Neuron, Vol. 39, 855–867, August 28, 2003, Copyright ©2003 by Cell Press

Encoding Predicted Outcome and Acquired Value in Orbitofrontal Cortex during Cue Sampling Depends upon Input from Basolateral Amygdala

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Summary

Certain goal-directed behaviors depend critically upon interactions between orbitofrontal cortex (OFC) and basolateral amygdala (ABL). Here we describe direct neurophysiological evidence of this cooperative function. We recorded from OFC in intact and ABL-lesioned rats learning odor discrimination problems. As rats learned these problems, we found that lesioned rats exhibited marked changes in the information represented in OFC during odor cue sampling. Lesioned rats had fewer cue-selective neurons in OFC after learning; the cue-selective population in lesioned rats did not include neurons that were also responsive in anticipation of the predicted outcome; and the cue-activated representations that remained in lesioned rats were less associative and more often bound to cue identity. The results provide a neural substrate for representing acquired value and features of the predicted outcome during cue sampling, disruption of which could account for deficits in goal-directed behavior after damage to this system.

Introduction

Orbitofrontal cortex (OFC) is critical for integrating the incentive value of outcomes with predictive cues to guide behavior. Humans with damage to this region are impaired in the capacity to plan an effective course of action and are often afflicted by poor judgment even when they apparently understand the likely outcome of their actions (Bechara et al., 1997), suggesting a deficiency in the power of incentive and motivational information to control behavior. Similarly, experiments in rats and monkeys have shown that damage to OFC produces an inability to control behavior according to the motivational significance of cues or to modify behavior when the outcomes predicted by those cues change in value (Baxter et al., 2000; Gallagher et al., 1999; Izquierdo and Murray, 2000, Soc. Neurosci., abstract; Pears et al., 2001).

A major source of input to OFC regarding the value of cues may be the basolateral complex of the amygdala (ABL) (Carmichael and Price, 1995; Ghashghaei and Barbas, 2002; Kita and Kitai, 1990; Krettek and Price, 1977; Ongur and Price, 2000; Shi and Cassell, 1998). The ABL is critically involved in affective functions including memory for emotional experiences and the formation of associations between neutral cues and outcomes (Davis, 1992; Gallagher and Chiba, 1996; Kluver and Bucy, 1939; LeDoux, 1996; McGaugh, 2002; Weiskrantz, 1956), and behavioral studies have shown that damage to ABL produces deficits similar to those observed with OFC damage (Baxter et al., 2000; Gallagher et al., 1999; Hatfield et al., 1996; Izquierdo and Murray, 2000, Soc. Neurosci., abstract; Malkova et al., 1997; Parkinson et al., 2001; Pears et al., 2001). The similarities in effects of OFC and ABL lesions in certain tasks suggest that these two structures form a functional system involved in the acquisition and use of incentive information to guide goal-directed behavior. Those reports, along with neurophysiological findings from awake, behaving animals (Schoenbaum et al., 1999), support the hypothesis that OFC accesses information regarding the significance or incentive value of predictive cues through connections with ABL.

To test this hypothesis, we recorded neural activity from OFC 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. We expected that ABL lesions would disrupt the associative encoding properties normally observed in OFC neurons in intact rats, so that neurons would be more sensitive to the sensory identity of the odor cues than to their acquired motivational significance. In addition, we examined whether OFC neurons in intact rats activated representations of the predicted outcomes during cue sampling, a function critically dependent on interactions between OFC and ABL (Baxter et al., 2000), and whether such stimulus-outcome representations would be disrupted by ABL lesions.

Results

Thirsty 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 appetitive sucrose solution, and the other "negative" odor signaled the availability of an aversive quinine solution. When presented with a new odor pair, the rats initially responded at the fluid well on every trial but subsequently learned to respond after sampling the positive odor and to refrain from responding after sampling the negative odor. Rats acquired the odor problem when they met a behavioral criterion of 18 correct responses in the last 20 trials.

After the rats had each acquired several such problems, they underwent surgery to implant a drivable bundle of microwires in OFC and to make a bilateral sham (n = 4) or neurotoxic lesions (n = 4) of ABL. After recovery from surgery, recording sessions were conducted in which neural data were acquired as the rats learned

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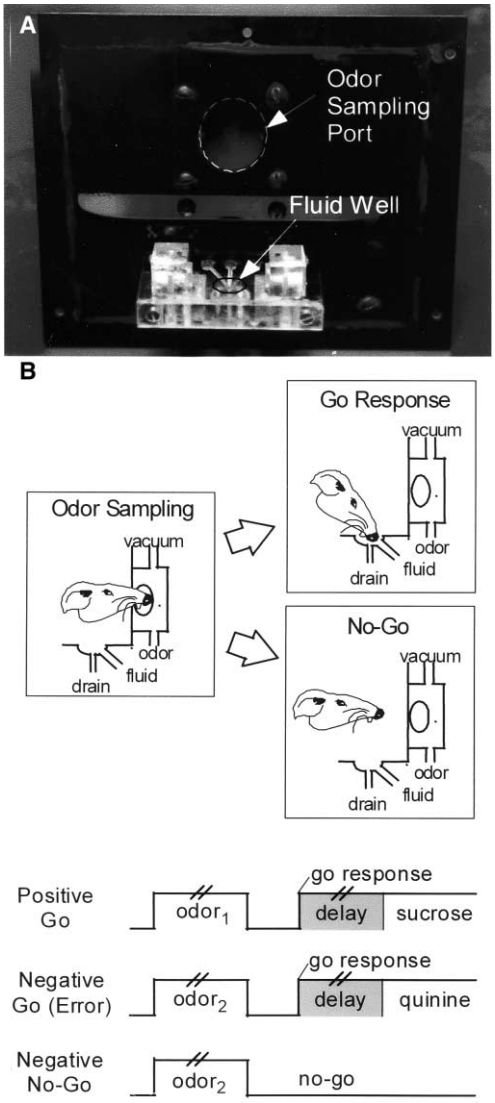

Figure 1. Illustration of Training Apparatus and Behaviors in the Task

(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.

