Published in final edited form as:

J Neurophysiol. 2006 March ; 95(3): 1888–1896.

Olfactory Cortical Adaptation Facilitates Detection of Odors Against Background

Mikiko Kadohisa and Donald A. Wilson

Department of Zoology, University of Oklahoma, Norman, Oklahoma

Abstract

Detection and discrimination of odors generally, if not always, occurs against an odorous background. On any given inhalation, olfactory receptor neurons will be activated by features of both the target odorant and features of background stimuli. To identify a target odorant against a background therefore, the olfactory system must be capable of grouping a subset of features into an odor object distinct from the background. Our previous work has suggested that rapid homosynaptic depression of afferents to the anterior piriform cortex (aPCX) contributes to both cortical odor adaptation to prolonged stimulation and habituation of simple odor-evoked behaviors. We hypothesize here that this process may also contribute to figure-ground separation of a target odorant from background stimulation. Single-unit recordings were made from both mitral/tufted cells and aPCX neurons in urethane-anesthetized rats and mice. Single-unit responses to odorant stimuli and their binary mixtures were determined. One of the odorants was randomly selected as the background and presented for 50 s. Forty seconds after the onset of the background stimulus, the second target odorant was presented, producing a binary mixture. The results suggest that mitral/tufted cells continue to respond to the background odorant and, when the target odorant is presented, had response magnitudes similar to that evoked by the binary mixture. In contrast, aPCX neurons filter out the background stimulus while maintaining responses to the target stimulus. Thus the aPCX acts as a filter driven most strongly by changing stimuli, providing a potential mechanism for olfactory figure-ground separation and selective reading of olfactory bulb output.

INTRODUCTION

The olfactory system faces at least two distinct information processing tasks to account for odor perception. First, most natural odors are composed of multiple, often hundreds of individual volatile components (Leon and Johnson 2003; Polak 1973). However, perception of these odors is synthetic or configural wherein a single odor object is perceived despite the presence of multiple components (Jinks and Laing 2001; Stevenson 2001). In fact, although simple binary mixtures can be perceived either analytically or configurally (Kay et al. 2003; Wiltrout et al. 2003), for mixtures composed of more than three components, the identity of individual components cannot be reliably determined (Jinks and Laing 2001; Laing and Francis 1989). Recent work from our lab suggests that a major contributor to configural coding and odor object synthesis may be the piriform cortex (Wilson 2003). It has been hypothesized that multiple, co-occurring odorant features, extracted by the periphery and refined by the olfactory bulb, are synthesized within piriform cortical circuits into perceptual odor objects (Granger and Lynch 1991; Haberly 2001; Hasselmo et al. 1990; Wilson and Stevenson 2003).

A second, seemingly contradictory olfactory perceptual phenomenon, however, is the ability to detect and identify odors despite the presence of background odor (Dalton 2000; Goyert et

al. 2005). Thus under some circumstances, the olfactory system must be able to selectively filter some odorant features (those contributing to background) while responding to others as distinct from background. In essence, in the presence of background odorants, the olfactory system must ‘read’ some components of the olfactory bulb input and “ignore” others. Piriform cortex sensory physiology shows characteristics that may contribute to this figure-ground separation. Thus for example, afferent synapses from olfactory bulb mitral/tufted cells to the anterior piriform cortex (aPCX) display homosynaptic short-term depression after several seconds of activation (Best and Wilson 2004). This synaptic depression contributes to both cortical odorant adaptation (Best and Wilson 2004; Wilson 1998b) and behavioral odor adaptation (Best et al. 2005). However, given that the depression is mediated presynaptically, cortical afferents activated by other odorants are unaffected (Best and Wilson 2004; Wilson 2000). This leads to the hypothesis that olfactory bulb output activated by relatively stable, background odorants could be filtered through cortical synaptic depression, whereas novel odorants experienced after some temporal delay could continue to drive cortical activity, leading to separation of target odorants from background.

This study compared mitral/tufted cell and aPCX neuron responses to odorants presented against known backgrounds in anesthetized rats and mice. Our previous work has demonstrated that after exposure and adaptation to one odorant, responses of aPCX neurons to other odorants are relatively unaffected, i.e., there is little cross-adaptation between odorants presented sequentially. This study asks a much different question. That is, once the cortex has adapted to one odorant and that odorant remains present in the background as a second, new odorant is presented, does the olfactory system treat the simultaneously presented odorants as a mixture (reflecting what is inhaled) or can the system treat new odorants as distinct from background? The results are consistent with the preceding hypothesis and suggest that mitral/tufted cells provide information regarding all odorant features present during an inhalation, whereas cortical neurons filter background odorants, responding to new odorants as if presented in isolation.

METHODS

Subjects

Male Long-Evans hooded rats (230–450 g), obtained from Harlan Lab Animals, were used as subjects. Food and water were available ad lib. In addition, a small number of male Swiss-Webster albino mice (28–41 g), obtained from a breeding colony at the University of Oklahoma and housed as described for rats were used for comparative purposes. Animal care and use conformed to National Institutes of Health guidelines and were in accordance with the University of Oklahoma IACUC.

