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Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons

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Abstract

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Both the nucleus accumbens (NAc) and basolateral amygdala (BLA) contribute to learned behavioral choice. Neurons in both structures that encode reward-predictive cues may underlie the decision to respond to such cues, but the neural circuits by which the BLA influences reward-seeking behavior have not been established. Here, we test the hypothesis that the BLA drives NAc neuronal responses to reward-predictive cues. First, using a disconnection experiment, we show that the BLA and dopamine projections to the NAc interact to promote the reward-seeking behavioral response. Next, we demonstrate that BLA neuronal responses to cues precede those of NAc neurons, and that cue-evoked excitation of NAc neurons depends on BLA input. These results indicate that BLA input is required for dopamine to enhance the cue-evoked firing of NAc neurons, and that this enhanced firing promotes reward-seeking behavior.

Introduction

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The ability to predict oncoming events based on environmental stimuli and to adjust behavior accordingly is a critical function of the central nervous system. Understanding the processes that link a conditioned stimulus (CS) to a motor response is therefore a fundamental question in neurobiology. The amygdala is a primary site of CS encoding (Everitt et al., 2003; LeDoux, 2003). For example, following aversive Pavlovian conditioning with an auditory CS, information from the thalamus is processed in the amygdala and elicits innate behavioral responses (e.g., freezing) via the central nucleus of the amygdala (LeDoux, 2003). The amygdala is also essential for conditioned appetitive stimuli to evoke goal-directed behavior (Everitt et al., 2003) but neither the amygdala neuronal firing properties nor the neural circuits underlying this process have been fully elucidated.

The circuits that generate goal-directed behavior in response to conditioned reward-predictive cues involve a neural connection between the lateral/basolateral amygdala (BLA) and the nucleus accumbens (NAc) (Di Ciano and Everitt, 2004; Johnson et al., 1994; McDonald, 1991; Setlow et al., 2002; Wright et al., 1996). Indeed, the NAc, which is commonly viewed as a limbic-motor interface (Mogenson et al., 1980), is ideally situated to translate

reward-predictive information from the amygdala into appropriate reward-seeking motor behavior. The NAc receives a prominent direct excitatory projection from the BLA (Brog et al., 1993; Christie et al., 1987; O'Donnell and Grace, 1995), and studies in behaving animals have shown that NAc neurons encode both the value of reward-predictive cues (Cromwell and Schultz, 2003; Hassani et al., 2001; Hollerman et al., 1998) and the action leading to the reward (Nicola, 2007; Nicola et al., 2004b; Taha et al., 2007). Thus the NAc, and possibly the BLA projection to the NAc, may act to select actions that optimize reward-seeking in response to sensory cues (Nicola, 2007; Yun et al., 2004a). Dopamine projections from the ventral tegmental area (VTA) may also play a role in this circuit: dopamine is released in the NAc in response to conditioned incentive cues (Day et al., 2007; Roitman et al., 2004) and VTA inactivation disrupts both cue-evoked firing and the behavioral response to the cue (Yun et al., 2004b).

Dopamine alone is insufficient to induce firing in NAc neurons and instead modulates neuronal excitability (Floresco, 2007; Nicola, 2007; Nicola et al., 2000). An intriguing possibility is therefore that dopamine facilitates excitatory cue-evoked BLA inputs to NAc neurons which in turn promotes the behavioral response to the cues. Consistent with this hypothesis, many BLA neurons are excited by reward-predictive cues (Paton et al., 2006; Schoenbaum et al., 1998; Sugase-Miyamoto and Richmond, 2005). Here, we test the hypotheses that BLA neurons encode the value of a reward-predictive cue in a discriminative stimulus (DS) task that requires dopamine release in the NAc, and that NAc neuronal responses to such cues depend on a projection from the BLA. In addition, we show that the reward-seeking response to incentive cues results from the integration of information from dopaminergic and BLA inputs to the NAc.

Results

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All rats in this study were trained on the DS task (Figure 1A). Two auditory cues, the discriminative stimulus (DS) and the non-rewarded stimulus (NS) were randomly presented on average every 30 sec. Each DS was presented for up to 10 sec; if the animal pressed a lever during DS presentation, the DS was turned off and a liquid sucrose reward was delivered in a nearby receptacle. Each NS was presented for 10 sec; responding during the NS or in the absence of cues was never rewarded and had no programmed consequence.

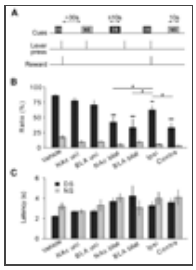

Figure 1

The BLA, and its relationship with dopamine transmission in the NAc, is required for the DS task. (**A**) Temporal organization of the DS task. Two cue tones (up to 10 sec for the DS; 10 sec for the NS) were randomly presented on a variable interval schedule **...**

BLA-NAc disconnection reduces behavioral performance in the DS task

We first investigated whether concomitant BLA input and dopamine receptor activation in the NAc promotes incentive cue responding. Bilateral inactivation of the BLA reduces responding to DSs (Ishikawa et al., 2008a; Yun and Fields, 2003) as does bilateral injection of dopamine receptor antagonists into the NAc (Yun et al., 2004a; Yun et al., 2004b). We reasoned that since the BLA to NAc projection is largely ipsilateral (Brog et al., 1993; Christie et al., 1987; Johnson et al., 1994), transient unilateral inactivation of the BLA, coupled with dopamine antagonist injection into the contralateral NAc, should disconnect the NAc from the BLA, because after these injections the NAc in neither hemisphere receives both BLA and dopamine inputs. If the dopamine released in the NAc facilitates BLA-driven firing that underlies cue responding, the result of the disconnection should be impaired performance of the DS task. Seven rats were trained on the DS task and implanted with microinjection cannulae bilaterally in both the BLA and NAc core (Figure S1). The BLA was transiently inactivated with bilateral or unilateral injections of a cocktail of the $GABA_A$ and $GABA_B$ agonists baclofen and muscimol (B/M,

12.5ng/0.5µl), and dopamine receptors were blocked bilaterally or unilaterally in the NAc with the D_1 receptor antagonist SCH23390 (0.5ug/0.5µl).

The different injections significantly affected the cue response ratio (Figure 1B, ANOVA injection × cue $F(6,180)=6.18$, $p<0.001$) but not the response latency (Figure 1C, ANOVA injection × cue $F(6,161)=1.03$, $p=0.40$). The effects on cue response ratio were due entirely to differences in DS response ratio, since none of the injections significantly affected the NS response ratio (Figure 1B). As previously shown (Yun et al., 2004a; Yun et al., 2004b), bilateral blockade of D1 receptors in the NAc reduced behavioral performance, as did bilateral BLA inactivation (Ishikawa et al., 2008a; Yun and Fields, 2003). However, neither unilateral injections of B/M into the BLA ($p=0.29$) nor unilateral injection of the D1 antagonist into the NAc ($p=0.07$) significantly reduced the DS response ratio. These results confirm that both the BLA and dopamine receptor activation in the NAc are required to respond to the DS, and that elimination of the BLA or dopamine receptor function in one hemisphere alone is insufficient to disrupt DS responding.

In the disconnection study, each structure was injected unilaterally either in the same or in different hemispheres. Combined unilateral injections into the same hemisphere (IPSI injections; left NAc, left BLA and right NAc, right BLA) reduced DS responding compared to the vehicle injections, but to a significantly smaller extent than did bilateral injections in the BLA alone or the NAc alone. In contrast, the CONTRA injections (left NAc, right BLA and right NAc, left BLA) reduced the DS response ratio to the same degree as bilateral injections in the BLA alone or the NAc alone. Furthermore, the DS response ratio was significantly more affected by the CONTRA injections than the IPSI injections. These data demonstrate that an ipsilateral connection between the BLA and the NAc, requiring dopamine transmission in the NAc, is necessary for rats to respond to reward predictive cues in the DS task.

BLA and NAc neurons differentially encode stimulus incentive value in the DS task

The disconnection study clearly establishes that a functional relationship between the BLA and dopamine receptor activation in the NAc underlies cue responding in the DS task. However, these results do not establish what kind of information neurons in these structures carry. We therefore recorded the activity of BLA and NAc neurons in rats performing the DS task.

