

Rapid Associative Encoding in Basolateral Amygdala Depends on Connections with Orbitofrontal Cortex

Michael P. Sadoris,^{1,*} Michela Gallagher,¹
and Geoffrey Schoenbaum^{2,*}

¹Department of Psychological and Brain Sciences
Johns Hopkins University
3400 North Charles Street
25 Ames Hall

Baltimore, Maryland 21218
²Department of Anatomy and Neurobiology
University of Maryland School of Medicine
20 Penn Street, HSF-2 S251
Baltimore, Maryland 21201

Summary

Certain goal-directed behaviors depend upon interactions between basolateral amygdala (ABL) and orbitofrontal cortex (OFC). Here we describe neurophysiological evidence of this cooperative function. We recorded from ABL in intact and OFC-lesioned rats during learning of odor discrimination problems and reversals. During learning, rats with ipsilateral OFC lesions exhibited a marked decline in the proportion of ABL neurons that fired differentially during cue sampling both before and after reversal and in the proportion of neurons that reversed odor preference when the odor-outcome associations were reversed. This decline appeared to reflect a loss of rapid flexibility in cue selectivity that characterized activity in intact rats. In addition, lesioned rats had fewer neurons that fired in anticipation of the predicted outcome during a delay period after responding but before outcome delivery. These findings support a role for OFC in facilitating the encoding of information about expected outcomes in ABL.

Introduction

Reciprocal connections between the basolateral complex of the amygdala (ABL) and the orbitofrontal cortex (OFC) provide a critical circuit for using incentive information to guide behavior (Carmichael and Price, 1995; Gallagher and Schoenbaum, 1999; Ghashghaei and Barbas, 2002; Kita and Kitai, 1990; Krettek and Price, 1977; Shi and Cassell, 1998). The function of this circuit is evident in settings that depend upon the acquisition and use of associations between predictive cues and outcomes. For example, humans with damage to either amygdala or OFC are impaired in the capacity to assess and use the value of predicted outcomes to guide their actions in the Iowa gambling task (Bechara et al., 1999). Similarly, monkeys with damage to these regions or disconnection of this circuit are unable to adaptively select behavior according to the motivational significance of cues and the value of the outcomes those cues predict (Baxter et al., 2000; Cousens and Otto, 2003;

Fanselow and Gale, 2003; Gallagher et al., 1999; Hatfield et al., 1996; Izquierdo et al., 2004; Malkova et al., 1997; Parkinson et al., 2001; Pears et al., 2001; Weiskrantz, 1956; Izquierdo et al., 2004).

While lesions in humans and monkeys often include many subregions within the amygdala, work in rats suggests that the ability to learn about and use the motivational significance of cues and the value of predicted outcomes depends critically upon ABL. In line with this proposal, several features of neural activity in ABL reflect a fundamental role in encoding associations between cues and the outcomes that those cues predict. It has been widely reported in both Pavlovian and instrumental tasks that amygdala neurons in general, and ABL neurons in particular, fire to cues predictive of rewards or punishments (Maren, 2000; Muramoto et al., 1993; Nishijo et al., 1988; Quirk et al., 1995; Sanghera et al., 1979). These neural correlates are characterized by a rapid development during learning and after reversal. For example, we have demonstrated, in an odor discrimination task, that cue-selective firing emerges in the first 10 to 15 trials during learning and typically reverses almost immediately when the contingencies between the cues and outcomes are switched (Schoenbaum et al., 1999). In addition, ABL neurons fire during delay periods after responding but before delivery of an expected outcome (Schoenbaum et al., 1998). Like neurons that acquire selective activity in the presence of predictive cues, these delay-selective neurons develop selectivity rapidly during learning and provide information about predicted outcomes. Similar findings have recently been reported from recordings in primate amygdala during discrimination reversal learning (M.A. Belova et al., 2004, Soc. Neurosci., abstract).

Many of the features just described also characterize neural activity in OFC (Hikosaka and Watanabe, 2000; Schoenbaum et al., 1998; Schoenbaum et al., 1999; Tremblay and Schultz, 2000; Wallis and Miller, 2003). Given the importance of the ABL-OFC circuit for behavior guided by the value of predicted outcomes, we sought to test whether the establishment of encoding associated with the outcome in ABL, either during the sampling of predictive cues or during the subsequent delay period after responding, might depend on input from OFC. To this end, we recorded neural activity from ABL in rats performing a go, no-go odor discrimination task. In this task, thirsty rats learn a series of discrimination problems in which one odor signals delivery of a rewarding sucrose solution and the other odor signals delivery of an aversive quinine solution. Neural encoding in ABL was compared in intact rats and rats with neurotoxic lesions of ipsilateral OFC.

Results

Water-deprived rats were trained on a series of two-odor go, no-go discriminations (Figure 1). In each problem, one “positive” odor signaled the availability of an

*Correspondence: sadoris@jhu.edu (M.P.S.); schoenbg@schoenbaumlab.org (G.S.)

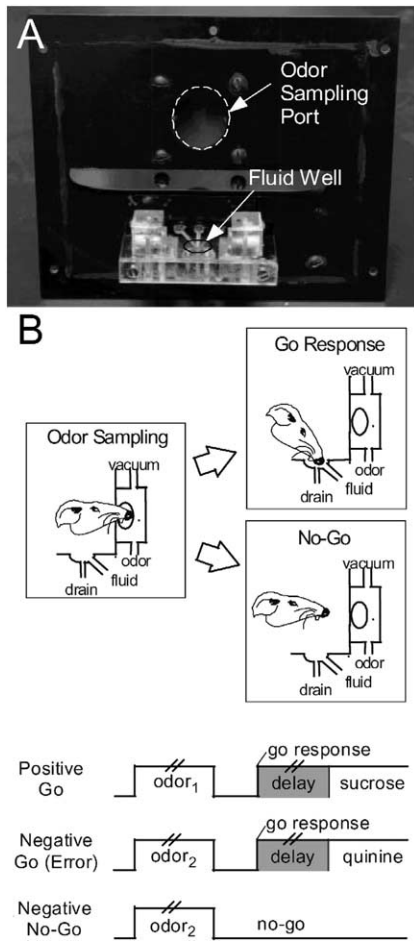


Figure 1. Illustration of Training Apparatus and Behaviors in the Task

(A) Photograph of the polycarbonate panel removed from the operant chamber to show the odor sampling port (white circle) and the fluid delivery well (black circle).

