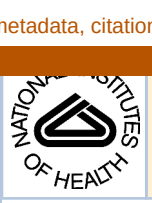

Published in final edited form as:

Biol Psychiatry. 2010 April 15; 67(8): 737–744. doi:10.1016/j.biopsych.2009.11.006.

Basolateral amygdala modulates terminal dopamine release in the nucleus accumbens and conditioned responding

Joshua L. Jones[1], **Jeremy J. Day**[1], **Brandon J. Aragona**[1], **Robert A. Wheeler**[1], **R. Mark Wightman**[2,3], and **Regina M. Carelli**[1,3]

[1]Department of Psychology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

[2]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

[3]Neuroscience Center University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Abstract

Background—Dopamine signaling in the nucleus accumbens (NAc) is essential for goal-directed behaviors and primarily arises from burst firing of ventral tegmental area (VTA) neurons. However, the role of associative neural substrates such as the basolateral amygdala (BLA) in regulating phasic dopamine release in the NAc, particularly during reward-seeking, remains unknown.

Methods—Male Sprague-Dawley rats learned to discriminate two cues; a discriminative stimulus (DS) that predicted sucrose reinforcement contingent upon a lever press, and a non-associated stimulus (NS) that predicted a second lever never reinforced with sucrose. Following training, a test session was completed in which NAc dopamine was measured using fast-scan cyclic voltammetry in conjunction with inactivation of the ipsilateral BLA (GABA agonists; baclofen/muscimol) to determine the contribution of BLA activity to dopamine release in the NAc core during the task.

Results—Under vehicle conditions, DS and NS presentation elicited dopamine release within the NAc core. The DS evoked significantly more dopamine than the NS. Inactivation of the BLA selectively attenuated the magnitude of DS-evoked dopamine release, concurrent with an attenuation of DS-evoked conditioned approaches. Other behavioral responses (e.g., lever pressing) and dopamine release concomitant with those events were unaltered by BLA inactivation. Furthermore, neither VTA electrically-stimulated dopamine release nor the probability of high concentration dopamine release events was altered following BLA inactivation.

Conclusions—These results demonstrate that the BLA terminally modulates dopamine signals within the NAc core under specific, behaviorally-relevant conditions, illustrating a functional mechanism by which the BLA selectively facilitates responding to motivationally salient environmental stimuli.

Keywords

behavior; ventral striatum; basolateral amygdala; reward; cue; learning

Corresponding author: Regina M. Carelli, Professor & Director, Behavioral Neuroscience Program, Department of Psychology, University of North Carolina, Chapel Hill, CB#3270 Davie Hall, Chapel Hill, NC 27599, rcarelli@unc.edu, (919) 962-8775.

Financial disclosures: The authors reported no biomedical financial interests or potential conflicts of interest.

Introduction

The ability of an organism to successfully pursue, procure and consume rewards is a critical determinant of survival. Organisms learn to assign value to relevant environmental stimuli that are associated with successful reward-seeking behavior. These associations can produce marked changes in an organism's ability to direct and guide future decisions. Numerous lines of research have demonstrated that reward learning is mediated by a distributed network of brain nuclei including the nucleus accumbens (NAc) and its midbrain dopaminergic input from the ventral tegmental area (VTA). Furthermore, dysfunction of associative learning processes within this network appears to underlie aspects of compulsive drug-seeking and addiction (1-3).

The importance of NAc dopamine to reward-seeking behaviors is clear (4), particularly in the acquisition and expression of learned associations (5,6), responding to reward-paired cues (7), and reward-related decision making (8). VTA dopamine neurons that project to the NAc signal critical determinants of expected reward value (9), and we have demonstrated terminal dopamine release within the NAc during the presentation of rewards and reward-predictive stimuli (10,11). Nevertheless, very little is known regarding neural substrates that regulate NAc phasic dopamine release during behavior.

Phasic dopamine release arises primarily from burst-firing of VTA dopamine neurons (12), and both its development (13,14) and disruption (15) correlate with changes in learned reward-related behaviors. Phasic dopamine signals are critical for motivated behaviors as VTA-mediated dopamine transmission within the NAc is both necessary for behavioral responses to reward-predictive cues (7) and sufficient for reward-related conditioning (16). However, post-synaptic signals in NAc neurons are not driven by dopamine alone (17), but through a complex integration of glutamatergic afferent input with concurrent dopaminergic signals (17,18). As such, a critical question is whether glutamatergic afferents influence NAc dopamine release at the terminal level to functionally alter responding to reward-predictive stimuli.

The basolateral amygdala (BLA), a structure linked with associative learning (19,20), is anatomically positioned to modulate the terminal release of dopamine (21). Furthermore, BLA-NAc interactions are critical for reward-seeking (22,23), and BLA activity can significantly alter NAc cellular responding (24,25). As such, it has been proposed that BLA activity contributes to phasic dopamine release within the NAc (26). However, BLA-dependent facilitation of dopamine within the NAc has been shown in anesthetized animals (27), or during longer temporal measurements (28) which may not reflect the rapid time-scale of behaviorally significant events. Further, studies in behaving animals have been unable to confirm that this modulation occurs in a behaviorally relevant manner (29,30).

Here, we used fast-scan cyclic voltammetry within the NAc coupled to microinfusion into the BLA of $GABA_A$ and $GABA_B$ agonists (muscimol 0.03 nmol and baclofen 0.3 nmol in 0.3μL) (31) to determine the functional contribution of the BLA to phasic dopamine release in the NAc during a discriminative-stimulus operant task. Our results provide a critical characterization of afferent modulation of phasic dopamine signaling at the terminal level in the NAc, and demonstrate a functionally relevant mechanism by which the BLA can selectively facilitate responding to motivationally salient events.

Methods

Subjects and surgery

Male Sprague-Dawley rats (n=9) (Harlan; 90–120 d; 260–350 g) were used and individually housed with a 12 h/12 h light/dark cycle. Rats were surgically prepared for voltammetric recordings as described previously (10). A guide cannula was stereotaxically positioned above the NAc core (1.3-1.5 mm anterior, 1.3 mm lateral from bregma) and a bipolar stimulating electrode in the VTA (5.2 mm posterior, 1.0 mm lateral from bregma, 7 mm ventral from brain). A Ag/AgCl reference electrode was placed contralateral to the stimulating electrode in the left forebrain. Another guide cannula (Plastics One) was implanted 2 mm above the BLA (2.7-3.1 mm posterior, 4.9-5.0 lateral from bregma). Body weights were maintained at no less than 85% of pre-experimental levels by food restriction (10–25 g of Purina laboratory chow daily, and approximately 1 g of sucrose consumed during task) except during the post-operative recovery period when food was given *ad libitum*. All procedures were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

Apparatus

Experimental sessions occurred in 43 × 43 × 53 cm Plexiglas chambers housed within sound-attenuating boxes (Medical Associates, St. Albans, VT, USA). Two symmetrically located retractable levers (Colburn Instruments, Allentown, PA, USA) were placed 17 cm apart on one wall of the chamber. Cue-lights were positioned above each lever. A food receptacle was centered between the levers, 2.5 cm from the floor. A house-light was centrally located on the wall opposite the food receptacle and levers, 2 cm from the ceiling.

