

Associative Encoding in Posterior Piriform Cortex during Odor Discrimination and Reversal Learning

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Recent proposals have conceptualized piriform cortex as an association cortex, capable of integrating incoming olfactory information with descending input from higher order associative regions such as orbitofrontal cortex and basolateral amygdala (ABL). If true, encoding in piriform cortex should reflect associative features prominent in these areas during associative learning involving olfactory cues. We recently reported that neurons in anterior piriform cortex (APC) in rats exhibited significant plasticity in their responses to odor cues during associative learning. Here, we have repeated this study, recording from neurons in posterior piriform cortex (PPC), a region of piriform cortex that receives much stronger input from ABL. If associative encoding in piriform cortex is driven by inputs from ABL, then we should see more plasticity in PPC neurons than we observed in APC. Consistent with this hypothesis, we found that PPC neurons were highly associative and appeared to be somewhat more likely than neurons recorded in APC to alter their responses to the odor cues after reversal of the odor-outcome associations in the task. Further, odor-selective PPC populations exhibited markedly different firing patterns based on the valence of the odor cue. These results suggest associative encoding in piriform cortex is represented in a topographical fashion, reflecting the stronger and more specific input from olfactory bulb concerning the sensory features of odors in anterior regions and stronger input from ABL concerning the meaning of odors in posterior regions.

Keywords: amygdala, olfactory, orbitofrontal, piriform, reversal

Introduction

Piriform cortex, the largest of the olfactory cortical areas, is part of a parallel, distributed, and bidirectional system involved in processing olfactory information (Haberly 2001). On one hand, piriform cortex has strong reciprocal connections with olfactory bulb and has traditionally been thought of as primary olfactory cortex. In fact, it is the largest recipient of afferent fibers from the bulb. On the other hand, piriform cortex also receives substantial descending input from downstream brain regions, such as orbitofrontal cortex (OFC) and basolateral amygdala (ABL), that are involved in processing multimodal input and in representing associative information (Datiche and Cattarelli 1996; Johnson and others 2000; Majak and others 2004; Illig 2005).

Based on these connections, it has been proposed that piriform cortex may function not as a primary sensory region, but as an olfactory association cortex, integrating sensory input from bulb with learned associative information from downstream regions (Haberly 2001). If this is true, then one might expect encoding in piriform cortex to reflect associative features prominent in these downstream areas during associa-

tive learning involving olfactory cues. Single-unit studies in awake rats provide some support for this hypothesis. For example, in rats trained to perform an 8-odor discrimination task, neuronal activity in lateral and ventrolateral orbital regions and piriform cortex is very similar, with firing in both areas reflecting both the identity and the valence of the odors (Schoenbaum and Eichenbaum 1995). More recently, we have reported that neurons recorded in anterior piriform cortex (APC) in rats learning and reversing odor discrimination problems exhibited associative features (Roesch and others 2006). For example, these neurons often changed their firing between 2 different odor cues as the rats learned that one predicted sucrose and the other predicted quinine. This is similar to what we have observed in areas of OFC, including the laterally located orbital areas as well as the dorsal and ventral agranular insular regions (Schoenbaum and others 1999, 2003, 2006). However, the neurons in APC failed to switch their firing from one cue to the other, when the cue-outcome associations were reversed in the task, and the overall population response evoked by the odor cues in APC appeared to reflect the sensory features of the cues. This pattern of sensory encoding was relatively unique to APC; we have not observed sensory encoding in OFC, ABL, or ventral striatum in any substantial number of neurons let alone the entire population (Schoenbaum and others 1999, 2003, 2006; Setlow and others 2003; Saddoris and others 2005).

These results suggest that although APC is influenced by associative information from downstream areas, this influence is secondary to sensory coding. This is not surprising because although APC receives input from OFC (Datiche and Cattarelli 1996; Illig 2005), inputs from ABL, a region implicated in encoding associations between cues and outcomes, are primarily concentrated in posterior piriform cortex (PPC) (Johnson and others 2000; Majak and others 2004). In contrast, afferents from olfactory bulb synapse primarily on neurons in APC and only send lighter, more distributed inputs to PPC (de Olmos and others 1978; Haberly and Price 1978; Shipley and Adamek 1984; Haberly 1998, 2001). Furthermore, the ratio of associative (layer Ib) to afferent (layer Ia) input is higher in more posterior regions of piriform cortex (Haberly 1998). This suggests that information in piriform cortex may be represented in a topographical fashion, with more sensory representations maintained in APC and more associative representations maintained in PPC (Zelano and Sobel 2005; Gottfried and others 2006). To test this hypothesis, we repeated our prior experiment, this time recording in PPC in rats learning and reversing novel 2-odor discrimination problems. This experiment was identical in all respects to the prior experiment except for the location of the recording electrode and the number of subjects and neurons sampled.

Materials and Methods

This research was conducted at the University of Maryland School of Medicine in accordance with university and the National Institutes of Health guidelines. Note that as in our prior experiment, these rats received saline injections, intraperitoneal, for 14 days, as part of another experiment that examined the effects of drug exposure on encoding in ABL.

Surgical Procedures

Five adult male Long-Evans rats served as subjects (Charles River Laboratories, Wilmington, MA; 3–6 months old during recording, identical to prior study). Procedures for implanting electrodes were identical to those used previously (Roesch and others 2006). A drivable electrode bundle was chronically implanted in the left hemisphere at 3.0 mm posterior to bregma, 5.0 mm laterally, and 7.5 mm ventral to the surface of the brain. This electrode bundle was composed of 10 25- μ m diameter FeNiCr wires (Stablohm 675, California Fine Wire, Grover Beach, CA) in a 27-gauge thin wall cannula (Small Parts, Miami Lakes, FL). Immediately prior to implantation, these wires were freshly cut with surgical scissors to extend ~1 mm beyond the cannula and electroplated with platinum (H_2PtCl_6 , Aldrich, Milwaukee, WI) to an impedance of ~300 kOhms. During recording, the electrode bundle was advanced in 40- μ m increments to acquire activity from new neurons for the following day. In a given session, neural activity was acquired from neurons in ABL or PPC.