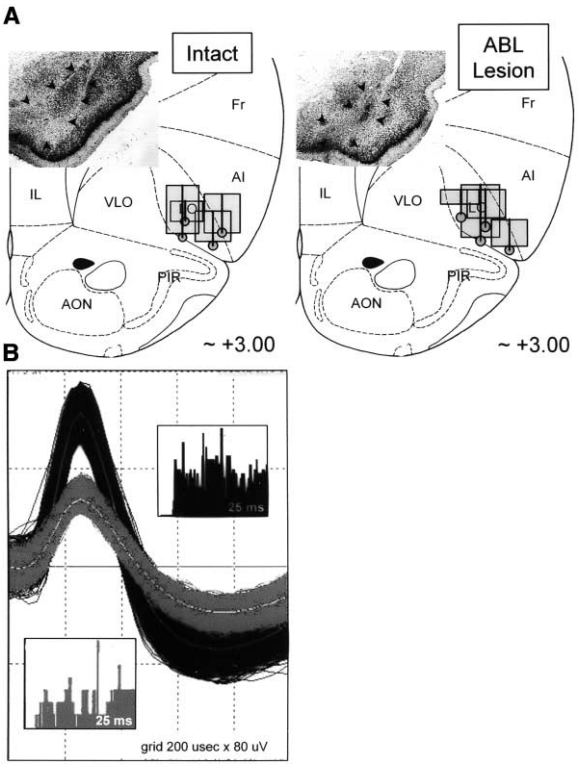

Figure 2. Electrode Placements, Histology, and Unit Waveforms in Intact and Lesioned Rats

(A) Drawings of electrode placements in OFC in intact (left panel) and ABL-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 OFC were similar in intact and lesioned rats and to those in an earlier study examining neural correlates in OFC during learning in this paradigm (Schoenbaum et al., 1998, 1999). In addition, the distribution and mean firing rates of the neurons were similar in intact (4.68 spikes/s) and lesioned rats (3.91 spikes/s). Insets show photomicrographs of coronal section taken through the junction between the basolateral and central nucleus in an intact rat (left panel) and in an ABL-lesioned rat (right panel). Note the large, darkly staining neuron bodies in the basal and lateral nuclei in the intact rat (arrows) and the absence of those neurons, replaced by gliosis, in the lesioned rat (arrows).

(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.

new odor problems and subsequent reversals of those problems. Neural recordings were obtained from 552 neurons in 58 sessions in the intact control rats and 512 neurons in 56 sessions in the ABL-lesioned rats (these numbers include all neurons recorded in these sessions). Figure 2 shows an example of an ABL lesion and also illustrates the recording sites in these sessions. Recordings were generally made in the lateral orbital areas or in ventral agranular insular regions. These areas are notable because they appear to receive overlapping projections from olfactory regions and ABL (Kita and Kitai, 1990; Price et al., 1991). Lesions were distinguished by an absence of neurons and extensive gliosis in the area of ABL, as well as by the presence of intact neurons at the lesion borders. Lesions generally encompassed >75% of ABL, and included the lateral, basal, and accessory basal nuclei, with some neuron loss in immediately adjacent areas of the endopiriform nucleus and piriform cortex in two cases. Aside from minor mechanical damage along the injection needle track, there was no damage evident in sham-lesioned rats.

Behavior in these recording sessions was similar to what we have reported in an independent study on the effects of ABL lesions in this task (Schoenbaum et al., 2003). Although intact and lesioned rats did not differ significantly in the rate at which they acquired the novel

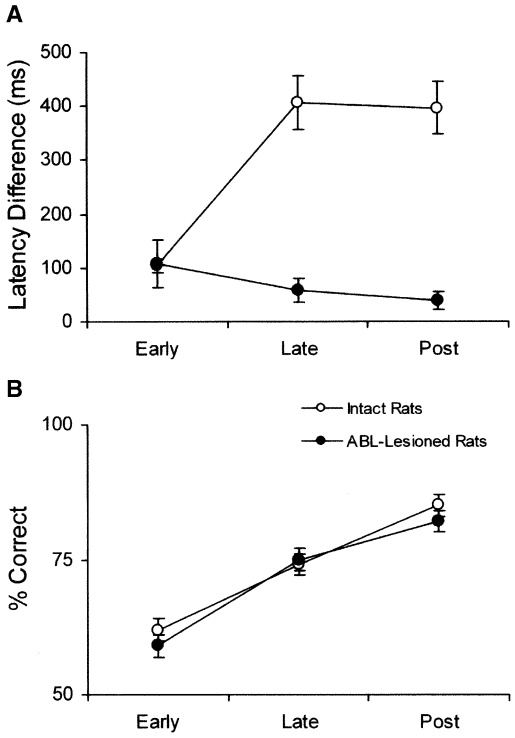

Figure 3. Changes in Response Latency and Choice Performance during Learning in the Recording Sessions

(A) Difference in latency (ms) to respond at the fluid well after the end of odor sampling for ABL-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. ABL-lesioned rats failed to develop the learning-related latency difference exhibited by intact rats.

(B) Choice performance during and after acquistion of the odor problems. ABL-lesioned rats did not differ from intact rats.

odor discrimination problems during recording [44 and 59 trials-to-criterion respectively; $F(1,106) = 3.44$, NS], ABL-lesioned rats failed to show normal changes in response latency during learning. As indicated by the data shown in Figure 3A, both groups of rats began each session responding to the well after odor sampling at similar latencies during the early precriterion trials [$F(1,106) = 0.04$, NS]. During the late precriterion trials, however, intact rats exhibited longer response latencies on negative than on positive trials; this difference did not develop in ABL-lesioned rats [$F_{int}(1,106) = 16.9$, $p < 0.001$]. Such latency changes are thought to reflect learning about the incentive value of the predicted outcome, whereas instrumental go, no-go behavior can be mediated, in part, by other mechanisms such as stimulus-response learning (Holland and Straub, 1979; Sage and Knowlton, 2000). Consistent with this distinction in the basis for these two measures, choice performance did not differ across these same phases (Figure 3B), although ABL-lesioned rats were mildly impaired at reacquiring the discriminations after reversal [49 and 60 trials-to-criterion in the intact and lesioned groups respectively, $F(1106) = 4.22$, $p < 0.05$]. A deficit in reversal learning after ABL damage is also consistent with our earlier results (Schoenbaum et al., 2003).

Predictive Cues Activated Fewer OFC Neurons in ABL-Lesioned Rats after Learning

Our analysis of neural activity during sampling of the predictive odor cues focused on the postcriterion trials, which included trials after the rats had met the behavioral criterion in each recording session but before reversal. On average, this block consisted of 92 trials in intact rats and 81 trials in lesioned rats and was characterized by highly accurate choice performance in both groups (Figure 3B). We compared firing during sampling of each of the two odors. As we have reported previously (Schoenbaum et al., 1999), many OFC neurons in intact rats exhibited differential activity during odor sampling during the postcriterion trials in this task (Table 1). Some neurons fired more to the positive cue; other neurons fired more to the negative cue. When the same comparison was made for OFC neurons recorded in ABL-lesioned rats, we found a significant reduction in the number of neurons that fired selectively to one or the other of the predictive cues (Table 1, $\chi^2 = 3.97$, $p < 0.05$). The magnitude of this reduction was similar in neurons selective for the positive and negative cues (Table 1, $\chi^2 = 1.39$, NS). Subsequent analyses focused on determining the significance of this reduction and on the encoding properties of the remaining neurons. For these analyses, we examined how firing to the cues in these populations developed during learning and across reversal, and how these cue-activated representations were related to encoding later in the trial as the rat awaited the outcome in the fluid well.