Experimental design

The design of this experiment is shown in Fig. 1A. The experimental protocol consisted of determining the cell’s responses to the 2-s test odorants twice including two single components (A and B) and binary mixtures (A + B) with at least a 60-s interstimulus interval. Then one of the odorants was randomly selected to serve as the background and was presented for 50 s. After the background stimulus was on for 40 s, the second stimulus was added (A + B) for 2 s (thus 1 target odorant presented with the other as background). If recording stability allowed, the protocol was repeated with the identity of the target and background odorants reversed. Further, when neurons responded to other different odorants, they were tested with them in the same way. For both binary mixtures and target odorants against background, airflow was equally divided between the two odorants, thus total volume of odorized air during mixtures (intensity) was the same as for a single stimulus, as in (Wilson 2000).

Recording and odorant stimulation

Details of single-unit recording and odorant-response characterization techniques for mitral/tufted and layer II/III aPCX neurons have been reported in detail elsewhere (Wilson 1998a). Briefly, animals were anesthetized with urethane (1.5 g/kg) and were freely breathing with the respiratory cycle monitored through a piezoelectric device. The single-unit nature of the recordings was verified by at least a 2-ms refractory period in interval histograms. Mitral/tufted cells were identified by antidromic stimulation of the lateral olfactory track (LOT) and layer II/III aPCX neurons were identified by LOT-evoked responses and/or histological confirmation. After isolation of a single-unit, 2-s test stimulus presentations were delivered for each odorant to test for responsiveness. Only cells that responded to at least two of the odorants were used here. Because the odorants selected were molecularly diverse, no attempt was made to direct main olfactory bulb (MOB) recording locations with reported spatial maps of glomerular layer activity, in contrast to our previous work using homologous series of ethyl esters (Fletcher and Wilson 2003).

Odorants were delivered with a flow-dilution olfactometer, with a constant, 1 liter per minute (LPM) flow of charcoal-filtered, humidified air presented 1–2 cm from the animal's nose. Saturated odorant vapor was added at 0.1 LPM to the clean air stream via computer-controlled solenoids to produce an approximate dilution of 1:10 of saturated vapor. Odorant stimulus onset was triggered off the respiratory cycle to coincide with the transition from inhalation to exhalation, and the test stimulus duration was 2 s. Background stimuli were presented for a total of 50 s with the 2-s target stimulus presented 40 s after background onset. Stimuli included peppermint (McCormick), isoamyl acetate, eugenol, and pentane (Sigma); and binary mixtures of odorants were within these four odorants. To deliver odorants in binary mixtures, airflow was equally divided between the two odorants, thus total volume of odorant mixture was the same as for a single stimulus (see (Wilson 2003)). This method of creating binary mixtures by splitting the airflow was deliberately chosen to reduce the possibility that neurons responding to an odorant presented against background were simply responding due to a change in airflow. This design, however, means that the volume of air saturated with each individual component during binary mixture stimulation, or target odorant against background presentation, is half of that experienced during single component stimulation. Odorized airflows entered a 3-cm-long mixing chamber prior to delivery to the animal to enhance uniformity of concentration and mixing.

Data analysis

The response magnitude of both mitral/tufted and aPCX neurons to odorants were quantified as the difference in number of spikes evoked during the 2-s stimulus compared with a 2-s prestimulus period. Response magnitude to target odorants were compared with firing rate prior to background odorant onset. Repeated-measures ANOVA's with post hoc Fisher tests where appropriate and *t*-test were performed to compare the responses between mitral/tufted and aPCX neurons, and to investigate whether the neurons had habituated during 40-s exposure to a background odorant.

Histology

After recording, animals were overdosed with anesthetic, transcardially perfused with saline and 4% paraformaldehyde, and the brains subsequently sectioned coronally at 40 μ and stained with cresyl violet for determination of electrode positions.

RESULTS

Data from a total of 61 mitral/tufted cells from 23 animals and 60 aPCX neurons from 38 animals are included in the analyses. Twenty-four mitral/tufted cells and 19 aPCX cells were

tested with a single protocol, while 37 mitral/tufted cells and 41 aPCX cells were tested more than once.

Responses to binary mixtures

As we have previously reported (Wilson 2003), aPCX neuron responses could be divided into two groups based on responses to the binary mixtures, similar to response classifications of olfactory receptor neurons (Cromarty and Derby 1998; Duchamp-Viret et al. 2003) and olfactory central neurons (Arbas et al. 1988; Tabor et al. 2004). Individual mitral/tufted cells and aPCX neurons were capable of expressing either mixture addition or mixture suppression, depending on the odorants used. One response type was defined as mixture addition in which the magnitude of response to the binary mixture was greater than or equal to that to the most effective component. The second classification was mixture suppression where the magnitude of response to the mixture was less than that to the most effective component. With the odorants, intensities and mixtures tested here, 67% of aPCX units showed mixture suppression and 33% showed mixture addition. Similar results were observed with mitral/tufted cells where 71% showed mixture suppression and 29% mixture addition (Fig. 2). Although the distribution of ratios of mixture response magnitude to most effective component response magnitude was largely uniform (Fig. 2), this categorization proved to identify potential differences in how cells responded to target odorants against background as described in the following text.

