptive fields can be described wherein a single receptor or mitral/tufted cell responds to a sequential series of chain lengths (e.g., 3–7 carbons) but not to chain lengths longer (≥8) or shorter (≤2). Mitral/tufted cell receptive fields are further frequently characterized by inhibitory surrounds (Katoh et al. 1993; Sato et al. 1994), with suppressive responses evoked by the outlier chain lengths (8 and 2 carbon chain lengths in this example). Receptive fields of aPCX neurons have been less thoroughly described but appear to be similar to those of mitral/tufted cells with responsiveness occurring within a sequential series of carbon chain lengths (Wilson 2000).

The breadth of receptive fields in the olfactory receptors and MOB mitral/tufted cells has been interpreted as due to coding of specific molecular features or odotopes that may be a component of many odors (Malnic et al. 1999; Mori et al. 1999; Shepherd 1994). In contrast, cross-habituation studies suggest that aPCX receptive fields may be due to relatively independent odor inputs, which can be selectively modified by experience, leaving responses to other odors stable (Wilson 1998a, 2000). The present study used a cross-habituation paradigm with alkane odors of varying carbon chain length to specifically compare receptive field dynamics and odor discrimination between MOB mitral/tufted cells and aPCX neurons. The results suggest that while mitral/tufted cells show cross-habituation between odors within their receptive fields, aPCX neurons show very little cross-habituation, significantly enhancing odor discrimination at the cortical level.

METHODS

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

Single-unit recordings from mitral/tufted cells and aPCX layer II/III

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neurons in urethan-anesthetized, freely breathing rats were obtained as previously described (Wilson 1998a, 2000). Briefly, electrical stimulation of the lateral olfactory tract (LOT) was used to identify mitral/tufted cells by antidromic activation and layer II/III aPCX neurons from extracellular evoked field potentials and synaptic activation (Fig. 1) (Haberly and Shepherd 1973). Respiratory activity was monitored via a piezoelectric device strapped to the animal’s chest. 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 at least a 2-ms refractory period.

**Odor stimulation**

A continuous stream (600 ml/min) of air, passed through an activated charcoal filter and humidified, was blown across the nares of the freely breathing animal. A computer controlled Picospritzer forced air through odorant saturated syringe filters (2.7 μm glass microfiber, Whatman), which mixed with the clean airstream to create odor concentrations of \(10^{-2}\) of saturated vapor. Odorants used were pentane, heptane, and \(n\)-nonane (Sigma-Aldrich). Stimulus onset was triggered on the respiratory cycle (at the inhalation/exhalation transition).

Mitral/tufted cell or aPCX neuron partial receptive fields to these three alkanes were mapped with 2-s odor pulses, separated by \(\geq 60\) s between each stimulus. Order of stimuli were randomly varied within and between cells. Stability of receptive fields were determined by a second presentation of each stimulus. In cells responding to at least two of the stimuli, one of the stimuli was pseudo-randomly chosen to serve as the habituating stimulus (50-s duration). The receptive field was then remapped 15–50 s after the termination of the habituation exposure and recovery was tested at least 2 min later. In most cases, only one habituating series was used for a single odor in individual animals.

Spike counts during the 2-s stimuli were compared with counts during the immediate 2 s preceding the stimulus. Response magnitude to posthabituation stimuli were expressed relative to the mean response magnitude prehabituation. Response magnitude during the 50-s odor stimuli were determined in 10-s bins, by comparing odor-evoked spike counts (difference from preodor activity) in each bin with those evoked during the first 10 s of odor stimulation. Data are plotted as means ± SE.

**RESULTS**

A total of 24 mitral/tufted cells and 21 aPCX neurons responded to at least two of the test odors and were included in the data set. Both mitral/tufted cells and aPCX layer II/III neurons displayed receptive field properties to alkanes similar to those described by Imamura et al. (1992) for rabbit olfactory bulb mitral/tufted cells (Fig. 1A). Individual cells responded to sequential series of chain lengths; that is, there was no cell observed that responded to both pentane and nonane without also responding to heptane. These responses to 2-s odor pulses were relatively stable as long as interstimulus intervals exceeded \(\geq 60\) s (Fig. 1B).