We recorded 242 neurons (5 rats, 24 sessions) in the BLA and, in a separate group of rats, 748 neurons (5 rats, 63 sessions) in the NAc; these were located almost exclusively in the core (see Figures S2 and S3 for histological reconstruction of electrode locations). In both structures, subpopulations of neurons were modulated during each of the task events we measured (cue onset, operant response, and reward receptacle entry and exit). Because BLA inactivation disrupts the rats' ability to respond behaviorally to cues, we focused on cue responses in the present study. Importantly, the behavioral performance did not differ in the 2 groups of rats (Figures 2A and 2B, ANOVA for response ratio: structure × cue, $F(1,170)=0.58$, $p=0.44$; ANOVA for response latency: structure × cue $F(1,161)=2.56$, $p=0.11$) and thus cannot account for differences we observed in the activity of BLA and NAc neurons.

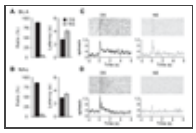

Figure 2

Behavioral performance during electrophysiological recording, and example of BLA and NAc neuronal responses to cues. (**A, B**) Response ratio (left) and behavioral latency to respond (right) during BLA (**A**) and NAc (**B**) recording sessions. (**C, D**) Example of **...**

In the BLA, neurons were primarily excited by cues (50 excited and 21 inhibited). Figure 2c shows an example BLA neuron that was activated by both the DS and the NS. Both cues excited this neuron with a sharp peak at very short latency, and the magnitude of this initial peak was equivalent for both cues. However, the DS caused a

prolonged elevation in firing, lasting several sec, whereas the NS-evoked firing rapidly returned to the pre-cue baseline. These characteristics were common to most BLA cue-excited neurons: they responded to both DS and NS (Figure 3A), and the distributions of onset latencies were similar for DS- and NS-evoked excitations (Kolmogorov-Smirnov test, $P>0.1$) with more than half the neurons beginning to fire 20 ms or less after cue onset (Figure 3B). During the first 100 ms, the magnitude of DS and NS excitations was similar (Figures 3G and 3I, ANOVA event × structure $F(1,330)=8.76$, $p<0.005$), indicating that in BLA neurons, the incentive value of the cue is not encoded by the initial peak. Finally, the cue-induced excitations were sustained only after the DS; NS-evoked excitations rapidly returned to baseline following the peak (Figures 3C, 3G and 3J, Kolmogorov-Smirnov test, $P<0.005$). Therefore, the magnitude of the late response (100–3000 ms) was larger for the DS than the NS (Figure 3J, ANOVA event × structure $F(1,330)=0.51$, $P>0.52$, event $F(1,330)=23.72$, $P<0.001$). Because the DS predicts reward and elicits a behavioral response whereas the NS does neither, these results indicate that the prolonged firing following cue presentation in BLA neurons either encodes the incentive value of the cue or reflects some aspect of neural processing related to the motor response to the cue. However, the initial response to the DS and NS was identical, indicating that the initial short latency response to the cue in BLA neurons does not encode the cue's incentive value.

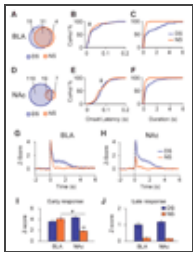

Figure 3

Excitations in BLA and NAc neurons encode cues differently. (**A, D**) Venn diagrams representing the proportion of neurons excited by the DS, the NS or both (overlapping section) in the BLA (**A**) and NAc (**D**). (**B, E**) Cumulative percentage of firing onset latencies **...**

In the NAc, we recorded 144 neurons that were excited and 54 that were inhibited by either the DS or the NS. In contrast to BLA neurons, a majority of NAc cue-excited neurons responded exclusively to the DS (Figure 3D) or only minimally to the NS (e.g., Figure 2D). However, as in the BLA, most neurons that responded to the NS tended also to respond to the DS (70% overlap). Similar to BLA cue-excited neurons, NAc cue-evoked excitations occurred at the same onset latency for both cues, but had longer durations for the DS (Figures 3E and 3F, Kolmogorov-Smirnov test, $P<0.001$). In contrast to BLA neurons, however, the magnitudes of both the early (100 to 200 ms after cue onset) and late (200–3000 ms) DS excitations were more than twice the size of NS-excitations (Figures 3H, 3I and 3J).

Cue-evoked excitations began at significantly longer latencies in the NAc than in the BLA (median onset latencies were 20 and 60 ms, respectively, Kolmogorov-Smirnov test, $P<0.001$, Figures 3B and 3E). These results are consistent with the hypothesis that BLA neurons drive NAc neuronal excitations to cues. On its own, however, this hypothesis cannot explain why the initial NAc neuronal response differentiates the DS from the NS whereas the initial BLA response does not. One possibility is that NAc neurons do not begin to fire after cue onset until after BLA neurons have begun to discriminate the DS from the NS, which would suggest that BLA neurons' encoding of stimulus value contributes to similar encoding among NAc neurons. To test this hypothesis, we compared the latency of onset of differential encoding in the BLA and NAc. For each neuron, we subtracted the NS-evoked from the DS-evoked firing rate and determined the latency at which the first significant difference from 0 occurred (see Methods). We call this the discrimination latency since it equals the time after cue onset at which DS and NS responses first differ (Figure 4A). About half of all cue-responsive BLA neurons (26/50), and fewer than one third of NAc neurons (42/144), had no discrimination latency because their firing rates did not discriminate among the cues at any time between 0 and 3 sec after cue onset (Figure 4B). The non-discriminative neurons tended to be those with shorter duration responses (Figure S4), consistent with the idea that differential responding to the two cues develops after the non-discriminative initial response. Among discriminative neurons, the distribution of discrimination latencies did not significantly differ between BLA and NAc neurons (Figure 4C, Kolmogorov-

Smirnov, P<0.1). Notably, the fastest 50% of discrimination latencies in the BLA and NAc were remarkably similar. This observation suggests that despite the rapid peak in firing that did not discriminate between the two cues (Figure 3G,I), the later differential firing to the two cues among this population of BLA neurons could contribute to the differential firing of the majority of cue-responsive NAc neurons.

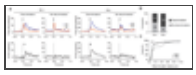

Figure 4

Timing of incentive value-encoding in NAc and BLA neurons. (**A**) Top row of graphs shows PSTHs of two example neurons in the BLA (left) and two neurons in the NAc (right) that were excited by both the DS and NS. Bottom graphs represent the PSTHs in the **...**

We also studied cue-evoked inhibitions in BLA and NAc neurons (Figure S5). For both structures, DS responses were stronger than NS responses and the onset latencies of the DS response did not differ between BLA and NAc inhibitions. Because of the similarities in onset latency, these results are most consistent with the hypothesis that the inhibitions are not due to projections between the two structures (and instead are due to inhibition arising from a third nucleus that projects to both).

The BLA is necessary for cue-induced responses in the NAc

The previous results are compatible with the hypothesis that BLA activity generates DS responses in the NAc, in particular excitations, which occurred earlier in the BLA than the NAc. To test this hypothesis explicitly, we analyzed the effect of BLA inactivation on DS-evoked firing in the NAc. Rats (n=8) trained on the DS task were implanted with recording electrodes in the NAc and bilateral microinjection cannulae in the BLA. A pre-injection period, used to obtain baseline cue-evoked responses of NAc neurons during the DS task, was then followed by a post-injection period in which we observed the consequence of either unilateral or bilateral reversible inactivation of the BLA on the same neurons.

The DS response ratio was dramatically reduced by bilateral inactivation of the BLA, but not by vehicle injection (Figure 5A, ANOVA interval × injection: DS response ratio, $F(2,57)=52.28$, $p<0.001$; NS response ratio $F(2,54)=0.71$, $p=0.49$). Unilateral BLA inactivation also reduced the DS response ratio, but to a significantly smaller degree than bilateral inactivation (unilateral: ~20% reduction; bilateral: ~70% reduction). Although the behavioral latency to respond to the DS was significantly affected by the injections (ANOVA interval $F(1,54)=19.75$, $P<0.001$), the interaction between interval and injection was not significant ($F(2,57)=1.33$, $p=0.27$), indicating that all injections slightly increased the latency (Figure 5B).

