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Norepinephrine and Dopamine Transmission in Two Limbic Regions Differentially Respond to Acute Noxious Stimulation

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Summary

Acute tail pinch induces distinct changes in limbic catecholamine neurotransmission, which may promote the physiological and behavioral responses necessary for survival.

Keywords

norepinephrine; dopamine; ventral bed nucleus of the stria terminalis (*v*BNST); fast-scan cyclic voltammetry; noxious stimuli; nucleus accumbens (NAc) shell

1. Introduction

The motivational, hedonic and associative aspects of pain are encoded by the brain's limbic system, a heterogeneous collection of structures positioned at the interface of the hindbrain and cortex. Limbic function is supported by catecholamine innervation from the dopaminergic cells of the ventral tegmental area (VTA) and periaqueductal gray (PAG) [24; 54], as well as the noradrenergic cells of the locus coeruleus (LC), nucleus of the solitary tract (NST, A2) and A1 group [20; 41]. Indeed, a number of animal studies have demonstrated that these cells respond to noxious stimuli [8; 23; 59]. The modulatory actions of catecholamine neurotransmission are moreover implicated in the physiological and behavioral reactions to pain [3; 46], and are associated with disorders often comorbid with pain dysregulation such as drug addiction and depression [13; 18; 64].

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The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the article.

Measuring the neurochemical changes induced by acute pain is challenging due to the temporo-spatial dynamics of rapid neurotransmitter release. Unsurprisingly, past studies have produced controversial results [2; 4; 27; 45]. *In vivo* fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes can capture real-time changes in extracellular catecholamines within the substructures of the brain [43; 50]. Recently we employed this methodology in anesthetized animals to observe striatal dopamine responses during a noxious 3 s tail-pinch [10]. We found that tail-pinch elevated extracellular dopamine throughout the nucleus accumbens (NAc), a VTA-innervated limbic structure involved in motivational salience and reward processing. The temporal aspects of this response, however, were subregion-dependent. Dopamine increased during the stimulation in the NAc core but only at the stimulus termination in the NAc shell, demonstrating that accumbal dopamine release differentially encodes information regarding the presence and cessation of a noxious stimulus.

Interestingly, we failed to identify a striatal region where dopamine activity decreased with tail-pinch. This is unexpected as electrophysiological recordings have identified a subset of VTA dopamine neurons that are inhibited by pain [8; 59; 65]. The absence of this response pattern in our previous data prompted this reinvestigation of the NAc shell, where no discernable dopamine change was observed during the tail-pinch. We furthermore extend our study to consider norepinephrine responses in the ventral bed nucleus of the stria terminalis (*v*BNST), a spatially contiguous limbic structure. Norepinephrine release in this region, arising primarily from NST and A1 cell groups, is involved in the affective component of pain and facilitates stress responses through upregulation of HPA axis activity [15; 19; 20].

Using pharmacological agents to enhance catecholamine signaling, we found that tail-pinch has diverse effects on dopamine concentrations in the NAc shell and that, in most locations, dopamine release is inhibited during the noxious stimulus. In the *v*BNST norepinephrine release increased with tail-pinch but, unlike dopamine responses in the NAc shell, did not respond to the termination of the stimulus in a time-locked manner. These results demonstrate that dopamine in the NAc shell and norepinephrine in the *v*BNST process noxious stimulation via disparate, and in the case of dopamine in the NAc shell non-uniform, signaling patterns. The integration of these responses may act to initiate survival behaviors.

2. Materials and Methods

2.1. Animals

Male Sprague-Dawley rats (300 – 400 g, Charles River Laboratories, Wilmington, MA) were used for these studies. Animals were housed in a controlled humidity and temperature environment with a 12:12 hour light:dark cycle. Food and water were available *ad libitum*. Experiments were conducted between 9:00 am and 5:00 pm. All procedures for handling and caring for the laboratory animals were in accordance with the NIH Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina and Wake Forest University.

2.2. Surgery

Animals were maintained at a body temperature of 37°C with a heating pad (Harvard Apparatus, Holliston, MA) during surgical procedures, which have been described previously [38; 41]. Briefly, animals were anesthetized with urethane (1.5 g/kg, i.p.), immobilized in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and their dorsal skulls exposed. Hole were drilled using coordinates relative to bregma obtained from the atlas of Paxinos and Watson [44]. Placement of the carbon-fiber microelectrode targeted either the NAc shell (anteroposterior [AP] +1.8 mm, mediolateral [ML] +0.8 mm) or the *v*BNST (AP 0.0 mm, ML +1.2 mm). In every animal, a bipolar, stainless-steel electrode (0.2 mm in diameter, Plastics One, Roanoke, VA) was lowered into the VTA/ventral noradrenergic bundle (VNB, AP −5.2 mm, ML +1.2 mm, dorsoventral [DV] −8.0 to −9.0 mm). Electrical stimulation (24–60 biphasic pulses, 300 μA, 2 ms/phase, 60 Hz) of these coordinates, which was applied via a pair of optically-isolated stimulators (NL 800A, Neurolog, Digitimer Ltd, Hertfordshire, UK) in this study, activates both the noradrenergic axons coursing through the VNB as well as the dopaminergic cell bodies of the VTA [42]. In the hemisphere contralateral to the carbon-fiber and stimulating electrodes a Ag/AgCl reference electrode was implanted and secured to the skull with a jeweler's screw.

2.3. Voltammetric procedures

Fast-scan cyclic voltammetry was computer-controlled as described in detail previously [25]. A triangular scan (−0.4 to +1.3 V, 400V/s) was applied to a glass-sealed carbon-fiber microelectrode (75−100 μm exposed tip length, 7 μm diameter, T-650; Amoco, Greenville, SC) [11] every 100 ms to electrochemically detect catecholamine changes. All electrodes were preconditioned with the waveform (15 min at 60 Hz, 15 min at 10 Hz) before voltammetric measurements commenced. Background-subtracted cyclic voltammograms were obtained by digitally-subtracting voltammograms collected during stimulation from those collected during baseline recording. Voltammetric responses were viewed as color plots with the abscissa as voltage, the ordinate as acquisition time, and the current encoded in color [34]. Currents were converted to concentration based on averaged *in vitro* calibration factors (6.9 ± 0.3 pA/(μM·μm^2 for dopamine, 4.5 ± 0.2 pA/(μM·μm^2) for norepinephrine) obtained from a separate set of electrodes previously used *in vivo*.

2.4. Chemicals and drugs

All chemicals and drugs were obtained from Sigma-Aldrich (St. Louis, MO, USA) and were used without further purification. Calibration of the carbon-fiber microelectrodes with pH, dopamine, and norepinephrine were made after *in vivo* recordings 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 in double distilled water (Mega Pure System, Corning Glasswork, Corning, NY). Desipramine-HCl, raclopride-HCl, and idazoxan-HCl were dissolved in sterile saline. GBR 12909 was dissolved in water and diluted in saline. Injected volumes were ~ 0.6 mL and were given intraperitoneally (i.p.).

