Opposing Catecholamine Changes in the Bed Nucleus of the Stria Terminalis during Intracranial Self-Stimulation and its Extinction

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Abstract

Background—While studies suggest that both dopamine and norepinephrine neurotransmission support reinforcement learning, the role of dopamine has been emphasized. As a result, little is known about norepinephrine signaling during reward learning and extinction. Both dopamine and norepinephrine projections innervate distinct regions of the bed nucleus of the stria terminalis (BNST), a structure that mediates behavioral and autonomic responses to stress and anxiety. We investigated whether norepinephrine release in the ventral (v) BNST and dopamine release in the dorsolateral (dl) BNST correlate with reward learning during intracranial self-stimulation (ICSS).

Methods—Using fast-scan cyclic voltammetry, norepinephrine concentration changes in the vBNST (n = 12 animals) during ICSS were compared to dopamine changes in the dlBNST (n = 7 animals) and nucleus accumbens (NAc) (n = 5 animals). Electrical stimulation was in the ventral tegmental area/substantia nigra region.

Results—Whereas dopamine release was evoked by presentation of a cue predicting reward availability in both dlBNST and NAc, cue-evoked norepinephrine release did not occur in the vBNST. Release of both catecholamines was evoked by the electrical stimulation. Extracellular changes in norepinephrine were also studied during extinction of ICSS and compared to results obtained for dopamine. During extinction of ICSS norepinephrine release in the vBNST occurred at the time where the stimulation was anticipated whereas dopamine release transiently decreased.

Conclusions—The data demonstrate that norepinephrine release in the vBNST differs from dopamine release in the dlBNST and the NAc in that it signals the absence of reward rather than responding to reward predictive cues.

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Introduction

Intracranial self-stimulation (ICSS) is an operant behavior in which animals are conditioned to press a lever to deliver an electrical stimulation to specific brain regions (1-3). Early research suggested that norepinephrine was a critical neurotransmitter involved in ICSS (reviewed in (4)). Anatomical sites that supported ICSS were found near norepinephrine processes (5), and radioactive norepinephrine and its metabolites were released during ICSS(6). However, subsequent research found evidence to the contrary (7-10), and supported a more important role for dopamine in ICSS reward (11). Norepinephrine has also been suggested to be involved in the extinction of reward based behaviors, although this role has also been disputed (12-14).

ICSS studies have shown that mesolimbic dopamine neurons projecting from the ventral tegmental area (VTA) are important in the neural circuitry mediating reward (15). Indeed, dopamine has been viewed as the primary neurotransmitter involved in the rewarding aspects of ICSS (3). Our previous studies demonstrated that reward learning can be investigated by quantifying dopamine changes in the nucleus accumbens (NAc) with carbon-fiber microelectrodes using fast-scan cyclic voltammetry (16). Transient surges of extracellular dopamine become time-locked to cues that predict ICSS reward and coincide with cues associated with cocaine or food reward (17; 18). Since dopamine transients are a direct result of burst firing of dopaminergic neurons (19), these results concur with electrophysiological recordings that demonstrated that the firing rate of dopamine neurons encode for a reward prediction error (20; 21).

In this work we measure the roles of dopamine and norepinephrine during ICSS in the bed nucleus of the stria terminalis (BNST) and compare them to dopamine responses in the NAc. The BNST is part of the extended amygdala and serves as a relay center between limbic brain regions and the hypothalamic paraventricular nucleus (22). The anterior portion of the BNST receives both dopaminergic and noradrenergic inputs, but they are differentially distributed (23). The ventral (v) BNST has the highest noradrenergic innervation in the brain (24) but has little dopamine content (25; 26). In contrast, the dorsolateral (dl) BNST receives dopaminergic innervation from the VTA and periaqueductal gray but contains negligible norepinephrine (27-31). The research reported here provides three new insights into the role of catecholamines in ICSS. First, dopaminergic responses in the dlBNST were found to resemble dopaminergic responses in the NAc. Second, unlike dopamine, norepinephrine in the vBNST was not released in response to cues that predicted lever availability. Third, during extinction, norepinephrine release occurred at the time of the anticipated electrical stimulation whereas there was a suppression of extracellular dopamine.

Materials and Methods

Animals

Male Sprague-Dawley rats (n = 24, Charles River, NC), aged 90-120 d and weighing 280-380 g, were used. They were individually housed in a controlled temperature environment with a 12:12 light:dark cycle. Food and water were available *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

Surgery

Rats were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg) administered intraperitoneally and placed in a stereotaxic frame. A guide cannula (Bioanalytical Systems, West Lafayette, IL) was positioned above the *dl*BNST (anteroposterior (AP) −0.1 mm, mediolateral (ML) +1.6 mm), the *v*BNST (AP 0.0 mm, ML +1.2 mm), or the NAc (AP 1.7 mm, ML 0.8 mm). Coordinates were from a stereotaxic atlas (32). An Ag/AgCl reference electrode was implanted contralateral to the guide cannula in the left forebrain. A bipolar stimulating electrode was implanted in the VTA/substantia nigra (VTA/SN) (AP −5.2 mm, ML +1.2 mm, dorsoventral (DV) −8.0 to −9.0 mm). Stimulation at these coordinates also activates the ventral noradrenergic bundle (VNB) (26). Stainless steel skull screws and dental cement secured all items.