Mixture addition

RESPONSE TO TARGET ODORANTS AGAINST BACKGROUNDS—After identification of single units that responded to components and their binary mixtures, one of the odorants was randomly selected to serve as the background odorant. Forty seconds after background onset, the target odorant was present against the background for 2 s, followed by return to the background alone. Because mixtures were produced by splitting the air stream between both odorants, total flow rate was constant throughout. Figure 1*B* shows an example of data from a mitral/tufted cell and an aPCX neuron stimulated in this manner. The mitral/tufted cell (111604#2–2660) responded to both peppermint and isoamyl acetate separately and as a binary mixture. After 40-s exposure to peppermint alone (background), the response magnitude to peppermint was not obviously decreased, suggesting minimal habituation to the background peppermint odorant by mitral/tufted cells, as previously described (Wilson 1998a). Addition of the target odorant isoamyl acetate 40 s after the onset of peppermint background further increased activity of this cell, though it occurred against the heightened background activity (Fig. 1*B*).

In contrast, aPCX neurons responded quite differently under these conditions. For example, an aPCX neuron (061604#1–6801) responded to both isoamyl acetate and peppermint separately and as a binary mixture. Presentation of isoamyl acetate as background produced rapid and nearly complete adaptation to that odorant. Addition of the target odorant after 40 s of isoamyl acetate background produced a reliable response against an activity rate nearly identical to spontaneous rates (Fig. 1*B*).

Figure 3 (*left*) shows mean data of mitral/tufted and aPCX neurons for spontaneous activity, response magnitude to the background odorant at onset (initial 2 s), response magnitude to background odorant immediately prior to target odorant addition (38–40 s post background odorant onset), and response magnitude to the target odorant against the background. There was a significant difference in the mean spontaneous activity between mitral/tufted and aPCX neurons with mitral/tufted cell spontaneous activity [mean = 6.7 ± 0.65 (SE) Hz] significantly higher than aPCX neurons [mean = 0.8 ± 0.2 Hz; $t(220) = 9.44$, $P < 0.001$]. At onset of background odorant, there was a significant increase in mean mitral/tufted cell firing rate. Furthermore there was no significant mitral/tufted cell adaptation over the 40-s background

stimulation [Fig. 3; repeated-measures ANOVA, $F(3,84) = 5.80$, $P < 0.01$; post hoc tests revealed a significant difference between the spontaneous activity and both the first 2 s of background odorant and the last 2 s with no significant difference between the first and last 2 s of background stimulation, $P < 0.05$]. In contrast, over the same period of background odorant exposure, aPCX neurons showed significant response adaptation [Fig. 3; repeated-measures ANOVA, $F(3,117) = 16.86$, $P < 0.01$; post hoc tests revealed a significant difference between the spontaneous activity and the 1st 2 s of background odorant and a significant difference between the first and last 2 s of background stimulation, $P < 0.01$]. Both mitral/tufted and aPCX neurons showed significant activity change when the target odorant was introduced against the background (evoked spikes during 2-s mixture vs. firing rate during 2-s background immediately preceding the mixture, mitral/tufted cells; post hoc tests, mitral/tufted neurons, $P < 0.05$; aPCX neurons, $P < 0.001$).

The critical question for the purposes here, however, was whether the responses of olfactory bulb and cortical neurons to the target odorant presented during background stimulation reflected the stimulus inhaled (binary mixture) or were more similar to the target odorant alone, with the background filtered out. As shown in Fig. 3 (*right*), based on evoked spike counts, mitral cells on average responded as if they were stimulated with a binary mixture (reflecting sensory afferent input), whereas aPCX neurons on average responded different from the binary mixture as if a single odorant alone was presented (reflecting adaptation to background). There was no significant difference in response magnitude of mitral/tufted neurons to the binary mixture between before and after 40-s exposure to the background odorants [repeated-measures ANOVA, $F(2,60) = 3.14$, $P = 0.05$; post hoc tests revealed a significant difference between the single odorant and both the binary mixture and the target odorant against background and no difference between the binary mixture and target odorant against background]. In contrast, there was a significant difference in response magnitude of aPCX neurons to the binary mixture before and the response to the target odorant presented against the background odorant [repeated-measures ANOVA, $F(2,78) = 17.11$, $P = 0.001$; post hoc tests revealed a significant difference between the binary mixture and both the single odorant and the target odorant against background, and no difference between the single odorant and the target odorant against background; Fig. 3]. After adaptation to one component of a binary mixture, aPCX neurons responded to that mixture as if it was a single, unadapted component despite the reduced intensity of that component due to airflow division to the background (see METHODS). These data do not allow us to determine whether the individual aPCX neurons treated the target odorant against background as identical the target odorant alone but are consistent with that interpretation (see following text).