2.5 Noxious Stimulation

As in our previous study [10], tail pinch was conducted with soft rubber gloves to avoid tissue damage and electrical noise artifacts. During each stimulus, the tail of the rat was pressed between the thumb and the index finger for 3 s with maximal pressure (P) of 3.12 ± 0.62 MPa. P was calculated by measuring the contact area between the fingers and the tail of the rat and by a measurement of the applied force using a Pasco CI-6537 Force Sensor (Roseville, CA).

2.6 Histology

At the end of the experiment, rats were euthanized with an overdose of urethane (2.0 g/kg) and electrode placements were verified by electrolytic lesions made by applying constant current (20 μA for 10 s) to the carbon-fiber microelectrodes [41]. Brains were removed and stored in 10 % formalin solution for at least a week before being coronally sectioned into 50 m thick slices on a cryostat. The sections were then mounted on slides and examined under a light microscope to verify carbon-fiber placements within the NAc shell or *v*BNST.

2.7 Data analysis

Catecholamine concentration changes in response to tail pinch were quantified using a locally-written principal component regression algorithm [28]. A residual analysis procedure was used to validate the predicted concentrations, and any trials containing uncharacteristic variance larger than 95% of the noise of the training set were discarded. All data were background subtracted from the time point of lowest concentration in the 5 s prior to tail pinch. Data within this 5 s time window were subsequently averaged to determine baseline concentrations and noise levels. A shorter, 1 s time frame was used as baseline for post-drug analysis in NAc experiments due to the presence dopamine transients (see section 3.2). In every experiment, peak catecholamine concentration changes were determined relative to baseline values for the period during and 5 s after noxious stimulation. Responses were considered significant if their magnitudes were larger than 3 times the standard deviation of the noise (S/N ≥ 3). Mean values were compared in GraphPad Software version 4.0 (San Diego, CA, USA) by using the two-tailed Student's *t*-test to calculate the level of significance. Analysis employing Fisher's exact test was conducted through GraphPad's QuickCalc website [1]. $P < 0.05$ was regarded as statistically significant. Data are represented as mean ± S.E.M. and '*n*' values indicating the number of rats.

2.8 Experimental overview

At the beginning of each experiment a fresh carbon-fiber microelectrode was lowered into the NAc shell (DV −5.8 to −7.4 mm) or the *v*BNST (DV −7.2 to −7.7 mm). After waveform preconditioning (see section 2.2), extracellular catecholamine changes were monitored voltammetrically during application of a 3 s tail pinch. Reactions such as ear or whisker twitches to the tail pinch were tested to ensure the animals were fully anesthetized before these measurements commenced. For dopamine experiments, the effects of noxious stimulation were assessed at different depths of the NAc shell by lowering the carbon-fiber microelectrode in 0.2 – 0.3 mm increments and repeating the tail-pinch procedure. For norepinephrine experiments, voltammetric measurements were confined to a single location

per animal given the limited depth range (~500 μm) of the *v*BNST. In both NAc and *v*BNST, tail pinch was repeated 3 – 6 times (each referred to as a 'trial') for each recording depth with a 2–3 minute rest period between noxious stimulations.

In some animals selective autoreceptor and transporter blockers (Rac+GBR for dopamine experiments, IDA+DMI for norepinephrine experiments) were administered as separate i.p. injections (5 min apart) after control responses were recorded. Catecholamine responses to tail pinch were again assessed after sufficient time had passed for the drugs to exert their maximum effects (20–80 min for Rac+GBR, 30–40 min for IDA+DMI) [4; 38]. Control and drug response magnitudes were only recorded at the depth in which the electrode was positioned when the drugs were administered. Subsequently, the effects of the drugs on tail-pinch were qualitatively examined as the electrode was raised in 0.2–0.3 μm increments in the NAc shell, but for only one depth of the vBNST.

At the end of each experiment catecholamine release was mapped for recording regions through electrical stimulation. The site of recording was lesioned before the animal was sacrificed and its brain fixed for histology (see section 2.6).

3. Results

3.1. Rapid extracellular dopamine changes in the NAc shell elicited by tail pinch

A fresh carbon-fiber microelectrode was lowered through the NAc shell to evaluate changes in extracellular dopamine concentration in response to the noxious stimuli (tail pinch). Figure 1A (left panel) shows the coronal plane in which measurements were made (AP ~ +2.0 mm) in the NAc shell with the approximate electrode track marked by the solid line. Our previous study showed that maximal dopamine release is not observed until the electrode is positioned within the depths of the NAc shell (− 6.0 mm to − 7.0 mm below the skull) [38]. Recording location was verified by electrolytic lesion at the end of the experiment (Fig. 1A, dashed white circle). Only dopamine signals recorded in the NAc shell were used in this study. To characterize the distribution of dopamine release sites in the NAc shell, dopamine release evoked by a bipolar electrical stimulation (60Hz, 24 pulses, 300 μA) of the VTA and the VNB was measured at different depths with fast-scan cyclic voltammetry.

Once the electrode was positioned in the NAc shell, changes in extracellular dopamine concentration in response to tail pinch were measured at multiple recording depths (Fig. 1B, single animal data). The time course of dopamine concentration changes was obtained from the oxidation peak of the voltammograms (~ +0.65 V). The dopamine responses to tail-pinch were much smaller than the concentration changes that occurred with electrical stimulation. At each recording depth tail pinch (at t = 0, 3 s duration, denoted as red bar) was repeated at least 3 times. In the example animal extracellular dopamine levels rapidly decreased on average during the tail pinch (t = 0 to 3 s). Once the stimulus ceased dopamine concentrations returned or, in some locations, spiked above pre-stimulus basal levels (t = 3 s). Apparent variation in the initial time of a dopamine response is most likely due to human error as the tail pinch was administered manually. At the end of each experiment electrical stimulation of the VTA was used to confirm dopamine release in each location. Tail pinch

data are only presented for depths that exhibited dopamine release with electrical stimulation.

Three distinct response types (Fig. 2) occurred with tail pinch among locations in the NAc shell, in which dopamine concentrations either decreased during the tail pinch (monophasic decrease), transiently increased at the cessation of the stimulation (monophasic increase), or exhibited both of these components (biphasic). The features of these responses could be observed in many (Fig. 2A) but not all (Fig. 2B) trials conducted at a single recording depth as the magnitudes of the dopamine changes were often close to or under the signal-to-noise threshold (S/N ≥ 3). However, the dopamine response pattern for a single recording location became more evident when data over several trials was averaged (Fig. 2C). It is important to note that many recording sites in the NAc shell exhibited no measurable dopamine response to tail pinch even though release could be evoked by electrical stimulation. This is likely due in part to the sedative effects of urethane anesthesia. In fact, significant dopamine responses to tail pinch were only found for half of the recording locations within the NAc shell under control conditions (n = 7 animals, summarized in Table 1).

3.2. Effects of dopamine autoreceptor and transporter inhibition on tail-pinch induced responses in the NAc shell

Effects of the selective dopamine uptake inhibitor GBR 12909 (GBR, 15 mg/kg) and the D2 autoreceptor antagonist raclopride (Rac, 2 mg/kg) on dopamine signaling to tail pinch were investigated. Figure 3 shows dopamine responses to tail pinch before (control) and after drug administration (Rac+GBR) for different recording locations in the NAc shell. Under each dopamine concentration profile, voltammetric data is shown in a false-color plot. The oxidation and reduction potentials for dopamine are indicated by dashed and solid white lines, respectively. The time course of dopamine concentration changes was obtained from the oxidation peak (~ +0.65 V) of the voltammograms.