Fast-scan cyclic voltammetry

Voltammetric recording procedures have been described previously (10). Briefly, on the test day, a carbon-fiber electrode was lowered into the NAc core. The potential of the electrode was held at -0.4 V versus the Ag/AgCl reference electrode. Voltammetric recordings were made every 100 ms by applying a triangular waveform that drove the potential to +1.3 V and back. The current arising from the double-layer capacitance and oxidation and reduction of surface functional groups on the carbon-fiber was removed by background subtraction. The background period (500 ms) was obtained at the minima for the dopamine signal 5 s prior to event onset. Prior to the session, dopamine release was electrically evoked by stimulating the VTA (24 biphasic pulses, 60 Hz, 120 μA, 2 ms per phase) to ensure that carbon-fiber electrodes were placed close to release sites. VTA stimulation was repeated following both treatments and following the experiment to verify electrode stability and to obtain the training set for principal component analysis. The sensitivity of the electrodes was determined upon completion of the session using a previously established protocol (32). The electrode was removed from the brain and placed in a flow injection apparatus employing TRIS buffer (TRIS buffer contained (in mm): TRIS, 15; NaCl, 126; KCl, 2.5; $NaHCO_3$, 25; $CaCl_2$, 2.4; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; Na_2SO_4, 2.0; and was adjusted to pH 7.4 at the electrode). Four concentrations of dopamine (0.5μM, 0.75μM, 1 μM, and 2 μM) were evaluated. The calibration factors averaged 15.0 nA/μM for dopamine.

Behavioral task

One week later, rats were trained on a discriminative stimulus task (8-12 days) followed by a single test session. Rats discriminated two tones associated with spatially distinct levers. One tone (2750 Hz or 1000 Hz; discriminative stimulus; DS) was presented for 500 ms accompanied by illumination of a cue-light above lever 1 (Fig. 1a). Three seconds after DS onset lever 1 was extended; depression (triangle; Fig. 1a) resulted in lever retraction, termination of the cue-light, and sucrose pellet delivery (45mg). A second distinctive tone (alternate Hz; non-associated

stimulus; NS) was presented for 500 ms with the associated cue-light above lever 2; 3 s later lever 2 was extended. Depression of this lever resulted in lever retraction, cue-light termination but no sucrose. Levers were extended into the chamber for 15 s and if no response was made, the lever was retracted and the cue-light terminated. DS or NS trials were semi-randomly presented on a variable inter-trial interval (vITI, average = 15s). Rats fully consumed all earned pellets.

Following training, one test session was completed in two phases (Fig. 1b). Phase 1 consisted of unilateral infusion of either vehicle (VEH; 0.3μL of sterile saline 0.9% NaCl) or baclofen/muscimol (BM; 0.3/.03nmol in 0.3μL VEH; Sigma Aldrich) directly into the BLA via a 28 gauge injection cannulae (Plastics One). This dose was chosen based on prior work showing: 1) that it effectively inhibits neural activity (33,34) and 2) that it allows the discrimination of specific neural substrates underlying reward-seeking behavior (31). Microinfusions were made over a 1 min period using a syringe pump (Harvard Apparatus), and injectors remained in place for 1 minute following infusion. Animals were then given 5 min recovery followed by five VTA stimulations (24 biphasic pulses, 60 Hz, 120 μA, 2 ms per phase) and a 60 trial period (30 DS, 30 NS), followed by a recovery phase. Phase 2 consisted of microinfusion of the alternate infusate into the BLA, stimulation collection, another 60 trial period and a subsequent recovery phase. Order of infusate was counter-balanced across animals.

Data analysis

Several behavioral measures were examined: the DS and NS approach response ratios, the DS response latency, and the number of overall responses on the DS- and NS-levers. Approach responses were analyzed through video analyses, wherein an approach was defined as a directed movement or orienting of the animals head into the cue-lever region of the chamber (2in. × 2in. surrounding lever) during the DS or NS presentation, but prior to the lever presentation. The latency to reach the sucrose delivery well, and the sucrose consumption period (defined as time in which the rats head remained over the sucrose delivery well) were also scored via video analysis. Response latencies and lever presses were recorded via computer. A within-subjects repeated-measures ANOVA was used to compare lever pressing across training sessions. Bonferroni *post hoc* tests compared replicate means across cue condition. Cue-evoked approach percentages were compared using paired t-tests across VEH and BM conditions. Cue-evoked lever responses were compared using paired t-tests.

Principal component regression was used to extract the dopamine component from the voltammetric data (35). Training sets constructed from representative, background-subtracted cyclic voltammograms for dopamine and pH allow for principal component regression on data collected during the behavioral session, described previously (10). Changes in NAc dopamine concentration ([DA]) were evaluated using a one-way repeated measures ANOVA with Dunnett's *post hoc* test for multiple comparisons of 100-ms time bins (7s post-cue) to a single baseline window (mean [DA] from 5 s pre-cue onset). The average peak change in [DA] was determined for both DS and NS for each animal and statistically compared via Student's paired t-tests.

The average peak change in [DA] was determined for the DS, NS, or stimulation for each animal across both VEH and BM treatments (i.e. all trials for a given animal were averaged to provide a single [DA] trace), and this average was statistically compared via Student's paired t-tests. Differences in [DA] relative to lever extension or lever press were similarly examined by determining the peak change in [DA] in the 2s following onset and comparing it to pre-cue baseline using Student's paired t-tests. For sucrose consumption, [DA] was averaged across the consumption period, then compared between treatments using Student's paired t-tests.

Dopamine release events occur independently of any overt behavioral stimuli (36,37). To determine the effect of BLA activity on the likelihood of high [DA] release events, every 100 ms sample from each trial for each rat was time-stamped if it contained a concentration increase of 40 nM or higher. This threshold represents the average value of spontaneous dopamine release events (36) that are a result of burst firing of VTA dopamine neurons (12). Furthermore, this [DA] is within the range of affinities for high-affinity D1 receptor (38). With these data, the probability of [DA] concentrations exceeding 40 nM was calculated ($Prob_{40}$). A two-way ANOVA was used to identify main effects of epoch (baseline versus DS) and treatment (VEH versus BM). Bonferroni *post hoc* tests for multiple comparisons were used to identify significant differences within epoch and treatment.

Statistical significance was designated at $p < 0.05$. All statistical analyses were carried out using Graphpad Prism 4.0 for Windows (Graphpad Software) or SPSS version 17.0 for Windows (SPSS).

For a description of the methods for histological verification of electrode and microinfusion placements and a diagram showing those placements see Figure S1 in Supplement 1.

Results

Acquisition of stimulus-controlled behavior

Rats learned the discriminative stimulus task over 8-12 sessions. A two-way repeated measures ANOVA of the final six sessions revealed a significant main effect of session ($F_{(5,60)} = 12.52$; $p<0.0001$) and cue ($F_{(1,60)} = 42.69$; $p<0.0001$) on the percentage of cue-trials with an operant response (Fig. 1c). *Post hoc* comparisons revealed a significant difference between percentage of cue-trials with an operant response over the final three days of training ($p<0.05$). The final training response ratios were 100 ± 0 for the DS and 27.8 ± 7.6 for the NS trials.

DS- and NS-evoked dopamine release in the NAc core

After training, both the DS and NS evoked dopamine signals in the NAc core under VEH conditions. Figure 2a and b shows [DA] changes during a single DS and NS trial from a representative animal following VEH treatment. Figure 2c shows the average [DA] for both cues across all animals. The DS produced an immediate increase in [DA] ($F_{(6,70)} = 11.92$, $p<0.0001$). Peak DS-evoked [DA] occurred 560 ± 50 ms after DS onset, reaching an average peak of 83 ± 16 nM and remained elevated throughout the operant response (Bonferroni post-hoc $p<0.05$), although there was no significant further increase at the lever extension (dashed line), lever press (triangle) or during sucrose consumption. NS presentation evoked a lesser, yet significant increase in [DA] ($F_{(6,70)} = 22.61$; $p<0.0001$). The peak NS-evoked [DA] of 38 ± 8 nM occurred 470 ± 40 ms after NS onset, and then rapidly returned to baseline levels, with no significant increase following the lever extension. The latency to peak [DA] was not different between cues ($t_{(6)} = 1.87$, $p=0.11$). Importantly, the average peak [DA] evoked by the DS was significantly larger than that evoked by the NS ($t_{(6)} = 3.55$, $p=0.012$; Fig. 2d).