Intracranial Self-Stimulation (ICSS) Training

After each animal recovered to their presurgery weight, ICSS training commenced as previously described (16). During recording sessions, an audiovisual cue was followed 2 s later by lever extension. Depression of the lever by the rat delivered an electrical stimulation to the animal. See *Methods and Materials in Supplement 1* for more detail.

On the day of recording, some animals also underwent ICSS extinction where the lever-press did not deliver the electrical stimulation. When the cue no longer elicited lever-press behavior, ICSS was reinstated with 1-2 operator-delivered stimulations.

Fast-scan Cyclic Voltammetry

Following successful training, a fresh, glass-sealed carbon-fiber microelectrode (75–100 μm exposed tip length, 7 m diameter, T-650; Amoco, Greenville, SC (33; 34)) was lowered into the brain with a micromanipulator inserted into the guide cannula. The microelectrode was lowered to the *dl*BNST (DV −6.0 to −7.0 mm), the *v*BNST (DV −7.2 to −7.7 mm) or the NAc shell (DV −6.4 to −7.4 mm (16)). The carbon-fiber and Ag/AgCl reference electrodes were connected to a head-mounted amplifier attached to a commutator (Med-Associates, St. Albans, VT). Fast-scan cyclic voltammetry was computer-controlled (35). A triangular scan (−0.4 to +1.3 V, 400 V/s) was repeated every 100 ms. Data were digitized and stored on a computer using software written in LABVIEW (National Instruments).

Histology

At the end of the experiment, electrode placements were verified by electrolytic lesions made with the carbon-fiber microelectrodes (Fig. S1 in Supplement 1) (26). Rats were euthanized with an overdose of urethane (2.0 g/kg) and a lesion was made at the recording site by applying constant current (20 μA for 10 s). Brains were removed and stored in 10 % formalin solution for a week before being sectioned into 40 μm coronal slices. The sections were mounted on slides and viewed with an optical microscope.

Chemicals and Drugs

Chemicals and drugs were reagent-quality and used without further purification. Drugs were obtained from Sigma-Aldrich (St. Louis, MO, USA). Calibration of the carbon-fiber microelectrodes with standards for pH, dopamine, and norepinephrine were made in a buffer (pH 7.4 containing 15 mM Tris, 140 mM NaCl, 3.25 mM KCl, 1.2 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 1.2 mM $MgCl_2$, and 2.0 mM Na_2SO_4).

At least one pharmacological agent selective for each catecholamine was administered intraperitoneally (i.p.) at the end of every experiment (0.6 mL volume) to verify the identity of the electrochemical signal (26). Cyclic voltammetry cannot be used to distinguish

dopamine from norepinephrine and thus the pharmacological distinction is required (36). Desipramine-HCl (DMI, 15 mg/kg), raclopride-HCl (2 mg/kg), and idazoxan-HCl (IDA, 5 mg/kg) were dissolved in saline. GBR 12909 (GBR, 15 mg/kg) was dissolved in water and diluted in saline. For sites reported in the *dl*BNST positive responses were obtained to dopaminergic drugs (GBR, raclopride) but not noradrenergic drugs (DMI, IDA) whereas the opposite was true in the *v*BNST (Fig. S2 in Supplement 1) as described in a previous study (23). The limited chemical selectivity of cyclic voltammetry precludes its use in brain regions that employ both catecholamines as neurotransmitters.

Data Analysis

Catecholamine concentration changes were quantified using principal component regression (37; 38). The post-calibration factors were from the average responses obtained with multiple electrodes in our previous studies (23; 39) (6.9 ± 0.3 pA/(μM$\cdot\mu$m^2) for dopamine, and 4.5 ± 0.2 pA/(μM$\cdot\mu$m^2) for norepinephrine). A residual analysis procedure was used to verify that the cyclic voltammograms of the trials being predicted were consistent with the cyclic voltammograms used for calibration (40).

Clampfit 8.1 (Axon Instruments, Foster City, CA, USA) was used to analyze maximal catecholamine concentration evoked by the cue or lever-press. The half-life ($t_{1/2}$) for catecholamine clearance was taken as the time to descend from its maximum to half of that value (41). Only catecholamine concentrations with signal to noise (S/N) > 3 were considered as cue- or lever-press evoked catecholamine release. Concentration changes were evaluated by subtracting the average baseline (-2.0 to 0 s relative to cue or lever-press) from events after the cue (0.1-2.0 s) or lever-press (2-15 s). Mean values were compared with the two-tailed Student's *t*-test to calculate the level of significance. The coefficient of variation (CV, the ratio of the standard deviation of the mean) was used to characterize the catecholamine maximum concentrations. Statistical analysis employed GraphPad Software version 4.0 (San Diego, CA, USA). Data are represented as mean $\pm$ S.E.M. and '*n*' values indicate the number of rats.

Results

Cue-evoked Dopamine Concentration Changes in the *dl*BNST during ICSS

Following ICSS training, catecholamine release was measured in the *dl*BNST. During training, the animal learned that a tone and house light were followed 2 s later by lever extension, and that a lever-press delivered ICSS stimulation to its brain (timing diagram in Fig. 1A). As shown for a single trial (Fig. 1B), dopamine release occurs both following the cues and the lever-press. The color plot shows the cyclic voltammograms recorded with the applied voltage as the ordinate and the abscissa as the acquisition time of the cyclic voltammogram. The current is encoded in color. The trace above the color plot is the dopamine concentration extracted from these data by principal component regression. The low residual confirms that the training set used to extract the dopamine concentrations describes the data appropriately (Fig. 1C).