Administration of Rac and GBR had two major effects. First, the combined administration of these drugs induced spontaneous dopamine transients (clearly seen in Fig. 3A), consistent with previous studies in the NAc shell of anesthetized animals [38; 42]. Secondly, D2/DAT inhibition enhanced dopamine overflow dynamics, significantly increasing the number of sites with detectable (S/N ≥3) responses to tail pinch (two-tailed Fisher's exact test, $P < 0.001$, Table 1). This enhancement additionally revealed biphasic dopamine signaling at many sites that appeared to respond monophasically under pre-drug conditions.

In the first animal of Figure 3 (A and B), dopamine responses were not evident during control recordings. However, with the signal gain provided by D2/DAT inhibition, a measurable dopamine response became apparent after drugs in the second (Fig. 3B) of the two sites shown. In these particular examples the length of noxious stimulation was increased from 3 to 10 s. As in other animals, the dopamine response remained time-locked to the duration of the stimulus even with extended stimulation.

In the second of the two animals shown, changes in dopamine concentration during tail pinch were small, but measurable before drug administration (Fig. 3C and D, left panels). In this animal, extracellular dopamine levels monophasically decreased to the stimulus at one

location (Fig. 3C) and changed in a biphasic manner at another (Fig. 3D). Once dopamine transients were induced through autoreceptor and transporter inhibition, these dopamine responses became pronounced (Fig. 3 C and D, right panels). Overall pre-drug dopamine responses to tail pinch (Fig. 3E, Δ[DA] = − 46.9 ± 3.9 nM during stimulus; Δ[DA] = 63.1 ± 7.0 nM post stimulus, n = 7) were significantly enhanced after dopamine drug administration (Rac + GBR: Δ[DA] = − 89.4 ± 12.9 nM during stimulus, $P < 0.05$; Δ[DA] = 630 ± 145 nM, $P < 0.01$ post-stimulus, n = 7).

3.3. Rapid extracellular norepinephrine changes in the vBNST elicited by tail pinch

A second set of experiments was conducted to investigate extracellular norepinephrine concentration changes in the *v*BNST in response to noxious stimulation. Figure 4A displays the electrode tract (left) for norepinephrine measurements in *v*BNST (shaded red), and a histological image of the *v*BNST (right) with the recording site marked by electrolytic lesion indicated by the dashed white circle. Only signals that were verified by histology to be recorded in the *v*BNST were used in this study (n = 7). Within the *v*BNST (DV ~ 7.3 mm – 7.7 mm from the skull) electrically-stimulated norepinephrine release reached a maximum at a depth of ~7.5 mm as described previously [26; 41]. Figure 4B displays norepinephrine release evoked by electrical stimulation and tail pinch (left and right panels respectively, denoted by red bar) at different depths in the *v*BNST. As norepinephrine release is confined to a narrow range of depths in the *v*BNST [41; 43], data from only one recording depth was obtained for each animal.

In contrast to dopamine trials in the NAc shell, norepinephrine concentrations in the *v*BNST consistently increased during tail pinch and remained elevated briefly even after the stimulus ended (Fig. 4C, Table 1). Again, apparent variation in the initial time of norepinephrine response is due to the fact that the tail pinch was administered manually. Similar to dopamine in the NAc shell, the norepinephrine changes observed during tail pinch were smaller than that elicited by the electrical stimulation (Fig. 4B and C), but were still detectable in single trial data (Fig. 5A). The magnitude of individual responses did not significantly change after multiple noxious stimulations (Fig. 5B). Clearer norepinephrine responses were found after data was averaged for multiple trials at a single location (Fig. 5C).

3.4. Effects of norepinephrine autoreceptor and transporter inhibition on tail-pinch induced responses in the vBNST

As in dopamine experiments, the effects of the selective norepinephrine uptake inhibitor, desipramine (15 mg/kg), and α2 receptor antagonist, idazoxan (5 mg/kg), on norepinephrine transmission in response to tail pinch were investigated. Pre- and post-drug norepinephrine concentration changes are shown for two animals in Figure 6. Under each norepinephrine concentration profile, data is shown in a false-color plot of voltammetric current. The time course of norepinephrine concentration changes was obtained from oxidation potential of norepinephrine denoted on the color plots by the dashed white lines. For some anesthetized animals prominent pH shifts coincided with changes in norepinephrine concentration in the *v*BNST (Fig. 6B, pH features indicated by yellow dashed lines) [56]. Concentration data for

such trials were obtained through the use of principle component analysis (see, **Data Analysis**).

Unlike dopamine, the combined effects of autoreceptor/transporter inhibition do not induce transient norepinephrine release [42], but did significantly increase the magnitude and duration of extracellular norepinephrine elevation with tail pinch in this study. Norepinephrine transmission continued to display the same response pattern to the tail pinch even after norepinephrine drug administration (Fig. 6C, Predrug Δ[NE] = 47.1 ± 3.8 nM, n = 7), Post IDA +DMI Δ[NE] = 119.8 ± 17 nM, $P < 0.01$, n = 7).

4. Discussion

Tail pinch is a classic noxious stimulus, which may result in pain depending on its intensity [20]. In rodents this mild stressor generates a state of arousal that can facilitate motivated behaviors such as feeding, copulation and maternal behaviors [5; 29; 55]. In anesthetized animals where affective and cognitive aspects of pain are dampened, noxious sensory input can still evoke measurable alterations in the neurons underling these behaviors, including those of the central catecholamine systems [8; 23].

Here we investigated the effects of a brief tail-pinch on catecholamine neurotransmission in two limbic terminal regions of the anesthetized rat brain, the NAc shell and the *v*BNST. Using FSCV and pharmacological agents to enhance release dynamics, we report new features in dopamine transmission for the NAc shell during tail pinch and, moreover, find that norepinephrine transmission in the BNST is oppositely regulated by noxious stimulation. While some intertrial variation was apparent at each recording site, neither dopamine nor norepinephrine responses showed sensitization over repeated stimulations.

4.1. Tail-pinch induced dopamine transmission in the NAc shell

As an important limbic-motor interface, the NAc has received considerable attention regarding its role during appetitive behaviors, where dopamine overflow increases during the presentation, seeking and anticipation of food reward, drugs of abuse, and intracranial self-stimulation [39; 47; 51]. While not as extensively characterized, accumbal dopamine also responds to various states of aversion [7; 36; 43; 51], and can inhibit pain [3; 58; 63]. In contrast to the general excitation of dopamine neurons by reward, electrophysiological recordings have established that the effects of noxious stimuli on VTA dopamine neurons are variable. Subpopulations of VTA dopamine neurons are excited, inhibited or unaffected by noxious stimuli such as electrical foot-shock and tail-pinch [8; 31; 59; 65]. Another subset of dopamine neurons exhibits a transient surge of activity at the offset of aversive stimuli [8; 62].