Histology

Following testing, rats were given an overdose of pentobarbital and prepared for perfusion. Immediately prior to perfusion, the final electrode position was marked by passage of a 15- μ A current through each microwire for approximately 10 s to create a small iron deposit. The rats were then perfused intracardially with 0.9% saline followed by 4% formaldehyde followed by 100 ml of 3% potassium ferrocyanide in perfusate to visualize the iron deposit. Brains were removed from the skulls and stored in a 30% sucrose/4% formaldehyde/3% potassium ferrocyanide solution for several days until sectioning. The brains were sectioned on a freezing microtome, and coronal sections (40 μ m) collected through APC. Sections were mounted on glass slides, stained with thionin, and coverslipped with Permount. Electrode placements were verified under a light microscope and drawn onto plates adapted from the atlases of Paxinos and Watson (1997; Roesch and others 2006).

Behavioral Methods

Odor discrimination training was conducted in aluminum chambers approximately 18" on each side with sloping walls narrowing to an area of 12" \times 12" at the bottom. An odor port and fluid well were located on a panel, which was located in the right wall of each chamber below 2 panel lights. Discrimination problems were composed of odor compounds obtained from International Flavors and Fragrances (New York, NY; e.g. iso-propyl hydratropic ald para, verbena oliffac, petinerol, ald C-8 orange fraction florex, cedryl acet trubek, vanoris, geranyl formate, auralva, camekol DH, dimeth phen eth carb acetate, hexenol B gamma extra, celeriax, cyclemone A, and phenoxanol; contact G.S. for full list). Discrimination problems were constructed from dissimilar odors, according to a subjective classification scheme (fruity, spicy, herbal, etc.) developed by Staubli and others (1987), and the odor discrimination sequence was arranged such that similar compounds were counterbalanced by valence and did not repeat across days. Task control and data acquisition were implemented by a custom program written in C++ and running in DOS on a Pentium II PC. During training, rats were maintained on water restriction. After each session, the rats were given ad lib access to water for 10–30 min depending on the fluid intake of each rat during the session.

The events in a trial are depicted in Figure 1. Trials were signaled by illumination of the panel lights inside the box. When these lights were on, nose poke into the odor port resulted in delivery of the preselected odor cue to a small hemicylinder located behind this opening. The rat terminated odor sampling by leaving the odor port then had 3 s to make a go response at the fluid well located below the port. If a response was made after sampling a positive odor, then a 0.05-ml bolus of an appetitive 10% sucrose solution was delivered to the well after a variable

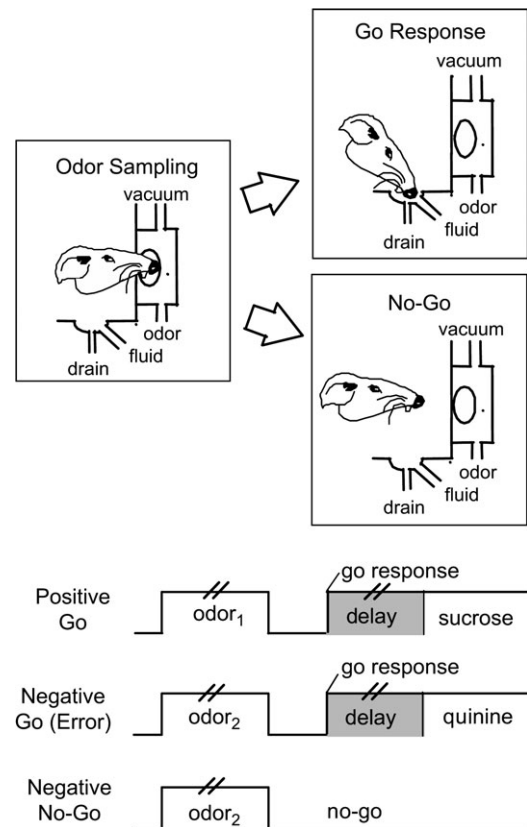


Figure 1. Schematic illustrating behaviors in the task. Pairs of vertical lines during odor presentation and the delay between ago response and fluid delivery denote the variable duration of these events; odor sampling typically lasted 250–750 ms and the delay was programmed to vary from 500–1500 ms.

(500–1500 ms). If the same response was made after sampling a negative odor, then a 0.05-ml bolus of an aversive 0.02-M quinine solution was delivered after a similar delay. If the rat did not respond within 3 s, the trial was counted as a no-go. A behavioral criterion was defined as 18 correct responses in a moving block of 20 trials.

The rats received training on several problems prior to surgery, and then neural data were collected as the rats acquired novel discriminations in sessions after surgery. In these sessions, the rats were trained until they met the behavioral criterion (~50 trials on average) and for an additional 60–100 trials after this criterion was achieved. In some sessions, the discrimination problem was also reversed and neural data were obtained as the rats acquired the reversal problem.

Data Acquisition and Analysis

Experimental recording sessions after surgery were conducted in 2 aluminum chambers identical in all respects to the set of chambers used for training prior to surgery. These chambers included a behavioral computer running the same program used in presurgical training; this computer was interfaced with the recording systems to signal events. For each recording session, the rat was placed in the training chamber, and the electrode wires were screened for neural activity while the rat explored the open chamber. If no activity was detected, the rat was removed, and the electrode assembly was advanced 40 or 80 μ m. Otherwise, active wires were selected for recording, and a training session was begun.