Animals were allowed multiple ICSS trials. As shown in this animal, the cue-evoked maximal dopamine concentration ([DA]$_{cue}$) was similar with each trial ([DA]$_{cue}$ = 36.7 ± 2.6 nM from the first 30 trials and 30.9 ± 1.8 nM from the last 30 trials, $t_{29} = 1.87$, $P > 0.05$, Fig. 1D). In contrast, lever-press-evoked dopamine ([DA]$_{stim.}$) decreased significantly over trials and fit to a parabolic curve ($r^2 = 0.81$, [DA]$_{stim.}$ = 278 ± 11 nM from the first 30 trials and 182 ± 3 nM from the last 30 trials, $t_{29} = 9.87$, $P < 0.0001$, Fig. 1D). In both respects, the responses of dopamine were similar to our findings in the NAc (16). The latency to press after lever extension was essentially constant indicating that the animal had learned the

behavior (Fig. 1E). Similar results were obtained in the *dl*BNST in 6 animals undergoing identical ICSS training. The average value of $[DA]_{cue}$ was 27.8 ± 5.3 nM and for $[DA]_{stim.}$ was 216 ± 51 nM.

Pharmacological Effects on Dopamine in the *dl*BNST

For some animals, dopamine release during ICSS was also monitored following administration of inhibitors of dopamine uptake (GBR 12909) and norepinephrine uptake (DMI) (n = 5 animals). When examined 20 minutes after GBR (n = 2), both cue- and lever-press-evoked dopamine concentrations significantly increased ($[DA]_{cue} = 34.8 \pm 1.1$ nM before and 63.3 ± 2.0 nM after GBR, $t_{49} = 8.85$, $P < 0.0001$; $[DA]_{stim.} = 118 \pm 7$ nM before and 427 ± 6 nM after GBR, $t_{49} = 78.05$, $P < 0.0001$, Fig. 2, data recorded when both responses had stabilized in amplitude). Interestingly, the maximal cue evoked dopamine shows greater relative variation than do the stimulus-evoked changes. The CV was found to be 0.281 for cue-evoked dopamine release and 0.109 during electrically-stimulated dopamine release. The latency to lever-press decreased (but not significantly) after administration of GBR (predrug = 0.64 ± 0.07 s, after GBR = 0.47 ± 0.05 s, $t_{49} = 1.98$, $P > 0.05$).

In contrast, DMI (n = 3) did not significantly change cue-evoked dopamine concentration in the *dl*BNST ($[DA]_{cue} = 45.1 \pm 2.5$ nM before and 41.6 ± 2.0 after DMI, $t_{49} = 0.98$, $P > 0.05$, representative example in Fig. 3A). There was also no significant change in stimulated dopamine release ($[DA]_{stim.} = 299 \pm 4$ nM predrug and $[DA]_{stim.} = 303 \pm 8$ nM after DMI, $t_{49} = 0.583$, $P > 0.05$, Fig. 3B). DMI did significantly increase the latency to lever-press (0.47 ± 0.05 s before and 0.76 ± 0.06 s after DMI, $t_{49} = 3.736$, $P < 0.001$). Following DMI, this animal was administered GBR. As with GBR alone, cue and lever-press-evoked dopamine concentrations were increased ($[DA]_{cue} = 99.4 \pm 4.8$ nM, $t_{49} = 10.52$, $P < 0.0001$ and $[DA]_{stim.} = 1226 \pm 64$ nM, $t_{49} = 14.55$, $P < 0.0001$) and the latency to lever-press was restored to its original value (0.49 ± 0.06 s).

Norepinephrine Responses during Maintenance-delay Trials

In a different group of animals, measurements were made in the *v*BNST to evaluate changes in extracellular norepinephrine concentration during an identical ICSS task. There was no response to the cue but norepinephrine increased during the electrical stimulation (Fig. 4A) that followed the lever-press. The residual (Fig. 4B) was below that predicted for 95 % of the noise from the training set. In this animal, maximum lever-press-evoked norepinephrine concentration ($[NE]_{stim.}$) decreased with subsequent trials ($[NE]_{stim.} = 288 \pm 8$ nM from the first 30 trials and 213 ± 4 nM from the last 30 trials, $t_{29} = 8.160$, $P < 0.0001$, Fig. 4C), although the latency to lever-press did not significantly change with trials (Fig. 4D). Similar results were obtained in 6 other animals. The average value across all trials of $[NE]_{stim}$ was 135 ± 25 nM. The average latency to lever-press from these animals over 100 trials was 1.25 ± 0.27 s. Norepinephrine release in response to the cue was never observed, even with extended training (Fig. S3 in Supplement 1).