(B) Schematic illustrating behaviors in the task. Pairs of vertical lines during odor presentation and the delay between a go 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 to 1500 ms.

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.

After the rats had each acquired several such problems, they underwent surgery to make a unilateral sham ($n = 3$) or neurotoxic lesion ($n = 4$) of OFC. A lesion of OFC was made in the left hemisphere, targeting the lateral orbital and dorsal/ventral agranular insular regions to disrupt input from OFC to ABL (McDonald, 1998). In no case did an OFC lesion extend medially to affect interactions between ABL and areas on the me-

dial wall of prefrontal cortex. In the same surgery, we also implanted a driveable bundle of microwires in the left ABL in both intact and lesioned rats. After recovery from surgery, recording sessions were conducted in which neural data were acquired in ABL as the rats learned new odor problems and subsequent reversals. We obtained data from 56 sessions in intact rats, including 35 reversal sessions, and from 71 sessions in OFC-lesioned rats, including 34 reversal sessions. Figure 2 shows an example of an OFC lesion and also illustrates the recording sites in these sessions.

It is important to emphasize that we made unilateral rather than bilateral OFC lesions to avoid the confounding effect of any behavioral impairment, which would have resulted from bilateral damage, on our recording results. Rats with bilateral OFC lesions would have had substantial difficulty acquiring reversals (Schoenbaum et al., 2003a), and a failure to acquire the reversals in lesioned rats would have made it difficult to compare neural recordings during reversal learning from intact and lesioned rats, since go, no-go responding would have differed dramatically between the groups. We expected to avoid such gross behavioral differences between our intact and lesioned rats by using unilateral OFC lesions. Since the projections between OFC and ABL are largely ipsilateral, with only sparse connections to the region in the contralateral hemisphere (Allen et al., 1991; Kita and Kitai, 1990; McDonald et al., 1996), this approach preserves an intact circuit in one hemisphere to support normal behavior, while allowing us to record neural activity from ABL neurons in a damaged circuit in the opposite hemisphere. Any remaining projections from contralateral OFC into our recording site would presumably bias against observing changes in encoding as a result of the much greater loss of input from ipsilateral OFC.

Ipsilateral OFC Lesions Did Not Disrupt Performance in the Recording Sessions

Behavioral data from the recording sessions indicated that the approach of making unilateral OFC lesions did spare behavioral performance. Intact rats and rats with OFC lesions ipsilateral to the recording site performed similarly on the discrimination problems during these recording sessions, achieving criterion at comparable rates in the initial discriminations (74 and 60 trials to criterion, respectively, $F_{(1, 125)} = 3.5$, NS) and in subsequent reversals (52 and 41 trials to criterion for intact and lesioned rats, respectively, $F_{(1, 67)} = 1.96$, NS). In addition, rats with ipsilateral OFC lesions showed normal changes in response latency during learning (Figure 3A). For this analysis, the acquisition of each problem was divided into an early block of trials, corresponding to trials before the sixth error, a subsequent block of trials during acquisition, corresponding to trials after the sixth error but before the rat met the behavioral criterion, and a postcriterion block of trials, corresponding to trials after the criterion was met. As shown in Figure 3A, a difference in latency to respond emerged during the precriterion phase of training, reflecting relatively shorter latencies after sampling the positive odor and increases in latencies after the negative odor (Schoen-

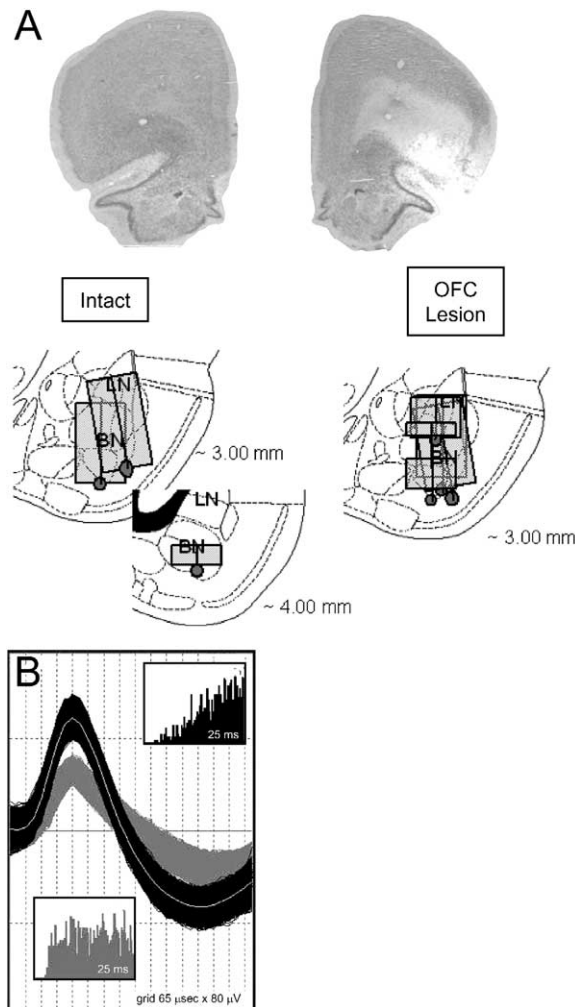


Figure 2. Electrode Placements, Histology, and Unit Waveforms in Intact and Lesioned Rats

(A) Drawings of electrode placements in ABL in intact (left panel) and OFC-lesioned rats (right panel). Vertical bars on the drawing indicate the center of the electrode track in each rat; shaded boxes indicate approximate extent of recording sessions vertically and give an estimate of lateral (and AP) spread of the wires (~1 mm). The recording sites within ABL were similar in intact and lesioned rats and to those in an earlier study examining neural correlates in ABL during learning in this paradigm (Schoenbaum et al., 1998; Schoenbaum et al., 1999). Photomicrographs show coronal sections taken through OFC in an intact rat (left panel) and in a rat with a unilateral lesion of OFC (right panel).