BLA modulation of DS-evoked behavior and NAc dopamine release

To determine the contribution of BLA activity to DS-evoked behavior and NAc dopamine signaling, we pharmacologically inactivated the BLA with BM. Figure 3a shows the percentage of DS trials in which an animal made a conditioned approach response to the cue (i.e., approached the DS-associated lever prior to extension), and paired t-tests demonstrate that BM significantly attenuated DS-evoked approaches ($t_{(8)} = 2.456$; $p=0.04$; VEH 95.7 ± 1.75; BM 67.9 ± 11.4). Despite the reduction in cue-evoked approach, BM did not alter the ability to perform the instrumental response once initiated, as the percentage of trials with a DS lever response was unaltered ($t_{(8)} = 1.43$; $p=0.19$; VEH 100.0 ± 0.0; BM 95.2 ± 4.8; Fig. 3b). The

latency to respond was not significantly altered following BLA inactivation, although there was an increase ($t_{(8)}$ = 1.95; p=0.09; VEH 489 ms ± 57; BM 832 ms ± 148; data not shown). Furthermore, BLA inactivation did not alter the ability of the rats to consume the sucrose, nor the sucrose consumption duration ($t_{(6)}$ = 1.68; p=0.14; VEH 2.23 s ± 0.23; BM 1.88 s ± 0.12).

Concomitant with decreased DS-evoked approaches, we found a significant attenuation of DS-evoked dopamine. Figure 3c shows the average [DA] traces aligned to DS-onset following VEH and BM treatment (left panel) and also aligned to the DS lever press (right panel) across all animals. Paired t-tests showed that BLA inactivation significantly decreased the peak magnitude of DS-evoked [DA] ($t_{(6)}$ = 2.587; p=0.04; VEH 83 ± 16 nM versus BM 61 ± 14 nM; Fig. 3d). However, [DA] following lever insertion (indicated by dashed line; left panel Fig. 3c) was unaltered by BLA inactivation ($t_{(6)}$ = 1.07; p=0.32). Furthermore, Figure 3c (right panel) shows the average [DA] traces aligned to the DS-evoked lever press (denoted by triangle) following VEH and BM treatment across all animals. BLA inactivation had no significant effect on peak [DA] following the DS-related lever press ($t_{(6)}$ = 0.214; p=0.83; VEH 42 ± 8 nM versus BM 43 ± 10 nM; Fig. 3e) nor did it alter [DA] during the sucrose consumption period (data not shown; $t_{(6)}$ = 0.79; p=0.45).

BLA modulation of NS-evoked behavior and NAc dopamine release

BLA inactivation had no effect on behavioral responses related to the NS. That is, neither the percentage of trials with an NS-evoked conditioned approach response ($t_{(8)}$ = 1.13; p=0.29; Figure 4a), nor the percentage of NS trials with a lever press were altered following BLA inactivation ($t_{(8)}$ = 0.99; p=0.35; Figure 4b). Figure 4c shows the average [DA] traces aligned to NS-onset (time 0) following VEH and BM treatment across all animals. No significant difference was observed in the peak [DA] evoked by the NS between VEH or BM treatments ($t_{(6)}$ = 1.73; p=0.13; VEH 38 ± 8 nM BM 27 ± 4 nM; Figure 4d).

BLA modulation of VTA-mediated NAc dopamine release

Next, we examined the mechanism of BLA modulation of dopamine release in the NAc. One possibility is that BLA modulation of NAc dopamine is mediated indirectly through actions on dopamine neurons in the VTA. If so, BLA inactivation should affect VTA electrically-stimulated dopamine release in the NAc (12). Figure 5a shows the average [DA] following electrical stimulation of the VTA (24 biphasic pulses, 60 Hz, 120 μA, 2 ms per phase), during both VEH and BM treatments. The peak VTA-evoked stimulated [DA] was not significantly different between VEH and BM treatments ($t_{(5)}$ = 0.479, p>0.05).

Likewise, if BLA modulation of NAc dopamine is mediated via the VTA, a concurrent reduction in the probability of large concentration (>40nM; $Prob_{40}$) dopamine release events in response to the DS should be observed, since dopamine release of this magnitude reflect synchronous burst firing of VTA neurons (12,39). Thus, we assessed the effect of BLA inactivation on the probability of high [DA] release events during two event-related time epochs (baseline: 5s period prior to DS onset; DS: 3s period following DS onset; Fig. 6a) across both VEH and BM treatments. An example of a representative trial is illustrated in Figure 6a. Note the occurrence of one naturally occurring large dopamine release event during the baseline period as well as a large DS-evoked dopamine release event. Across all animals, two-way repeated measures ANOVA (epoch × treatment) revealed a significant main effect of epoch ($F_{(1,12)}$ = 62.24; p<0.0001), but no main effect of treatment ($F_{(1,12)}$ = 1.16; p=0.30) on $Prob_{40}$ (Figure 6b) Further, *post hoc* analyses revealed that BLA inactivation did not alter the $Prob_{40}$ during either the baseline epochs (p>0.05; VEH 0.33 ± 0.05, BM 0.38 ± 0.02) or DS epochs (p>0.05; VEH .078 ± 0.06, BM 0.81 ± 0.05). These data indicate that BLA modulation of DS-related NAc dopamine release events are not the result of alterations in burst firing of VTA dopamine neurons.

Discussion

We examined the contribution of BLA activity to NAc core dopamine during a cued sucrose reinforcement task. The DS, which predicted access to the reinforced lever, evoked significantly higher phasic dopamine than the NS, which predicted access to a non-reinforced lever. Pharmacological inactivation of the BLA selectively attenuated DS-evoked dopamine, concurrent with an attenuation of DS-evoked conditioned approaches. However, dopamine measured following NS-onset, lever extensions, lever presses or during sucrose consumption was unaltered following BLA inactivation. Likewise, VTA electrically-stimulated dopamine release was unchanged following BLA inactivation. These findings demonstrate that BLA activity functionally modulates phasic dopamine release within the NAc, through terminally mediated mechanisms, to facilitate reward-seeking evoked by motivationally salient environmental stimuli.

The importance of NAc dopamine to reward-seeking behavior has been extensively described (40,41). The dopaminergic projection from the VTA to the NAc contributes significantly to cue-evoked behavioral responding (42), and phasic activation of dopaminergic neurons is sufficient to establish a conditioned place preference (16). Burst firing of dopamine neurons drives phasic dopamine release within the NAc (12) and these phasic dopamine signals are time-locked to reward predictive cues within the NAc core (10). Our data are consistent with previous findings, as we show DS-evoked dopamine release that was significantly higher than NS-related release (Fig. 2). While our measurements were made following learning, the differential behavioral responding to the DS and NS is consistent with prior studies showing that the development of cue-evoked phasic dopamine release correlates with successful learning of an appetitive task (13) and disruption of phasic dopamine release can selectively attenuate the acquisition of tasks guided by cues (15).

However, the primary goal of this study was to determine the functional contribution of BLA activity to ongoing phasic dopamine release within the NAc core. We demonstrate that transient unilateral reduction of BLA activity causes a selective attenuation in the magnitude of DS-evoked dopamine (Fig. 3). Previous findings have demonstrated that pre-synaptic glutamate receptors (43,44) can mediate dopamine release in the striatum in slices (45,46). Further, in anesthetized preparations (27) and awake microdialysis tests (28), stimulation of the BLA produces terminally mediated, glutamate-dependent increases in NAc dopamine. BLA neurons are activated by emotionally salient stimuli (47-50) which in turn drives post-synaptic excitatory responses in NAc neurons (24,25,51). Our findings indicate that BLA activation to cues, in addition to driving post-synaptic firing, augments concurrent VTA-mediated phasic dopamine release within the NAc.