Mixture suppression

RESPONSE TO ODORANTS AGAINST BACKGROUNDS—Mixture suppression is likely to be influenced by a number of subthreshold events not observable with extracellular recordings, and thus response to adaptation and mixtures is more complex and more difficult to interpret. Nonetheless, the effects of background stimulation on odor responses in both mitral/tufted neurons and aPCX neurons suggest these cells treat target odors against background differently than mixture addition cells (Fig. 4). As in mixture addition cells, mitral/tufted mixture suppression cells responded to background odor onset, showed no significant adaptation over the course of 40-s background exposure, and significantly responded to the target odor against background [repeated-measures ANOVA, $F(3,213) = 5.67$, $P < 0.01$, see Fig. 4 for pairwise comparisons]. In contrast to mixture addition mitral/tufted cells, however, there was no significant difference between responses to the target odor against background and either the binary mixture or target odor alone in mixture suppression mitral/tufted neurons [repeated-measures ANOVA, $F(2,142) = 2.25$, N.S.].

In the aPCX, mixture suppression cells responded to background odor onset, showed significant adaptation to the background and responded to target odors against the background [repeated-measures ANOVA, $F(3,240) = 40.37$, $P < 0.01$, see Fig. 4 for pairwise comparisons]. Again, in contrast to mixture addition aPCX neurons, however, the response to target odorant against background in mixture suppression cells was significantly different from the response to the target odorant alone and different from the response to the binary mixture. These results suggest that mixture suppression responses may provide distinctly different information to downstream sites about odorants against background than mixture addition cells and further support the finding that mitral/tufted neurons and aPCX neurons respond to these conditions differently.

Piriform cortical output: response to odorants against background

A final analysis was performed to extract some indication of the total output pattern of piriform cortex activity under these conditions. All units tested with the odorant combination of isoamyl acetate (IAA) as the target odorant and pentane (C5) as the background odorant were combined regardless of mixture addition/suppression classification or animal of origin. This odorant combination was chosen for additional analyses because it was the most common combination in this dataset ($n = 21$). The response magnitude to the target odorant presented against background (2-s stimulus, IAA against C5) was compared with the response magnitude of the same cells to the target odorant alone (2 s IAA), the binary mixture (2 s C5 + IAA), or the background odorant alone (2 s C5), using Pearson correlations (Friedrich and Laurent 2001). As expected based on the analyses in the preceding text, there was a significant correlation between the combined cellular responses to IAA alone and to IAA presented against a C5 background (Fig. 5; $P < 0.05$). There was no statistically significant correlation between response magnitudes to IAA presented against C5 background and the C5 + IAA binary mixture despite the fact that these two stimuli are identical during the 2-s test. Similarly there was no significant correlation between IAA presented against C5 background and the response to C5 alone.

Effect of odorant identity

Four different odorants and six different binary mixtures were used for this study (Table 1). With the odorants and intensities chosen here, when examining average responses across cells, no individual odorant (A or B) nor any specific binary mixture (A + B) was significantly more effective (or less effective) at driving single-unit activity than any other. Thus as shown in Table 2, ANOVA detected no significant differences in averaged responses to the four component odorants and the six binary mixtures were observed in either mitral/tufted or aPCX neurons. Therefore the results reported here are not due to the specific components or binary mixtures used.

Localization of recording in aPCX

The reconstructed positions of the neurons in aPCX are shown in Fig. 6. Most of cells were obtained in layer II/III between 0.0 and 0.9 mm anterior to Bregma. Most cells were in the dorsal region of the aPCX. There was no difference in location between neurons displaying mixture addition or mixture suppression.

Mice

Recordings from a small number ($n = 10$ mice) of urethan-anesthetized male Swiss-Webster mice were made for comparison purposes. Odorants and mixtures were the same as used in rats. Eleven aPCX neurons displayed mixture addition responses and 14 showed mixture suppression. As shown in Fig. 7, these mouse aPCX neurons were very similar to rat aPCX neurons in response to prolonged background odorant stimulation and in their ability to respond

to target odorants against background. Both mixture addition and mixture suppression mouse aPCX neurons showed significant initial responses to background odorant onset and adaptation to the background odorant over 40 s [repeated-measures ANOVA, mixture addition, $F(3,30) = 10.06$, $P < 0.01$; mixture suppression, $F(3,39) = 6.33$, $P < 0.01$, see Fig. 7 for pairwise comparisons].