Predictive Cues Fail to Activate Neurons Encoding Expected Outcome in OFC in ABL-Lesioned Rats

We previously reported that OFC neurons fire differently on positive and negative precriterion trials during a delay after responding but prior to outcome delivery in this task (Schoenbaum et al., 1998). Such neurons exhibited outcome-expectant firing as the rat awaited delivery of reinforcement in the fluid well. In that prior study, we did not determine whether any of *those neurons* subsequently became selective for the corresponding predictive odor cues. In the current dataset, we examined whether such outcome-expectant firing was present and whether neurons in this population also became active during sampling of the corresponding odor cues after learning in the postcriterion trials. As in our previous study (Schoenbaum et al., 1998), we found that many neurons ($n = 112$; 20%) recorded in intact rats fired during the delay as the rats awaited the delivery of sucrose or quinine in the fluid well (Figure 4A). Consistent with our own and others' work (Hikosaka and Watanabe, 2000; Schoenbaum et al., 1998; Tremblay and Schultz, 1999), the encoding properties of these neurons more strongly reflected the motivational value of the impending outcome than the identity of the preceding odor cue; many more of these neurons showed selective activity to the corresponding outcome than they did to the associated odor cue during the precriterion trials (Table

Table 1. Differential Firing to Odor in the Postcriterion Trials

	Intact Rats (n = 552)	ABL-Lesioned Rats (n = 512)
Total cue-selective neurons	137	101*
Neurons selective for + odor cue	82	68
Neurons selective for − odor cue	55	33

*$p < 0.05$ by χ^2.

2; Figure 4B, left panels). The proportion and characteristics of these outcome-expectant neurons in ABL-lesioned rats (n = 107; 21%) were similar to those in intact rats. As in intact rats, such neurons often fired in anticipation and during presentation of one of the two outcomes (Table 2; Figure 5B, left panels), and more rarely exhibited selectivity for the associated odor cue during the precriterion trials (Table 2; Figure 5B, left panels). Thus, OFC neurons in ABL-lesioned rats, like their counterparts in intact rats, represented features of the expected outcome in the fluid well after a response was made.

ABL-lesioned rats and intact rats, however, differed sharply in whether neurons with outcome-expectant encoding went on to develop selective firing to the corresponding odor cue after learning. In intact rats, many of the neurons with outcome-expectant activity during precriterion trials (21/112, 19%) developed selective firing to the corresponding cue during the postcriterion trials (Figure 3B, right panels, and Figure 6). This subpopulation was significantly larger than that expected by chance (chance = 9.7 neurons, $\chi^2 = 4.53$, $p < 0.05$, see Experimental Procedures for calculations) and accounted for a large proportion (19%) of the OFC neurons that developed selective activity to the odor cues during the postcriterion trials. To confirm these findings, we also reexamined our earlier dataset (Schoenbaum et al., 1998) using the current analysis. We found that 20% of the neurons with outcome-expectant activity in that report also went on to develop selective firing to the associated odor cue after the discriminations were learned. Thus, the activation of outcome-expectant neurons by the predictive odor cues is a reliable feature of neural activity in OFC in this task.

By contrast, in the ABL-lesioned rats, very few of the neurons with outcome-expectant activity developed selective firing to the associated odor cue after learning (4/107, 4%) (Figure 6). This proportion was significantly smaller than that in intact rats ($\chi^2 = 12.2$, $p < 0.001$) and in fact was somewhat less than expected by chance (chance = 5.8 neurons, $\chi^2 = 0.42$, NS). Instead, most of the outcome-expectant neurons exhibited no difference in firing to the odor cues in the postcriterion trials (Figure 5B, right panels). Notably, the failure of these neurons to become activated by the odor cues after learning accounts for the reduction in the total number of OFC neurons with selective firing to the odor cues observed in the lesioned rats (Table 1).

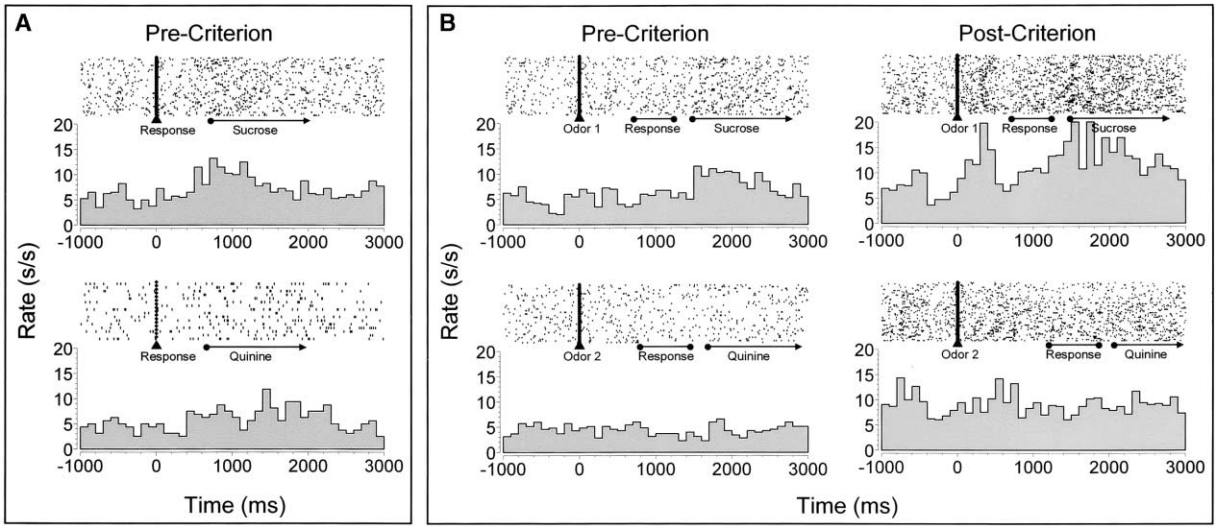

Figure 4. Activation of Outcome-Expectant Encoding during Cue Sampling in an Intact Rat

Example of an OFC neuron recorded in an intact rat that fires after responding in anticipation of and during sucrose delivery (A) in the precriterion trials and then develops a selective response to the associated odor cue during the postcriterion trials ([B], right column). Note that the neuron does not exhibit differential activity to the odor cues in the precriterion trials ([B], left column). Thus, this neuron activates a representation of the appetitive sucrose outcome during sampling of the positive odor cue after learning. Raster displays show neural activity on individual trials, and each histogram shows average activity in spikes/second in 100 ms bins. The timing of trial events is indicated beneath the rasters.

