Original Paper

Both N-methyl-D-aspartate and non-N-methyl-D-aspartate glutamate receptors in the bed nucleus of the stria terminalis modulate the cardiovascular responses to acute restraint stress in rats

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Abstract

The bed nucleus of the stria terminalis (BNST) is a forebrain structure that has been implicated on cardiovascular responses evoked by emotional stress. However, the local neurochemical mechanisms mediating the BNST control of stress responses are not fully described. In our study we investigated the involvement of glutamatergic neurotransmission within the BNST in cardiovascular changes evoked by acute restraint stress in rats. For this study, we investigated the effects of bilateral microinjections of selective antagonists of either N-methyl-D-aspartate (NMDA) or non-NMDA glutamate receptors into the BNST on the arterial pressure and heart rate increase and the decrease in tail skin temperature induced by acute restraint stress. Microinjection of the selective NMDA glutamate receptor antagonist LY235959 (1 nmol/100 nL) into the BNST decreased the tachycardiac response to restraint stress, without affecting the arterial pressure increase and the drop in skin temperature. Bilateral BNST treatment with the selective non-NMDA glutamate receptor NBQX (1 nmol/100 nL) decreased the heart rate increase and the fall in tail skin temperature, without affecting the blood pressure increase. These findings indicate a facilitatory influence of BNST glutamatergic neurotransmission via coactivation of local NMDA and non-NMDA receptors on the tachycardiac response to stress, whereas control of sympathetic-mediated cutaneous vasoconstriction is selectively mediated by local non-NMDA glutamate receptors.

Keywords

Bed nucleus of the stria terminalis, extended amygdala, autonomic activity, arterial pressure, heart rate, sympathetic activity

Introduction

A coordinated set of physiological changes occurs during aversive stimuli for the maintenance of homeostasis (Dampney et al., 2008; Sterling, 2012; Ulrich-Lai and Herman, 2009). Changes in cardiovascular function during stress include increases of blood pressure and heart rate (HR), hemodynamic alterations (e.g. vasoconstriction in cutaneous tissue and visceral beds and vasodilation in skeletal muscles), and resetting of the baroreflex toward higher arterial pressure (Crestani, 2016; Dampney, 2015; Dampney et al., 2008). The cutaneous vasoconstriction decreases local blood flow (Blessing, 2003) which, in turn, lowers the skin temperature (Busnardo et al., 2013; Vianna and Carrive, 2005). In spite of the importance of cardiovascular changes during aversive threats, the neurobiological mechanisms involved in these responses are not completely understood.

The physiological responses during emotional stress are coordinated by activation of overlapping limbic circuits (Dampney, 2015; Ulrich-Lai and Herman, 2009). The bed nucleus of the stria terminalis (BNST) is a limbic structure localized in the rostral prosencephalon that is activated during aversive stimuli (Cullinan et al., 1995) and has been implicated in the control of physiological and behavioral responses to stress (Crestani et al., 2013; Davis et al., 2010). Regarding the cardiovascular changes, both inhibitory and facilitatory roles of the BNST have been reported in these responses, depending on the type of aversive stimulus (e.g. conditioned versus unconditioned) (Crestani et al., 2013). For instance, reversible inactivation of the BNST enhanced the HR increase evoked by acute restraint stress without affecting the blood pressure increase (Crestani et al., 2009), whereas increases in blood pressure and HR induced by contextual fear conditioning were decreased (Resstel et al., 2008). Although these findings indicate a role of the BNST in the modulation of cardiovascular responses during aversive threats, the local neurochemical mechanisms involved in this control have not been fully elucidated.