Neural activity was recorded using 2 identical Plexon Multichannel Acquisition Processor systems (Plexon Inc, Dallas, TX). Signals from the wires were amplified 20 \times by an op-amp headstage (Plexon Inc, HST/8050-G20-GR) located on the electrode array. Immediately outside the recording chamber, the signals were passed through a differential preamplifier (Plexon Inc, PBX2/16sp-r-G50/16fp-G50). From there, the single-unit signals were amplified 50 \times , filtered at 150–9000 Hz,

and then sent to the Multichannel Acquisition Processor box, where they were further filtered at 250–8000 Hz, digitized at 40 kHz, and amplified at 1–32 \times . Waveforms (>2.5:1 signal-to-noise) were extracted from active channels and recorded to disk by an associated workstation along with time stamps from the behavior computer indicating when significant events occurred (odor onset, responding, fluid delivery, etc.).

Units were sorted off-line using software from Plexon Inc. For this analysis, files were first imported into Offline Sorter where waveforms on each channel were sorted using a template-matching algorithm. These waveforms were compared with notes regarding the waveforms made during the session, and the interspike interval histograms were inspected to ensure that spike events were separated by >1 ms. Typically, 1–3 waveforms could be isolated on an active channel.

Sorted files were then processed in Neuroexplorer to extract these unit time stamps and relevant event markers. These data were subsequently analyzed using statistical routines in Matlab (Natick, MA) to examine activity during odor sampling (from 50 ms after odor onset to 50 ms after odor offset), during the variable delay after a response at the fluid well (from 50 ms before the response until fluid delivery), and after fluid delivery (first 500 ms). Firing activity (spikes/second) in each time window was compared on positive and negative trials during pre- and postcriterion trial blocks using analysis of variance ($P < 0.05$), and neurons with a significant difference in activity were categorized as “selective” in that time window and phase.

A Pearson chi-square test was used to compare the proportions of neurons with different firing properties and to ask whether particular firing patterns (e.g. neurons that fired before sucrose delivery that became selective for the positive odor after learning) were observed at a greater frequency than expected by chance in the population of neurons. For these comparisons, chance was calculated based on the probabilities of neurons in the population exhibiting each type of response. This expected occurrence was compared with the actual proportion observed in our experimental groups.

Results

Neural activity was recorded from drivable arrays of microelectrodes. By moving the electrodes in 40- to 80- μ m increments overnight, we were able to acquire neural activity from a new location in PPC each day. Neural activity was recorded as rats learned and reversed a series of 2-odor go, no-go discrimination problems. In each problem, one “positive” odor signaled the availability of an appetitive sucrose solution, and the other “negative” odor signaled the availability of an aversive quinine solution. When presented with a novel odor pair, the rats initially responded at the fluid well on every trial but subsequently learned to respond only after sampling the positive odor. Rats acquired the odor problem when they met a behavioral criterion of 18 correct go, no-go responses in the last 20 trials. Data were acquired during this learning phase (pre-criterion) and after learning (postcriterion). In some sessions, we also recorded neural activity as the rats acquired a reversal of the discrimination problem (reversal).

We acquired data from 131 neurons located in the ventral part of the PPC in 44 sessions, including 21 sessions in which the rats also acquired a reversal of the problem. Figure 2 shows the locations in PPC from which recordings were obtained. The rats met a criterion of 18/20 correct on the initial discrimination problem in 122 trials on average and on the reversal in 72 trials in these sessions. Our initial analysis began by identifying neurons that fired differentially to the odor cues after the rats had learned the discrimination problem. As expected, we found that many neurons did show odor selectivity. A comparison of neural activity during odor sampling in trials from the post-criterion phase revealed that 27 (21%) of the 131 neurons recorded in PPC fired significantly more to one or the other odor cue, including 17 neurons that fired significantly more

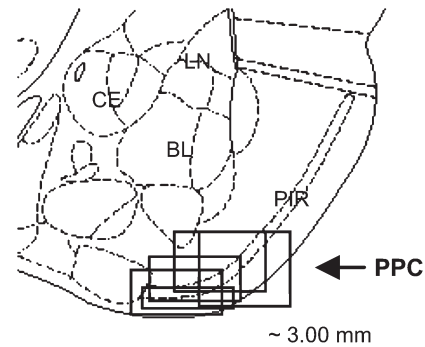


Figure 2. Location of recording sites in PPC. Vertical bars on the drawing indicate the center of the electrode track in each rat; boxes indicate approximate extent of recording sessions vertically during transition through each area and give an estimate of lateral (and AP) spread of the wires (~1 mm).

strongly to odor cue predicting sucrose and 10 neurons that fired significantly more strongly to the cue predicting quinine. These proportions were nearly identical to what we observed in APC, where we also observed more neurons that fired to the positive odor cue (Roesch and others 2006).

To dissociate sensory encoding of the olfactory cues from more associative representations, we examined activity in the odor-selective neurons during learning and after reversal. The various patterns and their interpretation are illustrated in Figure 3. For example, a purely sensory neuron should exhibit the same odor selectivity throughout a session, including before learning and even after reversal (Fig. 3A). A purely associative neuron—for example a neuron that encodes only what the odor means—should develop a cue-selective response with learning and reverse it during reversal learning (Fig. 3B). A neuron that encodes some combination of sensory and associative information—for example a neuron that represents the conjunction of a particular odor and a particular associative significance—might develop a selective response before reversal and then lose it after reversal or vice versa (Fig. 3C). In the analyses that follow, we analyzed neural activity PPC for firing that approximated these simple patterns, and then compared the proportions of these correlates across the 2 areas. This is the same analysis we applied to APC neurons in our earlier study (Roesch and others 2006).

The population responses of the cue-selective neurons in PPC are shown in Figure 4, along with data from APC neurons. These histograms provide a qualitative representation of the odor-evoked firing in PPC. The population response in PPC exhibited several unique features. First, the response to the odor cues in PPC was different in neurons selective for the positive (Fig. 4A, upper panel) and negative (Fig. 4A, bottom panel) odor cues. The former population exhibited a relatively simple phasic response to the odor cue, whereas the latter population did not become active until later in odor sampling and then only weakly. This valence specificity mirrored the disproportionate responding to the positive odor cue exhibited by the single units, discussed above, although this effect was not significant. In APC, although the single units were more likely to fire to the positive odor cue, the population response did not differ for the oppositely valenced cues.