These diverse firing patterns are thought to be due to a functional separation of VTA dopamine neurons into populations that encode motivational salience versus motivational value [9; 33]. Dopamine neurons associated with motivational salience increase firing to signify the relative importance of a stimulus, while the activity of dopamine neurons relaying motivational value directly correlates to the hedonic aspects of a stimulus. Though it is often difficult to discriminate between these two signaling modalities during reward—

where salience and value are weighted similarly—aversive stimuli are both salient and of negative valence, and therefore affect the activities of these neuronal populations inversely.

This dichotomy in motivational processing is maintained in the NAc, where the NAc core is more associated with salience and attentional-orientation while the NAc shell mediates valuation of external stimuli to drive approach-avoidance behaviors [9; 52]. Supporting this idea, we previously found regional differences in accumbal dopamine transmission to acute tail pinch [10]. During the tail pinch, a salient noxious event, dopamine increased in the NAc core. At the cessation of the tail pinch, which unarguably has rewarding attributes [57], dopamine transiently increased in the NAc shell.

Oddly, we did not observe a response during the tail-pinch in the NAc shell, where one would expect the aversiveness of the stimulus to be signaled by decreased dopamine release. Here we proposed that the absence of this response in our past study was due to an insufficient limit of detection. To increase signal strength, we pharmacologically blocked the D2 autoreceptor and the dopamine transporter (DAT) respectively with raclopride and GBR12909. These drugs act to increase the amount and duration of dopamine release that occurs with neuronal activation. In combination they also cause high-frequency dopamine transients within the NAc of anesthetized animals [38; 61], which provided background activity to clearly resolve decreases in release. Consistent with our theory, more response sites were found after drugs; however it is unclear whether D2/DAT inhibition simply amplified control signaling to detectable levels or altered the activity of nonresponsive dopamine neurons.

Regardless, this investigation found that tail pinch does suppress dopamine transmission in the shell, but not at all locations. Variability in tail-pinch induced dopamine signaling in the shell was not evident in our previous study as data was averaged across depths for each animal. Within this accumbal region, dopamine either decreased at the onset of the noxious stimulus or increased at the stimulus offset, and, at some locations, exhibited both of these responses. Other locations, however, showed no discernible dopamine change to tail-pinch even after pharmacological manipulation. The type of response observed was unlikely due to differences in synaptic proximity as all of the locations reported here exhibited similar electrically-stimulated dopamine release and drug-induced transient activity.

Together three distinct release patterns were identified, corresponding to the activities reported for VTA dopamine neurons during noxious stimulation [8; 59]. Our data suggests that the NAc shell is innervated by VTA dopamine neurons that differentially encode noxious input, in contrast to the NAc core where dopamine uniformly increased during tail pinch. Variation in dopamine signaling is not unexpected for this region given that appetitive and aversive processing are believed to be confined to subdomains of the NAc shell [48]. The temporo-spatial aspects of these responses may underlie the controversial results observed in past studies.

4.2 Tail-pinch induced norepinephrine transmission in the vBNST

The BNST, a major limbic target of the central norepinephrine system, integrates descending cortico-limbic and ascending sensory information to modulate HPA axis activity

and, in turn, glucocorticoid secretion in response to stress [17]. In general norepinephrine transmission is considered a major component of the central stress response, where it acts to heighten arousal and behavioral responsivity [6; 35]. Norepinephrine in the BNST, which is densely concentrated in the ventral region, specifically acts to facilitate HPA axis output during negative emotional states [19].

The noradrenergic innervation of the BNST originates principally from the NST and A1 cell groups, though a small contribution does arise from the LC through the dorsal noradrenergic bundle [20; 30]. The medullar (NST and A1) populations are positioned to relay sensory, visceral, cardiorespiratory and nociceptive information between the peripheral and central nervous systems [46; 49]. Studies have found a role for the NST in the baroreflex to noxious thermal stimulation [22] and in the tonic control of nociceptive thresholds [32]. Furthermore, medullar catecholamine neurons are activated by visceral pain [21]. The LC, a dense pontine population of norepinephrine neurons, is also excited by a wide-range of sensory stimuli including those that present physical or psychological threat including nociception [12; 53], and in the case of persistent pain provides feedback inhibition [46].

Consistent with electrophysiological work, norepinephrine release within the BNST is enhanced by a variety of stressors such as restraint, bitter taste, reward-omission and morphine withdraw [37; 39; 43; 60]. Pain can be a particularly potent form of stress and, accordingly, somatic and visceral nociception also stimulates BNST norepinephrine release [14; 15]. Functionally, increased BNST norepinephrine transmission through β adrenergic receptors is associated with the negative affective component of pain and is required for pain-induced conditioned-place aversion [16].

Together these reports describe a positive correlation between noradrenergic cell activity and states of aversion. Congruently, we found extracellular norepinephrine concentration in the *v*BNST transiently increased to acute tail-pinch (Fig. 5). Though it was not possible to map different recording depths within the *v*BNST due to its size, similar norepinephrine responses were observed across all animals. Unlike for dopamine in the NAc, no new features in *v*BNST norepinephrine responses were revealed after pharmacological enhancement of norepinephrine release with administration of the α2 autoreceptor antagonist idazoxan and the NET inhibitor desipramine.

Interestingly, extracellular norepinephrine remained elevated briefly after the noxious stimulation ceased, and the duration of the response became further extended after transporter blockade. While few non-voltammetric studies have considered extracellular norepinephrine responses to a single, brief noxious stimulation as we have done here, microdialysis measurements have recorded basal changes in BNST norepinephrine in awake animals lasting minutes to hours beyond the presentation of a stressor [35]. This may suggest that BNST norepinephrine plays a limited role in transmitting information regarding the temporal aspects of external stimuli, and rather promotes a general change in affective state to generate the appropriate coping response to environmental challenge.

4.3 Conclusion

In summary, we demonstrated that tail pinch, an acute noxious stimulus, triggers transient changes in extracellular catecholamine levels in the NAc shell and the *v*BNST of anesthetized rats. Throughout the NAc shell, a region associated with value-driven motivational behavior, dopamine transmission was overall attenuated by the stimulation and increased by its cessation. In the BNST, a key upstream regulator of adrenal stress hormone secretion, tail pinch caused an extended increase in extracellular norepinephrine levels. The opposing effects of tail pinch we observed align with the respective roles of accumbal dopamine and BNST norepinephrine as reward and stress neurotransmitters. Moreover, the temporal differences between their responses are in agreement with the respective involvement of these two limbic structures in motor versus hormonal aspects of pain processing. Together these data demonstrate that limbic catecholamine systems are divergently recruited during a physically threatening stimulation, and may be crucial in the expression of pain-related responses.