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Studies have indicated the presence of glutamatergic terminals in the BNST (Forray and Gysling, 2004; Puente et al., 2010), and this neurochemical mechanism has been demonstrated to be involved in the control of cardiovascular function (Crestani et al., 2013). For instance, microinjection of glutamate into the BNST has been reported to cause cardiovascular changes. Initial studies reported either an increase or a decrease in blood pressure and HR following microinjection of excitatory amino acids into the BNST of anesthetized animals, effects which were related to the region of stimulation within the BNST (Dunn and Williams, 1995; Gelsema and Calaresu, 1987). However, further studies consistently demonstrated depressor responses following glutamate microinjection into the BNST (Ciriello and Janssen, 1993; Gelsema et al., 1993; Hatam and Nasimi, 2007), which were mediated by co-activation of local N-methyl-D-aspartate (NMDA) and non-NMDA receptors (i.e. AMPA and kainate receptors) (Hatam and Nasimi, 2007). Additionally, an inhibitory role of BNST glutamatergic neurotransmission in baroreflex function was also reported (Alves et al., 2009). In spite of these pieces of evidence, to the best of our knowledge, possible involvement of BNST glutamatergic neurotransmission in cardiovascular responsiveness during aversive threats has not yet been investigated. Therefore, this study aimed to evaluate the hypothesis that glutamatergic neurotransmission within the BNST is involved in cardiovascular responses evoked by acute restraint stress in rats.

Material and methods

Animals

Male Wistar rats weighing approximately 250 g (60 days old) were used in the present study. Animals were obtained from the animal breeding facility of the São Paulo State University (UNESP) (Botucatu, São Paulo State, Brazil) and were housed in plastic cages in a temperature-controlled room (24ºC) in the Animal Facility of the Laboratory of Pharmacology (School of Pharmaceutical Sciences, UNESP). Animals were kept under a 12:12 h light-dark cycle (lights on between 07:00–19:00) and had free access to water and standard laboratory food, except during the experimental period. Experimental procedures were carried out following protocols approved by the Ethical Committee for Use of Animals of the School of Pharmaceutical Science, UNESP.

Surgical preparation

Five days before the trial, animals were anesthetized with tribromoethanol (250 mg/kg, intraperitoneal (i.p.)), scalp was anesthetized with 2% lidocaine, and the skull was exposed. Then, using a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA), stainless-steel cannulae (26 G, 12 mm long) were bilaterally implanted into the BNST. Stereotaxic coordinates were: anteroposterior=+8.6 mm from interaural; lateral=4.0 mm from the medial suture, ventral=−5.8 mm from the skull with a lateral inclination of 23º (Paxinos and Watson, 1997). Dental cement was used to fix cannulae to the skull. After surgery, the rats were treated with a poly-antibiotic containing streptomycins and penicillins to prevent infection (560 mg/mL/kg, i.m.) and the non-steroidal anti-inflammatory flunixine meglumine to provide post-operation analgesia (0.5 mg/mL/kg, subcutaneous (s.c.)).

One day before the experiment, animals were again anesthetized with tribromoethanol (250 mg/kg, i.p.) and a polyethylene cannula (a 4 cm segment of PE-10 bound to a 13 cm segment of PE-50) (Clay Adams, Parsippany, New Jersey, USA) was implanted into the abdominal aorta via the femoral artery for cardiovascular recording. The catheter was tunneled under the skin and exteriorized on the animal's dorsum. After surgery, the non-steroidal anti-inflammatory flunixine meglumine was administered to provide post-operation analgesia (0.5 mg/mL/kg, s.c.). The animals were kept in individual cages during the post-operative period and cardiovascular recording. No animal died during the surgical procedures.

Arterial pressure and HR recording

The cannula implanted into the femoral artery was connected to a pressure transducer (DPT100, Utah Medical Products Inc., Midvale, Utah, USA). Pulsatile arterial pressure (PAP) was recorded using an amplifier (Quad Bridge Amp, ML224, ADInstruments, New South Wales, Australia) and an acquisition board (PowerLab 4/30, ML866/P, ADInstruments, New South Wales, Australia) connected to a personal computer. Mean arterial pressure (MAP) and HR values were obtained from the PAP recordings.

Tail cutaneous temperature measurement

The tail cutaneous temperature was recorded using a thermal camera Multi-Purpose Thermal Imager (IRI4010, InfraRed Integrated Systems Ltd, Northampton, UK). For analyzing the images, the temperature was measured on five points along the animal's tail and a mean of the values was calculated for each recording (Busnardo et al., 2013; Gouveia et al., 2016).