Furthermore, our findings support the hypothesis that BLA modulation of dopamine signaling occurs at the terminal level within the NAc (26), rather than indirectly through actions on VTA dopamine neurons. First, any decrease in the global activity of VTA dopamine neurons that is a consequence of BLA inactivation would result in decreased dopamine release following electrical stimulation of the VTA (12). We clearly demonstrate that BLA inactivation has no effect on VTA-evoked stimulated dopamine release in the NAc (Figure 6). Second, if BLA modulation of NAc dopamine was mediated via the VTA, we would expect a concurrent reduction in the probability of large concentration dopamine release events in response to the DS or during the baseline epoch following BLA inactivation, as dopamine transients of this magnitude (i.e. >40nM) reflect synchronous burst firing of VTA neurons (12). However, BLA inactivation did not alter the $Prob_{40}$ during either of these epochs. Together, these data indicate that BLA modulation of DS-related NAc dopamine release is not the result of alterations in burst firing of VTA dopamine neurons, but rather mediated, whether monosynaptically or polysynaptically, through terminal mechanisms within the NAc.

Perhaps the most intriguing aspect of these data lies in the functional consequence of BLA modulation of terminal NAc dopamine release. It is thought that the BLA is critical for maintaining the assigned value of conditioned stimuli, and using this information to guide subsequent instrumental responding (52). Specifically, disruption of BLA activity does not alter instrumental conditioning (53) or simple Pavlovian autoshaping (54). However, BLA manipulation inhibits the formation of a conditioned place preference (55,56), Pavlovian-to-Instrumental Transfer (57), cue-induced reinstatement (58), second-order conditioning (59, 60), reward devaluation (61,62), and responding on high effort tasks (63). Additionally, BLA inactivation during an effort-based task biases rats towards low-cost low-reward responding and decreased the willingness to expend higher effort for rewards suggesting that the BLA contributes to ascertaining the value of behavioral options (64). Our data are consistent with those studies, as a transient decrease of BLA activity decreased DS-evoked conditioned responses, but had no impact on instrumental responding. It is possible that a bilateral manipulation may have caused a significant disruption of instrumental responding, but our task required a relatively simple FR1 lever response, which is typically maintained following BLA manipulation (53,65,66). Further, phasic dopamine may contribute to switching attention to a salient stimulus (67) since organisms must attend to a salient stimulus in order for it to guide subsequent behavior and the NAc may play a critical role in this process (68).

At the cellular level, our findings indicate that glutamatergic inputs from the BLA to the NAc drive post-synaptic signals underlying reward-seeking (25) and augment dopamine release at the terminal region. This concurrent activation may potentiate specific spatially- and temporally-linked synapses (69) providing a mechanism by which the BLA can confer motivational value to environmental stimuli, and thereby play a role in modulating ongoing behavior. Numerous studies have demonstrated that dopamine-glutamate interactions are essential for neuroplasticity within reward circuits (17), and interaction of BLA activity and NAc dopamine is necessary for conditioned responding to reward-predictive cues (25). Further, the BLA-NAc circuit may prove to be critical in understanding dysfunction associated with drug addiction as recent evidence suggests that cocaine exposure leads to severe deficits in associative learning during reversal tasks (70-72), mediated by persistent miscoding of information within the BLA (73). Our findings complement these results and provide a means for inflexible BLA activation to induce inappropriate reward-seeking behavior governed by NAc output in the addicted state.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH Grants F31 23745 to JLJ, DA10900 to RMW, and DA17318 to RMC. The authors thank Kate Fuhrmann, Jessica Briley, Laura Ciompi, and Lesley Macinnes for technical assistance and Michael P. Saddoris for comments on earlier versions of this manuscript.

References

1. Kelley AE. Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 2004;44:161–179. [PubMed: 15450168]
2. Berke JD, Hyman SE. Addiction, dopamine, and the molecular mechanisms of memory. Neuron 2000;25:515–532. [PubMed: 10774721]
3. Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 2006;29:565–598. [PubMed: 16776597]
4. Wise RA. Dopamine, learning and motivation. Nat Rev Neurosci 2004;5:483–494. [PubMed: 15152198]

5. Smith-Roe SL, Kelley AE. Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. J Neurosci 2000;20:7737–7742. [PubMed: 11027236]
6. 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: 11717381]
7. Nicola SM, Taha SA, Kim SW, Fields HL. Nucleus accumbens dopamine release is necessary and sufficient to promote the behavioral response to reward-predictive cues. Neuroscience 2005;135:1025–1033. [PubMed: 16165291]
8. Phillips PE, Walton ME, Jhou TC. Calculating utility: preclinical evidence for cost-benefit analysis by mesolimbic dopamine. Psychopharmacology (Berl) 2007;191:483–495. [PubMed: 17119929]
9. Schultz W. Behavioral dopamine signals. Trends in neurosciences 2007;30:203–210. [PubMed: 17400301]
10. Day JJ, Roitman MF, Wightman RM, Carelli RM. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nature neuroscience 2007;10:1020–1028.
11. 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: 14960596]
12. Sombers LA, Beyene M, Carelli RM, Wightman RM. Synaptic overflow of dopamine in the nucleus accumbens arises from neuronal activity in the ventral tegmental area. J Neurosci 2009;29:1735–1742. [PubMed: 19211880]
13. Stuber GD, Klanker M, de Ridder B, Bowers MS, Joosten RN, Feenstra MG, et al. Reward-predictive cues enhance excitatory synaptic strength onto midbrain dopamine neurons. Science (New York, NY 2008;321:1690–1692.
14. Owesson-White CA, Cheer JF, Beyene M, Carelli RM, Wightman RM. Dynamic changes in accumbens dopamine correlate with learning during intracranial self-stimulation. Proc Natl Acad Sci U S A 2008;105:11957–11962. [PubMed: 18689678]
15. Zweifel LS, Parker JG, Lobb CJ, Rainwater A, Wall VZ, Fadok JP, et al. Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. Proceedings of the National Academy of Sciences of the United States of America 2009;106:7281–7288. [PubMed: 19342487]
16. Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, et al. Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science (New York, NY 2009;324:1080–1084.
17. 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: 10845063]
18. Reynolds JN, Hyland BI, Wickens JR. A cellular mechanism of reward-related learning. Nature 2001;413:67–70. [PubMed: 11544526]
19. Everitt BJ, Cardinal RN, Parkinson JA, Robbins TW. Appetitive behavior: impact of amygdala-dependent mechanisms of emotional learning. Annals of the New York Academy of Sciences 2003;985:233–250. [PubMed: 12724162]
20. Pare D, Quirk GJ, Ledoux JE. New vistas on amygdala networks in conditioned fear. Journal of neurophysiology 2004;92:1–9. [PubMed: 15212433]
21. Kelley AE, Domesick VB, Nauta WJ. The amygdalostriatal projection in the rat--an anatomical study by anterograde and retrograde tracing methods. Neuroscience 1982;7:615–630. [PubMed: 7070669]
22. 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: 15306650]
23. Setlow B, Holland PC, Gallagher M. Disconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive pavlovian second-order conditioned responses. Behavioral neuroscience 2002;116:267–275. [PubMed: 11996312]
24. 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: 11487660]