Table 2. Differential Firing of Outcome-Expectant Neurons to the Associated Odor and Outcome in the Precriterion Trials

	Intact Rats (n = 112)	ABL-Lesioned Rats (n = 107)
Neurons selective for the associated odor	15	11
Neurons selective for the associated outcome	38	32

None of the comparisons are significant at $p < 0.05$ by χ^2.

Remaining Firing to the Predictive Odor Cues Is Less Associative and More Often Bound to Cue Identity in ABL-Lesioned Rats

The failure of outcome-expectant neurons to become activated by the associated odor cues after learning was only one of the effects of ABL-lesions on cue-selective firing in OFC; even when these neurons were excluded from the cue-selective population, significant differences in information represented in the remaining neurons were evident. In particular, cue-selective firing in ABL-lesioned rats was less strongly driven by the learned significance of the odor cues and more strongly driven by the sensory features or identity of the odor cues. This difference was evident in the effect of learning and reversal on firing during cue sampling.

Excluding neurons with outcome-expectant activity discussed above, there were 116 neurons in intact rats that exhibited cue-selective firing during the postcriterion trials. Consistent with our previously published observations (Schoenbaum et al., 1999), most of these neurons altered their odor preference during learning or reversal, indicating strong associative encoding in this population in intact rats. For example, 75% of these neurons developed a new odor preference between the precriterion and postcriterion trials ("New preference PRE-POST," Table 3). In addition, 89% of these neurons changed their postcriterion odor preference after reversal; some of these neurons reversed odor preference ("Reversed preference POST-REV," Table 3), while most stopped firing selectively to the odor cues when the contingencies were reversed. These neurons were in effect replaced by a new set of OFC neurons that became selective for the odors after reversal (n = 112/415 nonselective neurons).

The pattern of selectivity just described for intact rats is illustrated in Figure 7, which shows neurons that develop selective responses to odor cues either before or after reversal. Note that unlike the earlier example of an OFC neuron with cue-selective activity (Figure 4), these neurons do not fire differentially in anticipation or during sampling of the outcomes after a response was made; thus, they encoded the acquired significance of the odor cues independent of the features of the associated outcomes. Importantly, only two neurons (1.7%) in OFC in intact rats maintained the same odor selectivity across all three phases of training, suggesting very little encoding of the sensory qualities of the cues. That result agrees with our prior report in which no neurons exhibited such an encoding pattern (Schoenbaum et al., 1999).

In contrast to findings in intact rats, the remaining

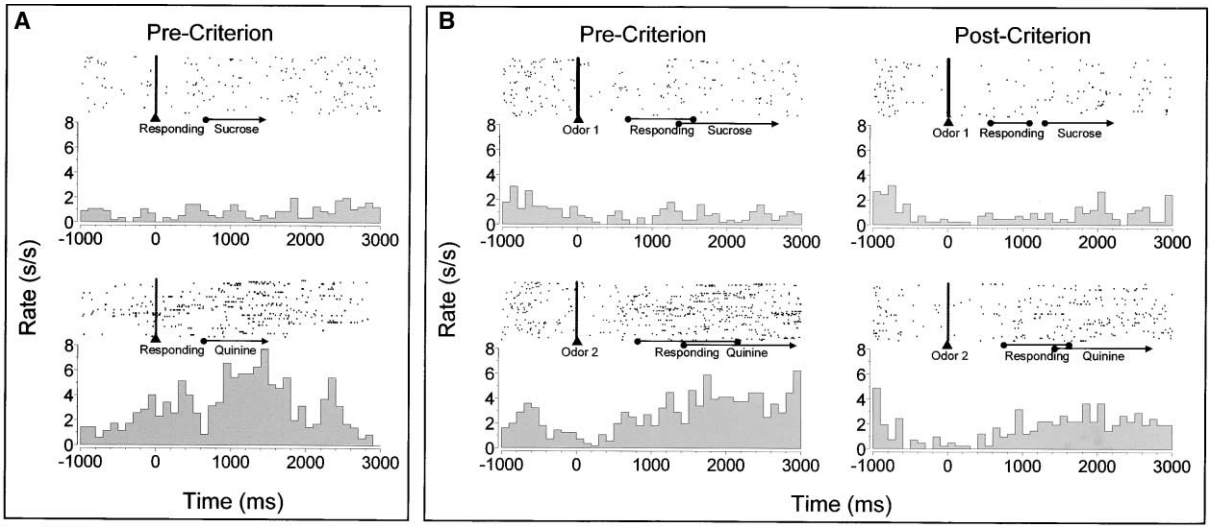

Figure 5. Outcome-Expectant Encoding in an ABL-Lesioned Rat

Example of an OFC neuron recorded in an ABL-lesioned rat that fires after responding in anticipation of and during quinine delivery (A) in the precriterion trials. Note that the neuron does not exhibit differential activity to the odor cues in the precriterion trials ([B], left column) nor does it become selective for the associated odor cue after learning in the postcriterion trials ([B], right column). Thus, unlike the neuron recorded in an intact rat depicted in Figure 3, firing of this neuron does not provide a representation of the outcome during cue sampling. Raster displays show neural activity on individual trials, and each histogram shows average activity in spikes/second in 100 ms bins. The timing of trial events is indicated beneath the rasters.

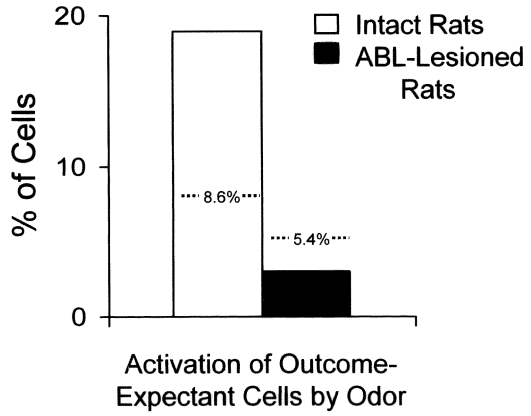

Figure 6. Proportion of Outcome-Expectant Neurons that Become Activated by the Associated Odor Cue in Intact and ABL-Lesioned Rats after Learning

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 this in the neural populations in each group. The proportion of these neurons in intact rats was significantly larger than that in ABL-lesioned rats ($\chi^2 = 12.2$, $p < 0.001$) and that expected by chance (chance = 9.7 neurons, $\chi^2 = 4.53$, $p < 0.05$); the proportion in ABL-lesioned rats was smaller than the number expected by chance (chance = 5.8 neurons, $\chi^2 = 0.42$, NS). See Experimental Procedures for description of these calculations.