(B) Example of two units sorted on one channel in an intact rat. The waveforms sorted for each unit are shown along with the interspike interval histograms of the waveforms in each unit. Note the refractory period in the histograms of both units. Interestingly, while the distribution and mean firing rates of the neurons were similar in intact (2.42 spikes/s) and lesioned rats (3.55 spikes/s), there were slightly more neurons with high baseline firing rates in the lesioned rats.

baum et al., 2003a). A 2×3 repeated-measures ANOVA of lesion and learning phase (early precriterion, late precriterion, and postcriterion) revealed a significant main effect of phase on this latency difference ($F_{(2, 250)} = 10.6, p < 0.001$), but no significant effect of lesion con-

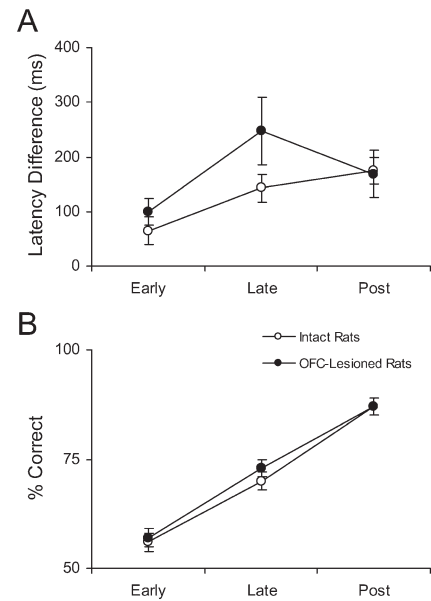


Figure 3. Changes in Response Latency and Choice Performance during Learning in the Recording Sessions

(A) Difference in latency (ms) to respond at the fluid well after the end of odor sampling for OFC-lesioned (black circles) and intact (white circles) rats. Difference was calculated as the average response latency on negative minus positive trials within each phase during and after acquisition of new go, no-go odor problems. No-go trials, in which the rat made no response for 3000 ms, were excluded from the analysis.

(B) Choice performance during and after acquisition of the odor problems for OFC-lesioned (black circles) and intact (white circles) rats.

dition ($F_{(1, 125)} = 2.70, NS$) nor any interaction between lesion condition and phase ($F_{(2, 250)} = 2.12, NS$). The groups also exhibited similar choice performance on the go, no-go discrimination across these training phases (Figure 3B); a 2×3 repeated-measures ANOVA revealed a significant main effect of phase on performance ($F_{(2, 250)} = 318, p < 0.001$), but no significant effect of lesion condition ($F_{(1, 125)} = 1.86, NS$) nor any interaction between lesion condition and phase ($F_{(2, 250)} = 1.11, NS$). These data indicate that circuits in the intact hemisphere were sufficient to support apparently normal performance, at least within the context of the recording sessions.

Ipsilateral OFC Lesions Delay Encoding of Cue Significance in ABL

The lack of effect of an ipsilateral OFC lesion on behavioral performance was in contrast to the effect on neural correlates observed in ABL in the lesioned hemisphere. Ipsilateral OFC lesions altered encoding in ABL related to predicted outcome throughout the trial. This effect was most evident in the development of activity in ABL related to outcome during sampling of the predictive odor cues.

For this analysis, we focused on firing in ABL neurons during sampling of the positive and negative odor cues during the postcriterion trial block, after the rats had

Table 1. Differential Firing during Odor Sampling

	Intact Rats (N = 376)	OFC-Lesioned Rats (N = 375)
Total odor-selective neurons	64	32*
Slowly selective	42	26
Rapidly selective	22	6*

*p < 0.01.

learned the discrimination. Our initial analysis examined whether a given neuron exhibited differential firing during this trial block, firing more during sampling of the positive or the negative odor cue after learning. A subsequent analysis then examined how differential firing changed during learning and after reversal. Thus, this analysis focused on the neurons recorded during sessions that included acquisition of the original discrimination problem and of a reversal of that problem. This population included 238 neurons in intact rats and 201 neurons in OFC-lesioned rats.

Of 238 neurons recorded in intact rats in reversal sessions, 64 neurons (27%) exhibited differential activity during odor sampling in the postcriterion trial block. Some cells fired more to the positive odor cue; other cells fired more to the negative odor cue. When we examined firing in these neurons during learning, in the precriterion block of trials, we found that many of these neurons became selective rapidly during learning (Table 1). Such *rapidly-selective* neurons are illustrated in Figures 4A and 5A. The early emergence of selective activity appeared to reflect rapid learning about the significance of the predictive cue rather than activity related to sensory features of the particular odor, since these neurons did not typically exhibit firing for the same odor cue at the start of the session or after reversal of the odor-outcome associations. At the same time, the emergence of selective activity early in training clearly occurred prior to the development of accurate discriminative performance. Thus, selective neural activity was not tied to motor responding (go, no-go). Other cue-selective neurons were more *slowly-selective*, developing differential activity only in the postcriterion phase (Table 1). This pattern is illustrated in Figure 5B, although the specific example is taken from a lesioned rat. In general, these different correlates were observed in proportions similar to what we have reported previously (Schoenbaum et al., 1999).