Following partial mapping of mitral/tufted and aPCX neuron receptive fields to alkanes, one of the three odors was pseudo-randomly chosen to be the 50-s habituating stimulus. Responses to the 50-s alkane stimuli revealed a marked difference...
between mitral/tufted cells and aPCX neurons. As previously reported for other odors (Wilson 1998a), aPCX neurons rapidly habituated to alkanes compared with mitral/tufted cells (Fig. 2). Thus although both cell types displayed some response decrement during the 50-s stimulation, there was a significant difference in odor-evoked response magnitude during the final 10 s of stimulation between cell types \( t(43) = 2.99, P < 0.01 \). This difference in habituation rate was apparent for all three alkane stimuli (Fig. 2B).

Interestingly, after the termination of the 50-s habituating stimulus, while aPCX responses began to recover almost immediately (Wilson 1998a), mitral/tufted cell responses to 2-s test stimuli continued to decline for \( \approx 30 \) s before recovery onset. This difference in posthabituation stimulus activity fortuitously allowed comparison of cross-habituation at a time when self-habituation levels in mitral/tufted cells and aPCX neurons were roughly similar (e.g., Fig. 4, 0 carbon chain length difference).

In addition to habituation rate/magnitude, the extent of cross-habituation between alkanes also differed significantly between MOB mitral/tufted cells and aPCX neurons. Figure 3 shows examples of odor receptive fields in a mitral/tufted cell and aPCX neuron before and after habituation. The mitral/tufted cell in Fig. 3A showed strong responses (number of odor-evoked spikes) to all three alkanes prior to habituation to heptane. Following heptane habituation, responses to all three alkanes were reduced, until recovery \( \sim 2 \) min later. In contrast, the aPCX neuron, which also responded to all three alkanes, showed a selective decrease in odor-evoked spiking to nonane following habituation to nonane (Fig. 3, B and D); responses to pentane and heptane were relatively unaffected.

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**FIG. 2.** A: habituation to alkanes was significantly enhanced in aPCX neurons during a 50-s stimulation compared with MOB mitral/tufted cells (means \( \pm \) SE). B: this differential habituation was observed for all 3 alkanes tested.

**FIG. 3.** Representative examples of MOB mitral/tufted cell (A and C) and aPCX neuron (B and D) receptive fields before and after habituation to a single stimulus (\( \downarrow \), habituating stimulus). Habituation to heptane reduced mitral/tufted cell responsiveness to all alkanes tested, while habituation to nonane selectively reduced aPCX neuron responsiveness to nonane, leaving responses to pentane and heptane relatively unaffected. Both cross- and self-habituation recovered within 2 min in the MOB and aPCX.
A plot of cross-habituation as a function of carbon chain length difference (Fig. 4) shows significantly greater cross-habituation between alkanes differing by two or four carbons in mitral/tufted cells compared with aPCX neurons [ANOVA, main effect of cell type, \( F(1,101) = 5.79, P = 0.01 \). Post hoc Fisher tests revealed a significant difference between mitral/tufted cells and aPCX neurons in cross-habituation between stimuli differing by two or four carbons, \( P < 0.05 \)]. Self-habituation levels were similar between mitral/tufted cells and aPCX neurons at this time point (15–50 s posttermination of the habituating stimulus).

The specific effects of habituation on MOB mitral/tufted and aPCX neuron odor receptive fields is shown in Fig. 5. Habituation to a single alkane had much more selective effects on aPCX receptive fields than on mitral/tufted cell receptive fields. Thus while habituation to one chain length produced marked self-habituation in aPCX neurons, responses to other chain lengths were relatively unaffected. Mitral/tufted cells, however, showed suppression of responses across the receptive field following habituation to a single odor.