Furthermore, as in rats, the mouse aPCX response magnitude of mixture addition cells to a target odorant presented against a background odorant was significantly different from the response to the binary mixture but not different from the response to the target odorant alone [Fig. 7; repeated-measures ANOVA, $F(2,20) = 6.35$, $P < 0.01$, post hoc tests revealed the response to the binary mixture was significantly different from response to either target odorant alone or to the target odorant against background, whereas target odorant alone and target odorant against background were not significantly different]. Similarly, as in rats, mouse aPCX response magnitude of mixture suppression cells responded to a target odorant against background differently than to the odorant alone, although the response was similar to a binary mixture [repeated-measures ANOVA, $F(2,26) = 5.10$, $P < 0.05$, post hoc tests revealed a significant difference between responses of odorant against background and odorant alone, $P < 0.05$].

DISCUSSION

The present results suggest that rapid cortical adaptation allows a subset of aPCX neurons to adapt to background odorants and selectively respond to new target odorants introduced against this background. Combined with previous work, these results suggest that cortical adaptation has two important functions in olfaction. First, it contributes to behavioral odor habituation (Best et al. 2005; Yadon and Wilson 2005). Second, it allows neurons within the piriform cortex to selectively respond to stimulus change; this may contribute to separation of target odorants from background. These results contribute to the growing evidence that the piriform cortex acts as a sensory gate in olfaction, allowing information throughput based on recent experience (Wilson 1998a) and behavioral state (Murakami et al. 2005) and thus shapes behavioral responses to the odor world (Best et al. 2005; Yadon and Wilson 2005).

It must be emphasized, however, that although the present results are strongly consistent with a role for the aPCX in detection and identification of odorants against background, the present techniques do not allow final determination of whether the target odorant against a background is actually identified, at the single-cell level, as specifically the target odorant alone. In fact, it is unlikely that firing rate of single cortical units summed over 2 s is sufficient to allow information about odorant identity to be extracted. Individual aPCX neurons most likely contribute to encoding of the quality of multiple odorants through participation in multiple, overlapping cortical ensembles. Replication of the current experiments, using ensemble recording techniques, may be required for a complete understanding of cortical coding of odorant quality and odorants against background. The correlation analyses present in Fig. 5, however, do provide some support for similarity in stimulus quality identification.

Rapid cortical adaptation allows the aPCX to function similar to a high-pass filter, responding primarily to stimulus change. This filtering effect could allow temporal separation of odorants based on differences in onset or inhalation time or potentially based on differences in absorption and transduction time across the olfactory epithelium. In fact, work by Laing and colleagues (Jinks and Laing 1999; Laing et al. 1994) has demonstrated that identification of odorants within complex mixtures can be facilitated in humans when the odorants vary in onset or diffusion characteristics. The time factor that affected odor identification in those studies (<1 s) was substantially shorter than the time scale here, although it suggests that further investigation at the physiological level is warranted.

It should be noted that the present results deal only with situations where background is relatively stable. If the background stimulus was fluctuating in intensity or quality, the mechanism described here may not allow filtering. However, a background fluctuating in such a way may itself provide important information and thus maintaining responsiveness may be beneficial to the animal in that case. In addition, these results are relevant only for short-term adaptation. Adaptation after long-term exposure to odorants (Dalton 2000; Dalton and Wysocki 1996) may involve distinctly different mechanisms and result in unique neurophysiological and perceptual consequences.

Cortical reading of olfactory bulb output

In contrast to mixture addition aPCX neurons, olfactory bulb mitral/tufted cells were unable to separate target odorants from background. Mixture addition mitral/tufted cell activity during odorant presentation against background was similar to responses to odorant mixtures. This difference between mitral/tufted cells and aPCX neurons supports the argument that odorant background separation may be a cortical phenomenon. The precise functions of mixture addition and mixture suppression cells at both the olfactory bulb and cortical levels are unclear, although the present results suggest that mixture addition cortical neurons may be particularly important in odorant background segmentation problems. Furthermore, the results strongly suggest different roles for mitral/tufted cells and aPCX neurons in odorant coding.

Mitral/tufted cell odorant responses are consistent with a feature detection role, wherein the spatiotemporal patterns of mitral/tufted cell activity encode the identity of odorant features (Araneda et al. 2000; Fletcher and Wilson 2003; Mori et al. 1999). In situations where more than one perceptual odor object is inhaled (i.e., most situations), the induced patterns of olfactory bulb activity reflect all features present. Interactions of odorant features at the olfactory receptor and within local olfactory bulb circuits may occur but most likely do not reflect natural groupings of features (i.e., odor objects). Thus olfactory bulb output provides information to higher centers about all features inhaled at any given time.

Analysis or synthesis?

Previous computational (Hasselmo et al. 1990), physiological (Wilson 2003), and psychophysical (Stevenson 2001) work has suggested that co-occurring odorants can be synthesized into unique odor objects, distinct from their components, but with perceptually merged qualities. Simultaneous, or near simultaneous presentation of the odorants (temporal association) facilitates this synthesis. The present results suggest that two odorants with distinctly different onset times are less likely to be synthesized and instead analyzed into background (early onset) and target (late onset) due to adaptation of cortical afferent synapses to the early onset odorant.