population of OFC neurons with cue-selective firing in ABL-lesioned rats (n = 97) was less dependent on the learned significance of the odor cues and more strongly driven by the identity of the odors. This was particularly evident in a significant increase in the proportion of neurons that maintained selectivity for the same odor both before and after reversal ("Same preference POST-REV," Table 3). Indeed a most striking phenomenon in ABL-lesioned rats was the large proportion of neurons that maintained the same odor preference across all three phases of training ("Same preference PRE-POST-REV," Table 3; Figure 8). The proportion of OFC neurons exhibiting such odor encoding increased by nearly an order of magnitude in ABL-lesioned rats (Table 3). In addition, there was a corresponding but nonsignificant decrease in the proportion of neurons that reversed during reversal trials ("Reversed preference POST-REV," Table 3), and fewer nonselective neurons were recruited (n = 57/411 nonselective neurons; $\chi^2 = 21.8$, $p < 0.001$) to become selective after reversal as compared to intact rats.

Discussion

Here we have identified two independent populations of cue-selective neurons in OFC in intact rats. One population encoded the associative activation of the expected outcome in the presence of a predictive cue. The second encoded a more general representation of the acquired significance of the cue independent of the expected outcome. ABL lesions prevented the first population from becoming activated in OFC during cue sampling, when information about the outcome could be used in guiding the decision to respond. In addition, ABL lesions significantly affected the encoding properties of the second population representing the acquired significance of the odor cues in OFC. As we will describe below, the failure of ABL-lesioned rats to develop such representations in OFC provides a possible basis for behavioral impairments produced by damage to this circuitry (Balleine et al., 2003; Gallagher et al., 1999; Hatfield et al., 1996; Izquierdo and Murray, 2000, Soc. Neurosci., abstract; Malkova et al., 1997; Parkinson et al., 2001; Pears et al., 2001).

Neural Correlates Supporting Behaviors Sensitive to the Value of the Predicted Outcome

Damage to ABL produces impairments in a number of settings that depend on the representation of outcomes (Blundell et al., 2001; Cardinal et al., 2002a; Hatfield et al., 1996; Malkova et al., 1997). Such deficits can be clearly demonstrated even though ABL lesions often have no effect on primary behavioral measures of learning. For example, after ABL damage, a variety of approach and orienting responses are acquired normally in appetitive Pavlovian conditioning (Everitt et al., 2000; Hatfield et al., 1996; Parkinson et al., 2000), and ABL lesions do not impair simple instrumental conditioning and discrimination learning that involves reward (Baxter et al., 2000; Malkova et al., 1997; Parkinson et al., 2001). Similarly, ABL lesions did not disrupt choice performance during acquisition in the current investigation.

Nevertheless, deficits after ABL damage are consistently reported in these settings when probe tests, using reinforcer devaluation procedures, are utilized to reveal stimulus-outcome associations formed during training. In such tests, the value of the outcome is experimentally changed after an association between a predictive cue and an outcome is learned, in order to probe the subject's ability to use a representation of the outcome in memory to guide behavior. For example, after learning

Table 3. Effect of Learning and Reversal on Differential Firing to the Odor Cues

	Intact Rats[a] (n = 116)	ABL-Lesioned Rats[a] (n = 97)
Learned significance		
New preference PRE-POST	87	59**
Reversed preference POST-REV	26	14*
Odor identity		
Same preference POST-REV	13	22**
Same preference PRE-POST-REV	2	13***

[a] Excluding neurons with corresponding odor and outcome-expectant encoding (Table 2).
*$p < 0.15$, **$p < 0.05$, ***$p < 0.001$ by χ^2.