Pharmacological Effects on Norepinephrine in the *v*BNST

In some animals norepinephrine changes during ICSS were evaluated after uptake inhibitors (n = 3 for GBR and n = 4 for DMI, example in Figure 5). DMI significantly increased stimulated release following the lever-press (from $[NE]_{stim} = 213 \pm 3$ nM to $[NE]_{stim} = 269 \pm 8$ nM after DMI, $t_{49} = 6.57$, P < 0.0001, 50 trials) and the evoked signal gradually declined to predrug levels (Fig. 5A and B). Even following DMI, cue-evoked norepinephrine did not occur. The latency to lever-press following DMI increased significantly (latency was 0.97 ± 0.16 s before drug and 1.96 ± 0.42 s after drug, $t_{49} = 2.609$, $P < 0.05$, Fig. 5C). In a single animal that was administered GBR following DMI, the latency to lever-press was restored to

its original value(0.85 ± 0.02 s). Administration of GBR alone had a slight effect on the norepinephrine responses to stimulation ($[NE]_{stim} = 105 \pm 3$ nM predrug and $[NE]_{stim} = 99 \pm 2$ nM after GBR, $t_{37} = 2.044$, $P < 0.05$, Fig. 6A and B), however it did shorten the latency to lever-press (1.74 ± 0.26 s before and 0.86 ± 0.19 s after GBR, $t_{37} = 2.801$, $P < 0.01$, Fig 6C).

Extracellular Changes of Dopamine and Norepinephrine during Extinction of ICSS

During extinction, the sequence of cues and lever extension was unchanged; however, depression of the lever had no consequence. Previous studies have demonstrated that cue-evoked dopamine release in the NAc decreased across extinction trials and this was accompanied by a decline in goal-directed behavior (16). In addition we found that dopamine in the NAc decreased during extinction at the time where the stimulation should have occurred by18.3 ± 1.4 nM (n = 5, individual example in Figure 7A, lever press indicated by dashed red line). Identical behavior was observed in the *dl*BNST in one animal (Figure 7B). However, due to the low success rate of *dl*BNST experiments, we primarily compare the behavior of norepinephrine in the *v*BNST with that of dopamine in the NAc shell (implantation success was approximately 17 % in the *dl*BNST versus 80 % in the NAc).

During extinction, norepinephrine was still unresponsive to the cue but it increased following the unrewarded lever-presses (example after the lever-press in one animal is shown in Fig. 7C). Data that was averaged and analyzed by principal component regression reveal an increase in norepinephrine concentration (n = 5 rats, 32.3 ± 3.5 nM) following the lever-press during extinction trials. In two animals, norepinephrine changes during extinction were examined after administration of DMI. The norepinephrine response to extinction following the lever-press was significantly enhanced (from 38.0 ± 3.6 to 85.6 ± 13.1 nM following DMI, $t_5 = 4.01$, *P* < 0.01, example in Fig. S4 in Supplement 1), but there was still no change to the cue. Electrically evoked norepinephrine and ICSS behavior were restored during subsequent reinstatement trials.

Discussion

Here we found that dopamine release in the *dl*BNST in response to cues that predict lever availability closely resembles dopamine changes in the NAc (16; 42). In contrast, norepinephrine in the *v*BNST does not respond to the cue, even after prolonged training. However, both catecholamines are released after the lever-press as a consequence of the VNB and VTA/SN electrical stimulation. During ICSS extinction, cue-evoked dopamine disappears in the *dl*BNST just as in the NAc shell (16). As shown here, extracellular dopamine actually decreases at the time of the lever-press when electrical stimulation is expected but not delivered. Remarkably, during extinction, norepinephrine increases at the expected time of electrical stimulation. The opposite responses of these two catecholamines in the extended amygdala are in accord with a proposed role of this system in distinct aspects of the addiction cycle that includes both positive and negative reinforcement mechanisms (43; 44). Dopamine relays information concerning positive hedonic states whereas the negative hedonic responses are mediated by norepinephrine (23).

Different Roles for Each Catecholamine in Subregions of the BNST during ICSS

Since the BNST is involved in the regulation of stress, homeostasis, and reward (25; 30; 45-48), activated catecholamine neurotransmission in this region during ICSS was anticipated. Indeed, previous experiments have demonstrated that dopamine neurotransmission is promoted in the *dl*BNST during reward-based behaviors (49; 50), including ICSS (51). The temporal resolution provided by fast-scan cyclic voltammetry

allowed us to distinguish catecholamine changes associated with the cues from those associated with the electrical stimulation. In the *dl*BNST dopamine was found to increase both in response to the cues that predict lever availability and during the self-administered (electrical) stimulation. The cue-evoked responses are similar to those observed in other dopaminergic regions during reward-based operant behaviors (18; 52-54). The greater variability of cue-induced dopamine transients when compared to stimulated release has been reported before (16). While the stimulation activates a uniform population of terminals on each trial, cue-induced dopamine appears to arise from a more variable activation of terminals.

This is the first time rapid recordings have been applied to norepinephrine during ICSS, and it has allowed us to reinvestigate the decades-old question of the role of norepinephrine in ICSS and extinction. Early studies suggested that the norepinephrine release was associated with reward function (5; 6). Although our work confirms that norepinephrine release is evoked by a site that supports ICSS, it is not evoked by the predictive cue. Thus it clearly plays a different role than dopamine during ICSS.