Thus the present results suggest that under the conditions of background stimulation, adaptive filtering by the aPCX allows the cortex to select a subset of the feature information encoded by olfactory bulb output based on temporal differences in odorant onset. Specific combinations of features activating the cortex at any given time can then be recognized as familiar odor objects through activation of learned patterns stored in intracortical association fiber synapses (Haberly 2001; Hasselmo et al. 1990). By combining short-term cortical afferent synaptic depression with learned patterns of feature combinations in intracortical association fiber synapses, the aPCX can identify odorants presented against backgrounds or analyze mixtures the components of which have delayed onset times.

Slow scale (seconds) temporal patterning in stimulus characteristics shapes central neuronal (present results; Heinbockel et al. 1999; Sobel et al. 2000) and perceptual (Goyert et al. 2005; Laing et al. 1994) odor responses. These slow scale patterns can lead to higher-order

processing such as figure-ground separation as suggested here. This leads to the conclusion that although the inhaled physicochemical stimulus and the resulting olfactory bulb output may place constraints on the ultimate sensory percept, under most real-world conditions, these two factors alone cannot predict that percept. Cortical processing and, as specifically demonstrated here, cortical adaptation, may allow selective reading of olfactory bulb activity, and ultimately greatly enhance analysis of the odor world.

Acknowledgements

GRANTS

This work was funded by grants from National Institute of Deafness and Other Communications Disorders and the National Science Foundation to D. A. Wilson.

References

- Araneda RC, Kini AD, Firestein S. The molecular receptive range of an odorant receptor. *Nat Neurosci* 2000;3:1248–1255. [PubMed: 11100145]
- Arbas EA, Humphreys CJ, Ache BW. Morphology and physiological properties of interneurons in the olfactory midbrain of the crayfish. *J Comp Physiol [A]* 1988;164:231–241.
- Best AR, Thompson JV, Fletcher ML, Wilson DA. Cortical metabotropic glutamate receptors contribute to habituation of a simple odor-evoked behavior. *J Neurosci* 2005;25:2513–2517. [PubMed: 15758159]
- Best AR, Wilson DA. Coordinate synaptic mechanisms contributing to olfactory cortical adaptation. *J Neurosci* 2004;24:652–660. [PubMed: 14736851]
- Cromarty SI, Derby CD. Inhibitory receptor binding events among the components of complex mixtures contribute to mixture suppression in responses of olfactory receptor neurons of spiny lobsters. *J Comp Physiol [A]* 1998;183:699–707.
- Dalton P. Psychophysical and behavioral characteristics of olfactory adaptation. *Chem Senses* 2000;25:487–492. [PubMed: 10944515]
- Dalton P, Wysocki CJ. The nature and duration of adaptation following long-term odor exposure. *Percept Psychophys* 1996;58:781–792. [PubMed: 8710455]
- Duchamp-Viret P, Duchamp A, Chaput MA. Single olfactory sensory neurons simultaneously integrate the components of an odour mixture. *Eur J Neurosci* 2003;18:2690–2696. [PubMed: 14656317]
- Fletcher ML, Wilson DA. Olfactory bulb mitral-tufted cell plasticity: odorant-specific tuning reflects previous odorant exposure. *J Neurosci* 2003;23:6946–6955. [PubMed: 12890789]
- Friedrich RW, Laurent G. Dynamic optimization of odor representations by slow temporal patterning of mitral cell activity. *Science* 2001;291:889–894. [PubMed: 11157170]
- Goyert H, Frank ME, Gent JF, Hettinger TP. Adaptation and identifying components of olfactory mixtures in humans. *Chem Senses* 2005;30:A112.
- Granger R, Lynch G. Higher olfactory processes: perceptual learning and memory. *Curr Opin Neurobiol* 1991;1:209–214. [PubMed: 1821183]
- Haberly LB. Parallel-distributed processing in olfactory cortex: new insights from morphological and physiological analysis of neuronal circuitry. *Chem Senses* 2001;26:551–576. [PubMed: 11418502]
- Hasselmo ME, Wilson MA, Anderson BP, Bower JM. Associative memory function in piriform (olfactory) cortex: computational modeling and neuropharmacology. *Cold Spring Harb Symp Quant Biol* 1990;55:599–610. [PubMed: 2132840]
- Heinbockel T, Christensen TA, Hildebrand JG. Temporal tuning of odor responses in pheromone-responsive projection neurons in the brain of the sphinx moth *Manduca sexta*. *J Comp Neurol* 1999;409:1–12. [PubMed: 10363707]
- Jinks A, Laing DG. Temporal processing reveals a mechanism for limiting the capacity of humans to analyze odor mixtures. *Brain Res Cogn Brain Res* 1999;8:311–325. [PubMed: 10556608]
- Jinks A, Laing DG. The analysis of odor mixtures by humans: evidence for a configurational process. *Physiol Behav* 2001;72:51–63. [PubMed: 11239981]