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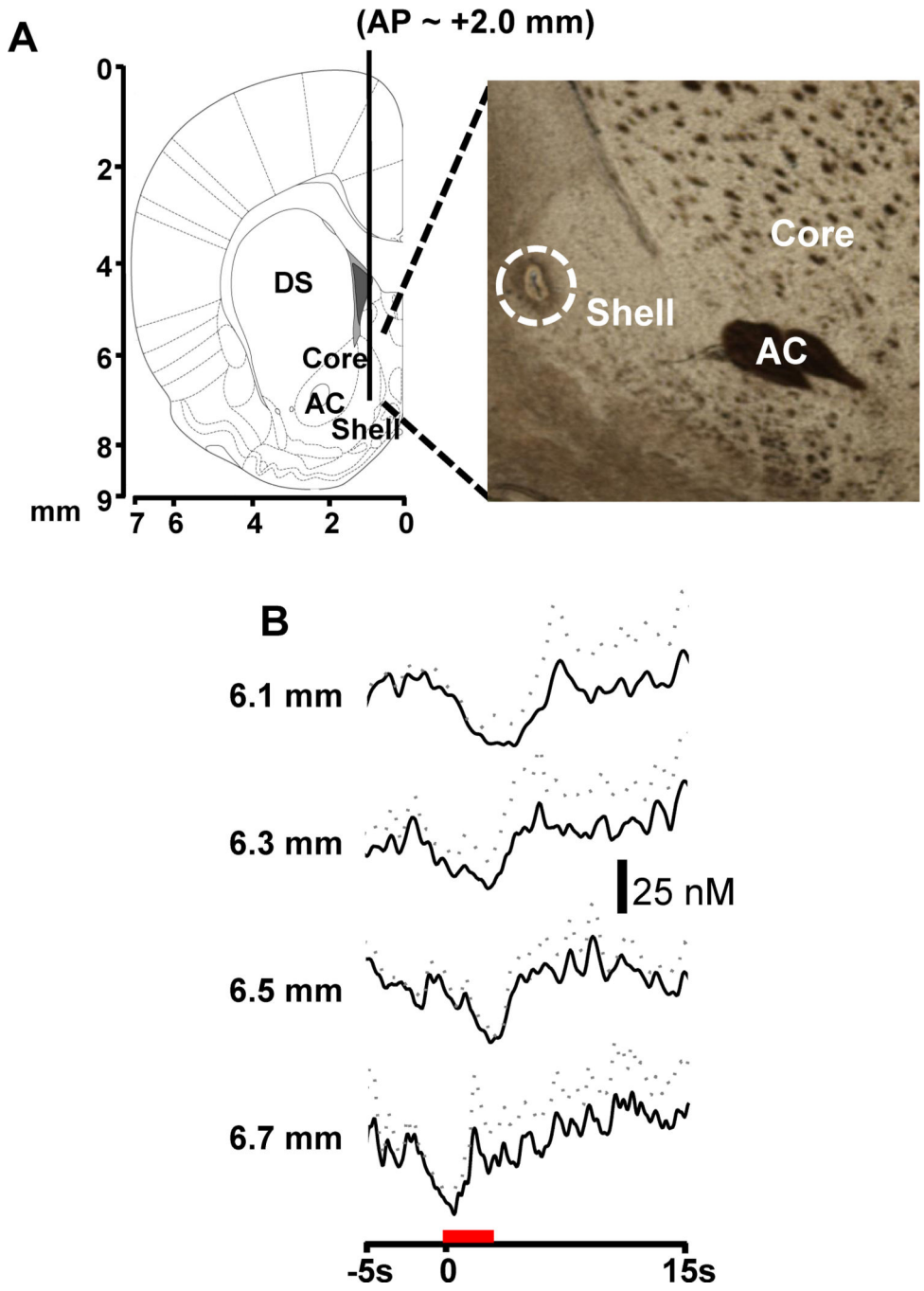

Figure 1.

Anatomical mapping of tail pinch induced and electrically evoked dopamine responses in the NAc shell. (A) Solid line in the schematic diagram illustrates the approximate path of the carbon-fiber microelectrodes in the NAc shell (left). The coronal section was modified from the atlas of Paxinos and Watson [44]. The placement of the carbon-fiber microelectrode tip is indicated by the dashed white circle, which provides histological evidence that the electrode was positioned in the NAc shell (right). AP, anterior-posterior; AC, anterior commissure. (B) Average dopamine concentration changes to tail-pinch as a function of

recording depth (n = 3 trials each). The time of tail pinch is indicated by the red bar. The dashed lines represent error as S.E.M.

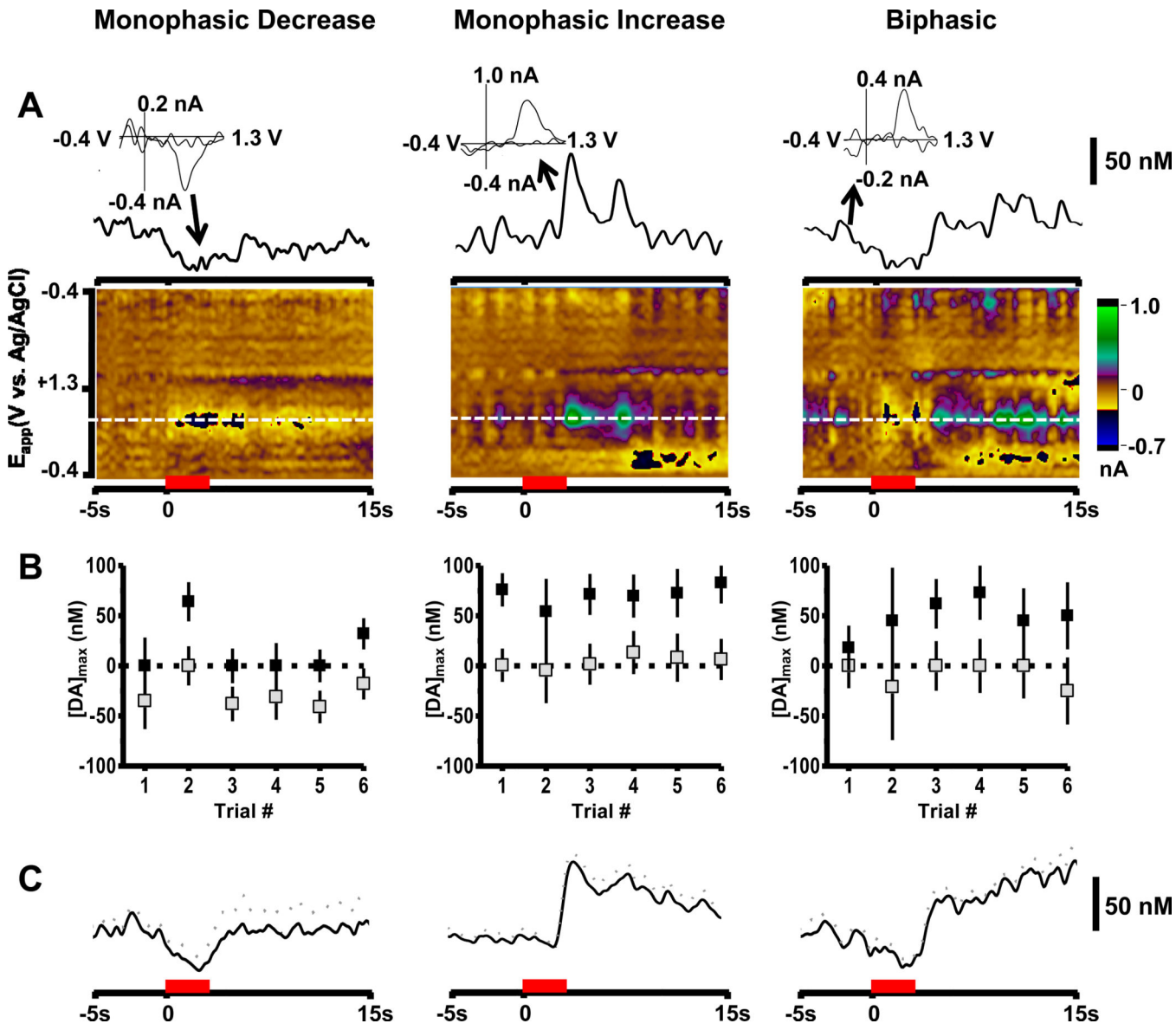

Figure 2.