When an animal acquires cue-reward associations, burst firing of midbrain dopaminergic neurons occurs at the onset of the cue (20; 21). Previously, we have shown that time-locked dopamine concentration transients also occur in terminal regions at such times (16; 55). Thus, dopamine release at the cue is one of the neurochemical responses that immediately elicits a goal-directed behavior (4; 56). In contrast, cue-evoked norepinephrine was not observed in the *v*BNST. This is unlikely to be a consequence of norepinephrine transmission restricted to a synaptic compartment because considerable evidence indicates that it communicates extrasynaptically in the BNST (31; 57). Indeed, electrophysiological studies have shown that noradrenergic neurons in the LC respond to novelty but lack a sustained response to stimuli (58;59).

To investigate whether selective uptake inhibitors affect ICSS, latency to press following DMI (NET inhibitor) or GBR 12909 (DAT inhibitor) was examined. In the absence of drug, the latency to lever-press was quite short because the animals were well trained. Overall, DMI significantly increased the latency to lever-press (Fig. 5C), consistent with the finding that acute DMI causes a decrement in ICSS reward (60). Following GBR administration, the latency to lever-press decreased but not significantly ($P > 0.05$, n = 5 animals), presumably because of a ceiling effect. Supporting this assumption, in rats with an average latency to press of more than1.0 s (example in Fig. 6) or who had previously received DMI, the latency was significantly decreased after GBR. Consistent with this finding, GBR has been shown to increase responding for ICSS reward (61).

Opposing Catecholamine Changes during Extinction of ICSS

Since identical dopamine responses were obtained during ICSS in the *dl*BNST as previously obtained in the NAc (16), we compared our previous dopamine results to those for norepinephrine in the *v*BNST. During ICSS extinction, the decrease in lever-pressing was accompanied by a significant decrease in cue-evoked phasic dopamine in the NAc shell across trials (16), similar to that seen during extinction of cocaine self-administration (53). In addition, extracellular dopamine decreased at the time when the electrical stimulation should have occurred, consistent with electrophysiological data demonstrating reward prediction error (62;63).

A role for norepinephrine in the behavior observed during extinction of ICSS has long been suspected. During extinction of ICSS, animals with lesions of the locus coeruleus pressed the lever with more vigor and over a prolonged period compared to intact animals (64). This response was attributed to a failure of locus coeruleus-lesioned rats to pay attention to

relevant cues. Subsequent research employing lesioned animals have provided support for this hypothesis (65), consistent with the view from electrophysiological studies in intact animals that norepinephrine neuronal systems are important in paying attention to the surrounding environment (66). Further, norepinephrine acting at central β-adrenergic receptors has been suggested to be important in the retrieval of drug-associated memories following extinction (67;68). Regardless, our data supports a role for norepinephrine during extinction. During ICSS, the 2 s cue predicts impending reward availability and results in a dopamine concentration transient without a change in norepinephrine. However, during extinction animals now learn that the 2 s cue predicts the absence of reward following a lever-press. This is associated with a norepinephrine surge. It is unlikely that this surge acts as a cue-associated signal since it occurs after the 2 s cue and any cues associated with the press itself (e.g., lever depression, click of the lever etc). A signal at the lever press is critical so that it can trigger a decline in goal-directed behavior; this requires new learning concerning the prediction of non-reward, a process that has been linked to norepinephrine (69; 70).

Alternative hypotheses must also be considered. For example, the *v*BNST receives noradrenergic input from the nucleus of the solitary tract as well as the locus coeruleus (71). These two nuclei have been shown to have quite different responses during aversive events such as opiate withdrawal (25), and, in the present experiments, we cannot distinguish which nuclei is the primary contributor to the measured norepinephrine release. In addition to its role in attention, norepinephrine release has also been associated with aversive stimuli. Using the same measurement approach, we previously showed that norepinephrine release was evoked by intraoral quinine infusion, an aversive tastant (23). Because the omission of reward can be considered aversive, this may also be the origin of the norepinephrine surge during ICSS extinction. In the prefrontal cortex, extracellular norepinephrine increases following both aversive and rewarding stimuli (72). However, those changes last for longer than an hour and it is unclear whether the rapid changes we report here have a similar origin.