25. Ambroggi F, Ishikawa A, Fields HL, Nicola SM. Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. Neuron 2008;59:648–661. [PubMed: 18760700]
26. Phillips AG, Ahn S, Howland JG. Amygdalar control of the mesocorticolimbic dopamine system: parallel pathways to motivated behavior. Neurosci Biobehav Rev 2003;27:543–554. [PubMed: 14599435]
27. 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. The European journal of neuroscience 1998;10:1241–1251. [PubMed: 9749778]
28. 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: 11826142]
29. Louilot A, Besson C. Specificity of amygdalostriatal interactions in the involvement of mesencephalic dopaminergic neurons in affective perception. Neuroscience 2000;96:73–82. [PubMed: 10683412]
30. Ahn S, Phillips AG. Independent modulation of basal and feeding-evoked dopamine efflux in the nucleus accumbens and medial prefrontal cortex by the central and basolateral amygdalar nuclei in the rat. Neuroscience 2003;116:295–305. [PubMed: 12535961]
31. McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 2001;21:8655–8663. [PubMed: 11606653]
32. Aragona BJ, Cleaveland NA, Stuber GD, Day JJ, Carelli RM, Wightman RM. Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is attributable to a direct increase in phasic dopamine release events. J Neurosci 2008;28:8821–8831. [PubMed: 18753384]
33. Arikan R, Blake NM, Erinjeri JP, Woolsey TA, Giraud L, Highstein SM. A method to measure the effective spread of focally injected muscimol into the central nervous system with electrophysiology and light microscopy. Journal of neuroscience methods 2002;118:51–57. [PubMed: 12191757]
34. Allen TA, Narayanan NS, Kholodar-Smith DB, Zhao Y, Laubach M, Brown TH. Imaging the spread of reversible brain inactivations using fluorescent muscimol. Journal of neuroscience methods 2008;171:30–38. [PubMed: 18377997]
35. Heien ML, Johnson MA, Wightman RM. Resolving neurotransmitters detected by fast-scan cyclic voltammetry. Anal Chem 2004;76:5697–5704. [PubMed: 15456288]
36. Stuber GD, Roitman MF, Phillips PE, Carelli RM, Wightman RM. Rapid dopamine signaling in the nucleus accumbens during contingent and noncontingent cocaine administration. Neuropsychopharmacology 2005;30:853–863. [PubMed: 15549053]
37. Robinson DL, Heien ML, Wightman RM. Frequency of dopamine concentration transients increases in dorsal and ventral striatum of male rats during introduction of conspecifics. J Neurosci 2002;22:10477–10486. [PubMed: 12451147]
38. Richfield EK, Penney JB, Young AB. Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. Neuroscience 1989;30:767–777. [PubMed: 2528080]
39. Roitman MF, Wheeler RA, Wightman RM, Carelli RM. Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. Nature neuroscience 2008;11:1376–1377.
40. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 1998;28:309–369. [PubMed: 9858756]
41. Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW. Associative processes in addiction and reward The role of amygdala-ventral striatal subsystems. Annals of the New York Academy of Sciences 1999;877:412–438. [PubMed: 10415662]
42. 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 2004;24:2923–2933. [PubMed: 15044531]
43. Gracy KN, Pickel VM. Ultrastructural immunocytochemical localization of the N-methyl-D-aspartate receptor and tyrosine hydroxylase in the shell of the rat nucleus accumbens. Brain research 1996;739:169–181. [PubMed: 8955937]

44. Tarazi FI, Baldessarini RJ. Regional localization of dopamine and ionotropic glutamate receptor subtypes in striatolimbic brain regions. Journal of neuroscience research 1999;55:401–410. [PubMed: 10723051]
45. Krebs MO, Trovero F, Desban M, Gauchy C, Glowinski J, Kemel ML. Distinct presynaptic regulation of dopamine release through NMDA receptors in striosome- and matrix-enriched areas of the rat striatum. J Neurosci 1991;11:1256–1262. [PubMed: 1851217]
46. Krebs MO, Desce JM, Kemel ML, Gauchy C, Godeheu G, Cheramy A, et al. Glutamatergic control of dopamine release in the rat striatum: evidence for presynaptic N-methyl-D-aspartate receptors on dopaminergic nerve terminals. Journal of neurochemistry 1991;56:81–85. [PubMed: 1824785]
47. 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. [PubMed: 16482160]
48. Tye KM, Janak PH. Amygdala neurons differentially encode motivation and reinforcement. J Neurosci 2007;27:3937–3945. [PubMed: 17428967]
49. Carelli RM, Williams JG, Hollander JA. Basolateral amygdala neurons encode cocaine self-administration and cocaine-associated cues. J Neurosci 2003;23:8204–8211. [PubMed: 12967981]
50. 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: 10024371]
51. 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: 7751934]
52. 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: 12034134]
53. Balleine BW, Killcross AS, Dickinson A. The effect of lesions of the basolateral amygdala on instrumental conditioning. J Neurosci 2003;23:666–675. [PubMed: 12533626]
54. 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: 10651899]
55. Everitt BJ, Morris KA, O'Brien A, Robbins TW. The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience 1991;42:1–18. [PubMed: 1830641]
56. McDonald RJ, White NM. A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. Behavioral neuroscience 1993;107:3–22. [PubMed: 8447956]
57. Corbit LH, Balleine BW. Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. J Neurosci 2005;25:962–970. [PubMed: 15673677]
58. Fuchs RA, Feltenstein MW, See RE. The role of the basolateral amygdala in stimulus-reward memory and extinction memory consolidation and in subsequent conditioned cued reinstatement of cocaine seeking. The European journal of neuroscience 2006;23:2809–2813. [PubMed: 16817884]
59. Everitt BJ, Cador M, Robbins TW. Interactions between the amygdala and ventral striatum in stimulus-reward associations: studies using a second-order schedule of sexual reinforcement. Neuroscience 1989;30:63–75. [PubMed: 2664555]
60. Setlow B, Gallagher M, Holland PC. The basolateral complex of the amygdala is necessary for acquisition but not expression of CS motivational value in appetitive Pavlovian second-order conditioning. The European journal of neuroscience 2002;15:1841–1853. [PubMed: 12081664]
61. Ostlund SB, Balleine BW. Differential involvement of the basolateral amygdala and mediodorsal thalamus in instrumental action selection. J Neurosci 2008;28:4398–4405. [PubMed: 18434518]
62. Johnson AW, Gallagher M, Holland PC. The basolateral amygdala is critical to the expression of pavlovian and instrumental outcome-specific reinforcer devaluation effects. J Neurosci 2009;29:696–704. [PubMed: 19158296]
63. Simmons DA, Neill DB. Functional interaction between the basolateral amygdala and the nucleus accumbens underlies incentive motivation for food reward on a fixed ratio schedule. Neuroscience 2009;159:1264–1273. [PubMed: 19344638]

64. Ghods-Sharifi S, St Onge JR, Floresco SB. Fundamental contribution by the basolateral amygdala to different forms of decision making. J Neurosci 2009;29:5251–5259. [PubMed: 19386921]

65. Burns LH, Everitt BJ, Robbins TW. Effects of excitotoxic lesions of the basolateral amygdala on conditional discrimination learning with primary and conditioned reinforcement. Behavioural brain research 1999;100:123–133. [PubMed: 10212059]

66. Whitelaw RB, Markou A, Robbins TW, Everitt BJ. Excitotoxic lesions of the basolateral amygdala impair the acquisition of cocaine-seeking behaviour under a second-order schedule of reinforcement. Psychopharmacology 1996;127:213–224. [PubMed: 8912399]

67. Pezze MA, Dalley JW, Robbins TW. Differential roles of dopamine D1 and D2 receptors in the nucleus accumbens in attentional performance on the five-choice serial reaction time task. Neuropsychopharmacology 2007;32:273–283. [PubMed: 16641946]

68. Floresco SB, Ghods-Sharifi S, Vexelman C, Magyar O. Dissociable roles for the nucleus accumbens core and shell in regulating set shifting. J Neurosci 2006;26:2449–2457. [PubMed: 16510723]

69. Arbuthnott GW, Wickens J. Space, time and dopamine. Trends Neurosci. 2006

70. Jentsch JD, Olausson P, De La Garza R 2nd, Taylor JR. Impairments of reversal learning and response perseveration after repeated, intermittent cocaine administrations to monkeys. Neuropsychopharmacology 2002;26:183–190. [PubMed: 11790514]

71. Calu DJ, Stalnaker TA, Franz TM, Singh T, Shaham Y, Schoenbaum G. Withdrawal from cocaine self-administration produces long-lasting deficits in orbitofrontal-dependent reversal learning in rats. Learning & memory (Cold Spring Harbor, NY 2007;14:325–328.