Restraint stress

Animals were submitted to restraint by placing each rat into a plastic cylindrical restraint tube (diameter 6.5 cm, length 15 cm), ventilated by holes (1 cm diameter) that comprised approximately 20% of the tube surface. Restraint lasted for 30 min, and immediately after the end of the session rats were returned to their home cages. Each rat was submitted to only one session of restraint in order to avoid habituation.

Drug microinjection into the brain

Microinjections were performed into the BNST using a 2 µL syringe (7002 KH, Hamilton, USA), which was connected to the microinjection needle (33 G, Small Parts, USA) via a PE-10 tubing. The needle used for microinjection into the BNST was 1 mm longer than the guide cannulas. Needles were carefully inserted into the guide cannulae without restraining the animals, and drugs were injected in a final volume of 100 nL (Crestani et al., 2009; Gouveia et al., 2016).

Drugs and solutions

LY235959 (TOCRIS, Westwoods Business, Park Ellisville, Missouri, USA), NBQX (TOCRIS), tribromoethanol (Sigma-Aldrich, St Louis, Missouri, USA) and urethane (Sigma-Aldrich)

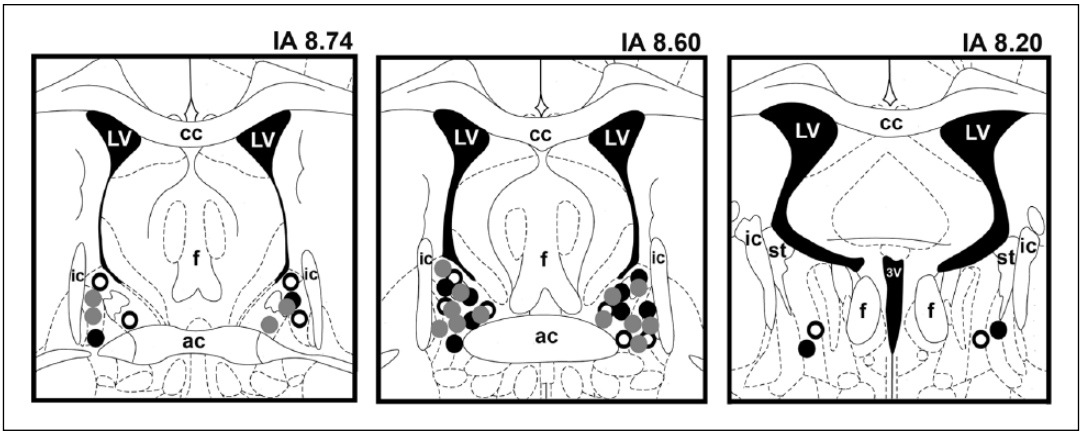

Figure 1. Diagrammatic representation based on the rat brain atlas of Paxinos and Watson (1997) indicating the microinjection sites of vehicle (white circles), the selective N-methyl-D-aspartate (NMDA) glutamate receptor antagonist LY235959 (black circles), and the selective non-NMDA glutamate receptor antagonist NBQX (gray circles) into the bed nucleus of the stria terminalis (BNST). 3V: third ventricle; ac: anterior commissure; cc: corpus callosum; f: fornix; IA: interaural coordinate; ic: internal capsule; LV: lateral ventricles; st: stria terminalis.

were dissolved in saline (NaCl 0.9%). Flunixine megulimine (Banamine, Schering Plough, Cotia, São Paulo State, Brazil) and the polyantibiotic preparation of streptomycins and penicillins (Pentabiotico, Fort Dodge, Campinas, São Paulo State, Brazil) were used as provided.

Experimental design

Rats were brought to the experimental room in their own cage. Animals were allowed at least 60 min to adapt to experimental room conditions, such as sound and illumination, before starting the experiment. The experimental room was temperature controlled (24°C) and acoustically isolated from other rooms.

Independent sets of animals received bilateral microinjections of the selective NMDA glutamate receptor antagonist LY235959 (1 nmol/100 nL), the selective non-NMDA glutamate receptor antagonist NBQX (1 nmol/100 nL), or vehicle (saline, 100 nL) into the BNST (Alves et al., 2009; Busnardo et al., 2013). Each animal received only one microinjection per brain side. Ten minutes after BNST pharmacological treatment, the animals underwent a 30-minute session of restraint stress.

