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MICRO REPORT

Exposure to hot and cold environments increases noradrenaline release in the bed nucleus of the stria terminalis in rats

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Abstract

Aims: Thermoregulatory responses in homeothermic animals, including humans, are classified into involuntary autonomous and voluntary behavioral thermoregulatory responses. Although behavioral thermoregulatory responses are probably driven by positive (pleasant) and/or negative (unpleasant) emotions, the neuronal mechanisms underlying the induction of negative emotions by hot and cold environments remain poorly understood. The bed nucleus of the stria terminalis is a brain region implicated in stress responses and negative emotions, such as fear, anxiety, and aversion. Various stimuli that cause negative emotions, such as immobilization stress, fox odor, gastric distension, and inflammatory pain, increase noradrenaline release in the rat bed nucleus of the stria terminalis, especially in the ventral bed nucleus of the stria terminalis. It has been reported that the negative emotional component of pain is mediated by noradrenergic neurotransmission in the ventral bed nucleus of the stria terminalis. However, the role of intra-ventral bed nucleus of the stria terminalis noradrenergic neurotransmission in the induction of negative emotion by exposure to hot and cold environments remains to be elucidated. For the first step to address this issue, the effects of hot and cold environments on noradrenaline release in the ventral bed nucleus of the stria terminalis were examined.

Methods: In vivo microdialysis analyses in unanesthetized, freely moving male Sprague-Dawley rats were performed to examine hot and cold environments-induced noradrenaline release in the ventral bed nucleus of the stria terminalis.

Results: Exposure to hot (38°C) and cold (8°C) environments significantly increased noradrenaline release in the ventral bed nucleus of the stria terminalis.

Conclusions: The results suggest that exposure to hot and cold environments enhances noradrenergic neurotransmission in the ventral bed nucleus of the stria terminalis, which may induce negative emotion, and thereby drive avoidance behaviors, that is, escape from hot and cold environments.

KEYWORDS
aversion, bed nucleus of the stria terminalis, emotion, noradrenaline, thermoregulation

1 | INTRODUCTION

Temperature affects various physiological functions and is one of the key factors maintaining homeostasis in living bodies.[1] Thermoregulatory responses in homeothermic animals, including humans, are classified into involuntary autonomous thermoregulatory responses (eg, sweating, skin vasodilation, and shivering) and voluntary behavioral thermoregulatory responses (eg, escape behaviors from hot and cold environments).[2] Although these escape behaviors are probably driven by negative emotions, the neuronal mechanisms underlying behavioral thermoregulatory responses, that is, underlying the induction of negative emotions by hot and cold environments, remain poorly understood.

The bed nucleus of the stria terminalis (BNST) is a brain region implicated in stress responses and negative emotions such as fear, anxiety, and aversion. Using a conditioned place aversion/avoidance (CPA) test, we previously showed that pain-induced aversion is mediated by noradrenergic (NAergic) neurotransmission in the ventral BNST (vBNST).[3] Specifically, (a) pain stimulation increased noradrenaline (NA) release in the vBNST, (b) avoidance responses to the place associated with pain stimulation were suppressed by intra-vBNST injection of a β-adrenoceptor antagonist, and (c) intra-vBNST injection of a β-adrenoceptor agonist induced avoidance responses to the place associated with the drug injection even in the absence of conditioning with pain stimulation. These findings suggest that pain stimulation induces negative emotions by enhancing NAergic neurotransmission in the vBNST and thereby induces avoidance responses to the place associated with pain stimulation, and that activation of NAergic neurotransmission in the vBNST is sufficient to induce such avoidance responses. However, the role of intra-vBNST NAergic neurotransmission in the avoidance behaviors against hot and cold environments remains to be elucidated. For the first step to address this issue, the effects of hot (38°C) and cold (8°C) environments on NA release in the vBNST were examined using an in vivo microdialysis technique.

2 | MATERIALS AND METHODS

2.1 | Animals

In total, 70 male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan; 210-380 g) were used. Different cohorts of animals were used for different temperature/humidity groups. The animals were housed in a room with a constant ambient temperature (23 ± 1°C) under a 12-hour light/dark cycle with food and water available ad libitum. All experiments were performed with the approval of the Institutional Animal Care and Use Committee of Hokkaido University.