Furthermore the cross-habituation expressed by MOB mitral/tufted cells was asymmetrical. For example, habituation to heptane produced more cross-habituation to pentane than to nonane (Fig. 5). In fact, significantly greater cross-habituation occurred to carbon chains shorter than the habituating stimulus compared with cross-habituation to carbon chains longer than the habituating stimulus in the MOB [Fig. 6; ANOVA, main effect of relative chain length; \( F(1,61) = 7.68, P = 0.01 \)]. Post hoc Fisher tests revealed significantly \((P < 0.05)\) greater cross-habituation to shorter chains than longer chain in mitral/tufted cell responses but no significant asymmetry in aPCX cross-habituation.

**DISCUSSION**

The present results demonstrate that while MOB mitral/tufted cells and aPCX neurons have superficially similar static odor receptive fields, examination of receptive field dynamics reveals important differences between these two cell types.
Neurons in the aPCX showed more rapid and odor-specific habituation than MOB mitral/tufted cells. The rapid habituation in the aPCX relative to its primary afferent, MOB mitral/tufted cells, may in part be due to synaptic depression of LOT afferents (Wilson 1998b) and could allow cortical filtering of prolonged stimuli. The enhanced habituation in cortex compared with more peripheral structures is similar to that reported for both vision (Miller et al. 1991; Sanchez-Vives et al. 2000) and audition (Condon and Weinberger 1991). The reduced cross-habituation apparent in the aPCX relative to MOB mitral/tufted cells suggests that computations occurring within the aPCX enhance odor discrimination, similar to the MOB enhancement of odor discrimination compared with the olfactory receptor sheet (Duchamp and Sicard 1984; Yokoi et al. 1995). Just as the rat aPCX is capable of discriminating between 2 carbons chain length differences, rats are capable of making behavioral odor discriminations of 1–2 carbon chain length differences (Linster and Hasselmo 1999). Given the differences in odor single-unit discrimination shown here, this behavioral discrimination may be more dependent on the aPCX than on the MOB.

In the rodent MOB, single mitral/tufted cells receive olfactory nerve input within a single glomerulus and, thus given the precise projection patterns of receptor cell axons, potentially receive direct input from receptor neurons all expressing the same receptor protein (Ressler et al. 1994; Vassar et al. 1994). Despite expressing a single receptor protein type, individual vertebrate olfactory receptor neurons respond to multiple odors (Duchamp-Viret et al. 1999; Firestein et al. 1993; Malnic et al. 1999; Sato et al. 1994; Sicard and Holley 1984; Touhara et al. 1999) and show cross-adaptation between odors (Caprio and Byrd 1984; Lipschitz and Michel 1999; although see Kashiwayanagi and Kurihara 1994) as reported here for mitral/tufted cells. Thus it appears likely that mitral/tufted cell odor receptive field dynamics stem, in part, from a loose coding of molecular features at the receptor level, such that exposure to one stimulus affects responses widely through the receptive field. Similar results have been reported in awake rats where repeated, 20-min stimulation with a single odor produced long-term decreased responding of mitral/tufted cells to that odor, as well as molecularly dissimilar odors (Buonviso et al. 1998). However, the specificity of habituation effects on aPCX neuron receptive fields is in sharp contrast to cross-habituation expressed at the receptor and MOB levels. The question arises then if more peripheral structures have broadly depressed receptive fields following exposure to a single odor, how can the aPCX display such selective habituation? While precise mechanisms of enhanced odor discrimination in the cortex remain to be clarified, feedback/association systems within the cortex and between the cortex and MOB are most likely involved (Bower 1991; Haberly 1985; Hasselmo and Barkai 1995).

At another level of analysis, perhaps the difference in cross-habituation is evidence that mitral/tufted cells and aPCX neurons respond to the same odors but for different reasons. For example, in the visual system, neurons in the lateral geniculate nucleus of the thalamus respond maximally to points of light that fall on their center-surround receptive fields. Visual cortex neurons with simple receptive fields respond maximally to bars of light or contrast edges (an ensemble of many points of light) in particular orientations. Thus both of these cell types would respond to bars of light if the bar overlaps with their specific receptive field but for different reasons.