Conclusion

Subsecond recordings of catecholamines in subregions of the BNST during ICSS reveal that time-locked catecholamine changes occur during this reward-seeking behavior. In a manner quite similar to events in the NAc shell, phasic dopamine release in the *dl*BNST was evoked by a cue that had become associated with ICSS. In contrast, there was no evidence of cue-evoked, phasic norepinephrine in the *v*BNST during ICSS. Both catecholamines were released by the stimulation as a consequence of the positioning of the stimulating electrode in the VTA/SN/VNB. During extinction, the activity of both catecholamines was dramatically altered. Dopamine release to the cue diminished during ICSS extinction and the dopamine concentration actually decreased following the lever-press. At this time, when the cue no longer predicts impending ICSS availability and there is new learning about the consequence of the lever-press, extracellular norepinephrine increased. These data support the hypothesis that norepinephrine plays a central role in responses to extinction. Furthermore, the data demonstrate that catecholamine neurons in subregions of the BNST encode opposite aspects of learning during goal-directed behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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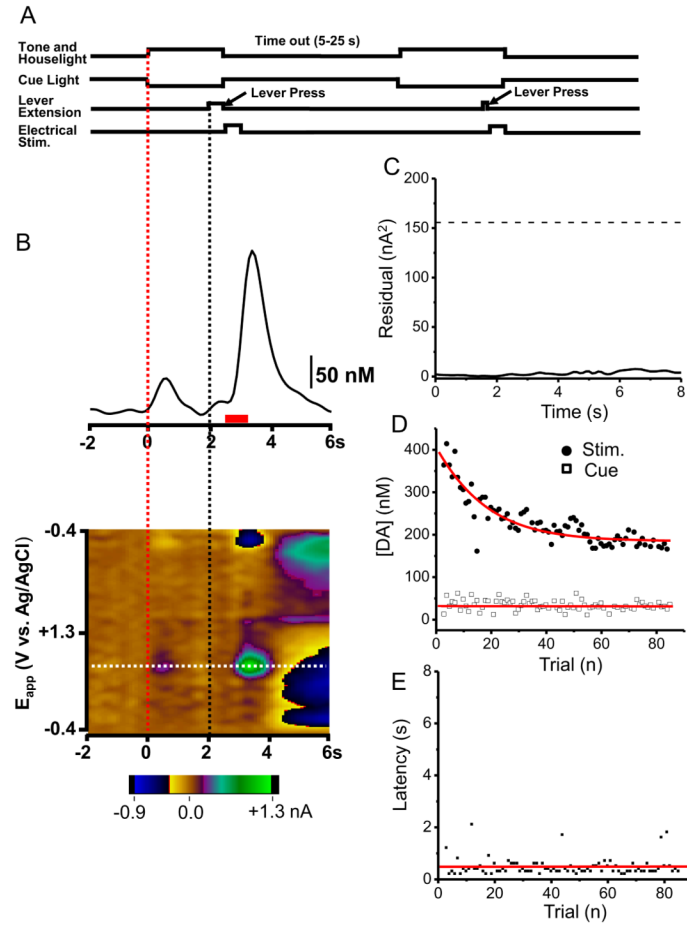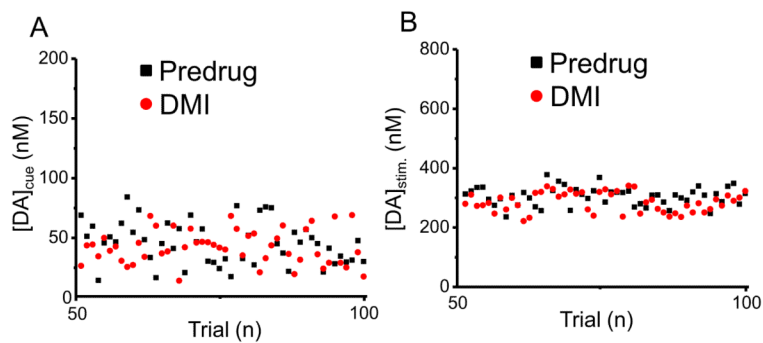
Figure 1.