2.2 | In vivo microdialysis

In vivo microdialysis experiments were carried out as described previously.[3–6] Briefly, at least 2 days after the surgery for unilateral implantation of a microdialysis guide cannula at 1.0 mm above the left vBNST (−0.3 mm rostral, 1.5 mm lateral, 6.7 mm ventral to the bregma),[7] microdialysis experiments were conducted in unanesthetized freely moving rats. A microdialysis probe was inserted through the guide cannula and continuously perfused with Ringer's solution at a constant flow rate of 1 μL/min. After a stabilization period, three 15-minute dialysate fractions were collected as baseline samples under the room temperature condition. Then, the animals were moved into a temperature- and humidity-controllable chamber (KCL-2000W; Tokyo Rikakikai Co., Ltd., Tokyo, Japan), with the temperature set to 8, 18, 28, or 38°C, and then twelve 15-minute fractions were collected. The noradrenaline contents in the dialysate samples were measured using an electrochemical detector (HTEC-500; Eicom; Kyoto, Japan). The baseline value of the NA level was calculated as the average of three baseline samples, and the percent change from the baseline value was calculated for each time point. Data from rats showing a baseline value less than 0.5 pg/μL (n = 2), an unstable baseline NA level (defined as a >30% difference among the three baseline samples, n = 4), or a bursting increase in serotonin level probably due to microhemorrhage (n = 2) were eliminated from statistical analyses. The area under the curve (AUC) values for the NA levels measured from 0 to 180 minutes were calculated.

2.3 | Histological analyses

Histological analyses were performed to examine the placement of the microdialysis probes. Briefly, rats were decapitated, and the brains were rapidly removed and frozen in powdered dry ice. Coronal sections (50 μm) were prepared using a cryostat, thaw-mounted onto slides, and stained with thionin. These sections were examined by light microscopy (40×). Data from the rats with correct placements of the microdialysis probe were used for the statistical analyses. In this study, no animals were excluded from the statistical analyses due to misplacement of the microdialysis probe or extensive tissue damage.

2.4 | Statistical analyses

Statistical analyses were performed using GraphPad Prism® software (ver. 6.00; GraphPad Software Inc., San Diego, CA, USA). The time courses data of intra-vBNST NA release were analyzed using two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons post hoc test. The AUC values were analyzed using one-way ANOVA followed by Bonferroni's multiple comparisons post hoc test. *P* values <0.05 were considered statistically significant.

3 | RESULTS

3.1 | Exposure to hot and cold environments increased NA release in the vBNST

Using an in vivo microdialysis technique in freely moving rats, intra-vBNST NA release under the hot (38°C, 50% relative humidity;