Pre-drug dopamine signaling in the NAc shell in response to tail pinch. Example data are shown for the three response types (monophasic decrease, monophasic increase, biphasic), representing three separate recording locations. (A) Single trial electrochemical data. The white dashed lines in the color plots indicate the potential of dopamine oxidation (~0.65 V). Current features at this potential become apparent with the administration of the tail-pinch stimulus (time denoted by red bars) and are converted to concentration by a post-calibration factor to produce the traces above the color plots. Representative cyclic voltammograms are provided for the times marked by the arrows. Negative oxidation currents at + 0.65 V are representative of a decrease in dopamine concentrations. (B) Peak dopamine changes by trial for the same animals and same recording locations in response to tail pinch. The vertical lines through the data points indicate the noise levels (3σ) at the dopamine oxidation potential for each trial. (C) Average dopamine concentration changes for the same animals

and same recording locations (n = 6 trials each). The time of tail pinch is indicated by the red bars. Error bars are S.E.M.

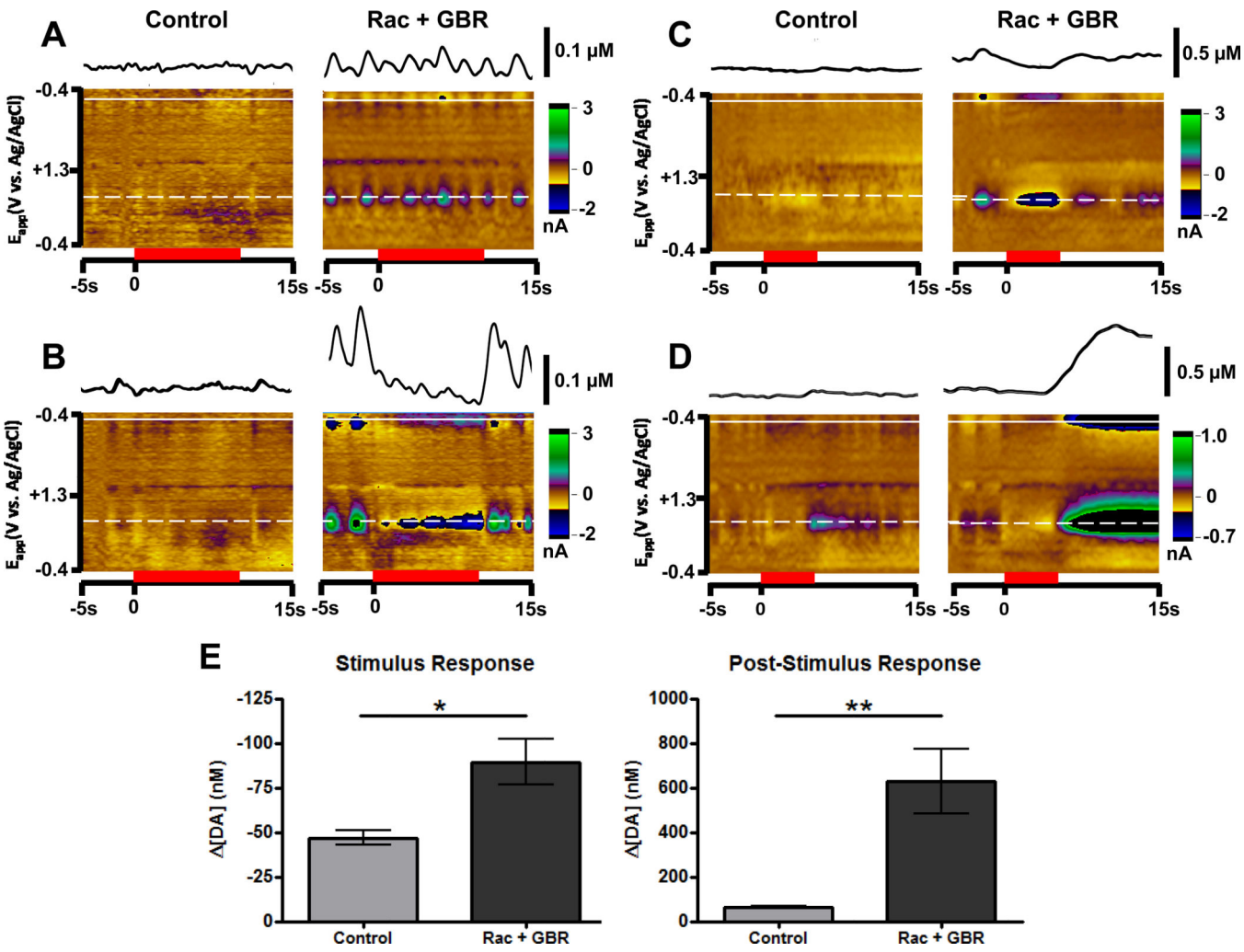

Figure 3.

Post-drug dopamine signaling in the NAc shell in response to tail pinch. Changes in extracellular dopamine were more clearly observed after administration of the selective dopamine drugs, raclopride (Rac, 2 mg/kg) and GBR 12909 (GBR, 15 mg/kg). (A and B) Tail-pinch induced dopamine responses for a single animal at two recording depths before (left) and after (right) pharmacological manipulation. Electrochemical data is provided in the color plots where the potential of dopamine oxidation (~0.65 V) is indicated by the white dashed lined and the potential of dopamine reduction (~−0.2 V) is indicated by the solid white line. Dopamine concentration changes over time are provided above the color plots. The time of tail pinch (10 s duration) is indicated by the red bars. (C and D) Tail-pinch induced dopamine responses for a separate animal at two recording depths before (left) and after (right) pharmacological manipulation. The time of tail pinch (3 s duration) is indicated by the red bars. (E) Average peak dopamine (DA) inhibition responses recorded during (left) and excitation responses after (right) tail pinch stimulation under control and post-drug conditions. *P<0.05, **P<0.01, paired student's t-test.