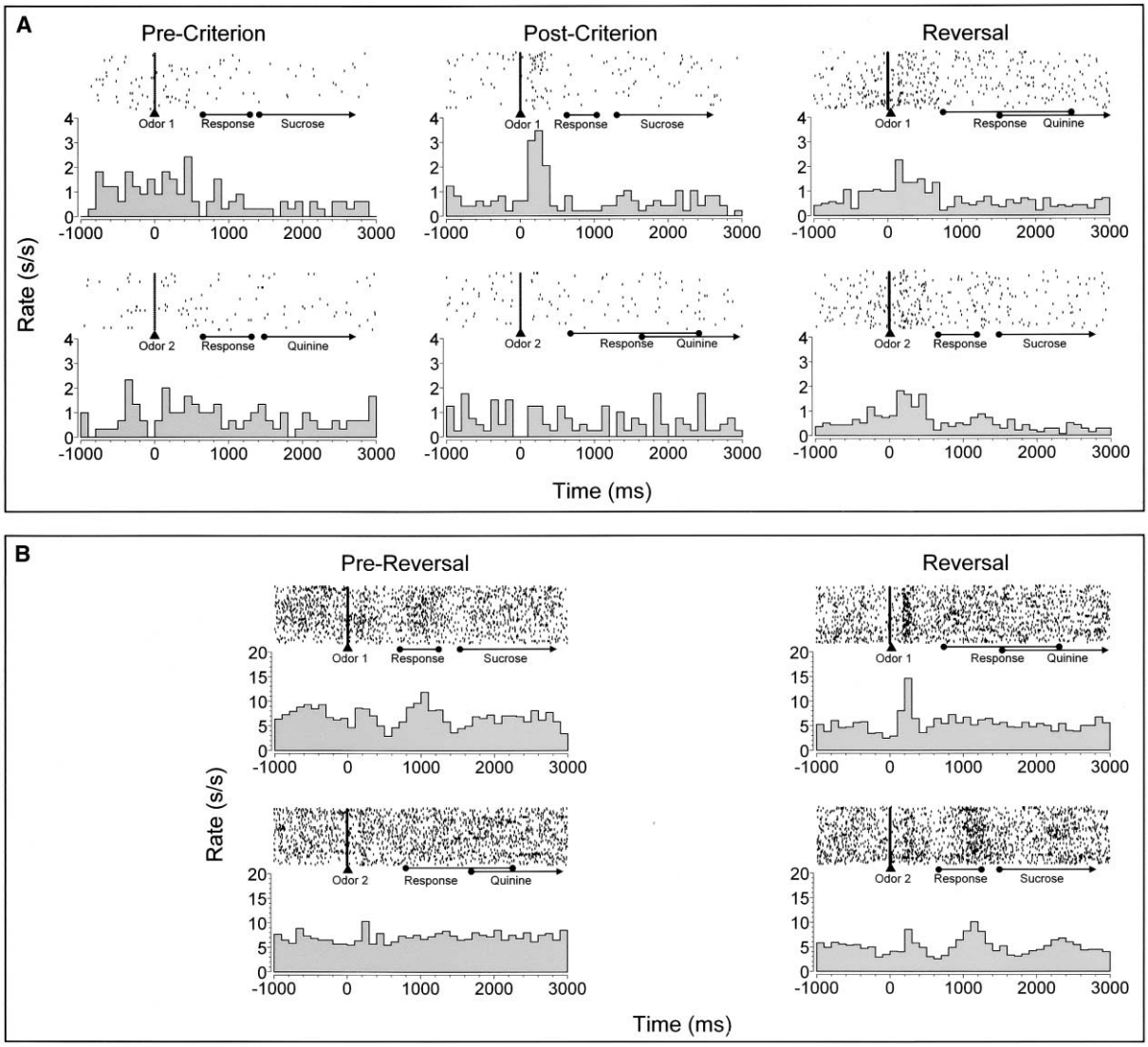

Figure 7. Encoding of Acquired Significance during Cue Sampling in an Intact Rat

(A) Example of an OFC neuron recorded in an intact rat that develops a selective response to one of the two odor cues in the postcriterion trials (middle column). Note that the neuron does not exhibit differential activity to the odor cues in the precriterion trials (left column) or after reversal (right column).

(B) Example of an OFC neuron recorded in an intact rat that develops a selective response to one of the odor cues after reversal (right column). Note that the neuron does not exhibit differential activity to the odor cues in the prereversal training (left column). Note that unlike the example in Figure 4, neither of these two neurons exhibit differential firing later in the trial in anticipation of the outcome or during outcome presentation; thus, the selective activity during cue sampling reflects the associative significance that the odor cue acquires during learning. Moreover, in both cases, there is some cue specificity in the firing, since the selective response fails to occur to the other odor when the contingencies are different (before or after reversal). Raster displays show neural activity on individual trials, and each histogram shows average activity in spikes/second in 100 ms bins. The timing of trial events is indicated beneath the rasters.

in a simple conditioning task in which a cue predicts food, the normally rewarding food can be devalued in the absence of the cue by pairing the food with illness. After devaluation, normal animals spontaneously reduce responding in the presence of the cue that predicts availability of the "devalued" food. Rats given fiber-sparing neurotoxic lesions of ABL exhibit apparently normal responding to the cue during learning but fail to modify this behavior after devaluation (Hatfield et al., 1996). These tests indicate that ABL-lesioned rats fail to form or cannot utilize associations between cues and outcomes to guide conditioned responding.

Similarly, in other settings, animals may form associations directly between cues and outcomes in much the same way that they do in explicit Pavlovian tasks. For example, monkeys trained on a set of visual discriminations subsequently bias responses to the discriminative cues after changes in the incentive value of the rewards they predict. As is the case with rats tested with Pavlovian devaluation procedures, monkeys with bilateral amygdala lesions acquire the discriminations normally in this task but are unable to appropriately modify their responses when the incentive value of the predicted reward is altered (Malkova et al., 1997). Thus, there ap-