Second, the PPC populations exhibited persistent firing after odor sampling during the response and outcome periods (Fig. 4A). This is evident starting several hundred milliseconds after

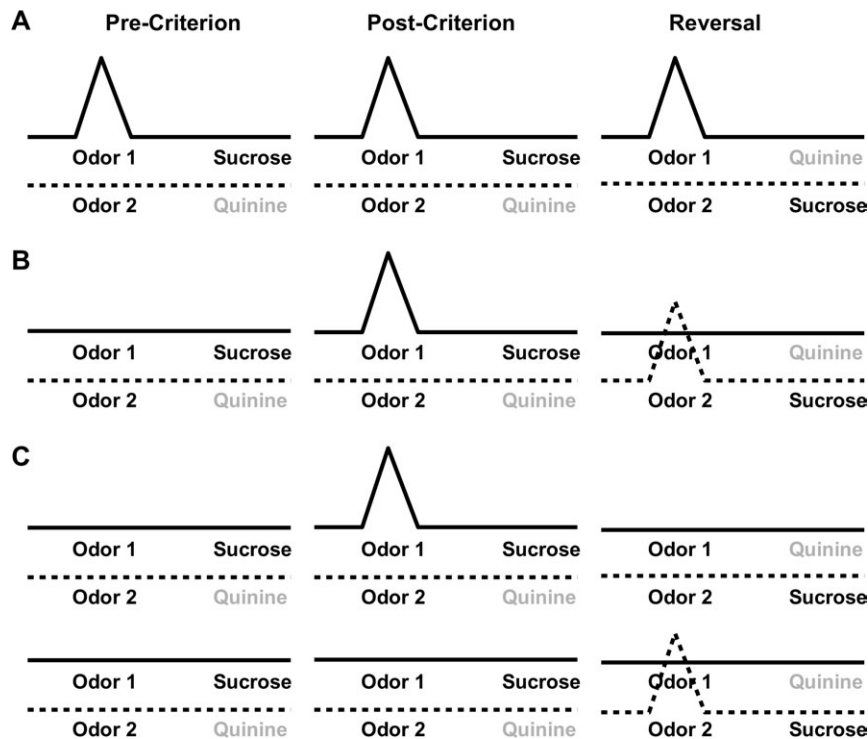


Figure 3. Predicted cue-selective patterns across learning and reversal. (A) Example of putative sensory encoding. (B) Example of putative associative encoding. (C) Two examples of putative conjunctive encoding of sensory + associative information.

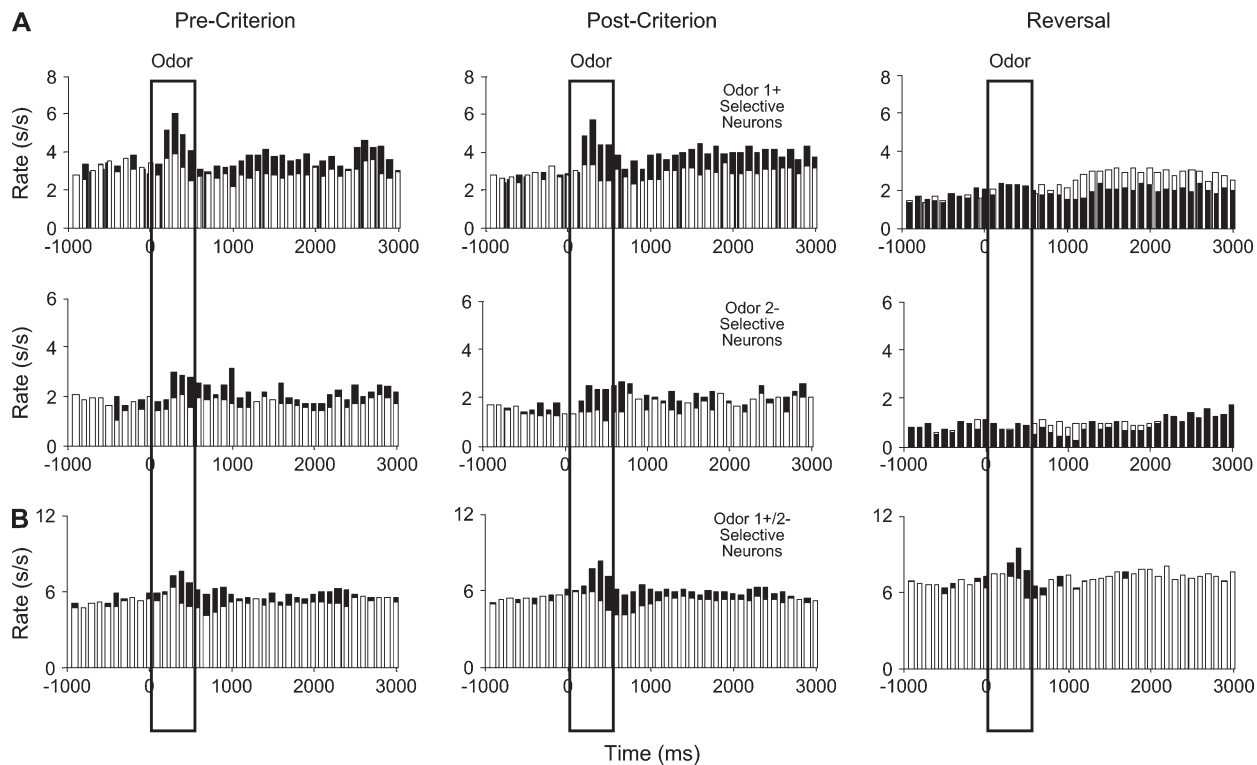


Figure 4. Effect of learning and reversal training on cue-selective firing in PPC. Population histograms show the average response of neurons that were selective in the postcriterion phase in (A). PPC—top panel: neurons that fired more for the positive odor postcriterion; bottom panel: neurons that fired more for the negative odor postcriterion. (B) For comparison, histograms are also shown for activity recorded in APC in an earlier study. Activity did not differ by odor valence, so they are shown together. Activity is shown separately for trials involving each odor cue during the precriterion, postcriterion, and reversal trial blocks. Black bars show the response to the preferred odor cue in the postcriterion phase. White bars show the response to the nonpreferred odor. Activity is synchronized to odor onset, displayed in 100 ms bins in spikes/second (s/s), and box overlays indicate the approximate duration of odor sampling, which was terminated by the rat.