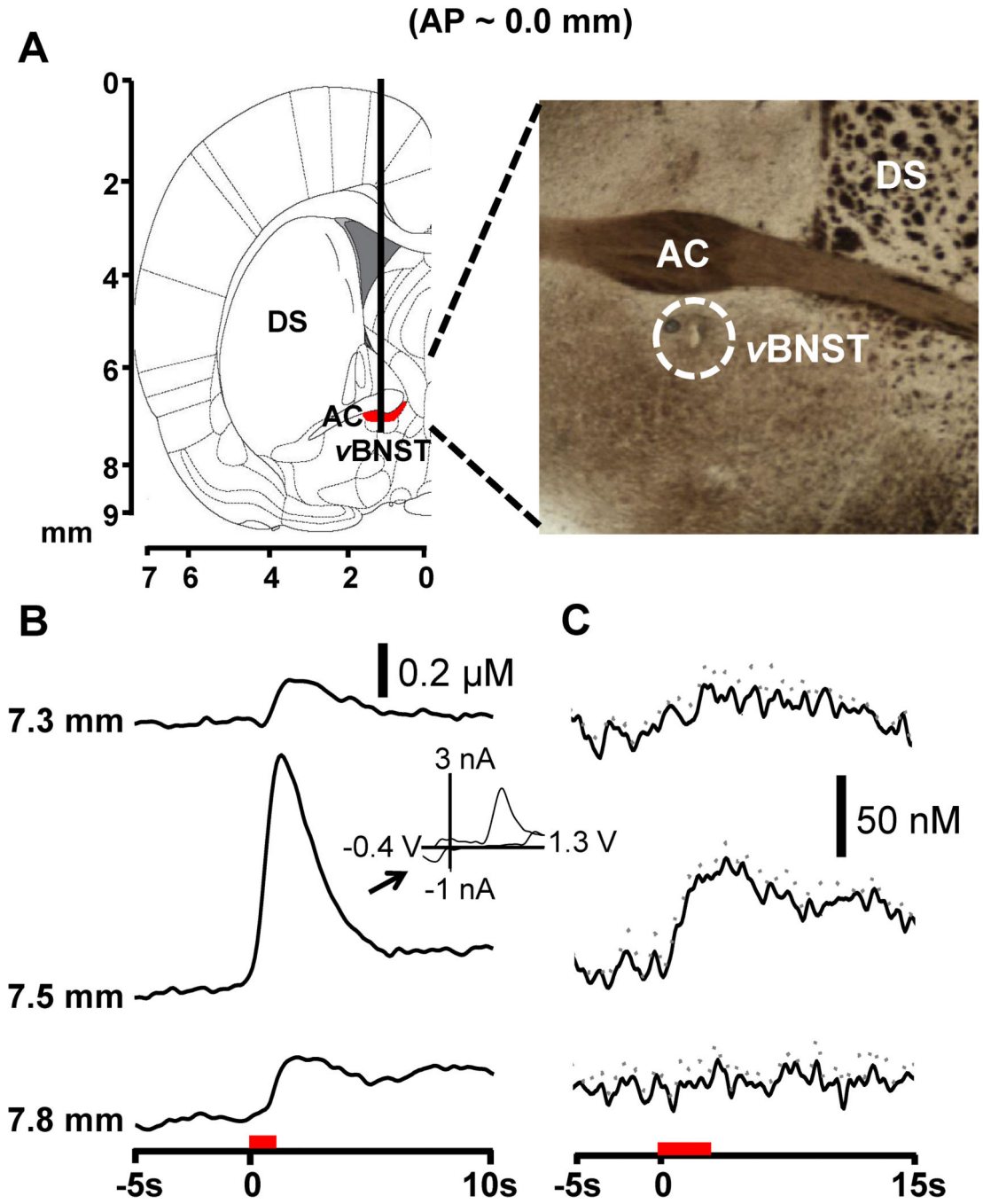

Figure 4.

Anatomical mapping of tail pinch induced and electrically evoked norepinephrine responses in the *v*BNST. (A) Solid line in the schematic diagram illustrates the approximate path of the carbon-fiber microelectrodes in the *v*BNST (shaded red, left). The coronal section was taken from the atlas of Paxinos and Watson [44]. The placement of the carbon-fiber microelectrode tip is indicated by the dotted white circle, which provides histological evidence that the electrode was positioned in the *v*BNST (right). AP, anterior-posterior; AC, anterior commissure; CPu, caudate-putamen; *v*BNST, ventral bed nucleus of the stria

terminalis. (B) Mapping of electrically evoked (60 Hz, 60 pulses, ±300 μA) norepinephrine release in the *v*BNST recorded at the depth indicated. The red bars under the current trace show the electrical stimulation time. (C) Average norepinephrine concentration changes to tail-pinch at the recording depth indicated (n = 3 trials each). The time of tail pinch is indicated by the red bar. The dashed lines represent error as S.E.M.

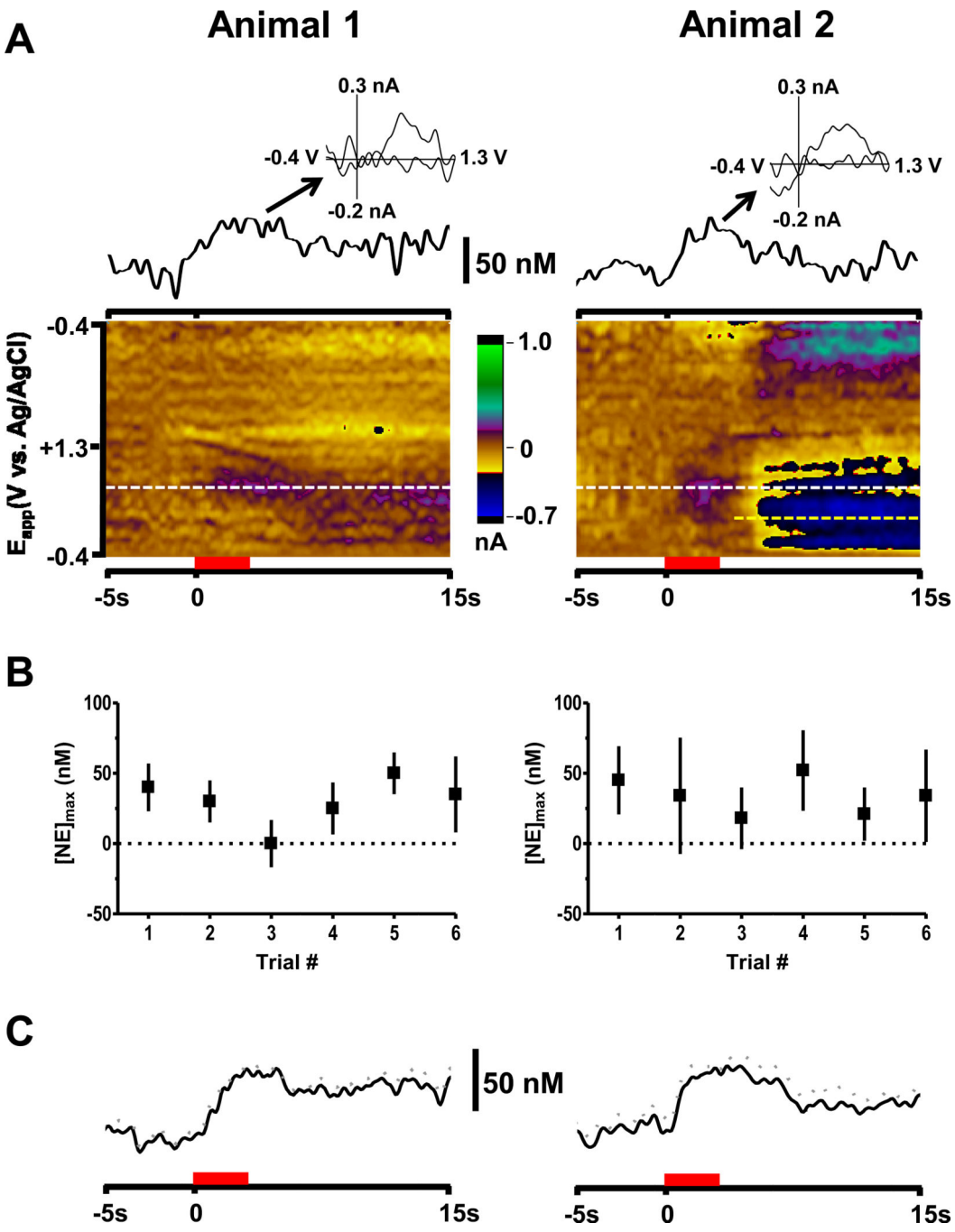

Figure 5.