- Kay LM, Lowry CA, Jacobs HA. Receptor contributions to configural and elemental odor mixture perception. *Behav Neurosci* 2003;117:1108–1114. [PubMed: 14570560]
- Laing DG, Eddy A, Francis GW, Stephens L. Evidence for the temporal processing of odor mixtures in humans. *Brain Res* 1994;651:317–328. [PubMed: 7922581]
- Laing DG, Francis GW. The capacity of humans to identify odors in mixtures. *Physiol Behav* 1989;46:809–814. [PubMed: 2628992]
- Leon M, Johnson BA. Olfactory coding in the mammalian olfactory bulb. *Brain Res Brain Res Rev* 2003;42:23–32. [PubMed: 12668289]
- Mori K, Nagao H, Yoshihara Y. The olfactory bulb: coding and processing of odor molecule information. *Science* 1999;286:711–715. [PubMed: 10531048]
- Murakami M, Kashiwadani H, Kirino Y, Mori K. State-dependent sensory gating in olfactory cortex. *Neuron* 2005;46:285–296. [PubMed: 15848806]
- Polak EH. Mutiple profile-multiple receptor site model for vertebrate olfaction. *J Theor Biol* 1973;40:469–484. [PubMed: 4754892]
- Sobel N, Prabhakaran V, Zhao Z, Desmond JE, Glover GH, Sullivan EV, Gabrieli JD. Time course of odorant-induced activation in the human primary olfactory cortex. *J Neurophysiol* 2000;83:537–551. [PubMed: 10634894]
- Stevenson RJ. Perceptual learning with odors: implications for psychological accounts of odor quality perception. *Psychon Bull Rev* 2001;8:708–712. [PubMed: 11848589]
- Tabor R, Yaksi E, Weislogel JM, Friedrich RW. Processing of odor mixtures in the zebrafish olfactory bulb. *J Neurosci* 2004;24:6611–6620. [PubMed: 15269273]
- Wilson DA. Habituation of odor responses in the rat anterior piriform cortex. *J Neurophysiol* 1998a;79:1425–1440. [PubMed: 9497422]
- Wilson DA. Synaptic correlates of odor habituation in the rat anterior piriform cortex. *J Neurophysiol* 1998b;80:998–1001. [PubMed: 9705487]
- Wilson DA. Odor specificity of habituation in the rat anterior piriform cortex. *J Neurophysiol* 2000;83:139–145. [PubMed: 10634860]
- Wilson DA. Rapid, experience-induced enhancement in odorant discrimination by anterior piriform cortex neurons. *J Neurophysiol* 2003;90:65–72. [PubMed: 12660351]
- Wilson DA, Stevenson RJ. The fundamental role of memory in olfactory perception. *Trends Neurosci* 2003;26:243–247. [PubMed: 12744840]
- Wiltout C, Dogra S, Linster C. Configurational and nonconfigurational interactions between odorants in binary mixtures. *Behav Neurosci* 2003;117:236–245. [PubMed: 12708520]
- Yadon CA, Wilson DA. The role of metabotropic glutamate receptors and cortical adaptation in habituation of odor-guided behavior. *Learn Mem* 2005;12:601–605. [PubMed: 16322361]

FIG. 1.

Top: experimental design for analysis of figure-ground separation by anterior piriform cortex (aPCX) and mitral/tufted neurons. Each component and the binary mixture were given twice for 2 s with at least a 60-s interval to determine initial response magnitude. Then 1 component of the binary mixture (background odorant) was presented for 50 s. Forty seconds after the onset of background, the other (target) odorant was added for 2 s, essentially creating a 2nd binary mixture. Binary mixtures and target against background stimuli were created by dividing the airstream toward both odors, thus total flow rate of odorized air did not differ between component and mixture stimuli. *Bottom:* representative examples of mitral/tufted and aPCX neuron responses during single stimulus and background stimulation. While both the mitral/tufted and aPCX neuron increased firing rate when the target odorant was presented against background, because the aPCX neuron had stopped responding to the background, its response was more similar to the target alone. The mitral/tufted cell response magnitude was more similar to the binary mixture under this same condition. Mean waveforms for both cells are shown to the right.

FIG. 2.

A: histograms of main olfactory bulb (MOB) mitral/tufted cell and aPCX neuron response magnitudes to a binary mixture as a ratio of the response magnitude to the most effective component of that mixture. Although the distribution was relatively continuous, cells were divided into mixture suppression and mixture addition groups based on previous work. B: example peristimulus time histograms (PSTHs) of single units showing mixture addition and mixture suppression. The number of evoked spikes/2-s stimulus are shown above each histogram. Mean waveforms for both cells are shown to the right. C: percentage of units demonstrating mixture addition and mixture suppression of mitral/tufted and aPCX neurons. Approximately 30% of units obtained in both MOB and aPCX showed mixture addition, and 70% mixture suppression. Right: standardized response magnitude of mitral/tufted and aPCX neurons to the target odorants and binary mixtures. The average response magnitude of mixture-addition neurons to the binary mixtures (A + B) was more than twice than that to the individual odorants (A or B), whereas that of mixture-suppression neurons to the binary mixture was less than that to the target odorants in both mitral/tufted and aPCX neurons.