By comparison, only 32 (16%) of 201 ABL neurons recorded in OFC-lesioned rats through reversal sessions exhibited differential activity during odor sampling in the postcriterion trial block. Comparison of these data with data from intact rats indicated that the lesioned rats had significantly fewer cue-selective neurons (Table 1, $\chi_2 = 7.68$, $p < 0.01$). As illustrated in Table 1, the decrease in cue-selective neurons in lesioned rats reflected a significant decline in the proportion of rapidly-selective neurons in lesioned versus intact rats (Table 1, $\chi_2 = 7.15$, $p < 0.01$). There was no significant difference in the proportion of slowly-selective neurons in intact and lesioned rats (Figure 5B and Table 1, $\chi_2 = 1.75$, NS). These results suggest that the rapid development of odor-outcome associations during cue sampling in ABL is specifically supported by OFC input (or interconnections with OFC).

Consistent with this idea, a substantial difference between intact and lesioned rats was also observed in how cue-selective encoding was affected by reversals of the odor-outcome associations. As in our prior study, we found that the majority of cue-selective neurons in intact rats (34/64, 53%) reversed their preference between the odor cues after reversal of the odor-outcome associations. By contrast, significantly fewer (5/32, 16%) cue-selective neurons reversed in OFC-lesioned rats ($\chi_2 = 5.87$, $p < 0.025$) (Figure 5C). Instead, these neurons became nonselective during odor sampling after reversal (59% in lesioned rats versus 25% in intact controls; $\chi_2 = 4.65$, $p < 0.05$). These patterns are illustrated in Figures 4A and 5A for intact rats and Figure 5B for OFC-lesioned rats.

The loss of cue selectivity after reversal indicates that the cue-selective neurons in the lesioned rats were nonetheless responsive to a change in contingencies that altered the original associations. Thus, cue selectivity was not simply representing sensory features of the odor cues. However, OFC lesions appeared to have made associative encoding less flexible. Indeed, other findings from the reversal sessions are consistent with a failure to rapidly modify previously established representations as well as develop encoding of new associations.

Over the course of reversal training, significantly fewer new neurons (15/169, 9%) became cue selective after reversal in OFC-lesioned rats than in intact rats (29/174, 17%; $\chi_2 = 4.65$, $p < 0.05$). Moreover, all of the “reversing” cue-selective neurons that were observed in the lesioned rats came from sessions with at least 60 postcriterion trials in the reversal phase (Table 2), while such cells were absent in sessions with a shorter duration of training. By contrast, cue-selective neurons in intact rats were equally likely to reverse in short and long reversal sessions ($\chi_2 = 0.90$, NS). Thus, OFC input appears to be needed to facilitate the rapid encoding of the predicted outcome during cue sampling in ABL during reversal training as it was during initial discrimination learning.

Ipsilateral OFC Lesions Reduce Encoding of Expected Outcome during Delays in ABL

A failure to encode information about the predicted outcome was also evident later in the trial, during a short delay after responding but before outcome delivery. Previously, we reported that neurons in both OFC and ABL develop outcome-expectant activity during this delay (Schoenbaum et al., 1998; Schoenbaum et al., 2003b). Here we again looked for this activity, comparing neural activity on positive and negative trials during the delay period before outcome delivery. We examined

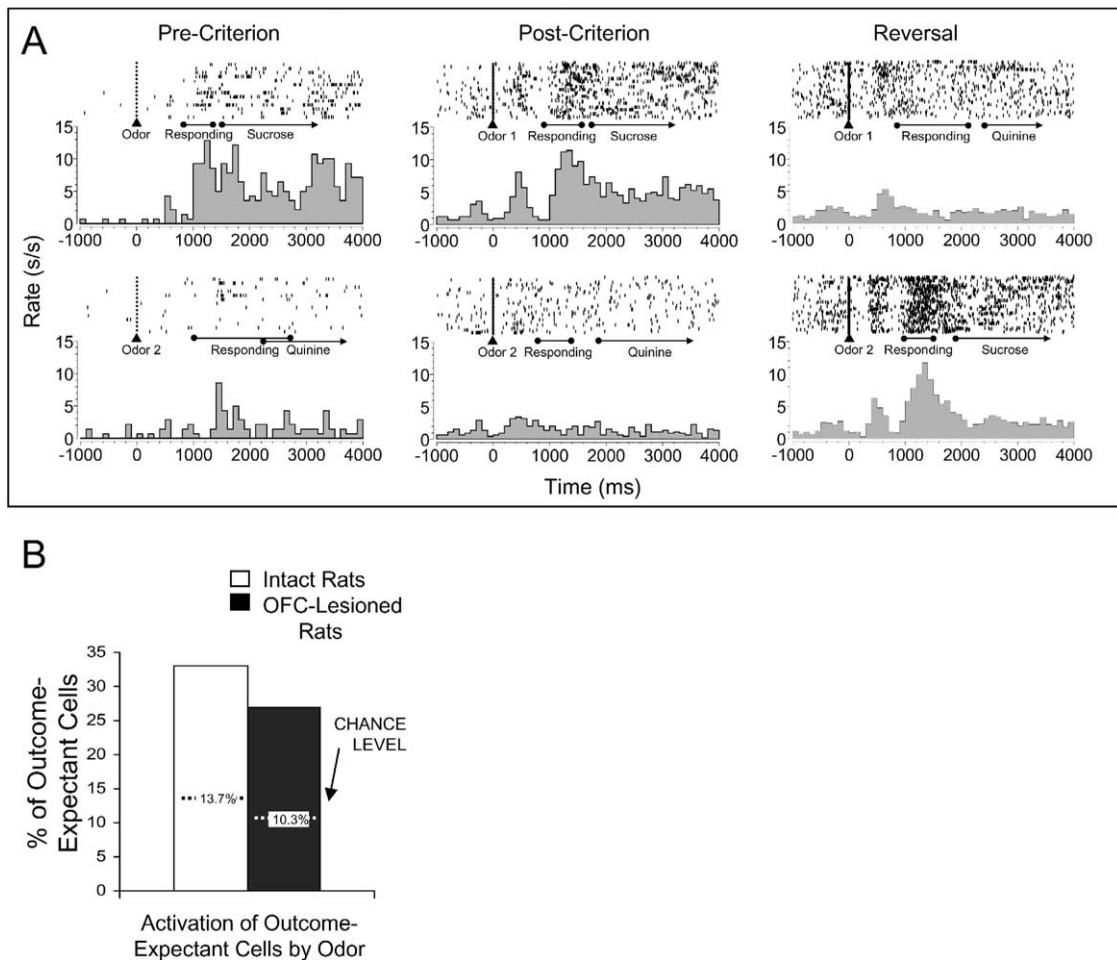


Figure 4. Activation of Outcome-Expectant Encoding during Cue Sampling in Intact and OFC-Lesioned Rats