72. Schoenbaum G, Saddoris MP, Ramus SJ, Shaham Y, Setlow B. Cocaine-experienced rats exhibit learning deficits in a task sensitive to orbitofrontal cortex lesions. The European journal of neuroscience 2004;19:1997–2002. [PubMed: 15078575]

73. Stalnaker TA, Roesch MR, Franz TM, Calu DJ, Singh T, Schoenbaum G. Cocaine-induced decision-making deficits are mediated by miscoding in basolateral amygdala. Nat Neurosci 2007;10:949–951. [PubMed: 17603478]

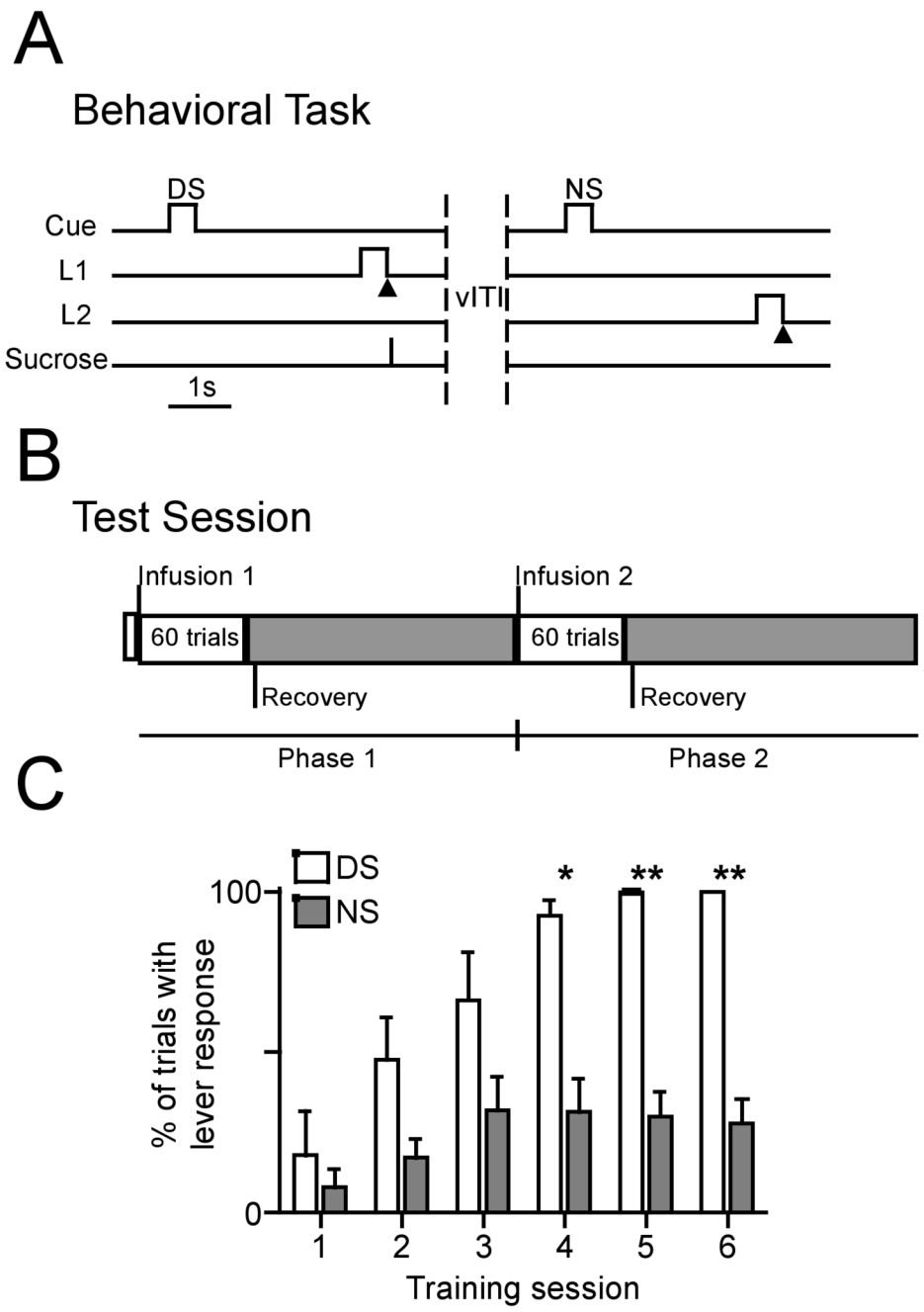

Figure 1.

Task, experimental protocol and behavior. (a) Schematic diagram of the behavioral task. Animals were semi-randomly presented one of two trial types (DS or NS), each distinguished by a unique auditory tone, and subsequent presentation of a spatially distinct lever (L1 or L2). Each response on the DS lever resulted in sucrose pellet delivery (FR1, schedule of reinforcement). Responses on the NS lever were never rewarded. Triangles denote lever presses. The variable ITI (vITI) averaged 15s. (b) The test session was divided into two phases in which delivery of either VEH or BM were microinfused into the BLA. Following Infusion 1, animals were given 60 trials (30 DS, 30 NS) and dopamine release was measured. Upon completion, a recovery period was initiated followed by Infusion 2, completion of a second

test session, and subsequent recovery period. (c) Animals successfully learned to discriminate cues. Plot shows the average percentage of trials in which rats pressed the lever following DS or NS presentation across the final six training sessions. Over the final 3 training sessions, rats responded more on DS trials versus NS trials. * denotes $p<0.05$; **, denotes $p<0.01$.