Dopamine changes in the *dl*BNST during maintenance-delay ICSS. (A) Temporal sequence of task. (B) Upper; a representative temporal dopamine concentration trace from a single trial during maintenance-delay ICSS. Principal component regression was used to extract the time course of the dopamine concentration transients. Dopamine release occurs immediately following the cue (t = 0, red dotted line) and again after the lever-press (lever out at 2 s, black dotted line). Red bar shows the stimulus duration. Lower: two-dimensional color plot representation of the background subtracted cyclic voltammograms collected 2 s before cue and 6 s after the lever extension. Catecholamine concentration changes are apparent in color plots at the potential for its oxidation (~ 0.65 V, white dashed line) and its reduction (~ −0.2 V). (C) Residual from principal component analysis. (D) Cue- and lever-press-evoked dopamine release across trials. (E) Latency to press the lever across trials. Stimulus parameters: 60 Hz, 40 biphasic pulses, 2 ms pulse width.
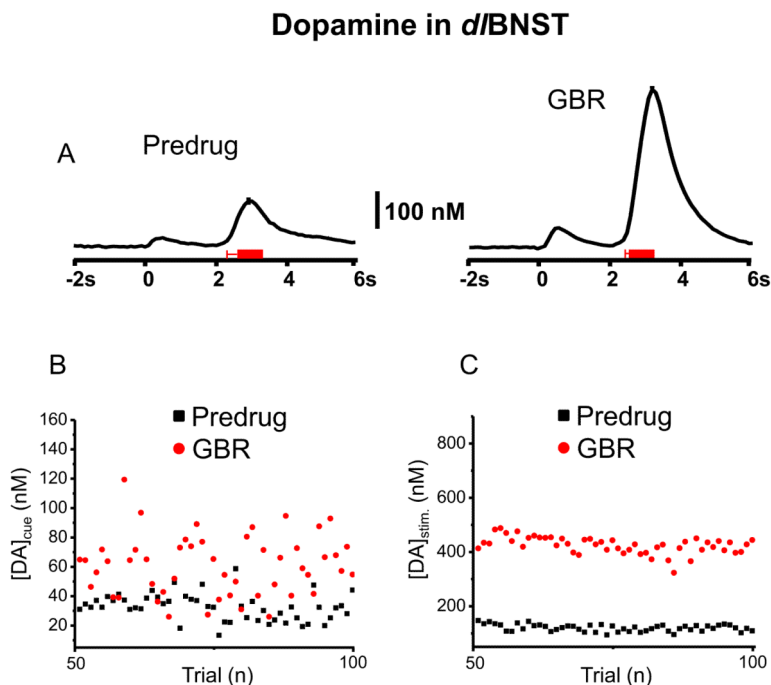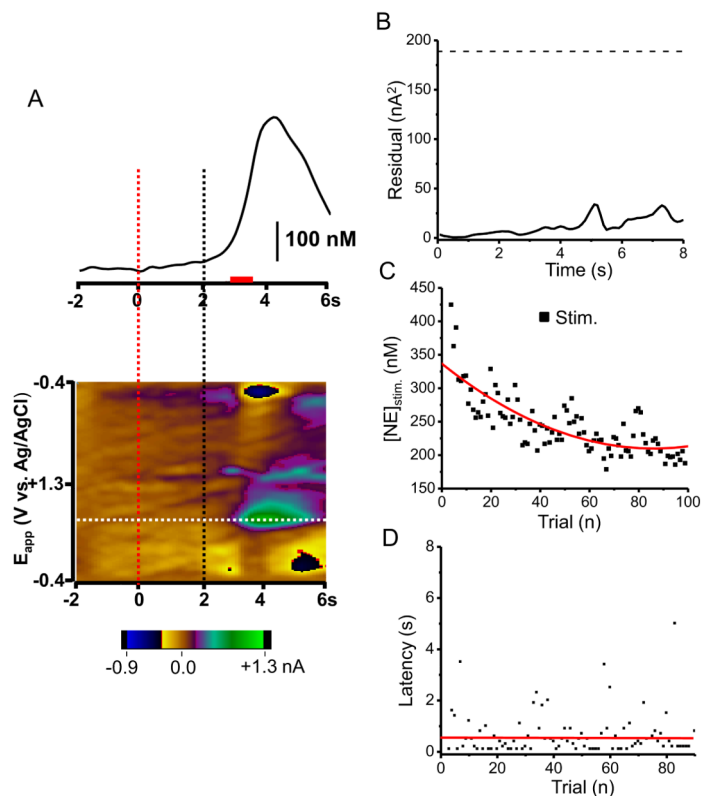
Figure 2.
Dopamine increase in the *dl*BNST during maintenance-delay ICSS after administration of GBR. (A) Average dopamine concentration traces with a representative ± S.E.M. and two-dimensional color plots from 50 trials before drug session (predrug, left) and 20 min after the administration of GBR (15 mg/kg, i.p., right). Principal component regression was used to extract time course of the dopamine concentration traces. The red bars under the current traces denote the average onset and duration of electrical stimulation. (B) Cue- and (C) lever-press-evoked dopamine concentration changes across trials.