Cardiovascular recording began at least 30 min before the onset of the restraint session and was performed throughout the period of exposure to the restraint stress. The tail skin temperature was measured 10, 5, and 0 min before the restraint for baseline values, and 10, 20, and 30 min after the onset of the restraint.

Histological determination of the microinjection sites

At the end of experiments, animals were anesthetized with urethane (250 mg/mL/200 g body weight, i.p.) and 100 nL of 1% Evan's blue dye was microinjected into the brain as a marker of the microinjection sites. Brains were removed and post-fixed in 10% formalin for at least 48 h at 4ºC. Then, serial 40 µm thick sections of the BNST region were cut with a cryostat (CM1900, Leica, Wetzlar, Germany). The actual placement of the microinjection needles was determined upon analysis of serial sections in a light microscopy according to the rat brain atlas of Paxinos and Watson (1997).

Statistical analysis

Data are presented as mean±standard error of the mean (SEM). The basal values of MAP, HR, and tail skin temperature were compared using Student's *t*-test. Effects of restraint stress on cardiovascular parameters are presented as changes from baseline values. Basal parameters were obtained by calculating the mean of the values recorded during the 10 min prior the restraint onset. The basal value was subtracted from the recorded values to obtain the restraint-evoked change. The time-course curves of cardiovascular changes during restraint stress were analyzed using two-way analysis of variance (ANOVA), with treatment as main factor and time as repeated measurement. The significance was set at $p<0.05$.

Results

Determination of the microinjection sites

Figure 1 presents diagrammatic representations showing microinjection sites into the BNST of LY235959, NBQX, and vehicle of all animals used in the present study.

Effect of bilateral microinjection of LY235959 into the BNST on cardiovascular responses to acute restraint stress

Bilateral microinjection of the selective NMDA glutamate receptor antagonist LY235959 into the BNST (1 nmol/100 nL; $n=6$) did not affect baseline values of either MAP (104±2 vs 108±5 mm Hg; $t=0.8$; $p>0.05$), HR (377±8 vs 359±12 bpm; $t=1.1$; $p>0.05$), or tail skin temperature (28.3±0.6 vs 27.9±0.3ºC; $t=0.9$; $p>0.05$), when compared with the vehicle-treated animals ($n=6$). However, acute restraint stress induced a marked and sustained