(A) Example of an ABL neuron recorded in an intact rat that fires after responding in anticipation of and during sucrose delivery in the precriterion trials and then develops a selective response to the associated odor cue several trials later (left column). This selective response continues in the postcriterion phase (middle column). After reversal, the anticipatory response re-emerges for sucrose almost immediately, and then the neuron again begins firing to the associated odor cue several trials later. Raster displays show neural activity on individual trials, and each histogram shows average activity in spikes/s in 100 ms bins. The timing of trial events is indicated beneath the rasters by range bars. (B) Proportion of outcome-expectant neurons that become activated by the associated odor cue in OFC-lesioned (black bar) and intact (white bar) rats. The dotted line indicates the proportions of outcome-expectant neurons that would have been expected to become activated by the associated odor cue by chance, given the probabilities of neurons that developed selectivity for each odor cue in the neural populations in each group.

activity in the precriterion trial block, since that is when comparable numbers of go, no-go responses occur on both trial types. Of 376 neurons recorded in intact rats during discrimination learning, 59 (16%) showed such differential activity during the delay during learning (Figures 4A and 6A). This population was similar to what we reported previously in ABL in two-odor learning sessions ($\chi_2 = 0.83$, $p = 0.36$) (Schoenbaum et al., 1998) and included 18 neurons that fired in anticipation of sucrose and 41 neurons that fired in anticipation of quinine. As illustrated in the figures, delay-selective firing in these neurons typically developed during acquisition of the discriminations. In OFC-lesioned rats, a similar analysis of activity in 375 ABL neurons identified only 38 (10 sucrose, 28 quinine) with outcome-expectant activity. This proportion (10%) was significantly

lower than that observed in intact rats in either this report ($\chi_2 = 5.16$, $p < 0.05$) (Figure 6B) or our prior study ($\chi_2 = 4.13$, $p < 0.05$).

Ipsilateral OFC Lesions Do Not Affect the Activation of Outcome Representations during Cue Sampling in ABL

Finally, we examined whether an ipsilateral OFC lesion would affect the relationship between the outcome-expectant activity just described and cue-selective firing. This analysis was of interest because we have found that, in OFC, such outcome-expectant neurons often develop selective responses to the predictive odor cues after learning, thereby activating a representation of the outcome during the cue (Schoenbaum et al., 2003b). Such cue-outcome representations failed to