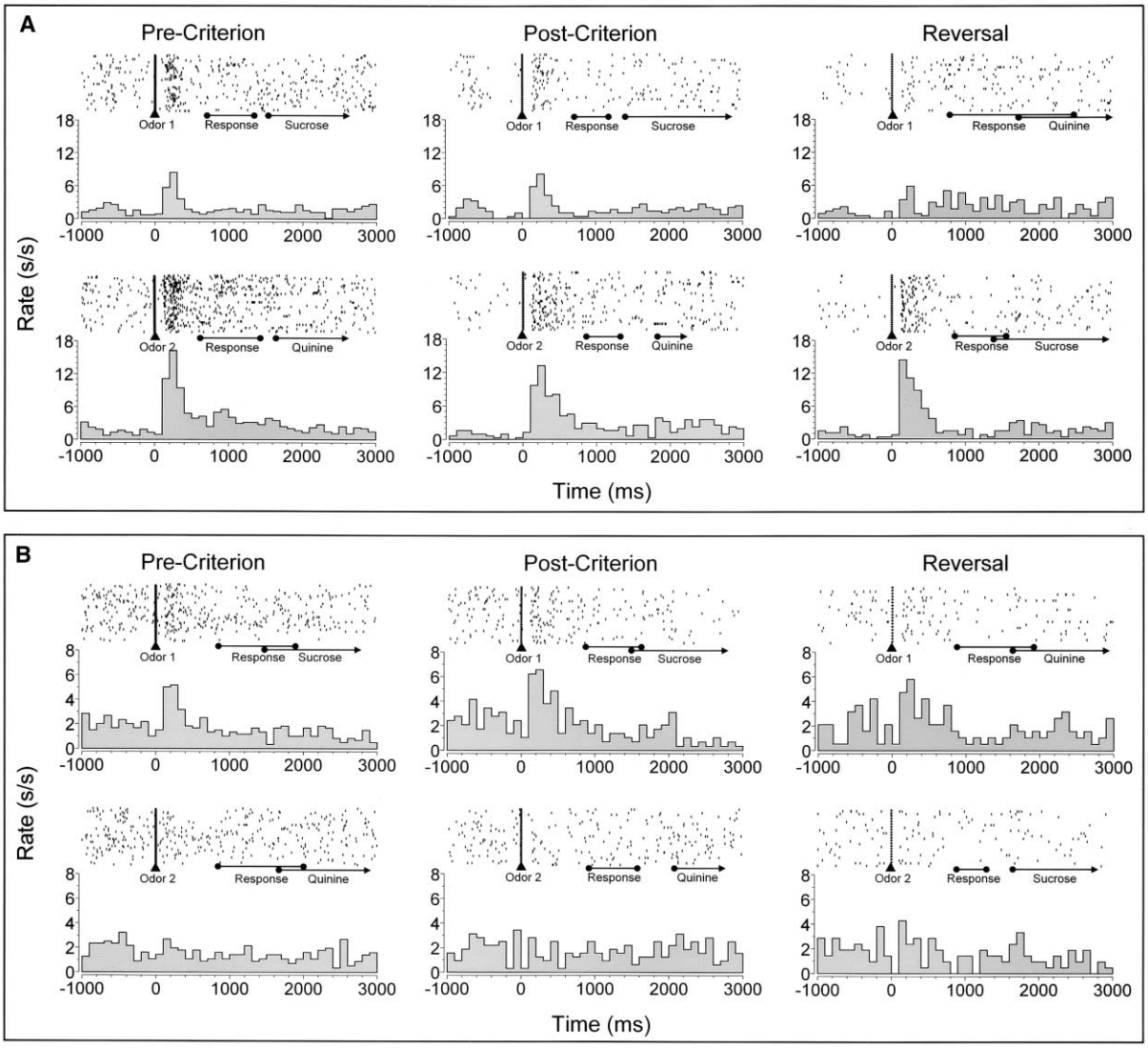

Figure 8. Encoding of Odor Identity during Cue Sampling in an ABL-Lesioned Rat

The figures show examples of OFC neurons recorded in ABL-lesioned rats that fired more to one of the odor cues throughout training.
(A) This neuron fired significantly more to odor 2 than to odor 1 during the precriterion (left column) and the postcriterion trials (middle column) and after reversal (right column).
(B) This neuron fired significantly more to odor 1 than to odor 2 during the precriterion (left column) and postcriterion trials (middle column) and after reversal (right column). In both cases, neural activity reflects identity of the odor cue rather than the value it acquires through training. Such neurons were nearly 10-fold more common in ABL-lesioned than in intact rats. Raster displays show neural activity on individual trials, and each histogram shows average activity in spikes/second in 100 ms bins. The timing of trial events is indicated beneath the rasters.

pear to be certain common amygdala-dependent mechanisms operating in both Pavlovian and instrumental settings to form associative structures linking cues to the incentive value of predicted outcomes. Notably, in these same experimental assessments, deficits are also produced by lesions of OFC (Gallagher et al., 1999; Izquierdo and Murray, 2000, Soc. Neurosci., abstract) or by disconnection of ABL from OFC (Baxter et al., 2000), indicating that ABL and OFC interact to encode and utilize such stimulus-outcome associations.

Here we report neural correlates of stimulus-outcome associations in OFC during discrimination learning. We found that a subset of OFC neurons that were responsive in anticipation of a given outcome in the task became activated during sampling of the cue that predicted that outcome once the rat had learned the predictive relationship. This population could provide information about the outcome to allow normal goal-directed responding to cues either in our recording setting or the aforementioned experimental paradigms.

Importantly, lesions of ABL selectively abolished the formation of stimulus-outcome correlates in OFC. These representations were constructed in OFC in intact rats during discrimination learning, but not in rats with ABL lesions. This finding is consistent with evidence from devaluation tests that ABL is also critical to behaviors that depend on such associative structures (Baxter et al., 2000; Hatfield et al., 1996; Malkova et al., 1997). In

addition, these findings provide insight into a possible basis for impairment after ABL lesions in settings that require animals to use representations of outcomes in memory to guide behavior. Previously it has been unclear whether the association linking the cue and outcome was not originally established in lesioned rats or whether this representation remained either immune to experimentally induced changes in value or inaccessible for use in memory to guide a response. The current data indicate an apparent deficit after ABL damage in establishing the associative representation of a predicted outcome in OFC during learning. This interpretation is consistent with recent behavioral evidence showing that ABL is particularly critical for the encoding of stimulus-outcome associations (Setlow et al., 2002), which may then be reflected in other brain regions after learning.

In the present experiment, rats with ABL lesions did show some evidence that they had failed to acquire an associative representation of the predicted outcome during cue sampling. This failure was evident in a lack of change in response latencies during learning. Changes in response latency are thought to reflect the acquisition of associations linking cues to outcomes (Holland and Straub, 1979; Sage and Knowlton, 2000; Salinas and White, 1998; Watanabe et al., 2001). For example, rats trained to enter a food cup to obtain a food reward signaled by an auditory cue exhibit longer latencies to enter the food cup after the incentive value of the food is devalued through pairing with illness (Holland and Straub, 1979). More recently, Sage and Knowlton (2000) reported that rats trained to complete trials to obtain food in a win-stay version of the radial arm maze showed longer trial completion times (latencies), but no change in choice accuracy, after devaluation of the food early in training. These data suggest that latency to respond reflects access to some representation of the incentive value of the associated outcome. Consistent with those data, we have found both here and as reported elsewhere (Schoenbaum et al., 2003) that these latency changes depend upon the integrity of the OFC/ABL system.

Interestingly, latency changes emerge at the same time that critical changes in cue-activated representations are observed in ABL in this task (Schoenbaum et al., 1998, 1999, 2000) but prior to the development of the odor-outcome encoding we have demonstrated in the current report. Thus, the latency changes depend upon encoding in ABL during learning, but it is unclear how OFC contributes to their emergence in this phase of training. One possibility is that outcome-expectant activity observed after responding, which is present in OFC early in training (Schoenbaum et al., 1998), may influence the development of cue-selective activity in ABL via reciprocal connections between these structures (Ghashghaei and Barbas, 2002).

Neural Correlates Supporting Behaviors Based on the Acquired Significance of Cues

In addition to the abolition of explicit stimulus-outcome representations in OFC, ABL-lesions also had a dramatic effect on the remaining cue-activated representations that were observed, which were less associative and more often bound to the identity of the odor cue. This effect is consistent with the connectivity of OFC, which receives sensory input from primary olfactory structures (Barbas, 1993; Haberly, 2001; Price et al., 1991), and with the locations of the recording electrodes, which straddled a region of OFC that receives afferent input from both olfactory structures and ABL (Kita and Kitai, 1990; Price et al., 1991). Without information from ABL regarding the affective significance of associated outcomes, control by the underlying sensory input might predominate the encoding characteristics of some neurons in OFC. More importantly, these findings also suggest that the acquired motivational significance of the odor cues established in OFC is sensitive to lesions of ABL.

Such findings fit well with indications that, in addition to becoming linked to representations of outcomes, otherwise neutral cues can acquire motivational significance or value through association with biologically significant events (Cardinal et al., 2002a; Gallagher, 2000; Gewirtz and Davis, 2000; Holland and Gallagher, 1999). Those associations can confer the ability for such cues to support new learning in both Pavlovian (second-order conditioning) and instrumental (conditioned reinforcement) paradigms. Behavior in these paradigms is sensitive to lesions of ABL (Amorapanth et al., 2000; Everitt and Robbins, 1992; Hatfield et al., 1996; Killcross et al., 1997; Parkinson et al., 2001), and ABL appears to be necessary only for encoding but not the use of information regarding the acquired motivational significance of cues in at least one of these settings (Setlow et al., 2002). That finding suggests that brain regions outside ABL, such as OFC, can support the use of such information after learning. A role for OFC in the use of acquired value for otherwise neutral cues is consistent with our report here of ABL-dependent encoding of acquired motivational significance in OFC and with recent reports that OFC lesions impair conditioned reinforcement (Pears et al., 2001). These data further suggest that impairments in other tasks, such as second-order conditioning, may occur as a result of OFC lesions.