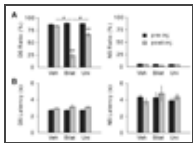

Figure 5

Behavioral performance during the BLA microinjection/ NAc recording study is less affected by unilateral than bilateral BLA inactivation. Response ratio (**A**) and behavioral latency (**B**) before and after bilateral vehicle (Veh), unilateral (Uni) or bilateral **...**

As we showed previously (Figure 2, Figure 3), DS-evoked excitations typically exhibited a sharp peak followed by sustained firing. Therefore, we analyzed the early (100 to 200 ms) and late (200 to 3000 ms) components of the NAc neuron DS response separately. Both components were reduced by bilateral BLA inactivation (Figures 6A and 6B, ANOVA interval × injection $F(3,146)=6.38$, $p<0.005$ and $F(3,146)=4.97$, $p<0.005$ respectively) whereas bilateral vehicle injections were without effect (n=51, 44 and 55 DS-excited neurons recorded during bilateral vehicle injections, bilateral BLA inactivation, and unilateral BLA inactivation, respectively). Critically, ipsilateral, but not contralateral BLA inactivations significantly reduced the early and sustained NAc DS-evoked excitations. Because unilateral BLA inactivations had only minimal effects on behavior

(Figure 5), yet reduced DS excitations in the ipsilateral NAc substantially, these results argue strongly that the DS-evoked excitations are not a consequence of behavior. Rather, these results indicate that an ipsilateral projection from the BLA to the NAc drives NAc cue-evoked excitations, and that these excitations are required for the behavioral response.

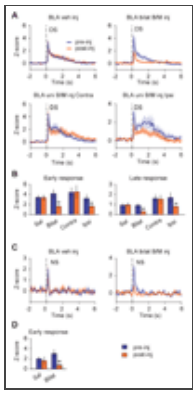

Figure 6

Inactivation of the BLA reduces DS-evoked excitation of NAc neurons. (**A**) Effects of the different injections on DS excitations recorded in NAc neurons. Top graphs represent vehicle injection (top left, n=51) and bilateral BLA inactivation with B/M (top **...**

To further validate this conclusion, we examined NS-evoked excitations, which begin with similar latencies, and occur mostly in the same neurons as DS-evoked excitations. Because NS presentation elicits virtually no behavioral response (Figure 5), a reduction in NS firing after bilateral inactivation of the BLA is unlikely to be an indirect result of the behavioral effects of BLA inactivation. Consistent with this hypothesis, bilateral BLA inactivation almost completely abolished NS-evoked excitations in NAc neurons (Figures 6C and 6D, ANOVA $F(1,18)=4.51$, $P<0.05$).

We also analyzed the effects of BLA inactivation on DS-evoked inhibitions in the NAc (Figure S6). Inhibition onsets occurred at longer latencies than excitations and lasted longer (Kolmogorov-Smirnov test, $P<0.05$ for both onset and duration), often persisting during the reward consumption period (Nicola et al., 2004c; Roitman et al., 2005; Taha and Fields, 2006; Taha et al., 2007). DS inhibitions were slightly reduced by vehicle injection and almost completely abolished by bilateral inactivation. In contrast to DS excitations, unilateral BLA inactivation reduced inhibitory responses of neurons recorded on both the ipsilateral and contralateral sides of the injection only to the same extent as bilateral vehicle injections. Therefore, NAc inhibitory responses are not strongly dependent on an ipsilateral projection from the BLA. They may, however, track the behavioral response, since both behavioral responding and the cue-evoked inhibitions were concomitantly reduced by bilateral BLA inactivation.

An ipsilateral excitatory projection from the BLA drives incentive cue-induced activation in the NAc

The BLA inactivation results are consistent with the fact that the direct ipsilateral projection form the BLA to the NAc is glutamatergic (Christie et al., 1987; Floresco et al., 2001; O'Donnell and Grace, 1995). If this projection underlies DS-evoked responses, then DS-excited (but not necessarily DS-inhibited) neurons in the NAc should be excited by BLA inputs. To test this, we electrically stimulated the BLA while recording from NAc neurons. Single pulses at low frequency and low intensity (see Methods) were given in the left and right BLA of awake animals after completion of a DS task recording session.

We found that 94/587 (16%) of NAc neurons were excited with <60 ms onset latency by ipsilateral BLA stimulation, and that 41/509 (8%) were excited by contralateral stimulation (Figures 7A and 7B). We categorized (Figure 7C) NAc neurons as ipsilaterally responsive to BLA stimulation ($ipsi^+$), contralaterally responsive ($contra^+$), or non-responsive. The proportion of neurons within the $contra^+$ population that was excited by DS presentation was similar to the proportion of DS-excited neurons among stimulation non-responsive neurons (Chi-square, $P=0.96$). In contrast, within the population of $ipsi^+$ neurons, DS-excited neurons were over-represented compared to non-responsive and $contra^+$ neurons (Chi-square, $P<0.001$). DS-inhibited neurons, however, were distributed similarly over the 3 populations. Therefore, NAc DS-excited (but not DS-inhibited) neurons tended to be excited

by ipsilateral BLA stimulation (but not by contralateral stimulation), consistent with the hypothesis that a BLA to NAc excitatory projection drives NAc DS-evoked excitations.

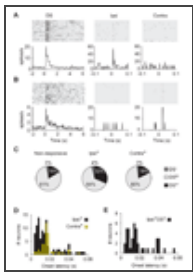

Figure 7

DS-excited NAc neurons are over-represented in the population of neurons excited by electrical stimulation of the BLA. (**A,B**) Example NAc neurons excited by the DS and ipsilateral (**A**) or contralateral (**B**) stimulation of the BLA. Bins are 20 and 2 ms for **...**

Onset latencies of both ipsi^{+} and contra^{+} responses were bimodally distributed (Kolmogorov-Smirnov test, $P<0.01$), with most latencies 4–20 ms after the stimulus, and a wide distribution of latencies >20 ms (Figure 7D). A similar bimodal distribution was observed for ipsilaterally responsive DS-excited neurons (Figure 7E). Since the monosynaptic projection is thought to result in responses with latencies of < 20 ms (Finch, 1996; Mulder et al., 1998; O'Donnell and Grace, 1995), it is likely that latencies in the tail of the distribution are due to a polysynaptic projection. Thus, these distributions suggest that NAc DS-excited neurons receive both mono- and polysynaptic excitatory input from the BLA. DS-excited neurons were over-represented among NAc neurons that were excited by ipsilateral BLA stimulation resulting in presumably monosynaptic responses (<20 ms latency) (Figure S7), supporting the idea that DS-evoked excitations in NAc neurons could be driven by the monosynaptic BLA to NAc projection.

Discussion

Go to:

The amygdala participates in neural circuits underlying behavioral responses to cues predictive of aversive events (LeDoux, 2003; Maren and Quirk, 2004). Amygdala neurons also encode cues predictive of reward (Belova et al., 2007; Paton et al., 2006; Schoenbaum et al., 1998; Sugase-Miyamoto and Richmond, 2005; Uwano et al., 1995), but the circuits underlying the behavioral response to such cues have not been as extensively studied. The BLA sends glutamatergic efferents to the NAc (Brog et al., 1993; Christie et al., 1987; Floresco et al., 2001; Johnson et al., 1994; O'Donnell and Grace, 1995), and subpopulations of NAc neurons encode reward-predictive cues (Ghitza et al., 2003; Ghitza et al., 2004; Nicola et al., 2004b; Day et al, 2006; Wan and Peoples, 2006). There is evidence that this encoding, which depends on dopamine, contributes to reward-seeking behavioral responses (Yun et al., 2004b). However, the importance of the BLA to NAc projection for behavioral and NAc neuronal responses elicited by incentive cues has not yet been investigated.