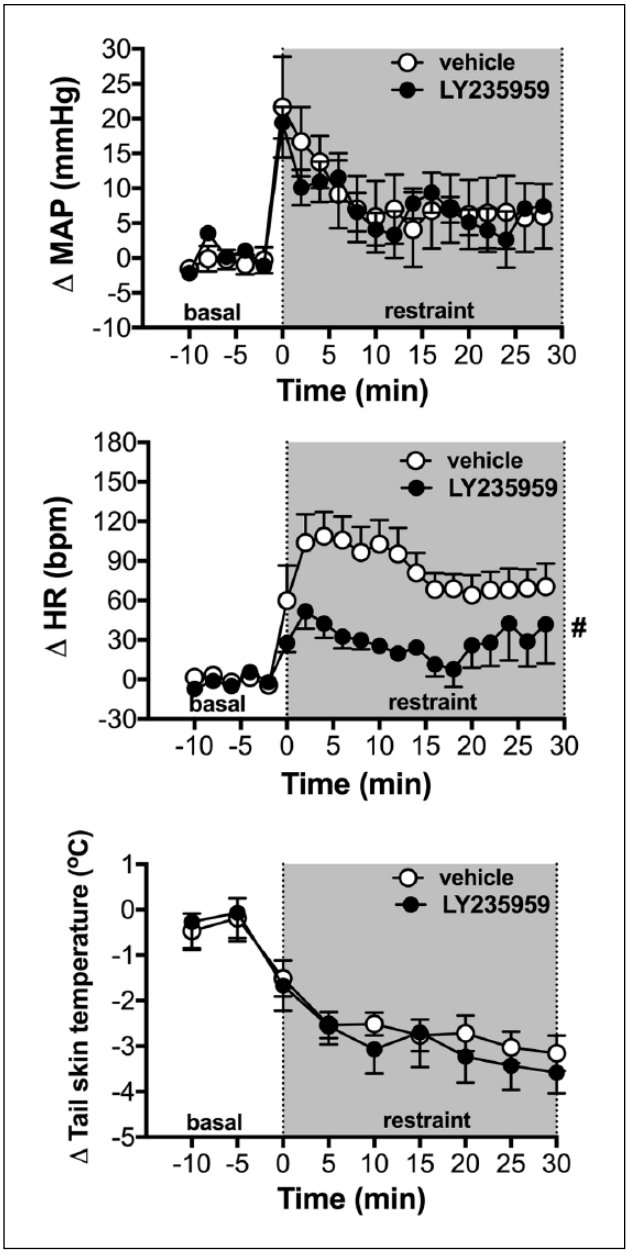

Figure 2. Time course of changes in mean arterial pressure (ΔMAP), heart rate (ΔHR), and tail skin temperature (Δtail skin temperature) induced by restraint stress in animals that received bilateral microinjection of vehicle (100 nL; n=6 ○) or the selective N-methyl-D-aspartate (NMDA) glutamate receptor antagonist LY235959 (1 nmol/100 nL; n=6 ●) into the bed nucleus of the stria terminalis (BNST). Shaded area indicates the period of restraint. Circles represent the mean and bars the standard error of the mean (SEM). #p<0.05 over the whole restraint period compared to vehicle-treated animals, analysis of variance (ANOVA) followed by Bonferroni post-hoc test.

increase in both MAP (factor time: $F_{(19,200)=}5$; p<0.0001) and HR (factor time: $F_{(19,200)=}8$; p<0.0001); and decreased the tail skin temperature (factor time: $F_{(8,90)=}12$; p<0.0001) (Figure 2). Additionally, bilateral BNST treatment with LY235959 decreased restraint-evoked increase on HR (factor treatment: $F_{(1,200)=}80$; p<0.0001), without affecting the pressor response (factor treatment: $F_{(1,200)=}0.1$; p>0.05) and the drop in tail skin temperature (factor treatment: $F_{(1,90)=}0.7$; p>0.05) (Figure 2). Analysis also indicated a significant treatment×time interaction for HR ($F_{(19,200)=}2$; p<0.02), but not for MAP ($F_{(19,200)=}0.2$, p>0.05) and tail skin temperature ($F_{(8,90)=}0.2$; p>0.05). Microinjection of LY23595 into structures surrounding the BNST such as the anterior commissure, fornix, and internal capsule did not affect restraint-evoked changes on arterial pressure (p>0.05), HR (p>0.05), and tail skin temperature (p>0.05) (Table 1).

Effect of bilateral microinjection of NBQX into the BNST on cardiovascular responses to acute restraint stress

Bilateral microinjection of the selective non-NMDA glutamate receptor antagonist NBQX into the BNST (1 nmol/100 nL; n=8) did not affect baseline values of either MAP (109±1 vs 108±5 mm Hg; t=0.2; p>0.05), HR (374±6 vs 359±12 bpm; t=1.1; p>0.05), or tail skin temperature (28.4±0.4 vs 27.9±0.3ºC; t=0.8; p>0.05), when compared with the vehicle-treated animals (n=6). However, acute restraint stress induced a marked and sustained increase in both MAP (factor time: $F_{(19,240)=}7$; p<0.0001) and HR (factor time: $F_{(19,240)=}11$; p<0.0001); and decreased the tail skin temperature (factor time: $F_{(8,108)=}6$; p<0.0001) (Figure 3). Moreover, bilateral BNST treatment with NBQX decreased the tachycardiac response (factor treatment: $F_{(1,240)=}21$; p<0.0001) and the drop in tail skin temperature (factor treatment: $F_{(1,108)=}8$; p<0.005) evoked by restraint stress, without affecting the increase on MAP (factor treatment: $F_{(1,200)=}3$; p>0.05) (Figure 3). Analysis did not indicate a treatment×time interaction for either MAP ($F_{(19,240)=}0.1$; p>0.05), HR ($F_{(19,240)=}0.8$; p>0.05), or tail skin temperature ($F_{(8,108)=}0.3$; p>0.05). Microinjection of NBQX into structures surrounding the BNST such as anterior commissure, fornix, and internal capsule did not affect restraint-evoked changes on arterial pressure (p>0.05), HR (p>0.05), and tail skin temperature (p>0.05) (Table 1).