the end of odor sampling and encompassed the response and outcome delivery periods, particularly in the precriterion phase when the rats were responding on most trials. Persistent firing after odor sampling was not evident in cue-selective APC neurons (Fig. 4B; although it was evident in APC neurons that were not cue selective). An analysis of single-unit activity, which compared firing on positive and negative precriterion trials during an ~1-s delay after responding prior to outcome delivery, indicated that 21 neurons or 16% of the PPC population exhibited differential firing in anticipation of the outcomes. This population included 4 neurons that fired more during the delay on positive trials and 17 neurons that fired more during the delay on negative trials. Analysis of cue selectivity in these neurons indicated that 2 of the former and 4 of the latter population also were selective for the appropriate odor cue. This proportion (6/21) was higher than one would expect by chance ($\chi^2 = 4.29$, $P < 0.05$), given the proportions of these correlates in the population, and it was also greater than we observed when the same analysis was applied to neurons recorded in APC ($\chi^2 = 3.81$, $P = 0.051$), where this correlate was not present at levels above chance (Roesch and others 2006).

Notably, these neurons differed from “cue-outcome” correlates that we have previously identified in OFC and ABL (Schoenbaum and others 2003, 2006; Saddoris and others 2005) in that the activity to the outcomes did not typically precede cue-selective firing, instead, often developing in

parallel. This is evident in the population histograms in Figure 4 and in the single-unit examples in Figure 6. It is not clear from the small sample size whether this is because the cue selectivity was more likely to develop early in these neurons or because the outcome-related firing developed later.

Third, whereas the population response in APC was relatively unchanged across learning and reversal, consistent with the pattern in Figure 3A for sensory encoding, the population responses in PPC were highly associative. This difference was not evident during learning; however, after reversal, selectivity for the odor cues in the cue-sampling period disappeared in these neurons, consistent with the pattern illustrated in Figure 3C.

A statistical analysis of cue selectivity in the single units largely supported the conclusions regarding associative encoding drawn from the population responses. Of the 27 cue-selective neurons recorded in PPC, 18 of them became selective only after training (Fig. 5, “slowly selective”), indicating an important contribution of learning to encoding in PPC. This correlate is illustrated by the single units in Figure 6, which developed cue selectivity in the postcriterion phase. The proportion of such neurons in the cue-selective population was slightly higher than observed in APC (Fig. 5), although not significantly so ($\chi^2 = 0.30$, $P = 0.58$).

More interesting was the effect of reversal. We recorded 68 neurons in sessions in which the rats successfully acquired a reversal of the novel odor problem. As illustrated in Figure 5,