Our results show that incentive cues promote reward-seeking behavior through a circuit that requires concomitant BLA input and dopamine acting at the D_1 dopamine receptor in the NAc. We have demonstrated that neurons in both structures respond to the same auditory cues, but that excitations in the BLA occur significantly earlier than those in the NAc. Furthermore, we show that DS-evoked excitations of NAc neurons, which depend on the VTA (Yun et al., 2004b), are also dependent on the BLA, and that neurons that are excited by cues receive both mono- and polysynaptic inputs from the BLA. These results demonstrate that an excitatory BLA projection to the NAc is critical for incentive cues to evoke excitations in NAc neurons. Moreover, this projection is required for appropriate behavioral responding to reward-predictive cues

We first tested whether an ipsilateral connection between the BLA and the NAc is required for responding to reward-predictive cues presented during the DS task. Consistent with our earlier findings (Ishikawa et al., 2008a; Yun et al., 2004a; Yun et al., 2004b), both bilateral BLA inactivation with B/M and blockade of dopamine D_1 receptors bilaterally in the NAc substantially reduce behavioral responding to incentive cues. Furthermore, unilateral inactivation of the BLA coupled with contralateral blockade of NAc D_1 receptors resulted in substantial reduction in DS responding, whereas unilateral injections made ipsilaterally in these structures were without

significant effect. This disconnection experiment strongly implicates an ipsilateral connection between the BLA and the NAc in cue responding. At the doses used, neither B/M infused bilaterally in the BLA nor the D_1 antagonist infused into the NAc affects operant responding on a fixed ratio 1 schedule (Ishikawa et al., 2008a; Yun et al., 2004a). Therefore, the reduction in DS response ratio in the disconnection experiment is not due to a general decrease in motivation or a motor impairment, but rather to the specific inability of the animal to respond to incentive cues. These results support the idea that the BLA to NAc projection contributes to the assessment of the value of incentive cues (Cardinal et al., 2002; Everitt et al., 2003).

Our electrophysiological results provide strong support for this hypothesis. DS-excited subpopulations of neurons were found in both the NAc and the BLA, and these excitations occurred earlier in BLA neurons than in NAc neurons. Bilateral inactivation of the BLA reduced cue-evoked firing in NAc neurons, suggesting that the BLA triggers NAc firing in response to cues. An alternative possibility is that the cue-evoked firing of NAc neurons depends on behavioral performance, which is profoundly disrupted by bilateral BLA inactivation (Figure 1, Figure 5). Two results render this hypothesis unlikely. First, NAc excitation evoked by the NS, to which rats do not respond behaviorally, was also reduced by bilateral BLA inactivation. Second, unilateral inactivation of the BLA substantially reduced ipsilateral (but not contralateral) DS-evoked excitations in NAc neurons even though unilateral injections had only minimal behavioral effects. These results demonstrate that the ipsilateral BLA is essential for NAc neurons to respond to the DS during normal behavioral performance.

The results of the BLA stimulation experiment further support the hypothesis that the BLA projection to the NAc drives the DS-evoked firing of NAc neurons. NAc neurons were excited by stimulation of the BLA, with a range of firing onset latencies consistent with both mono- and polysynaptic connections. Moreover, NAc neurons responding to ipsilateral BLA stimulation were more likely to be excited by the DS than non-responsive neurons or neurons found to be responsive only to contralateral BLA stimulation. We conclude that excitatory projections from the BLA to the NAc, which likely include the direct, monosynaptic projection observed by others (Brog et al., 1993; Christie et al., 1987; Floresco et al., 2001; O'Donnell and Grace, 1995; Yim and Mogenson, 1986), drive NAc neuronal firing to reward-predictive cues, and that these BLA-dependent NAc responses are essential for the reward-seeking behavioral response.

In BLA neurons, the latency to onset of DS-evoked excitation was <20 ms, similar to the rapid onset of excitation following auditory cues predicting aversive events (Collins and Pare, 2000; Goosens et al., 2003; Maren and Quirk, 2004; Repa et al., 2001). Such short latencies imply that cue information reaches the BLA through a small number of relays from sensory pathways, probably involving thalamic and/or cortical inputs to the amygdala (Maren and Quirk, 2004). After conditioning, cues predictive of aversive events often elicit greater excitations in BLA neurons than neutral cues (Collins and Pare, 2000; Goosens et al., 2003; Repa et al., 2001). However, the magnitudes of the differences, especially at short latencies, are often small, and large responses to neutral stimuli tend to persist even across extended habituation (Collins and Pare, 2000; Goosens et al., 2003; Repa et al., 2001). These observations suggest that, at short latency, a sizeable proportion of BLA neurons signals only that a salient sensory cue has been detected, without signaling the cue's hedonic valence. Consistent with this idea, BLA neurons did not differentiate between DS and NS in the first 100 ms after cue onset, in contrast to the late response which clearly encoded the value of these cues.

Other studies reported that BLA neurons encode the value of reward-predictive stimuli (Belova et al., 2007; Paton et al., 2006; Saddoris et al., 2005; Schoenbaum et al., 1999; Sugase-Miyamoto and Richmond, 2005). Notably, in studies where the time course of the value-encoding responses were systematically analyzed, response latencies were usually ~100–400 msec after cue onset (Belova et al., 2007; Paton et al., 2006; Sugase-Miyamoto and Richmond, 2005), consistent with the discrimination latencies we observed in BLA neurons. However, these studies found only minimal early, non-discriminative responses to cues, which may be due to several factors. The primate amygdala, which may encode cues differently from rodent amygdala, was recorded in some studies (Belova et al., 2007; Paton et al., 2006; Sugase-Miyamoto and Richmond, 2005). Alternatively, the different

sensory modality of the cues used (visual or olfactory, rather than the auditory cues used here), or the fact that in prior studies the cue value was assessed during learning or contingency reversal, could result in differences in the time course of encoding. Finally, the structure of the task could also have contributed to this difference. The long and variable inter-trial interval in our task results in temporally unpredictable stimulus presentation, conditions under which even neutral auditory stimuli elicit strong excitation of BLA neurons (Collins and Pare, 2000; Herry et al., 2007).

In contrast with BLA neurons, NAc neurons differentially encode the DS and NS throughout the time course of cue-evoked excitation. An important question is that of which inputs to NAc neurons determine this differential encoding. Although BLA neurons initially do not fire differentially to the DS and NS, many BLA neurons begin to discriminate between these cues prior to the onset of discrimination in most NAc neurons. Therefore, the encoding of cue value by BLA neurons could contribute to this encoding by NAc neurons. However, other inputs to the NAc likely also play a role. In particular, the observation that responding to the DS was reduced by disconnection of the BLA from the NAc, using a D1 dopamine receptor antagonist injected in the NAc, strongly suggests that the BLA and dopamine inputs to the NAc interact to produce reward-seeking behavior.

An intriguing possibility is that more dopamine is released in the NAc in response to the reward-predictive DS than to the NS, and this dopamine facilitates excitation from the BLA. In addition to the disconnection experiment described here, several lines of evidence support this hypothesis. First, putative dopamine neurons in the midbrain are excited at short latency by reward-predictive cues (Morris et al., 2004; Nakahara et al., 2004; Schultz, 2007; Schultz et al., 1997) and by other salient stimuli (Dommett et al., 2005; Horvitz, 2000); in the rat VTA, the excitation onset latency is ~40 ms (Pan et al., 2005). Second, these excitations cause the release of dopamine in the NAc, which is greater for reward-predictive than for neutral stimuli (Day et al., 2007; Roitman et al., 2004). Third, injection of dopamine receptor antagonists into the NAc abolishes the behavioral response (Yun et al., 2004b). Finally, inactivation of the VTA abolishes cue-evoked excitations in the NAc (Yun et al., 2004b), which we show here to be dependent on the BLA as well. This is consistent with the idea that dopamine increases the effect of strong relative to weak excitatory input on NAc neuronal firing (Floresco, 2007; Hjelmstad, 2004; Nicola et al., 2004a). Although this evidence is suggestive, further work is required to establish more precisely how the BLA and dopamine inputs interact to influence the firing of cue-responsive NAc neurons.