Discussion

The present study provides the first evidence for the involvement of BNST glutamatergic neurotransmission in the control of cardiovascular responsiveness to psychological stress. The results showed that bilateral microinjection of the selective NMDA glutamate receptor antagonist LY235959 into the BNST decreased the tachycardiac response evoked by restraint stress, without affecting the MAP increase and the drop in tail skin temperature. Additionally, bilateral BNST treatment with the selective non-NMDA glutamate receptor antagonist NBQX decreased both the tachycardiac and the sympathetic-mediated cutaneous vasoconstriction responses to restraint stress, but without affecting the blood pressure increase.

We have previously reported that acute bilateral BNST neurotransmission inhibition caused by local treatment with the nonselective synaptic blocker $CoCl_2$ enhanced tachycardia evoked by restraint stress without affecting the pressor response (Crestani et al., 2009). Likewise, restraint-evoked tachycardia was also enhanced following BNST treatment with either a selective α_1-adrenoceptor or muscarinic cholinergic receptor antagonists

Table 1. Two-way analysis of variance (ANOVA) results of time-course curve analysis of changes in mean arterial pressure (ΔMAP), heart rate (ΔHR), and tail skin temperature (Δskin temperature) induced by restraint stress in animals that received microinjection of the selective N-methyl-D-aspartate (NMDA) glutamate receptor antagonist LY235959 (1 nmol/100 nL; *n*=3) or the selective non-NMDA glutamate receptor antagonist NBQX (1 nmol/100 nL; *n*=4) into structures surrounding the bed nucleus of the stria terminalis (BNST).

Analysis	Response	Time	Factor treatment	Interaction
Vehicle versus LY235959				
	ΔMAP	$F_{(19,140)}$=3 p<0.0001	$F_{(1,140)}$=0.5 p<0.05	$F_{(19,140)}$=0.08 p<0.05
	ΔHR	$F_{(19,140)}$=12 p<0.0001	$F_{(1,140)}$=2 p<0.05	$F_{(19,140)}$=0.2 p<0.05
	ΔSkin temperature	$F_{(8,63)}$=12 p<0.0001	$F_{(1,63)}$=0.05 p<0.05	$F_{(8,63)}$=0.7 p<0.05
Vehicle versus NBQX				
	ΔMAP	$F_{(19,160)}$=4 p<0.0001	$F_{(1,160)}$=0.3 p<0.05	$F_{(19,160)}$=0.06 p<0.05
	ΔHR	$F_{(19,160)}$=8 p<0.0001	$F_{(1,160)}$=0.2 p<0.05	$F_{(19,160)}$=0.3 p<0.05
	ΔSkin temperature	$F_{(8,72)}$=20 p<0.0001	$F_{(1,72)}$=3 p<0.05	$F_{(8,72)}$=1 p<0.05

(Crestani et al., 2009; Gouveia et al., 2016), thus suggesting that the inhibitory influence of the BNST on tachycardiac response to restraint stress is mediated, at least in part, by local noradrenergic and cholinergic neurotransmission. Conversely, the present data indicate that BNST glutamatergic neurotransmission plays a facilitatory influence in the restraint-evoked tachycardiac response. Noradrenaline has been reported to play an inhibitory influence on the activity of neurons within the BNST (Casada and Dafny, 1993), which is likely mediated by both facilitation of local GABAergic signals (via local α_1-adrenoceptor) and inhibition of glutamatergic inputs (via local α_2-adrenoceptor) (Dumont and Williams, 2004; Egli et al., 2005; Krawczyk et al., 2011). An action of acetylcholine within the BNST suppressing local glutamatergic neurotransmission was also reported (Guo et al., 2012). These pieces of evidence support the findings indicating an opposite role of glutamatergic receptors (i.e. facilitatory) and noradrenergic and cholinergic neurotransmissions (i.e. inhibitory) within the BNST in the control of restraint-evoked cardiovascular responses.