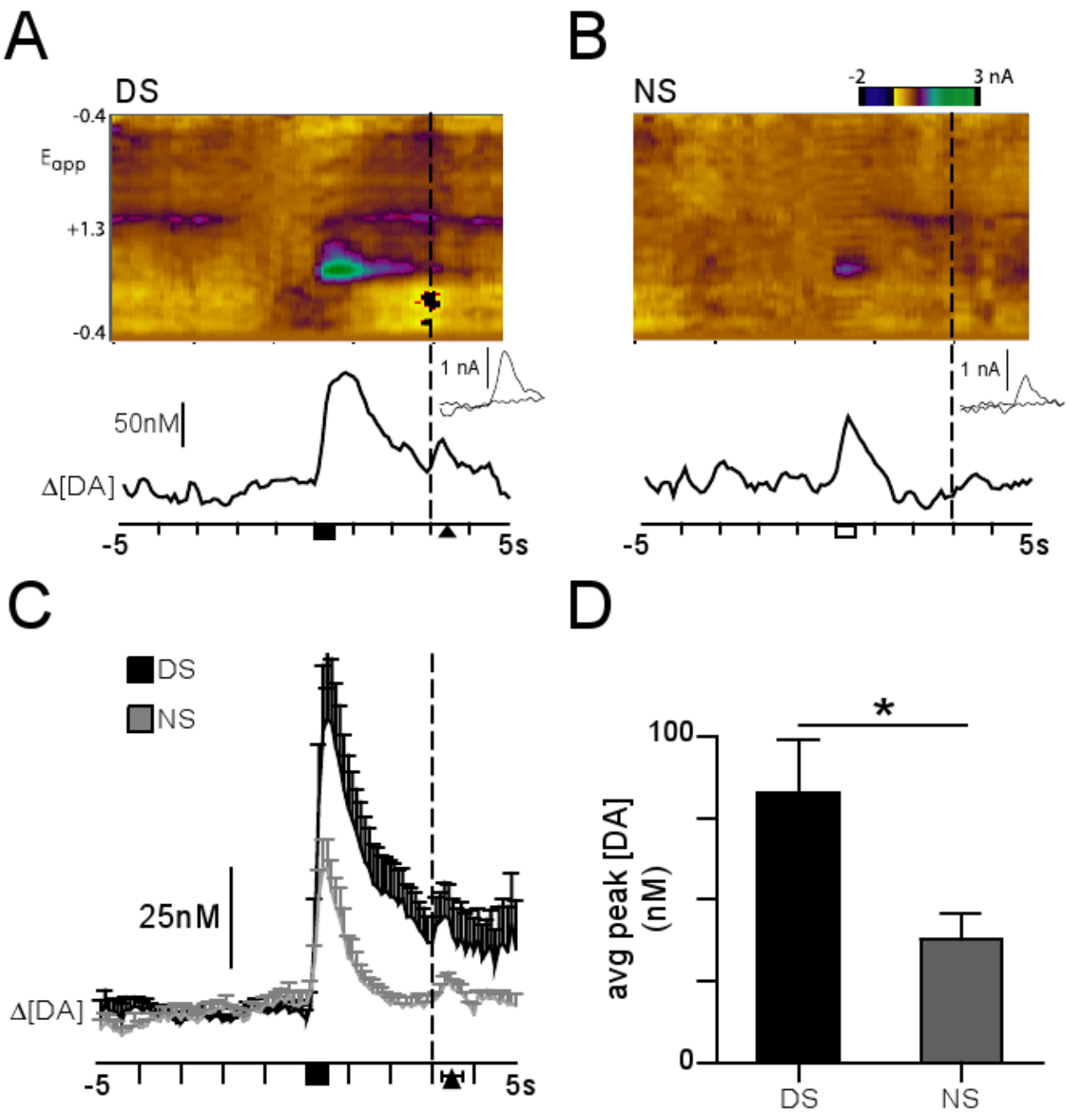

Figure 2.

Reward predictive cues evoke phasic dopamine release in the NAc core. (a) Dopamine release during one representative DS trial. (Top) Voltammetric plot (time × voltage × current) for DS trial and (Bottom) corresponding [DA] determined by principal component analysis. Black bar denotes DS period, dashed line denotes the DS-lever insertion, and the triangle represents the lever press. Inset: cyclic voltammagram taken from peak [DA]. (b) Dopamine release during one representative NS trial. (Top) Voltammetric plot (time × voltage × current) for NS trial and (Bottom) corresponding [DA] determined by principal component analysis. Open bar denotes NS period, and dashed line denotes NS-lever insertion. Inset: cyclic voltammagram taken from peak [DA]. (c) Average Δ[DA] (n=7) relative to DS (black line) or NS (grey line)

onset (at time 0) under VEH conditions. Lever extension is denoted by dashed line. Triangle represents mean ± range of DS lever presses across all animals. (d) There was a significant difference in the average peak [DA] between the DS and NS. Error bars show mean ± SEM; * denotes $p<0.05$.

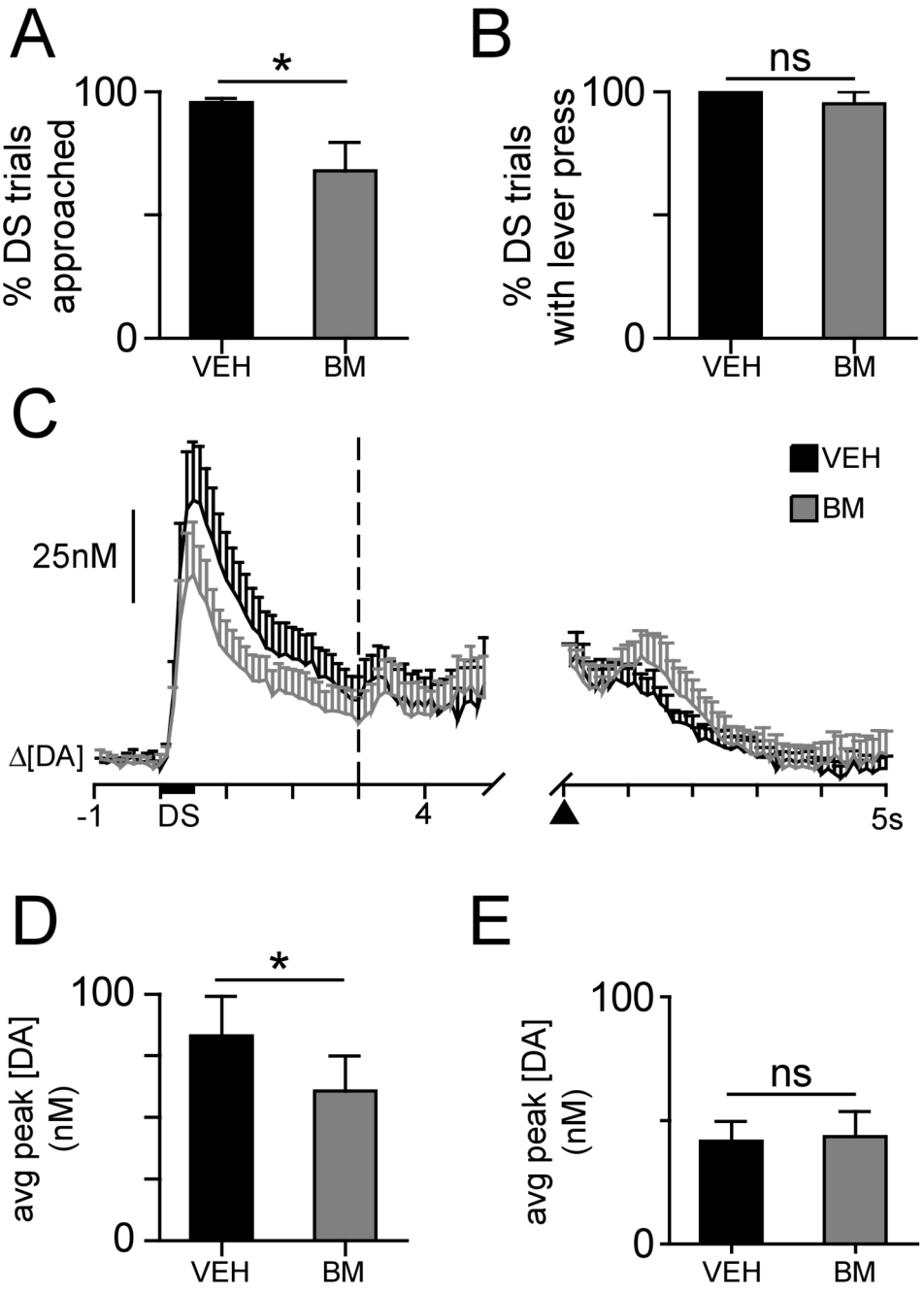

Figure 3.