The effects we observed could also be due to an indirect effect of BLA inactivation on dopamine release in the NAc and not an interaction between dopamine and BLA inputs. Inactivation of the BLA with B/M could reduce dopamine release in the NAc by two mechanisms. First, activation of BLA neurons can excite dopamine release in the NAc, an effect that is independent of VTA dopamine neuron firing and is therefore likely due to direct projections to dopamine terminals in the NAc (Floresco et al., 1998; Howland et al., 2002). However, we find that DS-excited neurons in the NAc are also excited by BLA stimulation very reliably and at short latency, an effect that cannot be due to dopamine released in the NAc by stimulation. Indeed, D1 antagonists do not alter the firing of NAc neurons evoked by low frequency BLA stimulation (Floresco et al., 2001), further confirming that activation of BLA neurons can excite NAc neurons independently of dopamine. Dopamine receptor activation may facilitate the BLA-evoked excitatory responses of NAc neurons, but it is clearly not an absolute requirement.

A second mechanism by which BLA inactivation could reduce NAc dopamine release is by diffusion of the B/M to the neighboring central nucleus of the amygdala (CeN), which contains neurons projecting to the VTA that may excite dopamine release in the NAc. Arguing against this hypothesis, muscimol doses nearly 10 times greater than those used here diffuse only 1 mm from the injection site (Corcoran et al., 2005; Martin, 1991). Nevertheless, approach to Pavlovian conditioned stimuli requires both the CeN (but not the BLA) (Parkinson et al., 2000) and dopamine release in the NAc core (Di Ciano et al., 2001; Parkinson et al., 2002). Because Pavlovian approach and DS responding are similar behaviors (both involve cue-evoked locomotion towards and responding on an operandum), it is conceivable that a CeN – VTA – NAc core circuit is required for DS responding as well. However, a critical difference is that in Pavlovian approach behavior, the operandum itself serves as the CS to be

approached, whereas in our task the auditory DS is not itself approached, but sets the occasion for approach to the lever. Different circuits may underlie these similar behaviors; for instance, Pavlovian approach driven by the CeN does not require an assessment of the reinforcer's motivational value (Cardinal et al., 2002; Everitt et al., 2003), whereas approach elicited by the DS may require such an assessment and may therefore depend on the BLA (Cardinal et al., 2002; Everitt et al., 2003; Hatfield et al., 1996; Holland et al., 2001). Consistent with this idea, selective lesions of BLA cell bodies, using a toxin that spares cells in the CeN, reduces behavioral responding to a DS (Yun and Fields, 2003), strongly supporting the idea that the effects of our BLA injections were independent of the CeN.

In addition to the BLA and dopaminergic projections to the NAc, the excitatory projection from the prefrontal cortex (PFC) could also contribute to the NAc neuronal response to incentive cues. Indeed, many NAc neurons receive convergent input from PFC and amygdala (Goto and O'Donnell, 2002; O'Donnell and Grace, 1995) and neurons in the PFC respond to reward-predictive cues (Jodo et al., 2000; Kobayashi et al., 2006; Leon and Shadlen, 1999; Takenouchi et al., 1999). Furthermore, inactivation of the PFC reduces both the behavioral DS response ratio and the cue-evoked excitation of NAc neurons (Ishikawa et al., 2008b) and PFC neurons that both project to the NAc and receive BLA inputs are excited by conditioned stimuli (McGinty and Grace, 2008). This raises the possibility that BLA neurons project to the NAc polysynaptically via the PFC. In the present study, NAc excitations resulting from BLA stimulation usually began with rapid (<20 ms) onset, consistent with a monosynaptic projection. However, many neurons were excited with longer latencies, and one possibility is that these excitations were relayed from the BLA through the PFC to the NAc. Therefore, the existing evidence is consistent with roles for both monosynaptic and polysynaptic projections from the BLA to the NAc in driving NAc cue-evoked excitations; further work should focus on elucidating their relative contributions.

Dopamine release in the NAc controls the decision to become engaged in reward-seeking behavior (Nicola and Fields, In preparation), a phenomenon that underlies NAc dopamine's ability to invigorate behavioral responding in many tasks (Robbins and Everitt, 2007), including cue responding tasks (Yun et al., 2004a). Indeed, blockade of dopamine in the NAc disrupts cue-driven reward seeking only in tasks where animals cannot predict when cue presentation will occur (Nicola, 2007). However, dopamine neurons respond rapidly to cues even when their occurrence is expected and animals are continuously engaged in the task (Morris et al., 2004; Nakahara et al., 2004; Pan and Hyland, 2005; Pan et al., 2005; Schultz, 2007; Schultz et al., 1997). BLA signals provide an explanation for this apparent discrepancy. Information that an unexpected cue of potential predictive value has been detected is rapidly processed by BLA neurons. In the case of reward-predictive cues, this information is integrated with dopaminergic (and other) inputs to the NAc to produce increased firing of a subpopulation of NAc neurons, which promotes the decision to initiate a reward-seeking behavioral response. Until reward is obtained, NAc neurons continue to encode either the predictive value of the cue or some aspect of the motor response, or both (Nicola et al., 2004b); our results suggest that the similar sustained encoding in BLA neurons drives this response in the NAc.

Methods

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Animals

Four experiments were performed in 3 separate groups of rats, all trained on a discriminative stimulus (DS) task. Seven rats were used for the disconnection study, 5 rats were used to record BLA neurons and 9 rats were used to record NAc neurons during the DS task. Of the latter 9, 8 were used for the BLA inactivation/NAc recording study and 5 were used for the BLA stimulation study. After the training concluded, rats were stereotaxically implanted with microinjection cannulae in the NAc and the BLA; recording electrode arrays in the BLA; or microinjection cannulae (with attached stimulating electrodes) in the BLA and electrode arrays in the NAc. The subjects were male Long–Evans rats (Harlan Sprague Dawley Indianapolis, IN) weighing ~350 g on arrival and individually housed on a 12 hr light/dark cycle. Experiments were conducted during the dark phase. After receipt, rats were allowed at least 1 week of *ad libitum* food and water, followed by 1 week of restricted food and water before

training. Throughout all experiments, food and water restriction was adjusted daily at the end of experimental manipulations to maintain the rats at ~90% of their initial body weight. Animal handling and experiments conformed to National Institutes of Health and Ernest Gallo Clinic and Research Center animal care and use policies.

DS task

The disconnection study was conducted in standard operant chambers (23.5 × 30.5 cm) and the electrophysiological experiments were conducted in larger cages (40.6 × 40.6 cm). All cages contained two retractable levers located on one wall of the chamber (one on each side of a reward receptacle), two house lights, a white noise speaker and a tone speaker (Med Associates, St. Albans, VT). Liquid sucrose reward was delivered into a well in the reward receptacle by a syringe pump. The DS task structure was based on that used in our previous experiments (Nicola et al., 2004b; Yun et al., 2004a; Yun et al., 2004b). Rats were run daily on the DS task for one or two hours (for behavioral and electrophysiological studies, respectively). Two tones, the DS and the NS, were presented on a variable interval schedule with an average interval of 30 sec. Pressing one of the two levers (designated the active lever, randomly chosen across rats) during DS presentation resulted in the delivery of 50 µl of 10% sucrose into the reward receptacle and termination of the DS tone. Each DS lasted for up to 10 sec, and each NS lasted 10 sec. Responding during the NS or in the absence of the DS was never rewarded. The cues were either an intermittent 4 kHz tone (40 ms on and 50 ms off), or a siren tone (ramped from 4 to 8 kHz with a 400 ms period). Tones were randomly assigned to be the DS or the NS across rats.

Surgeries were performed when the rats reached criterion performance of >90% DS response ratio and <20% NS response ratio (defined as the proportion of all DSs or NSs in the session to which the animal responded).