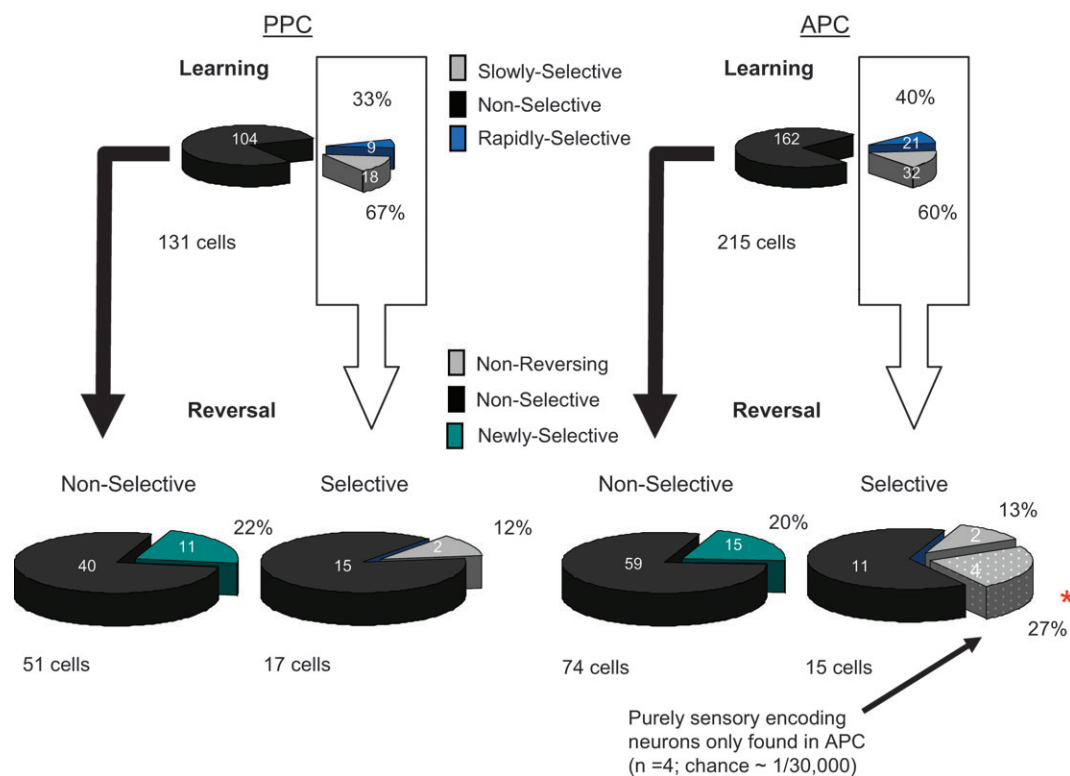


Figure 5. Effect of learning and reversal training on cue-selective firing in PPC. For comparison, pie charts are also shown for activity recorded in APC in an earlier study. The upper pie charts show the proportions of all neurons recorded that developed cue-selective firing during odor discrimination learning. Rapidly selective neurons (blue) developed an odor preference in the precriterion phase, which persisted postcriterion; slowly selective neurons (gray) developed an odor preference only in the postcriterion phase. The lower pie charts show how the odor preferences in selective and nonselective neurons change during reversal learning. Note that these numbers differ from those from “Learning” because while all sessions included learning, not all sessions progressed to reversal. Nonreversing neurons (gray) maintained the same odor preference during reversal learning. Some neurons in APC but not PPC maintained the same odor preference throughout the entire session (gray speckled). White numbers on the pie charts show actual numbers of neurons.

this population consisted of 28 neurons, including 15 neurons that fired selectively only before reversal (Fig. 6, bottom panel), 11 neurons that fired selectively to the odor cues only after reversal, and 2 neurons that kept the same cue selectivity across reversal (Fig. 6, top panel). Both of these neurons exhibited plasticity during learning (Fig. 6, top panel); thus, none of the cue-selective cells in PPC maintained the same odor preference across all phases of training. These patterns are consistent with conjunctive encoding illustrated in Figure 3C. By contrast, 6/15 cue-selective APC neurons maintained the same odor preference across reversal (Fig. 5; $\chi^2 = 2.62$, $P = 0.10$), and 4 of the 30 APC neurons that exhibited cue selectivity in the reversal sessions maintained the same odor preference across all 3

phases of training (Fig. 5; $\chi^2 = 4.01$, $P < 0.05$). These patterns are consistent with sensory encoding as illustrated in Figure 3A.

Discussion

Although piriform cortex receives sensory input from olfactory bulb, recent proposals have conceptualized this area as an association cortex, capable of integrating incoming olfactory information with descending input from higher order associative regions such as OFC (Haberly 2001). We recently reported that neurons in APC, a region which receives associative information from OFC but few inputs from ABL, exhibited some associative characteristics but were overall heavily influenced

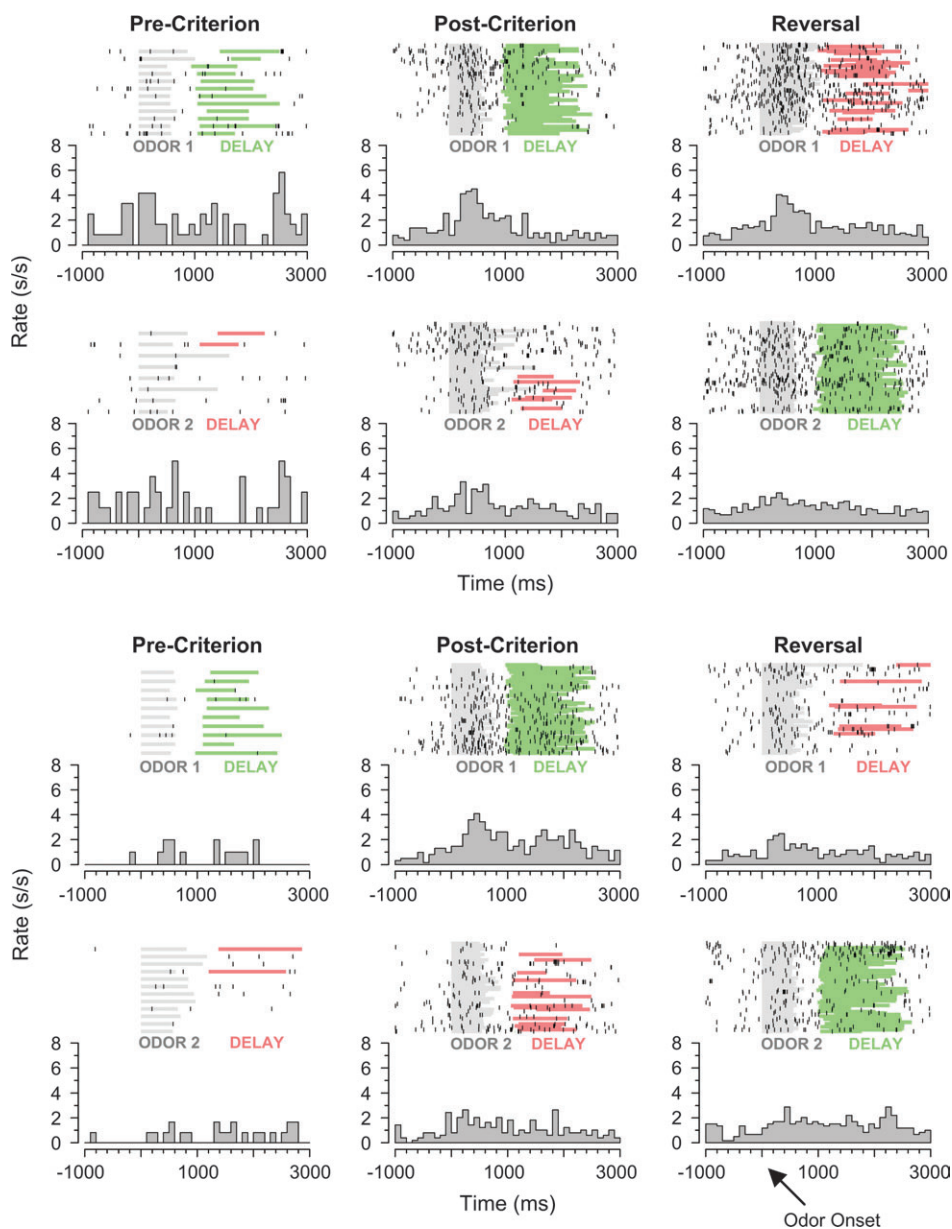


Figure 6. Neural activity during cue sampling during learning and reversal training. Neural activity is shown in 2 PPC neurons with selective responses to the positive odor cue in the postcriterion trials (middle column). Neither neuron exhibited a selective response to the odor cues precriterion (left column), but the neuron in the top panel did continue to fire to the same odor cue after reversal (right column). By contrast, the neuron in the bottom panel lost selectivity after reversal (right column). Raster displays show neural activity on individual trials, and each histogram shows average activity in spikes/second (s/s) in 100 ms bins. The timing of odor sampling and the delay after responding are shown by shading on the rasters (odor, gray; delay before sucrose, green; delay before quinine, red).

by the sensory attributes of the odor cues (Roesch and others 2006).