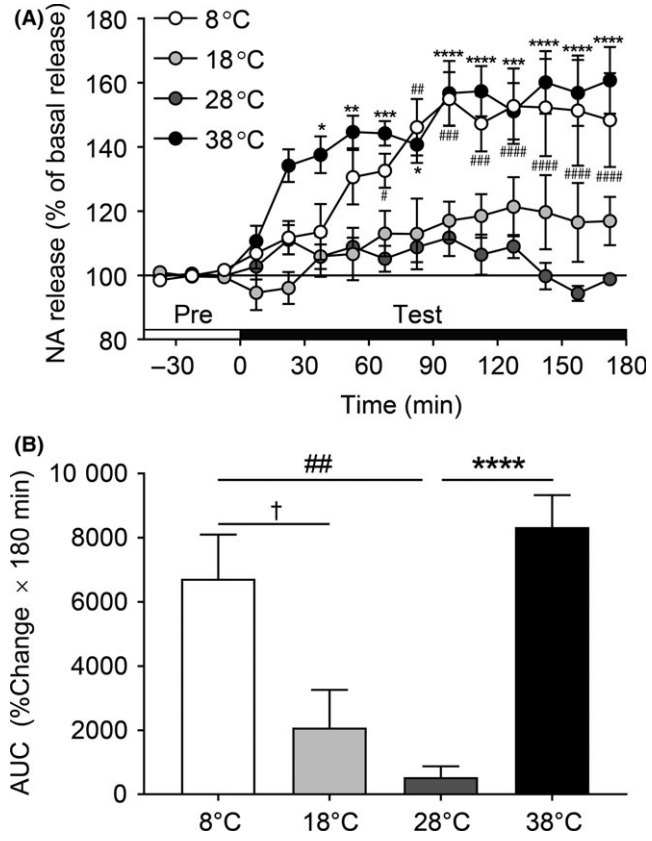

FIGURE 1 Hot and cold environments increase noradrenaline (NA) release in the ventral bed nucleus of the stria terminalis (vBNST). A, Time courses of intra-vBNST NA levels in the 8°C (n = 11), 18°C (n = 10), 28°C (n = 11), and 38°C (n = 10) groups. $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, hot (38°C) vs control (28°C); $^{\#\#\#\#}P < 0.0001$, $^{\#\#\#}P < 0.001$, $^{\#\#}P < 0.01$, $^{\#}P < 0.05$, cold (8°C) vs control (28°C). B, Area under the curve values for the changes in intra-vBNST NA levels measured from 0 to 180 min. Data are expressed as the mean ± SEM. $^{****}P < 0.0001$, hot (38°C) vs control (28°C); $^{\#\#}P < 0.01$, cold (8°C) vs control (28°C); $^{\dagger}P < 0.05$, cold (8°C) vs cool (18°C)

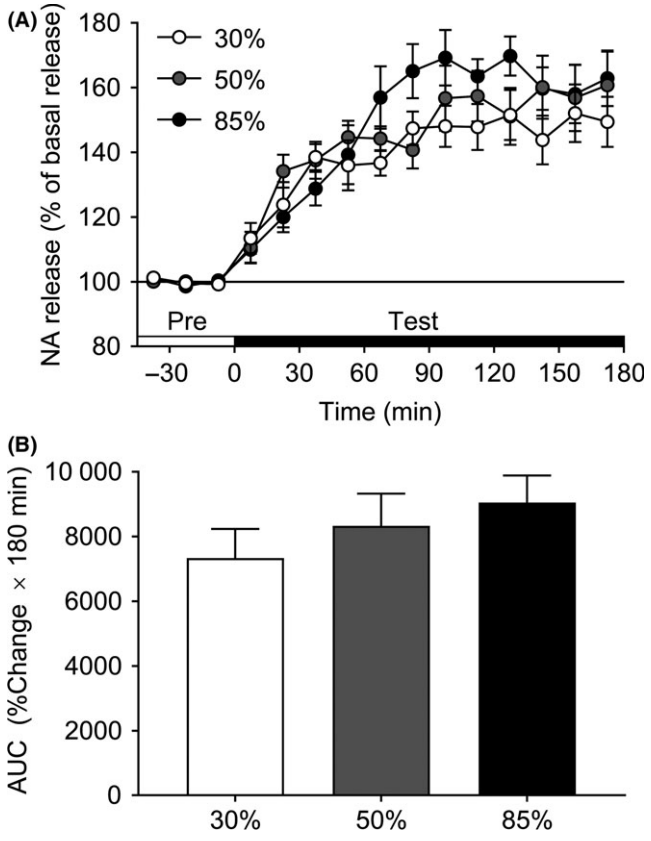

FIGURE 2 High humidity tends to enhance hot (38°C) environment-induced noradrenaline (NA) release in the ventral bed nucleus of the stria terminalis (vBNST). A, Time courses of intra-vBNST NA levels in the 30% (open circle, n = 10), 50% (shaded circle, n = 10), and 85% (closed circle, n = 10) groups. B, Area under the curve values for the changes in intra-vBNST NA levels measured from 0 to 180 min. Data are expressed as the mean ± SEM