Surgeries

For the behavioral (disconnection) study, rats were bilaterally implanted with microinjection guide cannulae (27 gauge, Plastics One, Roanoke, VA, USA) in the BLA (AP −3.0, ML ±4.8, DV −7.0 mm relative to bregma) and NAc core (AP +1.5, ML ±2 and DV −6 mm vertically or AP +1.5, ML ±3.5 and DV −6 mm with a 14° angle from vertical in the coronal plane). For electrophysiological recordings of BLA neurons, 2 rats received bilateral BLA fixed arrays of 8 electrodes each (NB Labs, Denison, TX, 50 µm stainless steel wires arranged in 2 rows of 4) and the remaining 3 rats received the same type of arrays but attached to 2 microdrive devices that allowed the entire array to be lowered by 80 or 160 µm increments. Target coordinates of the medioposterior electrode of each array were as follows: AP, −3.2; ML, ±4.9; DV, 8 to 9 mm relative to bregma. For electrophysiological recording of NAc neurons, rats received movable electrode arrays in the NAc core (medioposterior electrode: AP +1.2, ML ±1.5 and DV −6 to −8.5 mm). For 5 of these rats, bipolar stimulating electrodes (two 75 µm tungsten insulated wires separated by ~0.4 mm) were glued along guide cannulae (protruding 1.5 mm below the tip of the cannula) and lowered bilaterally in the BLA region (AP −3.0, ML ±4.8, DV −7.0 mm relative to bregma). The 4 remaining rats received bilateral cannulae, without stimulating electrodes, at the same coordinates.

Animals were anesthetized with isoflurane (5%) and placed in a stereotaxic apparatus. Anesthesia was maintained with isoflurane (0.5–2.0%) during surgery. Guide cannulae, electrodes and microdrives were secured to the skull with bone screws and dental acrylic, and wire obturators were inserted into the guide cannulae; the ends of the obturators were flush with the ends of the guide cannulae. Rats were given at least 7 days of recovery before being retrained on the DS task and habituated to the handling procedures.

Drugs

To block D1 receptors, we used the selective D_1 antagonist SCH23390 (0.5 µg/side in 0.5 µl saline). To inactivate the BLA, we used a mixed solution of the $GABA_A$ and $GABA_B$ agonists baclofen and muscimol (B/M, 12.5 ng of each drug per side for the behavioral study and 7.5, 12.5 and 25 ng/side for the electrophysiological study, in 0.5

μl saline).

Microinjections (behavioral study)

After retraining, animals received microinjections every other day in random order. To inject animals, the obturators were removed and 30 gauge injector cannulae were inserted into the guides. Injectors extended 1.5 mm below the tip of the cannula. After a 1 min pre-injection period the entire volume was injected over 2 min. After a 1 min post-injection period, the injectors were gently removed, the obturators were replaced, the animal was immediately placed into the behavioral chamber, and the behavioral session began. The following injections were performed on different days, in random order, in the same animals: unilateral and bilateral injection of SCH23390 in the NAc; unilateral and bilateral injection of B/M in the BLA; ipsilateral inactivation (unilateral injection of SCH23390 in the NAc and injection of B/M in the ipsilateral BLA); and contralateral inactivation (unilateral injection of SCH23390 in the NAc and injection of B/M in the contralateral BLA). Three types of vehicle injections were also performed: bilaterally in the NAc, bilaterally in the BLA, and contralaterally (unilateral injections in the NAc and the BLA). Because we found no differences among these vehicle injections (ANOVA event × injection type, response ratio: $F(3, 48) = 0.112$, $P=0.95$; latency: $F(3, 46) = 0.669$, $P=0.57$), the data from all vehicle injections were pooled. With this design, each structure received 6 microinjections. There were no effects of injection order (ANOVA injection order × cue: Response ratio $F(20, 152)=0.583$, $P=0.91$; Response latency $F(20, 133)=0.97$, $p=0.501$).

Electrophysiology

Electrophysiological recording was conducted as described previously (Nicola et al., 2004b). Animals were connected to the recording apparatus, which consisted of a head stage with operational amplifiers, cable, commutator to allow the animal free movement within the chamber, and Plexon Inc. (Dallas, TX) spike sorting hardware and software.

BLA recordings Rats were run for 2 hr daily sessions of the DS task. For rats with fixed arrays, we insured that each neuron contributed only one time to the analysis by selecting for analysis the session with the maximum number of isolated neurons for each of the 16 electrodes; all others were discarded for that electrode. For rats with movable electrodes, the microdrive was lowered by 80 or 160 μm at the end of each session to get a new set of neurons every day.

NAc recordings coupled to BLA stimulation After obtaining a 1 hr DS task performance baseline, a stimulation cable (Plexon Inc., Dallas, TX) connected to a stimulator (A–M Systems, Inc., Carlsborg, WA) was attached to the rat. The rats were presumably aware that the DS task ended because the houselights were off and the door of the recording cage was open as an additional cue. Single pulses (0.1 ms duration, 0.29 +/− 0.01 mA intensity) were applied at 0.5 Hz; trains of pulses lasting 90 sec were given to the right and the left BLA. The stimulation strength was adjusted to the maximal current (<0.7 mA) that did not produce a field potential in the NAc.

NAc recordings coupled with BLA inactivation Rats were allowed to perform the DS task every other day. After a pre-injection period of 1 hr during which neuronal signals were recorded as animals performed the task, animals were removed from the recording chamber and injected in the BLA with one of 4 injection types: bilaterally with vehicle, bilaterally with B/M, unilaterally in the left BLA with B/M, or unilaterally in the right BLA with B/M. After the injection, the animals were immediately reconnected to the recording apparatus and placed in the chamber for the post-injection session for 1 hr. These injections were done in batches such that for each batch, the 4 injection types (with the same dose of B/M) were completed on consecutive recording days. After the recording session on the last day of each batch, the microdrive carrying the electrode arrays was advanced 80 or 160 μm in order to obtain a new set of neurons for the next batch. In some cases, few neurons were recorded across electrodes and the array was advanced before completion of a batch of injections.

We conducted both unilateral and bilateral inactivation in order to investigate the contribution of their different

behavioral effects on NAc neuronal responses. Although the dose of B/M was the same for all injections within a batch, we used somewhat different doses for each rat and each batch to optimize the contrast in DS response ratio between bilateral and unilateral injections. We selected from 1 to 3 batches per rat for analysis (average 1.6±0.3 batches/rat). The mean dose of B/M was calculated for each injection type (bilateral, ipsilateral and contralateral) by first obtaining the dose used for each neuron that contributed to an injection type, and then averaging these doses. Mean doses did not differ across injection type for both DS-excited neurons ($F(2, 96)=1.42$, $p=0.25$; doses were: bilateral mean=11.0±0.7, mode=12.5 ng; ipsilateral 9.7±1.0, mode=7.5 ng; contralateral 9.8±0.9, mode=7.5 ng) and DS-inhibited neurons ($F(2, 38)=0.39$, $p=0.68$; doses were: bilateral mean=11.5±1.4, mode=7.5 ng; ipsilateral 10.2±1.4, mode=7.5 ng; contralateral 12.3±2.1, mode=7.5 ng).

Data analysis of electrophysiological recordings Discrimination of individual units was performed off-line with Offline Sorter (Plexon, Inc., Dallas, TX) using principal component analysis. Only units with well defined waveforms that were clearly distinct from noise were included in this study. The minimum peak-to-peak spike amplitude was 75 μV with noise of ~25 μV. Interspike interval distribution, cross-correlograms and autocorrelograms were used to insure that single units were isolated. Additionally, only units in which waveform characteristics were constant over the entire recording session were included in this study.

Peri-stimulus time histograms (PSTHs) constructed around cue (DS and NS) onset, with 20 ms time bins, were used to detect excitations and inhibitions and the time at which they occurred. The 10 sec period before cue onset was used as a baseline period. For the effect of electrical stimulation of the BLA on NAc neurons, PSTHs were made with 1 ms time bins and the baseline period consisted of 1 sec before the stimulation. Excitation and inhibition onset latencies were defined as the time of the first bin of N consecutive bins that were above (for excitations) or below (for inhibitions) a threshold defined as: baseline firing + X * SD, where SD is the standard deviation of the baseline firing rate and X is a constant. The threshold for response offset was computed by using the same formula to find the first bins that were below (for excitations) or above (for inhibitions) the calculated threshold. We used different N and X parameters for cue-evoked and BLA stimulation-evoked excitations and inhibitions. For cue responses, excitation parameters were: N=2/X=6 or N=3/X=2 for onset (i.e., the firing had to exceed the baseline plus 6 times the SD for 2 consecutive bins, or baseline plus 2 times the SD for 3 bins), and N=6/X=1 for offset (i.e., the firing had to be less than the baseline plus the SD for 6 consecutive bins). Inhibition parameters were: N=6/X=1 or N=3/X=2 for onset and N=6/X=1 for offset. For BLA electrical stimulation responses, we used a smaller bin width (1 ms) to detect early responses as accurately as possible. As a consequence, we had to use more stringent parameters to compensate for the noise introduced by this procedure. Parameters were N=2/X=7.5 or N=3/X=2.5 for onset and N=6/X=2 for offset.