Here, we repeated the same experiment, this time recording from single neurons in PPC in rats learning and reversing novel 2-odor discrimination problems. Unlike APC, PPC has heavy inputs from ABL and much lighter projections from olfactory bulb (Datiche and Cattarelli 1996; Johnson and others 2000; Majak and others 2004; Illig 2005). Furthermore, the ratio of associative to sensory input increases as one moves more posterior in piriform cortex (Haberly 1998). Accordingly, we expected that representations in PPC would be highly associative and perhaps even more associative than those in APC. Consistent with this hypothesis, we found that neural activity in response to odor cues in PPC was highly plastic during reversal learning. This associative encoding was evident in the population responses and also in the proportion of cue-selective single units that changed their odor preference during learning and after reversal. In addition, the population responses distinguished between the differently valenced odor cues, exhibiting both earlier and more pronounced phasic activity to positive odor cues than to negative odor cues. This was perhaps the most striking feature of the data set and shows clearly that sensory encoding was subjugated to representations of the associative significance of the cues in PPC. Note that valence specificity of responding is also seen in ABL, where neurons are more often activated by the negative odor cues (Schoenbaum and others 1999; Saddoris and others 2005). By comparison, encoding in APC in this setting, reported previously, was substantially less associative. This was evident in the population response, which did not change cue preference across reversal and in a small but highly significant number of cue-selective neurons that fired strictly based on the sensory features of the odor cues. In addition, the cue-selective populations in PPC also included a significant number of neurons that were also active in anticipation of the outcome predicted by the odor. Such higher order associative single-unit correlates are often observed in OFC and ABL during this task (Schoenbaum and others 2003; Saddoris and others 2005) but were not observed in APC.

Although the numbers of neurons sampled in the current and prior study were relatively small, these 2 studies together provide strong evidence that piriform cortex transforms sensory input in light of the behavioral relevance of the incoming information. This transformation almost certainly reflects descending influence from areas such as OFC and ABL. Further, a comparison of the 2 studies, although far from conclusive due to the small cell numbers, provides statistically significant evidence that PPC exhibits more associative encoding than APC. The strongest evidence in this regard is the neurons in APC that fire based strictly on the sensory features of the odor cues. The likelihood of observing such correlates by chance, using the likelihood of finding the same odor selectivity in each phase of training ($P < 0.025$), is approximately 1/30 000 (1/60 000 for each odor). If instead, one uses the probability of finding selectivity for one of the odor cues in piriform neurons after learning, which is approximately 10% for each odor, then the likelihood of finding such correlates is still only about 1/500 (1/1000 for each odor). Although these back-of-the-envelope calculations do make assumptions about the independence of activity across phases, they demonstrate that such correlates are likely to be relatively rare, unless some aspect of the cue that is constant across phases is driving activity. Finding 4/30 purely sensory neurons in APC seems highly unlikely by either

comparison. Indeed, although we have recorded ~2500 neurons during reversal learning in 5 different brain areas, including studies in young and old rats, lesioned rats, and drug-experienced rats, only twice before have we observed significant numbers of these neurons. These 2 exceptions were recordings in APC and recordings in OFC in rats with ABL lesions.

The suggestion that PPC may be more associative than APC is consistent with proposals showing important differences between APC and PPC (Zelano and Sobel 2005; Gottfried and others 2006). As in the current report, Gottfried and others (2006) have reported that blood oxygen level-dependent signals in human APC are most closely related to olfactory cue structure, whereas signals in PPC are more closely related to the quality of the cue. These results suggest that, in both rodent and primate species, there may be an anterior-posterior gradient in the representation of ascending sensory information versus descending associative information in piriform cortex. This gradient may reflect the pattern of descending input from ABL, which primarily projects to PPC. ABL has been shown to be the critical site for learning about associations between cues and outcomes (Setlow and others 2002; Pickens and others 2003; Schoenbaum and Roesch 2005). Indeed, in humans, ABL is highly responsive to the value or valence of olfactory cues (Zald and Pardo 1997, 2000; Gottfried and others 2002, 2003). Damage to ABL before olfactory learning (including reversal learning) disrupts the formation of associative neural correlates in OFC, suggesting that input from ABL is necessary for neurons in associated brain regions to encode odor-outcome associations (Schoenbaum and others 2003). Similarly, direct input to PPC from ABL during learning might account for the associative encoding observed here.

Of course, this does not minimize the importance of plasticity at earlier stages in the olfactory system. Clearly, there is substantial plasticity and associative encoding in APC and even olfactory bulb (Wilson 1998, 2000, 2003; Kay and Laurent 1999; Roesch and others 2006). Feedback from PPC to APC and back to bulb could allow these valence-related categories to influence sensory processing in precisely the fashion indicated in our earlier study (Roesch and others 2006) and recent work in anesthetized rodents (Kadohisa and Wilson 2006).