Effects of BLA inactivation on DS-evoked behavioral responding and dopamine release. (a) Percentage of DS trials with a DS-evoked conditioned approach response was significantly attenuated by BM compared to VEH (n=9). (b) Percentage of DS trials with an instrumental response was not altered by BM treatment (n=9). (c) Average Δ[DA] across NAc core recordings (n=7) on DS trials under VEH (black line) and BM (grey line) conditions. The left panel is aligned to DS-onset (at time 0); lever extension denoted by dashed line. The right panel is aligned to the DS lever-press response (triangle). (d) Significant reduction in peak [DA] to the DS following BM treatment compared to VEH (n=7). (e) No significant difference in peak [DA] after the lever press between BLA treatments (n=7). ns denotes $p>0.05$; * denotes $p<0.05$.

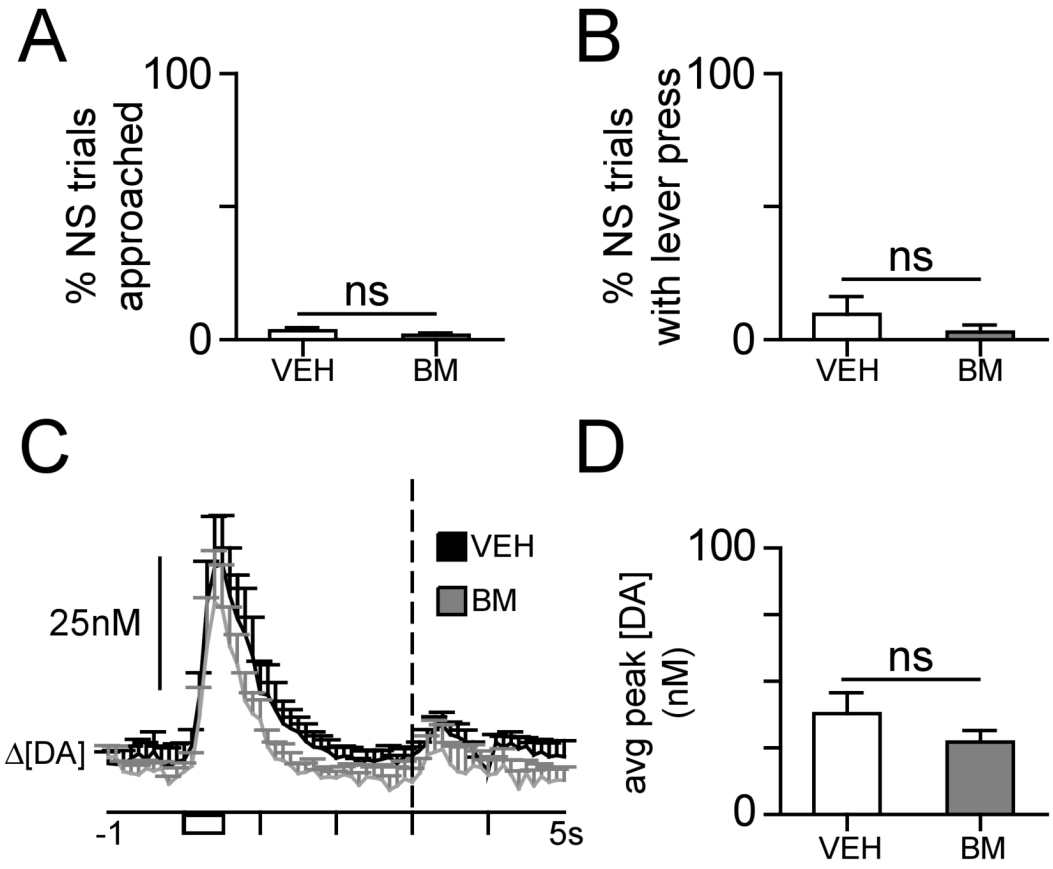

Figure 4.

Effects of BLA inactivation on behavior and NS-evoked dopamine (a) No significant difference in the percentage of NS trials with a cue-evoked behavioral approach toward the NS lever (n=9). (b) No significant difference in the percentage of NS trials with an associated lever press response (n=9). (c) Average Δ[DA] across NAc core recordings (n=7) on NS trials under VEH (black line) and BM (grey line) conditions. White bar denotes NS period; lever extension denoted by dashed line. (d) No significant difference in peak [DA] to the NS across BLA treatment (n=7). ns denotes $p>0.05$.

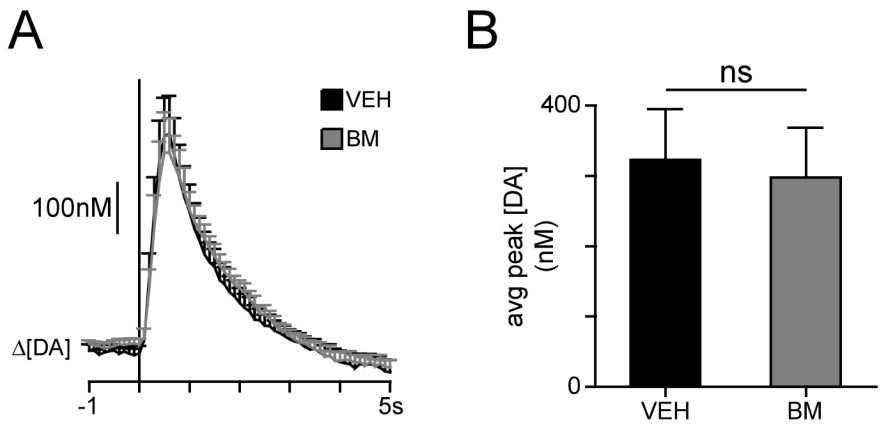

Figure 5.

BLA inactivation does not alter VTA-evoked stimulated dopamine release in the NAc. (a) Average Δ[DA] from NAc recordings (n=6) following VTA electrical stimulation (solid vertical line, time 0) during VEH (black line) or BM (grey line) treatments (b) Average peak [DA] following VTA electrical stimulation is not significantly different across BLA treatments (n=6). Error bars show mean ± SEM; ns denotes $p>0.05$.

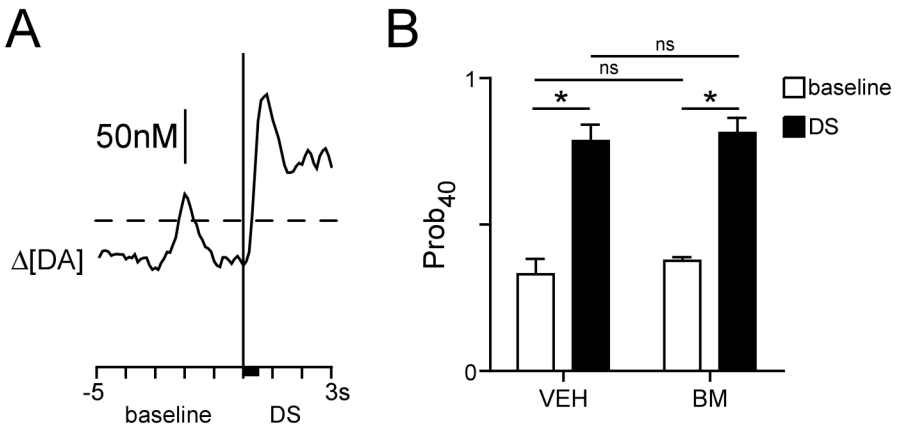

Figure 6.

BLA inactivation does not alter high [DA] release probability in the NAc. (a) A single trial trace of Δ[DA]. Event-related epochs are denoted on the x-axis, divided by the solid vertical line at DS onset (black bar denotes DS period). Dashed horizontal line represents the 40nM threshold. (b) $Prob_{40}$ significantly differs as a function of event epoch, but neither the baseline nor DS time epochs differ across VEH or BM treatment (n=7). Error bars show mean ± SEM; ns denotes $p>0.05$; * denotes $p<0.05$.