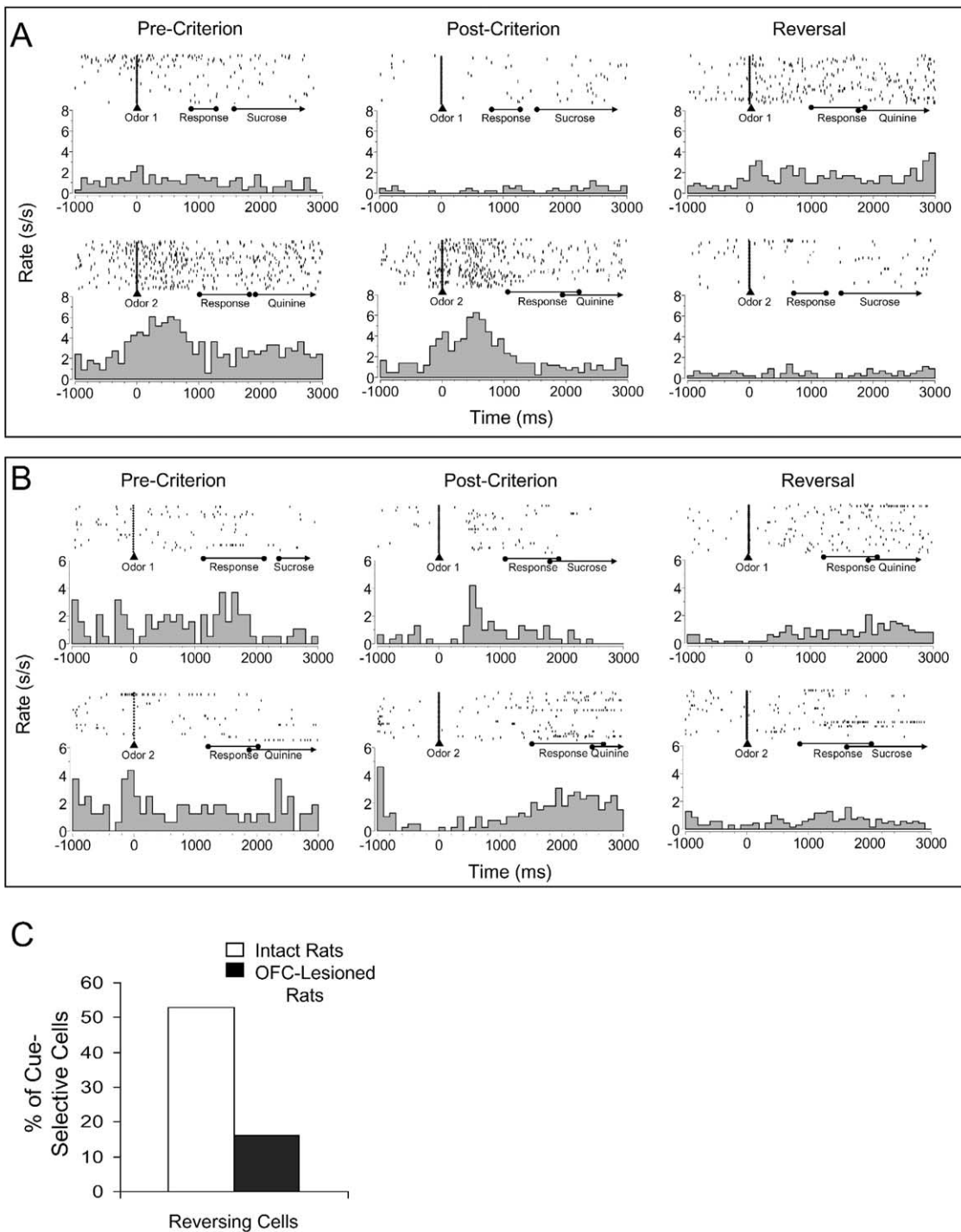


Figure 5. Encoding of Acquired Significance during Cue Sampling in Intact and OFC-Lesioned Rats

(A) Example of an ABL neuron recorded in an intact rat that exhibits a selective response to one of the two odor cues in the postcriterion trials (middle column). Note that this selective response develops rapidly in the precriterion trials (left column) and switches to the other odor cue rapidly after reversal (right column). This tendency to reverse was typical in intact rats, but was virtually absent in OFC-lesioned rats.

(B) Example of an ABL neuron recorded in an OFC-lesioned rat that develops a selective response to one of the odor cues in the postcriterion trials (middle column). This neuron differs from the example in (A) in two respects. First, this neuron becomes selective more slowly during learning and is not selective in the precriterion trials (left column). Second, although the odor preference disappears after reversal (right column), the neuron does not rapidly develop selectivity for the other odor. Though these nonreversing cells were seen in both lesioned and unlesioned rats, they comprised the vast majority in lesioned rats, but a minority in intact rats. (n.b. the neuron in (B) was not selective for quinine; the increased activity late in the trials in the postcriterion phase was only present on negative no-go trials, when the rat correctly withheld its response, and was not present during quinine delivery.) Raster displays show neural activity on individual trials, and each histogram shows average activity in spikes/s in 100 ms bins. The timing of trial events is indicated beneath the rasters by range bars.

(C) Proportion of cue-selective neurons that reversed odor preference after reversal in OFC-lesioned (black bar) and intact (white bar) rats. Chance = 5%.

Table 2. Reversal of Firing to Odor in Short versus Long Reversals

	Intact Rats (N = 238)	OFC-Lesioned Rats (N = 201)
Short reversal (Post-Crit \leq 60)	13/28	0/12*
Long reversal (Post-Crit $>$ 60)	21/36	5/20

*p < 0.06.

develop in OFC in rats with ABL lesions, resulting in an apparent decline in the number of cue-selective neurons in OFC in ABL-lesioned rats. Here, we examined whether similar representations are, in fact, formed in ABL during learning, whether there is any impact of OFC lesions on their formation, and whether such an effect might explain the reduction in cue-selectivity that we have discussed above.

For this analysis, we examined cue-selective firing in the outcome-expectant neurons identified by our

earlier analysis. Excluding neurons that fired to the odor cues before outcome-expectant activity was observed, we found that 15 of 59 outcome-expectant neurons (25%) recorded in intact rats became selective for the associated odor cue during learning (Figure 4A). These cue-selective cells were part of a larger population, discussed earlier, that developed selective firing for one of the two odor cues. Thus, cue-selective neurons in ABL were comprised of two independent populations. One population developed from the outcome-expectant neurons, thereby providing an associative activation of the expected outcome in the presence of a predictive cue. The second population encoded the acquired significance of the cue independently. Although the outcome-expectant population was smaller in lesioned rats, a comparable proportion of these neurons (7/38; 18%) became activated by the associated odor cue during learning (Figure 4B). Thus, although ipsilateral OFC lesions reduced outcome-expectant encoding in ABL, the lesions did not disproportionately affect activation of outcome-expectant cells during cue sampling once the rat had learned the cue-outcome associations.

Discussion

The current findings extend our understanding of associative neural encoding in ABL during learning and further identify a distinct role for the connections between OFC and ABL in supporting these correlates. Consistent with our previous reports (Schoenbaum et al., 1998; Schoenbaum et al., 1999), neurons in ABL developed selective firing that reflected outcome-related information both during cue sampling and during a delay after responding. Here we have shown that some cue-selective neurons in ABL, like those in OFC in this task (Schoenbaum et al., 2003b), develop from outcome-expectant neurons, thereby providing an associative activation of the expected outcome in the presence of a predictive cue. Furthermore, the current results provide evidence of the importance of projections from OFC to ABL in supporting outcome-related encoding; rats with OFC lesions failed to exhibit the rapid associative encoding during cue sampling that characterizes neural activity in ABL in a variety of settings and also failed to generate outcome-expectant neural activity during responding.