Finally, we conclude with 2 important caveats. First, our findings do not demonstrate that ABL provides associative information to PPC directly; certainly, other important candidates may contribute to associative encoding in this region. It would be of great interest to disrupt encoding in afferent areas such as OFC or ABL and repeat this experiment. This approach would show whether descending input from these areas is influencing encoding in piriform cortex as proposed. Second, it is important to acknowledge that our results reflect a small sampling of the neurons that comprise the piriform area. Although they are in accord with other reports and with proposals about how these areas interact, it is not possible to say with certainty that the encoding patterns in 28 cue-selective neurons in a single study are fully representative of the entire region, containing thousands of neurons. Further work in different paradigms will be necessary to confirm whether the anterior/posterior distinctions evident here are valid.

Notes

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References

- Datiche F, Cattarelli M. 1996. Reciprocal and topographic connections between the piriform and prefrontal cortices in the rat: a tracing study using the B subunit of the cholera toxin. *Brain Res Bull* 41:391-398.
- de Olmos J, Hardy H, Heimer L. 1978. The afferent connections of the main and the accessory olfactory bulb formations in the rat: an experimental HRP-study. *J Comp Neurol* 181:213-244.
- Gottfried JA, O'Doherty J, Dolan RJ. 2002. Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. *J Neurosci* 22:10829-10837.
- Gottfried JA, O'Doherty J, Dolan RJ. 2003. Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science* 301:1104-1107.
- Gottfried JA, Winston JS, Dolan RJ. 2006. Dissociable codes of odor quality and odorant structure in human piriform cortex. *Neuron* 49:467-479.
- Haberly LB. 1998. The synaptic organization of the brain. Oxford: Oxford University Press, Inc.
- Haberly LB. 2001. Parallel-distributed processing in olfactory cortex: new insights from morphological and physiological analysis of neuronal circuitry. *Chem Senses* 26:551-576.
- Haberly LB, Price JL. 1978. Association and commissural fiber systems of the olfactory cortex of the rat. *J Comp Neurol* 178:711-740.
- Illig KR. 2005. Projections from orbitofrontal cortex to anterior piriform cortex in the rat suggest a role in olfactory information processing. *J Comp Neurol* 488:224-231.
- Johnson DM, Illig KR, Behan M, Haberly LB. 2000. New features of connectivity in piriform cortex visualized by intracellular injection of pyramidal cells suggest that "primary" olfactory cortex functions like "association" cortex in other sensory systems. *J Neurosci* 20:6974-6982.
- Kadohisa M, Wilson DA. 2006. Olfactory cortical adaptation facilitates detection of odors against background. *J Neurophysiol* 95:1888-1896.
- Kay LM, Laurent G. 1999. Odor- and context-dependent modulation of mitral cell activity in behaving rats. *Nat Neurosci* 2:1003-1009.
- Majak KS, Ronkko S, Kempainen S, Pitkanen A. 2004. Projections from the amygdaloid complex to the piriform cortex: a PHA-L study in the rat. *J Comp Neurol* 476:414-428.
- Paxinos G, Watson C. 1997. The rat brain in stereotaxic coordinates. New York: Academic Press.
- Pickens CL, Setlow B, Saddoris MP, Gallagher M, Holland PC, Schoenbaum G. 2003. Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. *J Neurosci* 23:11078-11084.
- Roesch MR, Stalnaker TA, Schoenbaum G. 2006. Associative encoding in anterior piriform cortex versus orbitofrontal cortex during odor discrimination and reversal learning. *Cereb Cortex*. 10.1093/cercor/bhk009.
- Saddoris MP, Gallagher M, Schoenbaum G. 2005. Rapid associative encoding in basolateral amygdala depends on connections with orbitofrontal cortex. *Neuron* 46:321-331.
- Schoenbaum G, Chiba AA, Gallagher M. 1999. Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. *J Neurosci* 19:1876-1884.
- Schoenbaum G, Eichenbaum H. 1995. Information coding in the rodent prefrontal cortex. II. Ensemble activity in orbitofrontal cortex. *J Neurophysiol* 74:751-762.
- Schoenbaum G, Roesch MR. 2005. Orbitofrontal cortex, associative learning, and expectancies. *Neuron* 47:633-636.
- Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. 2003. Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. *Neuron* 39:855-867.
- Schoenbaum G, Setlow B, Saddoris MP, Gallagher M. 2006. Encoding changes in orbitofrontal cortex in reversal-impaired aged rats. *J Neurophysiol* 95:1509-1517.
- Setlow B, Gallagher M, Holland P. 2002. The basolateral complex of the amygdala is necessary for acquisition but not expression of CS motivational value in appetitive Pavlovian second-order conditioning. *Eur J Neurosci* 15:1841-1853.
- Setlow B, Schoenbaum G, Gallagher M. 2003. Neural encoding in ventral striatum during olfactory discrimination learning. *Neuron* 38:625-636.
- Shiple MT, Adamek GD. 1984. The connections of the mouse olfactory bulb: a study using orthograde and retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase. *Brain Res Bull* 12:669-688.
- Staubli U, Fraser D, Faraday R, Lynch G. 1987. Olfaction and the "data" memory system in rats. *Behav Neurosci* 101:757-765.
- Wilson DA. 1998. Habituation of odor responses in the rat anterior piriform cortex. *J Neurophysiol* 79:1425-1440.
- Wilson DA. 2000. Comparison of odor receptive field plasticity in the rat olfactory bulb and anterior piriform cortex. *J Neurophysiol* 84:3036-3042.
- Wilson DA. 2003. Rapid, experience-induced enhancement in odor discrimination by anterior piriform cortex neurons. *J Neurophysiol* 90:65-72.
- Zald DH, Pardo JV. 1997. Emotion, olfaction, and the human amygdala: amygdala activation during aversive olfactory stimulation. *Proc Natl Acad Sci* 94:4119-4124.
- Zald DH, Pardo JV. 2000. Functional neuroimaging of the olfactory system in humans. *Int J Psychophysiol* 36:165-181.
- Zelano C, Sobel N. 2005. Humans as an animal model for systems-level organization of olfaction. *Neuron* 48:431-454.