Dopamine in *dl*BNST

Figure 3.
Dopamine concentration changes in the *dl*BNST and latency to lever-press during maintenance-delay ICSS before and after administration of DMI. Responses are only shown for 50 trials after the initial decay in stimulated release had occurred. Maximum dopamine concentrations following (A) the cue and (B) the lever-press as a function of trial number.
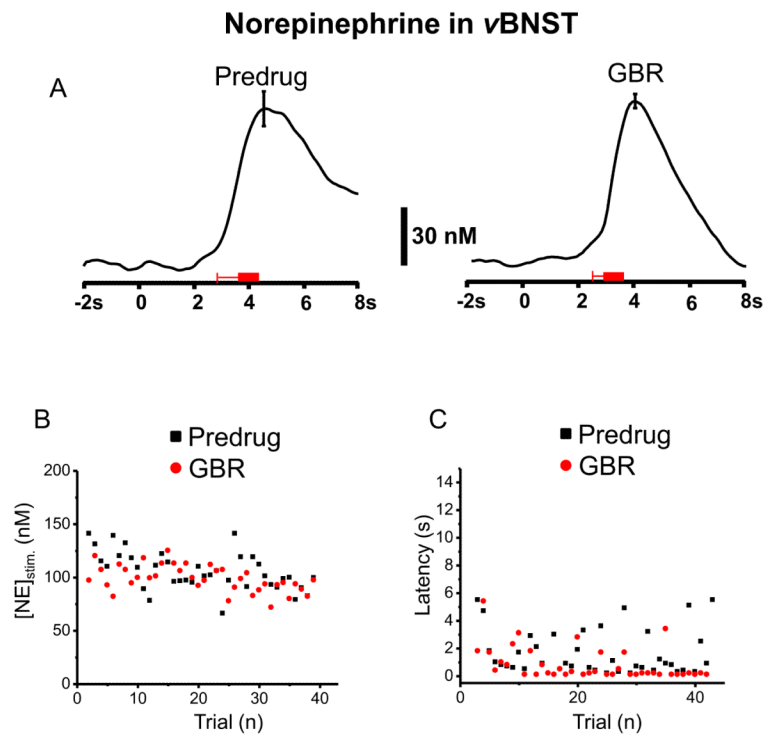
Figure 4.
Norepinephrine changes in the *v*BNST during maintenance-delay ICSS. (A) Upper; a representative temporal norepinephrine concentration trace from a single trial during the task. Principal component regression was used to extract time course of the norepinephrine concentration transients. Norepinephrine was evoked after the lever-press (lever out at 2 s, black dotted line) but not after the cue (t = 0, red dotted line). Red bar shows the stimulus duration. Lower; Two-dimensional color plot representation of the background subtracted cyclic voltammograms collected 2 s before cue and 6 s after the lever extension. Catecholamine concentration changes are apparent in color plots at the potential for its oxidation (~ 0.65 V) and its reduction (~ −0.23 V). (B) Residual from principal component regression. The dashed line shows the level where 95% of the noise is anticipated. (C) Norepinephrine concentration changes evoked by self-delivered stimulation in consecutive trials. (D) Latency to press the lever in consecutive trials.
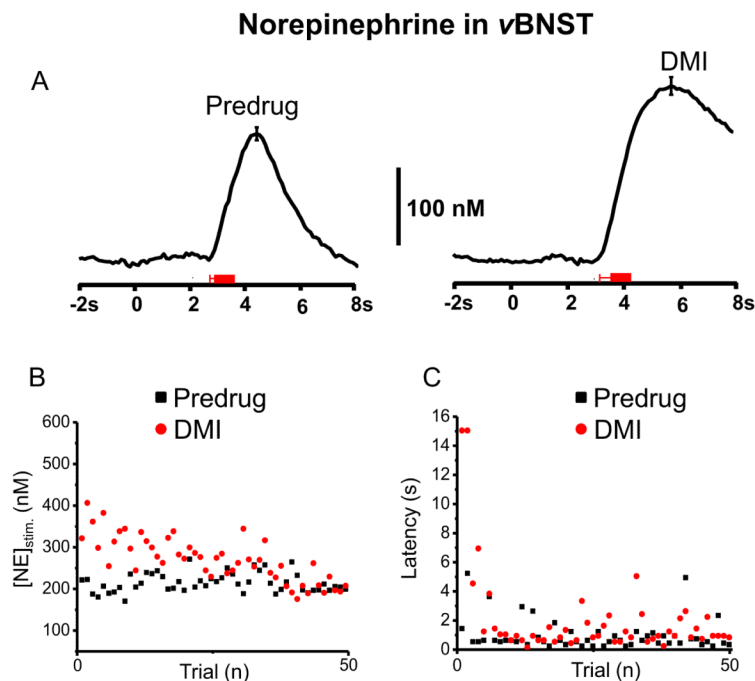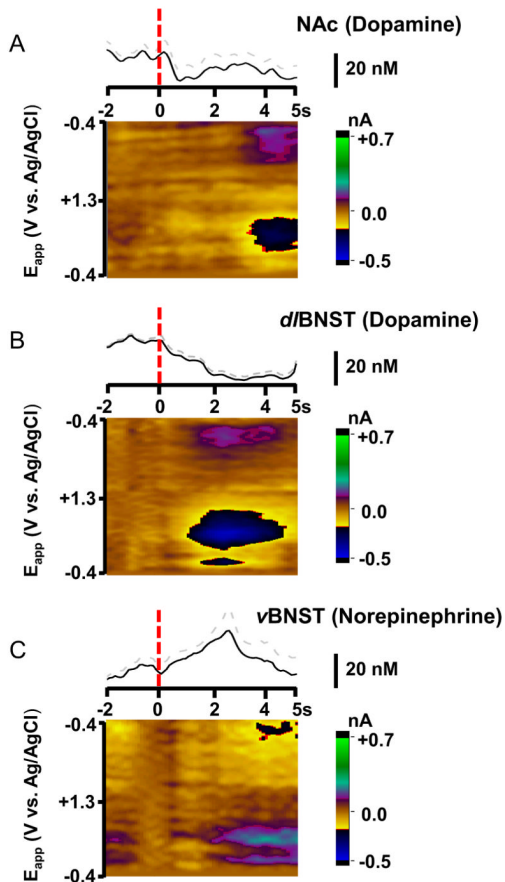
Figure 5.
Norepinephrine increase in the *v*BNST during maintenance-delay ICSS after administration of DMI. (A) Average norepinephrine concentration traces with a representative ± S.E.M. from ~ 50 trials before drug session (predrug, left) and 20 min after the administration of DMI (DMI, 15 mg/kg, i.p., right). The red bars under the current traces denote the average onset and duration of electrical stimulation. Principal component regression was used to extract the time course of the norepinephrine concentration traces. (B) Lever-press-evoked norepinephrine concentration change and (C) the latency of lever-press across trials.

Figure 6.
Norepinephrine concentration change in the *v*BNST and latency of the lever-press during maintenance-delay ICSS after administration of GBR. (A) Average norepinephrine concentration traces with a representative ± S.E.M. and from ~ 50 trials 20 min after the administration GBR (15 mg/kg, i.p.). The red bars under the current traces denote the average onset and duration of electrical stimulation. Principal component regression was used to extract time course of the norepinephrine concentration traces. (B) Lever press-evoked norepinephrine concentration change and (C) the latency of lever-press across trials.

Figure 7.
Catecholamine responses in the NAc (A), *dl*BNST (B) and *v*BNST (C) during ICSS extinction. Each data set is averaged from a single animal. The concentration profiles (displayed as mean and S.E.M.) above the 2D color plots were extracted using principal component analysis. Time of lever-press is indicated by the red dotted line. In extinction trials lever-press was not rewarded with electrical stimulation.