These findings, together with those in an earlier report (Schoenbaum et al., 2003b), demonstrate that the ability to represent information about expected outcomes in neural encoding depends on the cooperative function of the orbitofrontal-amygdalar circuit. This cooperative function is apparent in a comparison of the current findings, concerning the role of ABL input to

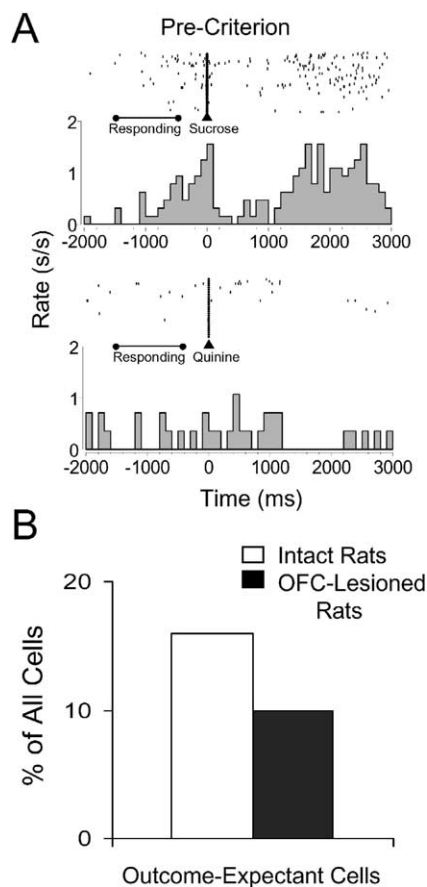


Figure 6. Outcome-Expectant Encoding in Intact and OFC-Lesioned Rats during Learning

(A) Example of an ABL neuron recorded in an intact rat that fires after responding in anticipation of and during delivery of sucrose but not quinine. Raster displays show neural activity on individual trials in the precriterion training phase, and each histogram shows average activity in spikes/s in 100 ms bins with activity synced to outcome onset. The timing of trial events is indicated beneath the rasters by range bars.

(B) Proportion of outcome-expectant neurons in OFC-lesioned (black bar) and intact (white bar) rats. Chance = 5%.

OFC, and the earlier report, concerning the role of OFC input to ABL. In the current report, we found that OFC lesions impaired outcome-expectant encoding in ABL during the delay interval after a response was made. By contrast, using the same task and methods, our earlier study showed that similar numbers of OFC neurons developed outcome-expectant encoding during the delay in intact rats and rats with an ABL lesion. Thus, connections with OFC appear to play a role in activating an expectancy of an impending outcome in ABL neural activity. Yet to provide a guide for action, outcome information is needed prior to responding; such information can be provided by predictive cues that activate outcome-related representations during learning. Here the roles of OFC and ABL are reversed; OFC lesions did not affect the proportion of outcome-expectant cells that became activated by the relevant predictive cue, whereas in our prior report, ABL damage virtually eliminated the emergence of such encoding in OFC.

The interdependence of encoding information about expected outcomes in OFC and ABL provides a basis for behavioral impairments after lesions of these brain regions in settings where appropriate behavior requires such information. One such setting uses devaluation procedures to alter the motivational value of a predicted outcome. Rats learn that a cue predicts a food reward, after which the motivational value of the food is reduced by pairing with illness. Rats with lesions of either ABL or OFC are subsequently unable to alter their responding to the predictive cue (Gallagher et al., 1999; Hatfield et al., 1996). Similar findings have been obtained in primates with lesions of either the amygdala or OFC or disconnection lesions that interrupt this circuit (Baxter et al., 2000; Izquierdo et al., 2004; Malkova et al., 1997). Another setting that is similarly sensitive to damage to either ABL or OFC is the differential outcome expectancy task (DOE) (Trapold, 1970). Here, rats learn to discriminate between two instrumental responses to obtain reward. When the two responses lead to different outcomes, acquisition of the discriminative response is facilitated in normal rats. This facilitation is thought to reflect the contribution of outcome-expectancies to learning. Rats with lesions to either ABL or OFC fail to show this facilitated learning in the presence of different outcomes (Blundell et al., 2001; McDannald et al., 2005).

The prominent effect of OFC lesions on the rapid development of cue selectivity in ABL also provides a possible neural substrate for a number of other behaviors that depend on OFC and ABL, particularly behaviors that reflect the motivational properties that a cue acquires during learning. For example, in the discrimination task employed here, we have found that intact rats exhibit changes in their latency to respond at the fluid well after sampling the odor cues, responding more slowly on quinine trials and more quickly on sucrose trials as they learn. These latency changes emerge at the same time as the rapidly developing cue-selective firing in ABL (Schoenbaum et al., 1999) and are abolished by either ABL or OFC lesions (Schoenbaum et al., 2003a).

The motivational value acquired by cues also supports conditioned reinforcement. Conditioned reinforcement refers to a change in the power of an originally neutral

cue after pairing with reward, which allows the cue to serve as a reinforcer either in support of new learning or in maintaining operant responding. Animals with either amygdala or OFC damage lose the ability to use a cue's acquired value as a conditioned reinforcer (Cousens and Otto, 2003; Parkinson et al., 2001; Pears et al., 2003). The inability to rapidly signal the value of the predictive cue in ABL after OFC lesions in the current study could provide a basis for this deficit.

Finally, our findings also have implications for how OFC and ABL interact in other settings that are thought to reflect learning for outcomes, but in which their roles appear to differ. For example, one setting that distinguishes the effects of OFC and ABL lesions is found in reversal learning. Reversal impairment has been reliably found after manipulations of the orbital region across species and tasks, even as the manipulations have become more selective and definitions of the orbital region more circumscribed (Bohn et al., 2003; Butter, 1969; Chudasama and Robbins, 2003; Dias et al., 1997; Fellows and Farah, 2003; Iversen and Mishkin, 1970; Izquierdo et al., 2004; Jones and Mishkin, 1972; Meunier et al., 1997; Rolls et al., 1994; Schoenbaum et al., 2002; Schoenbaum et al., 2003a; Teitelbaum, 1964). This impairment is thought to reflect an inability to use outcome-related information to control behavior (Dias et al., 1996; Hauser, 1999), yet recent studies using fiber-sparing neurotoxic lesions have shown that amygdala itself is not necessary for rapid reversal learning (Baxter and Murray, 2000; Schoenbaum et al., 2003a; A.D. Izquierdo et al., 2003, Soc. Neurosci. abstract).