At the same time, ABL-lesions did not entirely eliminate conditioned neural responses to the odor cues. Even in lesioned rats, some OFC neurons developed cue-selective responses with training. Similarly, there remained neurons with differential firing during the delay after a response was made at the fluid well. The persistence of these populations after ABL lesions suggests that ABL may not be the source of all outcome-related information afferent to OFC. Indeed, such encoding could be based on nonspecific motivating effects of outcomes that do not seem to be ABL dependent (Blundell et al., 2001). These nonspecific attentional or activating aspects of the outcome may be dependent on other areas of amygdala (Cardinal et al., 2002b; Gallagher et al., 1990; Holland and Gallagher, 1993); outflow from such areas to other brain regions could subsequently impact activity in OFC. For example, we have demonstrated that neurons in the nucleus accumbens rapidly develop conditioned firing to the odors and during the delay in this task (Setlow et al., 2003). Such information could come to influence OFC via indirect feedback from accumbens through ventral pallidum and mediodorsal thalamus (O'Donnell, 1999).

Alternatively, firing in OFC neurons in ABL-lesioned rats may represent information that differs from that in intact controls. For example, the conditioned neural activity that remains after ABL lesions may reflect certain sensory (rather than motivational) features of the expected outcomes or the associations between the cues and anticipated behavioral responses. The latter representations could serve as a basis for the relatively preserved performance of the lesioned rats in the task by providing stimulus-response associations that do not directly incorporate representations of motivational properties of the outcome.

From Rats to Primates: Modeling Orbitofrontal Function

It has become clear that a defining feature of prefrontal cortex is its rich network of interconnections with other brain systems, including other "association" areas of posterior and temporal neocortex, limbic structures such as the hippocampal formation and amygdala, and major efferent projections to striatum (Goldman-Rakic, 1987; Ongur and Price, 2000; Preuss, 1995). This connectional anatomy has provided an important basis for further subdividing regions of prefrontal cortex and guiding functional analysis of prefrontal systems. For example, the primate orbitofrontal region (areas 13 and 47, and inferior aspects of areas 10, 11, and 13) receives input from sensory areas including gustatory and olfactory regions and also interacts with the basolateral amygdala and ventral striatum (Fuster, 2000; Ongur and Price, 2000). This pattern of connectivity is also observed for the rat OFC, including the ventral and lateral orbital regions and the dorsal and ventral agranular insular cortices, and neurophysiological and behavioral findings demonstrate a remarkable degree of similarity between the critical functions of this prefrontal region in rats and the orbitofrontal area in primates (for review, see Schoenbaum et al., 2002).

Such similarities that have been identified across species suggest that findings in rat OFC may provide insight into fundamental processes in primate prefrontal regions. Thus, the ABL-dependent encoding properties of OFC neurons demonstrated in the current study may also develop in the prefrontal cortex in primates in support of certain representational functions. In humans, functional imaging studies report activation of this region of prefrontal cortex in anticipation of rewards and punishments (Breiter et al., 1997; Elliott et al., 2000; Nobre et al., 1999; O'Doherty et al., 2001). Similarly, many OFC neurons in monkeys encode the incentive value of impending rewards during a delay interval before reward delivery (Hikosaka and Watanabe, 2000; Tremblay and Schultz, 1999). This encoding resembles that seen in rats during a delay after responding but before outcome presentation, as described in the current investigation and as previously reported (Schoenbaum et al., 1998).

Many OFC neurons in primates also acquire selective responses when animals are presented with cues that predict the outcome on a trial (Rolls et al., 1996; Thorpe et al., 1983; Tremblay and Schultz, 1999; Wallis et al., 2001), and these neurons reflect the relative preference of the monkey for associated rewards (Tremblay and Schultz, 1999). Based on the current findings, those cue-responsive neurons may include a subpopulation that activates a representation of the predicted outcome. Such encoding could provide a basis for psychological processes in which outcome representations are required to guide behavior. Furthermore, the elimination of that encoding would account for behavioral impairments produced in monkeys after damage to the ABL/OFC system, such that actions fail to be appropriately guided by the modified incentive value of predicted outcomes (Baxter et al., 2000; Izquierdo and Murray, 2000, Soc. Neurosci., abstract; Malkova et al., 1997). A comparable function of the ABL/OFC circuit in humans would also explain certain similarities observed after damage to ventromedial prefrontal cortex and the amygdala. For example, in the so-called "gambling task," patients with damage to either of these two brain regions fail to use outcome information about rewards and penalties to make adaptive choices (Bechara et al., 1999). Lacking an effective guide for action may well contribute to impairment in patients with prefrontal damage and to a deficiency in functional encoding in cortex after amygdala damage.

Experimental Procedures

All procedures were conducted at Johns Hopkins University in accordance with University and NIH guidelines.

Surgical Procedures

Eight adult male Long-Evans rats served as subjects (Charles River Laboratories, Wilmington, MA). Procedures for creating ABL lesions and implanting electrodes were identical to those used previously (Hatfield et al., 1996; Schoenbaum et al., 1999). Neurotoxic lesions of ABL (n = 4) were made by intracerebral infusions of *N*-methyl-D-aspartic acid (NMDA, 12.5 μg/μl; Sigma, St. Louis, MO) in phosphate buffer vehicle bilaterally at 2.8 mm posterior to bregma, 5.0 mm lateral to the midline, and 8.4 (0.1 μl) and 8.7 mm (0.2 μl) ventral from skull. Sham lesions (n = 4) were made by lowering the infusion needle to the same coordinates, without infusing any solutions.

A driveable electrode bundle was chronically implanted dorsal to OFC in the left hemisphere at 3.0 mm anterior to bregma, 3.2 mm laterally, and 4.0 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 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 μm) 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 approximately 18″ on each side with sloping walls narrowing to an area of 12″ × 12″ 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, 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 5% 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 ($\sim$50 trials on average) and for an additional 60–100 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. In all sessions presented here, the rats met a criterion of 18 correct responses in a moving block of 20 trials on this reversal before the session ended.

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, bandpass filtered at 300–3000 kHz, and amplified differentially (relative to a silent reference electrode) at 5000× (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. Tyically one to three waveforms could be isolated on an active channel. An example of two units sorted on a single channel is shown in Figure 2.

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/second) 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 Chi-square 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 sampling 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.

Acknowledgments

This work was supported by MH12699 (B.S.) and MH60179 (M.G.) from the NIMH and AG00882 (G.S.) from the NIA. We thank Dr. Stephen Warrenburg at International Flavors and Fragrances for his assistance.

Received: May 5, 2003
Revised: May 29, 2003
Accepted: July 17, 2003
Published: August 27, 2003

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