To determine the time at which DS responses were different from NS responses, we subtracted, for each neuron that was excited by both cues, the value of each 20 ms bin of the firing rate PSTHs constructed around the NS from the corresponding bin in the PSTH constructed around the DS. We then ran the same analysis described above to find the discrimination onset latency. If no response was found within 3 sec after cue onset, the neuron was considered non-discriminative.

Average PSTHs across neurons were constructed with 100 ms bins. Prior to averaging, the firing rate of each neuron during each PSTH bin was transformed to a Z-score: $(F_i - F_{mean}) / F_{sd}$ where F_i is the firing rate of the i^{th} bin of the PSTH, and F_{mean} and F_{sd} are, respectively, the mean and the SD of the firing rate during the 10 sec preceding cue onset.

Histology

Animals were deeply anesthetized with pentobarbital and perfused intracardially with saline and 4% formalin (plus 3% ferrocyanide for rats with electrode arrays). Brains were removed, sectioned (40um), and stained for Nissl substance in order to locate injection or recording sites (labeled by passing a DC current through each electrode

before perfusion).

Statistical analysis

Paired *t*-tests or ANOVAs followed by Duncan post hoc tests were used when appropriate. Distributions were compared with the Kolmogorov-Smirnov test, and proportions were compared with χ^2 tests. All results were considered significant at $P<0.05$.

Supplementary Material

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Click here to view. (306K, pdf)

Acknowledgments

Go to:

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Footnotes

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References

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1. Belova MA, Paton JJ, Morrison SE, Salzman CD. Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. Neuron. 2007;55:970–984. [PMC free article] [PubMed]
2. Brog JS, Salyapongse A, Deutch AY, Zahm DS. The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. J Comp Neurol. 1993;338:255–278. [PubMed]
3. Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev. 2002;26:321–352. [PubMed]
4. Christie MJ, Summers RJ, Stephenson JA, Cook CJ, Beart PM. Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[3H]aspartate and [3H]GABA. Neuroscience. 1987;22:425–439. [PubMed]
5. Collins DR, Pare D. Differential fear conditioning induces reciprocal changes in the sensory responses of lateral amygdala neurons to the CS(+) and CS(−) Learn Mem. 2000;7:97–103. [PMC free article] [PubMed]
6. Corcoran KA, Desmond TJ, Frey KA, Maren S. Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction. J Neurosci. 2005;25:8978–8987. [PubMed]
7. Day JJ, Roitman MF, Wightman RM, Carelli RM. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci. 2007;10:1020–1028. [PubMed]
8. Day JJ, Wheeler RA, Roitman MF, Carelli RM. Nucleus accumbens neurons encode Pavlovian approach behaviors: evidence from an autoshaping paradigm. Eur J Neurosci. 2006;23:1341–1351. [PubMed]
9. Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ. Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance

of pavlovian approach behavior. J Neurosci. 2001;21:9471–9477. [PubMed]

10. Di Ciano P, Everitt BJ. Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. J Neurosci. 2004;24:7167–7173. [PubMed]
11. Dommett E, Coizet V, Blaha CD, Martindale J, Lefebvre V, Walton N, Mayhew JE, Overton PG, Redgrave P. How visual stimuli activate dopaminergic neurons at short latency. Science. 2005;307:1476–1479. [PubMed]
12. Everitt BJ, Cardinal RN, Parkinson JA, Robbins TW. Appetitive behavior: impact of amygdala-dependent mechanisms of emotional learning. Ann N Y Acad Sci. 2003;985:233–250. [PubMed]
13. Finch DM. Neurophysiology of converging synaptic inputs from the rat prefrontal cortex, amygdala, midline thalamus, and hippocampal formation onto single neurons of the caudate/putamen and nucleus accumbens. Hippocampus. 1996;6:495–512. [PubMed]
14. Floresco SB. Dopaminergic regulation of limbic-striatal interplay. J Psychiatry Neurosci. 2007;32:400–411. [PMC free article] [PubMed]
15. Floresco SB, Blaha CD, Yang CR, Phillips AG. Dopamine D1 and NMDA receptors mediate potentiation of basolateral amygdala-evoked firing of nucleus accumbens neurons. J Neurosci. 2001;21:6370–6376. [PubMed]
16. Floresco SB, Yang CR, Phillips AG, Blaha CD. Basolateral amygdala stimulation evokes glutamate receptor-dependent dopamine efflux in the nucleus accumbens of the anaesthetized rat. Eur J Neurosci. 1998;10:1241–1251. [PubMed]
17. Ghitza UE, Fabbricatore AT, Prokopenko V, Pawlak AP, West MO. Persistent cue-evoked activity of accumbens neurons after prolonged abstinence from self-administered cocaine. J Neurosci. 2003;23:7239–7245. [PubMed]
18. Ghitza UE, Fabbricatore AT, Prokopenko VF, West MO. Differences between accumbens core and shell neurons exhibiting phasic firing patterns related to drug-seeking behavior during a discriminative-stimulus task. J Neurophysiol. 2004;92:1608–1614. [PubMed]
19. Goosens KA, Hobin JA, Maren S. Auditory-evoked spike firing in the lateral amygdala and Pavlovian fear conditioning: mnemonic code or fear bias? Neuron. 2003;40:1013–1022. [PubMed]
20. Goto Y, O'Donnell P. Timing-dependent limbic-motor synaptic integration in the nucleus accumbens. Proc Natl Acad Sci U S A. 2002;99:13189–13193. [PMC free article] [PubMed]
21. Hatfield T, Han JS, Conley M, Gallagher M, Holland P. Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. J Neurosci. 1996;16:5256–5265. [PubMed]
22. Herry C, Bach DR, Esposito F, Di Salle F, Perrig WJ, Scheffler K, Luthi A, Seifritz E. Processing of temporal unpredictability in human and animal amygdala. J Neurosci. 2007;27:5958–5966. [PubMed]
23. Hjelmstad GO. Dopamine excites nucleus accumbens neurons through the differential modulation of glutamate and GABA release. J Neurosci. 2004;24:8621–8628. [PMC free article] [PubMed]
24. Holland PC, Hatfield T, Gallagher M. Rats with basolateral amygdala lesions show normal increases in conditioned stimulus processing but reduced conditioned potentiation of eating. Behav Neurosci. 2001;115:945–950. [PubMed]
25. Horvitz JC. Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. Neuroscience. 2000;96:651–656. [PubMed]
26. Howland JG, Taepavarapruk P, Phillips AG. Glutamate receptor-dependent modulation of dopamine efflux in the nucleus accumbens by basolateral, but not central, nucleus of the amygdala in rats. J Neurosci. 2002;22:1137–1145. [PubMed]
27. Ishikawa A, Ambroggi F, Nicola SM, Fields HL. Dissociable excitatory and inhibitory roles for amygdala, dorsal and ventral prefrontal cortex in reward-seeking behavior. Neuroscience. 2008a In Press.
28. Ishikawa A, Ambroggi F, Nicola SM, Fields HL. Dorsomedial prefrontal cortex contribution to behavioral and nucleus accumbens neuronal responses to incentive cues. J Neurosci. 2008b;28:5088–5098.