The facilitation of cue-selective firing in ABL by OFC provides a neural correlate of this classic orbitofrontal-associated deficit. In an OFC-lesioned animal, these abnormally slow and inflexible representations would provide incomplete or erroneous incentive information for guiding behavior. This effect would be particularly evident during reversal learning when such erroneous output would presumably influence other brain areas, thereby slowing the rate at which old response patterns are abandoned in favor of new strategies. Importantly, this account would be consistent with findings that amygdala damage by itself does not cause reversal impairments, since the associative encoding in ABL is not postulated to enhance normal reversal learning in the intact animal.

In conclusion, these data provide new information on how OFC and ABL interact in representing outcome-related information. In keeping with their close anatomical relationship, we found an interdependent basis for certain aspects of associative encoding. One aspect in particular, the ability to prospectively represent information about an impending outcome, relies on interconnections between these structures, thus providing a basis for behavioral impairments in tasks that require this associative representational function. At the same time, OFC connections were found to play an important and somewhat unanticipated role in the feature of rapid and flexible associative encoding that is characteristic of ABL. This latter finding suggests the interesting possibility that such impaired associative encoding provides a substrate that contributes to the syndrome that characterizes patients with prefrontal damage.

Experimental Procedures

This research was conducted at Johns Hopkins University in accordance with University and NIH guidelines for animal research; data were analyzed at the University of Maryland School of Medicine.

Surgical Procedures

Seven adult male Long-Evans rats served as subjects (Charles River Laboratories, Wilmington, MA). Procedures for creating OFC lesions and implanting electrodes were identical to those used previously (Gallagher et al., 1999; Schoenbaum et al., 1999). Ipsilateral (left) neurotoxic lesions of OFC ($n = 4$) were made by intracerebral infusions of *N*-methyl-D-aspartic acid (NMDA, 20 $\mu\text{g}/\mu\text{l}$, Sigma, St. Louis, MO) in phosphate buffer vehicle. Each lesion required four injections of neurotoxin. Two injections of 0.1 μl were made 4.0 mm anterior to bregma at 3.7 mm and 2.2 mm lateral to the midline at a depth of 4.2 mm ventral from skull. A third injection of 0.05 μl was made at 3.0 mm anterior to bregma, 4.2 mm lateral and 5.2 mm ventral from skull, and a fourth injection of 0.05 μl was made at 3.0 mm anterior to bregma, 3.2 mm lateral and 5.2 mm ventral from skull. Sham lesions ($n = 3$) were made by lowering the infusion needle to the same coordinates without infusing any solution.

A driveable electrode bundle was chronically implanted dorsal to ABL in the left hemisphere at 2.8 mm posterior to bregma, 5.0 mm laterally, and 6.7 mm ventral to the surface of the brain. This electrode bundle was composed of ten 25 μm 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 μm increments to acquire activity from new neurons for the following day.

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 μA current through each microwire for ~ 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 μm) were collected through the areas of ABL and OFC. Sections were mounted on glass slides, stained with thionin, and coverslipped with Permount. Lesion and electrode placements were verified under a light microscope and drawn onto plates adapted from the atlases of Paxinos and Watson (1997) and Swanson (1992).

Behavioral Methods

Odor discrimination training was conducted in aluminum chambers ~ 18 inches on each side with sloping walls narrowing to an area of 12 inches \times 12 inches at the bottom. An odor port and fluid well were located on a panel (Figure 1), which was located in the right wall of each chamber below two panel lights. Odor discrimination problems were composed of odor pairs chosen from compounds obtained from International Flavors and Fragrances (New York, NY). Discrimination problems were constructed from dissimilar odors, and the odor discrimination sequence was arranged such that similar compounds were counterbalanced by valence and did not repeat across days. 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.

Trials were signaled by illumination of the panel lights inside the box. When these lights were on, nosepoke into the odor port (Figure 1) 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 and then had 3 s to make a go

response at the fluid well located below the port (Figure 1). 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 delay (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 (Figure 1). 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 to 100 trials after this criterion was achieved. After these postcriterion data were obtained, the discrimination problem was 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 a single aluminum chamber identical in all respects to the set of chambers used for training prior to surgery. The recording chamber was mated to a commutator (Crist Instrument Co, Damascus, MD) and equipment from Datawave Technologies (Longmont, CO) for gathering neurophysiological data. 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 μm . Otherwise, active wires were selected for recording, and a training session was begun.

Neural activity was recorded using a single Datawave Enhanced Discovery system, capable of recording neural waveforms on up to eight channels. Signals from active wires were passed through a unity-gain JFET headstage, band-pass filtered at 300–3000 Hz, and amplified differentially (relative to a silent reference electrode) at 5000X (Neuralynx). Waveforms ($>2.5:1$ signal-to-noise) were digitized at 25 kHz and recorded to disk by the data acquisition software along with timestamps indicating when significant events occurred (odor onset, responding, fluid delivery, etc).

These files were analyzed later using software from Plexon Inc (Dallas, TX). 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 to 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, one to three waveforms could be isolated on an active channel.

Sorted files were then processed in Neuroexplorer to extract these unit timestamps and relevant event markers. These data were subsequently analyzed using statistical routines in Matlab (Natick, MA) to examine firing 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/s) in each time window was compared on positive and negative trials during pre- and postcriterion trial blocks using ANOVA ($p < 0.05$), and neurons with a significant difference in activity were categorized as “selective” in that time window and phase.

A Pearson χ^2 test ($p < 0.05$) was used to compare the proportions of neurons with different firing properties in intact and lesioned rats 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 actual proportion of neurons in the population that exhibited each type of response. For example, if 50 of 100 neurons fired selectively during sampling of the positive odor in a given phase, and 50 of 100 neurons fired selectively while the rat was waiting for sucrose delivery in that same phase, then the chance occurrence of neurons with this combination of selective activity (e.g., selective activity both during sam-

pling of the positive odor and prior to sucrose delivery) would be (0.5)(0.5)(100) or 25 neurons. This expected occurrence was compared to the actual proportion observed in our experimental groups.

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