Recent reports have indicated a facilitatory influence of corticotropin-releasing factor (CRF) neurotransmission within the BNST in restraint-evoked cardiovascular responses (Oliveira et al., 2015). Additionally, the activation of cannabinoid receptor type 1 (CB_1) in the BNST was demonstrated to play an inhibitory role in tachycardiac response to restraint stress (Gomes-de-Souza et al., 2016). Previous reports have indicated a relevant interaction between BNST glutamatergic neurotransmission and local CRFergic and endocannabinoid signalings. For instance, CRF as well as related peptides (e.g. urocortin 1), enhanced excitatory postsynaptic currents (Kash et al., 2008; Silberman et al., 2013). Conversely, the CB_1 cannabinoid receptor was identified in glutamatergic terminals within the BNST (Puente et al., 2010) and activation of this receptor suppressed local excitatory synaptic neurotransmission (Massi et al., 2008; Puente et al., 2010). These pieces of evidence are consistent with an interaction between BNST glutamatergic neurotransmission and local CRFergic and endocannabinoid signalings in the control of stress-evoked cardiovascular responses. However, further studies are necessary to directly investigate these interactions.

Both the sympathetic and parasympathetic nervous system are involved in cardiovascular responses during aversive threats (Crestani, 2016). For instance, stress-evoked tachycardia is abolished following blockade of cardiac sympathetic activity while inhibition of parasympathetic tone to the heart increases this response (Baudrie et al., 1997; Carrive, 2006; Dos Reis et al., 2014). These results indicate an increase in both sympathetic and parasympathetic tone to the heart during emotional stress (Crestani, 2016). The vasoconstriction in cutaneous beds, which in turn causes a drop in skin temperature (Busnardo et al., 2013; Vianna and Carrive, 2005), is mediated by an increase in sympathetic vasomotor tone and activation of α-adrenoceptors (Blessing, 2003).

Projections from the BNST reach medullary structures controlling autonomic activity, such as the nucleus of the solitary tract, nucleus ambiguus, and ventrolateral regions (Dong and Swanson, 2004; Gray and Magnuson, 1987). Accordingly, the BNST has been reported to modulate both vascular and cardiac sympathetic activity, as well as parasympathetic tone to the heart (Ciriello and Janssen, 1993; Crestani et al., 2008, 2009; Hatam et al., 2009; Nijsen et al., 2001). The control of sympathetic-mediated cutaneous vasoconstriction by non-NMDA receptors within the BNST indicates activation of sympathetic circuits. Therefore, control of restraint-evoked cutaneous vasoconstriction and tachycardia by BNST non-NMDA glutamate receptors may be mediated by stimulation of sympathetic outflow. Previous findings from our group provided evidence of an inhibitory influence of the BNST NMDA glutamate receptor in parasympathetic outflow (Alves et al., 2009), thus suggesting that the control of HR response to stress by this receptor may be mediated by inhibition of parasympathetic activity. Thus, these pieces of evidence indicate that NMDA and non-NMDA glutamate receptor may differently modulate autonomic responses to stress. However, further studies investigating the autonomic mechanisms mediating the control of cardiovascular responses to stress by BNST glutamate receptors are necessary to confirm this idea.

The BNST has been proposed as a relay station involved in processing of limbic information coming from forebrain structures such as the hippocampus, amygdala. and medial