[PMC free article] [PubMed]
29. Jodo E, Suzuki Y, Kayama Y. Selective responsiveness of medial prefrontal cortex neurons to the meaningful stimulus with a low probability of occurrence in rats. Brain Res. 2000;856:68–74. [PubMed]
30. Johnson LR, Aylward RL, Hussain Z, Totterdell S. Input from the amygdala to the rat nucleus accumbens: its relationship with tyrosine hydroxylase immunoreactivity and identified neurons. Neuroscience. 1994;61:851–865. [PubMed]
31. Kobayashi S, Nomoto K, Watanabe M, Hikosaka O, Schultz W, Sakagami M. Influences of rewarding and aversive outcomes on activity in macaque lateral prefrontal cortex. Neuron. 2006;51:861–870. [PubMed]
32. LeDoux J. The emotional brain, fear, and the amygdala. Cell Mol Neurobiol. 2003;23:727–738. [PubMed]
33. Leon MI, Shadlen MN. Effect of expected reward magnitude on the response of neurons in the dorsolateral prefrontal cortex of the macaque. Neuron. 1999;24:415–425. [PubMed]
34. Maren S, Quirk GJ. Neuronal signalling of fear memory. Nat Rev Neurosci. 2004;5:844–852. [PubMed]
35. Martin JH. Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. Neurosci Lett. 1991;127:160–164. [PubMed]
36. McDonald AJ. Topographical organization of amygdaloid projections to the caudatoputamen, nucleus accumbens, and related striatal-like areas of the rat brain. Neuroscience. 1991;44:15–33. [PubMed]
37. McGinty VB, Grace AA. Selective Activation of Medial Prefrontal-to-Accumbens Projection Neurons by Amygdala Stimulation and Pavlovian Conditioned Stimuli. Cereb Cortex. 2008 In Press. [PMC free article] [PubMed]
38. Mogenson GJ, Wu M, Jones DL. Locomotor activity elicited by injections of picrotoxin into the ventral tegmental area is attenuated by injections of GABA into the globus pallidus. Brain Res. 1980;191:569–571. [PubMed]
39. Morris G, Arkadir D, Nevet A, Vaadia E, Bergman H. Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. Neuron. 2004;43:133–143. [PubMed]
40. Mulder AB, Hodenpijl MG, Lopes da Silva FH. Electrophysiology of the hippocampal and amygdaloid projections to the nucleus accumbens of the rat: convergence, segregation, and interaction of inputs. J Neurosci. 1998;18:5095–5102. [PubMed]
41. Nakahara H, Itoh H, Kawagoe R, Takikawa Y, Hikosaka O. Dopamine neurons can represent context-dependent prediction error. Neuron. 2004;41:269–280. [PubMed]
42. Nicola SM. The nucleus accumbens as part of a basal ganglia action selection circuit. Psychopharmacology (Berl) 2007;191:521–550. [PubMed]
43. Nicola SM, Fields HL. Initiation of reward-seeking after a pause requires nucleus accumbens core dopamine. (In preparation)
44. Nicola SM, Surmeier J, Malenka RC. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annu Rev Neurosci. 2000;23:185–215. [PubMed]
45. Nicola SM, Woodward Hopf F, Hjelmstad GO. Contrast enhancement: a physiological effect of striatal dopamine? Cell Tissue Res. 2004a;318:93–106. [PubMed]
46. Nicola SM, Yun IA, Wakabayashi KT, Fields HL. Cue-evoked firing of nucleus accumbens neurons encodes motivational significance during a discriminative stimulus task. J Neurophysiol. 2004b;91:1840–1865. [PubMed]
47. Nicola SM, Yun IA, Wakabayashi KT, Fields HL. Firing of nucleus accumbens neurons during the consummatory phase of a discriminative stimulus task depends on previous reward predictive cues. J Neurophysiol. 2004c;91:1866–1882. [PubMed]
48. O'Donnell P, Grace AA. Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. J Neurosci. 1995;15:3622–3639. [PubMed]
49. Pan WX, Hyland BI. Pedunculopontine tegmental nucleus controls conditioned responses of midbrain

dopamine neurons in behaving rats. J Neurosci. 2005;25:4725–4732. [PubMed]
50. Pan WX, Schmidt R, Wickens JR, Hyland BI. Dopamine cells respond to predicted events during classical conditioning: evidence for eligibility traces in the reward-learning network. J Neurosci. 2005;25:6235–6242. [PubMed]
51. Parkinson JA, Dalley JW, Cardinal RN, Bamford A, Fehnert B, Lachenal G, Rudarakanchana N, Halkerston KM, Robbins TW, Everitt BJ. Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behaviour: implications for mesoaccumbens dopamine function. Behav Brain Res. 2002;137:149–163. [PubMed]
52. Parkinson JA, Robbins TW, Everitt BJ. Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. Eur J Neurosci. 2000;12:405–413. [PubMed]
53. Paton JJ, Belova MA, Morrison SE, Salzman CD. The primate amygdala represents the positive and negative value of visual stimuli during learning. Nature. 2006;439:865–870. [PMC free article] [PubMed]
54. Repa JC, Muller J, Apergis J, Desrochers TM, Zhou Y, LeDoux JE. Two different lateral amygdala cell populations contribute to the initiation and storage of memory. Nat Neurosci. 2001;4:724–731. [PubMed]
55. Robbins TW, Everitt BJ. A role for mesencephalic dopamine in activation: commentary on Berridge (2006) Psychopharmacology (Berl) 2007;191:433–437. [PubMed]
56. Roitman MF, Stuber GD, Phillips PE, Wightman RM, Carelli RM. Dopamine operates as a subsecond modulator of food seeking. J Neurosci. 2004;24:1265–1271. [PubMed]
57. Roitman MF, Wheeler RA, Carelli RM. Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. Neuron. 2005;45:587–597. [PubMed]
58. Saddoris MP, Gallagher M, Schoenbaum G. Rapid associative encoding in basolateral amygdala depends on connections with orbitofrontal cortex. Neuron. 2005;46:321–331. [PubMed]
59. Schoenbaum G, Chiba AA, Gallagher M. Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. Nat Neurosci. 1998;1:155–159. [PubMed]
60. Schoenbaum G, Chiba AA, Gallagher M. Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci. 1999;19:1876–1884. [PubMed]
61. Schultz W. Multiple dopamine functions at different time courses. Annu Rev Neurosci. 2007;30:259–288. [PubMed]
62. Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. Science. 1997;275:1593–1599. [PubMed]
63. Setlow B, Holland PC, Gallagher M. Disconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive pavlovian second-order conditioned responses. Behav Neurosci. 2002;116:267–275. [PubMed]
64. Sugase-Miyamoto Y, Richmond BJ. Neuronal signals in the monkey basolateral amygdala during reward schedules. J Neurosci. 2005;25:11071–11083. [PubMed]
65. Taha SA, Fields HL. Inhibitions of nucleus accumbens neurons encode a gating signal for reward-directed behavior. J Neurosci. 2006;26:217–222. [PubMed]
66. Taha SA, Nicola SM, Fields HL. Cue-evoked encoding of movement planning and execution in the rat nucleus accumbens. J Physiol. 2007;584:801–818. [PMC free article] [PubMed]
67. Takenouchi K, Nishijo H, Uwano T, Tamura R, Takigawa M, Ono T. Emotional and behavioral correlates of the anterior cingulate cortex during associative learning in rats. Neuroscience. 1999;93:1271–1287. [PubMed]
68. Uwano T, Nishijo H, Ono T, Tamura R. Neuronal responsiveness to various sensory stimuli, and associative learning in the rat amygdala. Neuroscience. 1995;68:339–361. [PubMed]
69. Wan X, Peoples LL. Firing patterns of accumbal neurons during a pavlovian-conditioned approach task. J Neurophysiol. 2006;96:652–660. [PubMed]
70. Wright CI, Beijer AV, Groenewegen HJ. Basal amygdaloid complex afferents to the rat nucleus

accumbens are compartmentally organized. J Neurosci. 1996;16:1877–1893. [PubMed]

71. Yim CY, Mogenson GJ. Mesolimbic dopamine projection modulates amygdala-evoked EPSP in nucleus accumbens neurons: an in vivo study. Brain Res. 1986;369:347–352. [PubMed]
72. Yun IA, Fields HL. Basolateral amygdala lesions impair both cue- and cocaine-induced reinstatement in animals trained on a discriminative stimulus task. Neuroscience. 2003;121:747–757. [PubMed]
73. Yun IA, Nicola SM, Fields HL. Contrasting effects of dopamine and glutamate receptor antagonist injection in the nucleus accumbens suggest a neural mechanism underlying cue-evoked goal-directed behavior. Eur J Neurosci. 2004a;20:249–263. [PubMed]
74. Yun IA, Wakabayashi KT, Fields HL, Nicola SM. The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. J Neurosci. 2004b;24:2923–2933. [PubMed]