FIG. 3.

Left: mean activity of mixture-addition mitral/tufted (*top*) and aPCX (*bottom*) neurons before (1st 2 s) and after 40-s exposure (last 2 s) to the background odorant. Mitral/tufted neurons did not significantly adapt to the background odorant, whereas aPCX neurons did. In both sites, presentation of the target odorant against the background evoked an increase in activity.

Right: mean response magnitudes of mitral/tufted (*top*) and aPCX (*bottom*) neurons to the target (nonbackground) odorant alone, the binary mixture and the target odorant against the background (same stimulus as the binary mixture). Response magnitudes are calculated as odor-evoked spikes during the 2-s stimulus minus spike rate during 2 s of spontaneous activity. Mitral/tufted cell response magnitude to the target odorant against background was similar to binary mixture and significantly different from the response to the nonadapted component alone. In contrast, aPCX neuron response magnitude to the target odorant against background was similar to the nonadapted component alone and significantly different from the binary mixture. The results are consistent with a role for aPCX neurons in filtering out background odorants and responding to new target odorants presented against the background as distinct.

FIG. 4.

Left: mean activity of mixture-suppression mitral/tufted (*top*) and aPCX (*bottom*) neurons before (1st 2 s) and after (last 2 s) 40-s exposure to the background odorant. Mitral/tufted neurons did not significantly adapt to the background odorant, whereas aPCX neurons did. In both sites, presentation of the target odorant against the background evoked an increase in activity. *Right:* mean response magnitudes of mitral/tufted (*top*) and aPCX (*bottom*) neurons to the target (nonbackground) odorant alone, the binary mixture before, the target odorant against the background odors. Mitral/tufted cells responded to the target odorant against background with a level of activity no different from their response to the binary mixture or a single odor component alone. In contrast, aPCX neurons responded to the odorant against background with a level of activity significantly different from either the binary mixture or the single component alone.

FIG. 5.

Response correlations for units tested with isoamyl acetate (IAA) as the target odorant and pentane (C5) as the background odorant ($n = 21$ units). Response magnitudes to IAA presented against C5 background were significantly correlated with the response of the same units to IAA alone. There was no significant correlation between IAA against C5 background and the binary mixture of IAA and C5 despite the fact that these two stimuli are identical at the nose.

FIG. 6.

Reconstructed positions of aPCX neurons recorded in this study. Distances noted are anterior to Bregma. These neurons were located in aPCX layer II/III. There was no significant difference in distributions among neurons with mixture addition, mixture suppression, and mixture addition/suppression. All cells were located in the dorsal region of the aPCX. AI, agranular insular cortex; CC, corpus callosum; CI, claustrum; CPu, caudate putamen; DEn, dorsal endopiriform nucleus; DI, dysgranular insular cortex; GI, granular insular cortex; LOT, lateral olfactory track; Pir, piriform cortex; Tu, olfactory tubercle; VP, ventral pallidum

FIG. 7.

A: example of an aPCX single-unit odor response in urethan-anesthetized mouse. *B, top*: mean evoked activity of mixture-addition mouse aPCX neurons before (1st 2 s) and after (last 2 s) 40-s exposure to the background odorant and in response to presentation of the target odorant against the background. Mouse data mirrors data obtained in rat (cf. Fig. 3). *Bottom*: mouse aPCX neurons responded to the target odorant against background similarly to the nonadapted component alone, and significantly different from the binary mixture. Again, this data are similar to rat data shown in Fig. 3. *C*: similar presentation of data from mouse aPCX mixture suppression cells. Note similarity with rat data in Fig. 4.

TABLE 1

Number of experiments for each pair of binary mixtures tested

	Iso, Pen	Iso, Pep	Iso, Eug	Pen, Pep	Pen, Eug	Pep, Eug	Total
MOB	30	19	19	10	11	12	101
aPCX	49	10	12	18	11	21	121

Iso, isoamyl acetate; Pen, pentane; Pep, peppermint; Eug, eugenol; MOB, main olfactory bulb; aPCX, anterior piriform cortex.

TABLE 2

No significant effect of odor identity

		F value	P value
MOB	Odor A	$F(5,95) = 1.553$	0.181
	Odor B	$F(5,95) = 0.797$	0.555
	Mix(A + B)	$F(5,95) = 2.017$	0.083
APCX	Odor A	$F(5,115) = 0.66$	0.65
	Odor B	$F(5,115) = 0.69$	0.63
	Mix(A + B)	$F(5,115) = 1.17$	0.32

A one-way ANOVA was performed to show differences in neural responses to each component and binary mixture tested in this study.