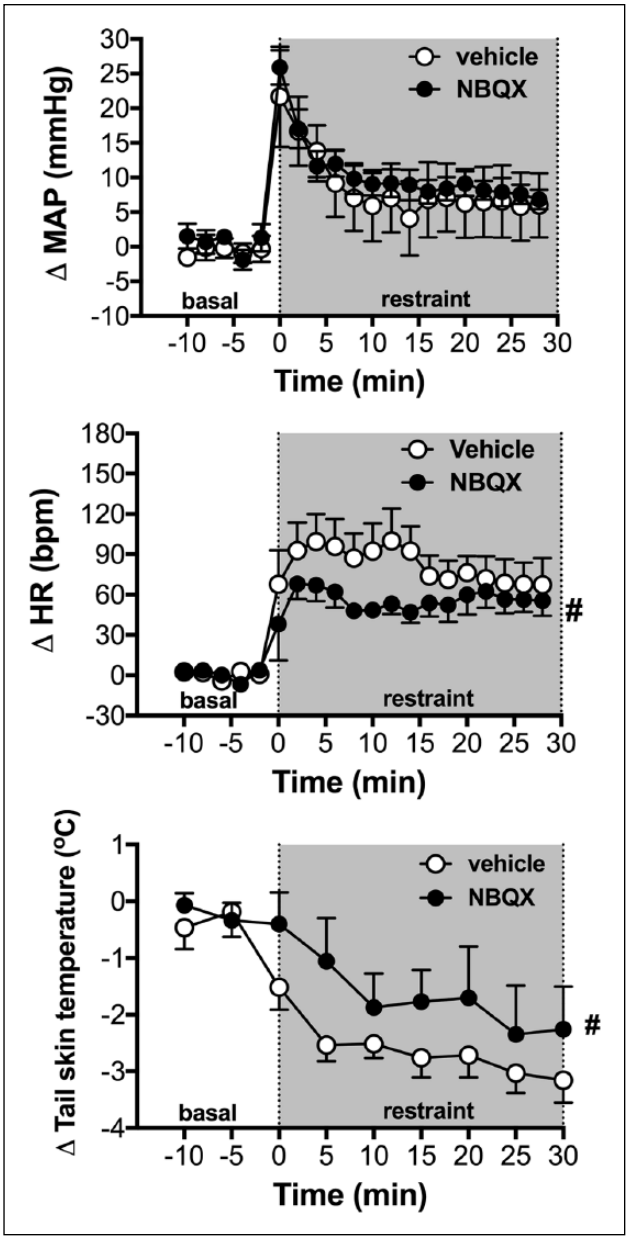

Figure 3. Time course of changes in mean arterial pressure (ΔMAP), heart rate (ΔHR), and tail skin temperature (Δtail skin temperature) induced by restraint stress in animals that received bilateral microinjection of vehicle (100 nL; n=6 ○) or the selective non-N-methyl-D-aspartate (NMDA) glutamate receptor antagonist NBQX (1 nmol/100 nL; n=8 ●) into the bed nucleus of the stria terminalis (BNST). Shaded area indicates the period of restraint. Circles represent the mean and bars the standard error of the mean (SEM). #p<0.05 over the whole restraint period compared to vehicle-treated animals, analysis of variance (ANOVA) followed by Bonferroni post-hoc test.

prefrontal cortex (MPFC) (Crestani et al., 2013; Myers, 2017; Ulrich-Lai and Herman, 2009). In this regard, limbic inputs to the BNST from the basolateral and basomedial amygdala, hippocampus, and MPFC have been reported to be primarily glutamatergic in nature (Guo et al., 2012; Massi et al., 2008; Myers et al., 2014). Accordingly, an MPFC lesion decreased restraint-evoked activation of neurons within the BNST (Figueiredo et al., 2003). Excitatory presynaptic terminals from the MPFC to the anterior BNST originate mainly from the infralimbic (IL) region (Massi et al., 2008; Vertes, 2004). Inhibition of the IL region reduced tachycardia to restraint stress (Tavares et al., 2009), thus suggesting a facilitatory role of this MPFC region in cardiovascular responses during aversive threats. The hippocampus was also demonstrated to play a facilitatory role in restraint-evoked cardiovascular responses (Moraes-Neto et al., 2014; Scopinho et al., 2013). Involvement of the basolateral and basomedial amygdala in the control of stress-evoked cardiovascular responses was also recently reported (Mesquita et al., 2016; Oscar et al., 2015). Taken together, these findings provide evidence of possible sources of glutamatergic inputs to the BNST involved in the control of cardiovascular responses to stress. Nevertheless, further studies are necessary to assess the specific limbic circuits in which BNST glutamatergic neurotransmission is part of modulating stress-evoked cardiovascular responses.

In summary, the present results indicate an involvement of glutamatergic neurotransmission within the BNST in the control of cardiovascular responses to emotional stress. Specifically, our data provide evidence of a facilitatory influence of BNST glutamatergic neurotransmission via coactivation of local NMDA and non-NMDA receptors on the tachycardiac response to stress, whereas control of sympathetic-mediated cutaneous vasoconstriction is selectively mediated by local non-NMDA glutamate receptors.

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