Pre-drug norepinephrine signaling in the *v*BNST in response to tail pinch. (A) Example norepinephrine responses to tail-pinch for two separate animals. Single trial electrochemical data is shown. The white dashed lines in the color plots indicate the potential of norepinephrine oxidation (~0.7 V). Current features at this potential become apparent with the administration of the tail-pinch stimulus (time denoted by red bars) and are converted to concentration by a post-calibration factor to produce the traces above the color plots. Representative cyclic voltammograms are provided for the times marked by the arrows. (B)

Peak norepinephrine changes by trial for the same animals and same recording locations in response to tail pinch. The vertical lines through the data points indicate the noise levels (3σ) at the norepinephrine oxidation potential for each trial. (C) Average norepinephrine concentration changes for the same animals and same recording locations (n = 6 trials each). The time of tail pinch is indicated by the red bars. Error bars are S.E.M.

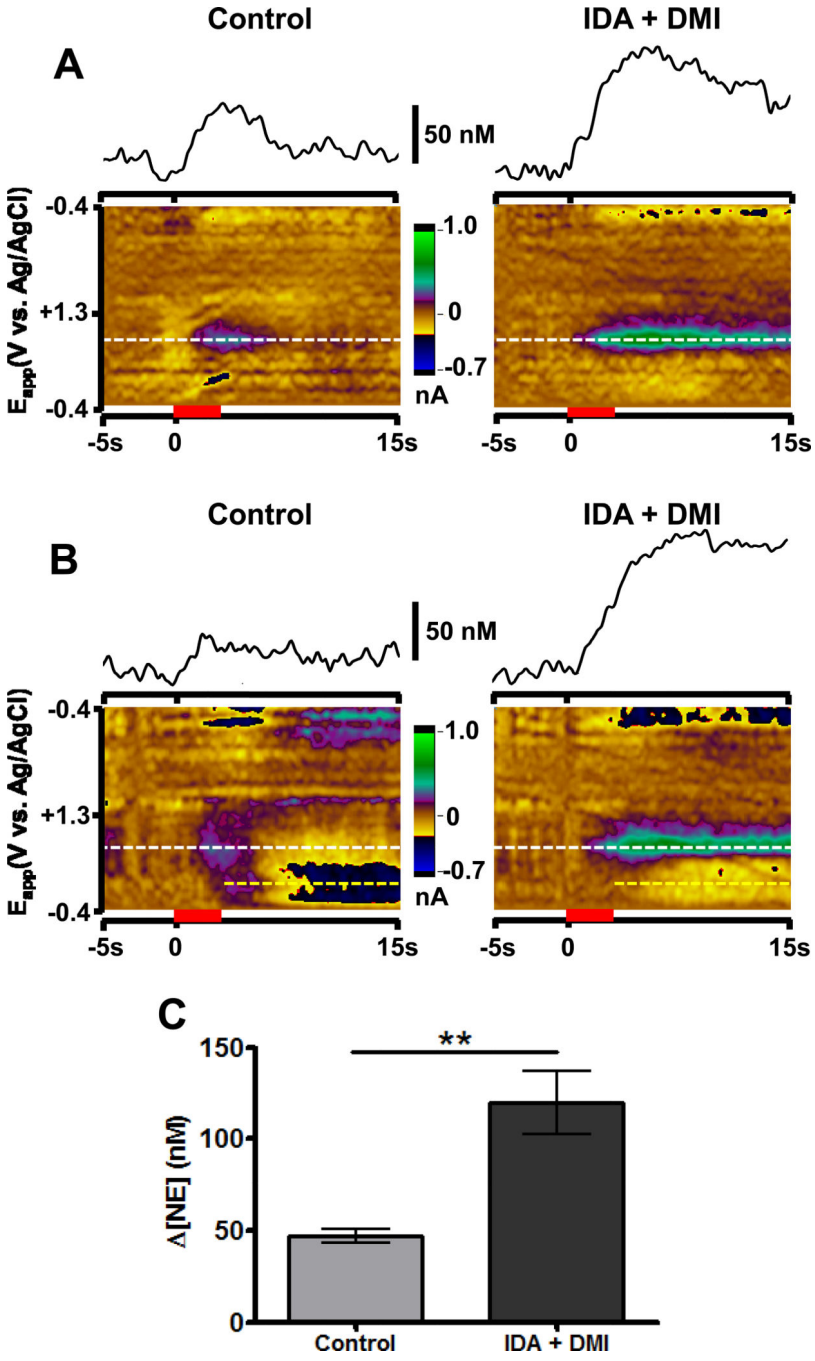

Figure 6.

Post-drug norepinephrine signaling in the *v*BNST in response to tail pinch. Norepinephrine signaling patterns were more clearly observed after administration of the selective norepinephrine drugs, idazoxan (IDA, 5 mg/kg) and desipramine (DMI, 15 mg/kg). (A and B) Tail-pinch induced norepinephrine responses two separate animals before (left) and after (right) pharmacological manipulation. Electrochemical data is provided in the color plots where the potential of norepinephrine oxidation (~0.65 V) is indicated by the white dashed lined. Norepinephrine concentration changes over time are provided above the color plots.

The time of tail pinch (3 s duration) is indicated by the red bars. (C) Average peak norepinephrine (NE) responses recorded with tail-pinch stimulation under control and post-drug conditions. **P<0.01, paired student's t-test.

Table 1

Distribution of catecholamine responses in the NAc shell (dopamine) and the vBNST (norepinephrine) before and after administration of selective autoreceptor and transporter inhibitors.

	# of sites (%)	
Response Type	**Control**	**Post-Drug[1]**
Dopamine		
No Change	26 (49)	6 (14)[§]
Monophasic Decrease	12 (23)	16 (36)
Offset Monophasic Increase	10 (19)	11 (25)
Biphasic Decrease-Increase	5 (9)	11 (25)
Norepinephrine		
Increase	7 (100)	7 (100)

[1] Post-Drug: raclopride (5 mg/kg) and GBR12909 (15 mg/kg) for dopamine, idazoxan (5 mg/kg) and desipramine (15 mg/kg) for norepinephrine.

[§] Significantly different from control. Two-tailed Fisher's exact test, $P < 0.001$.