A similar difference in the level of analysis performed by MOB and aPCX neurons may occur in the olfactory system with mitral/tufted cells responsive to specific odorant features expressed by many odorants and aPCX neurons responsive to feature ensembles (Bower 1991; Haberly 1985; Lynch 1986; Mori et al. 1999). This difference in coding between the two cell types could account for the observed difference in cross-habituation. The schematic shown in Fig. 7 shows three odors each composed of three odorant/molecular features. Individual odorant receptor neurons and MOB mitral/tufted cells respond to particular features. Thus mitral/tufted cell A responds to all three odors because they each contain the A feature. The aPCX neuron, however, responds to all three odors because it is driven by the feature ensembles ABC, ADE, and AFG due to patterns of mitral/tufted cell afferent convergence and/or association fiber convergence.
With this difference in the underlying basis of receptive fields, habituation to odor 2, for example, would produce effects in the mitral cells and in the aPCX neuron similar to that shown in the data reported here. Mitral cell A would show cross-habituation to both odors 1 and 3 because they both contain the A feature to which this cell is primarily responsive and to which it is now habituated. The aPCX neuron, on the other hand, while habituated to the ADE ensemble should continue to respond well to both the ABC and AFG ensembles given that many of the features in those odors are still effective. The reduction in input from the A component of these ensembles could be compensated for by cortical processing. Recent models of piriform cortex suggest that association circuitry and neuromodulatory inputs enhance the cortical ability to appropriately interpret partially degraded signals (Hasselmo and Barkai 1995). The minimal cross-habituation between binary odor mixtures and their components previously reported (Wilson 2000) further supports this cortical ensemble coding hypothesis.

Asymmetry in cross-habituation

The cross-habituation expressed by MOB mitral/tufted cells was asymmetrical with chain lengths shorter than the habituating stimulus expressing greater cross-habituation than chain lengths longer than the habituating stimulus. Although in the present study the aPCX did not demonstrate asymmetry, a similar asymmetry in cross-habituation between carbon chains of different length has been demonstrated in human psychology. Habituation to the alcohol pentanol (C5) produces greater cross-habituation to the shorter alcohol propanol (C3) than visa versa (Cain 1970).

Olfactory receptor neurons discriminate differences in carbon chain length (Kaluza and Breer 2000; Sato et al. 1994), and as chain length increases, the probability of receptor cell activation increases (Sato et al. 1994). Similarly as carbon chain length increases, there is an increase in the complexity of odor-evoked spatial patterns of MOB glomerular layer activation (Johnson et al. 1999). In fact, examination of the published spatial maps of glomerular activation to aliphatic acids (Johnson et al. 1999) suggests that odors with shorter chain lengths may activate a subset of the same glomeruli activated by longer chains (e.g., the response to a 7 carbon chain stimulus may include within it the response elements for a 5 or 6 carbon chain). If this is the case, then habituation to the longer chain stimulus might be expected to produce strong cross-habituation to shorter chain stimuli, as observed here in mitral/tufted cells.

What does the aPCX contribute to odor processing?

The present results, combined with previous work (Wilson 1998a, 2000), suggest that the aPCX is a rapidly adapting system with excellent odor-discrimination capabilities. Rapidly adapting systems are maximally responsive to change, allowing filtering of background or currently nonrelevant stimuli. In addition, the aPCX demonstrates significantly greater odor discrimination capabilities than its primary afferent, MOB mitral/tufted cells, as determined by cross-habituation (Fig. 4). This combination of rapid habituation and precise odor discrimination would allow the aPCX to filter background stimuli yet maintain responsiveness to similar, novel odors or change.

In addition to allowing precise odor habituation, the highly specific modulation of odor receptive fields in aPCX could enhance precision of odor discrimination, identification, and memory. Experience can produce either decreases or increases in cortical responsiveness to specific stimuli. For example in the auditory system, habituation causes highly frequency-specific decreases in cortical responses to the repeated tone (Condon and Weinberger 1991), while associative conditioning produces highly frequency-specific increases in both thalamic and cortical responses to the conditioned tone (Edeline and Weinberger 1992; Weinberger 1993). It is hypothesized that aPCX receptive fields could similarly be modulated in either direction with experience, and receptive field dynamics could play a major role in olfactory discrimination and memory (Bower 1991; Haberly 1985; Lynch 1986).

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REFERENCES