n = 10), cool (18°C, 50%; n = 10), and cold (8°C, 50%; n = 11) environments were compared to NA release under the control temperature (28°C, 50%; n = 11; Figure 1A). Two-way repeated measures ANOVA revealed significant effects of exposure to hot and cold environments on extracellular NA levels in the vBNST (temperature: $F_{3,38} = 11.7$, $P < 0.0001$; time: $F_{14,532} = 22.4$, $P < 0.0001$; interaction: $F_{42,532} = 4.17$, $P < 0.0001$). According to Bonferroni's multiple comparisons post hoc test, there were significant increases in intra-vBNST NA release between 30 and 180 minutes in the hot (38°C) environment-exposed group compared with the control (28°C) group. Bonferroni's multiple comparisons post hoc test also revealed that intra-vBNST NA release was significantly increased between 60 and 180 minutes in the cold (8°C) environment-exposed group. However, there was no significant increase in NA release in the cool (18°C) environment-exposed group.

The AUC values also showed the significant effects of hot and cold environments on intra-vBNST NA release (Figure 1B, one-way ANOVA: $F_{3,38} = 11.7$, $P < 0.0001$). Bonferroni's multiple comparisons post hoc test showed that intra-vBNST NA release was significantly increased in the hot (38°C) and cold (8°C) environment-exposed groups compared with the control (28°C) group (hot vs control: $P < 0.0001$; cold vs control: $P = 0.0016$). Bonferroni's multiple comparisons post hoc test also revealed a significant increase in intra-vBNST NA release in the cold (8°C) environment-exposed group compared with the cool (18°C) environment-exposed group ($P = 0.0194$). There was no significant increase in intra-vBNST NA release in the cool (18°C) environment-exposed group compared with the control (28°C) group ($P > 0.9999$).

3.2 | Influence of humidity on hot environment-induced NA release in the vBNST

The influence of humidity on hot (38°C) environment-induced NA release in the vBNST was examined. Humidity in the chamber was set to low (30%; n = 10), medium (50%; n = 10), or high (85%; n = 10). Figure 2A shows an enhancement of hot (38°C) environment-induced NA release by high humidity. However, two-way repeated measures ANOVA revealed no significant effect of ambient

humidity on hot (38°C) environment-induced NA release (humidity: $F_{2,27}$ = 0.882, P = 0.4257). The AUC values also showed that high humidity tended to enhance hot (38°C) environment-induced NA release in the vBNST, although the difference was not significant (Figure 2B, one-way ANOVA: $F_{2,27}$ = 0.882, P = 0.4257).

4 | DISCUSSION

Using an in vivo microdialysis technique, the current study showed that exposure to hot (38°C) and cold (8°C) environments significantly increased extracellular NA levels in the vBNST. Although high humidity (85%) tended to enhance hot (38°C) environment-induced NA release compared with lower (30% and 50%) humidity, the influence of humidity was not significant.

In addition to the hot (38°C) and cold (8°C) environments, we previously reported that inflammatory pain increased extracellular NA levels in the vBNST.[3] Cecchi et al[8] showed that NA release was increased in the BNST of male Sprague-Dawley rats during immobilization stress. Fendt et al[9] showed that NA release in the BNST was strongly increased during exposure to trimethylthiazoline (TMT), a component of fox odor. More recently, we showed that distension of gastrointestinal tract increased extracellular NA levels in the vBNST.[5,6] These findings suggest that stimulations that induce negative emotions increase NA release in the vBNST.

The vBNST receives dense innervation of NAergic fibers primarily from the nucleus of the solitary tract (NTS; including the A2 cell group) and caudal ventrolateral medulla (including the A1 cell group).[10,11] Since cold (4°C)-restraint is reported to increase c-fos expression in the NTS,[12] enhanced release of NA under the cold (8°C) environment is likely due to the activation of NTS NAergic neurons. Recently, Yahiro et al[13] reported that activation of parabrachial nucleus (PBN) neurons is required for eliciting thermoregulatory behaviors to escape from excessive heat and cold. The BNST receives the innervation from the PBN,[14] and optogenetic stimulation of the PBN-BNST pathway evokes excitatory postsynaptic currents (EPSCs).[15] We previously reported that activation of the NMDA receptor-neuronal nitric oxide (NO) synthase signaling within the vBNST mediates the negative emotional component of pain.[16] Using whole-cell patch-clamp recordings, we also showed that application of a NO donor to the vBNST slices induced depolarization in hyperpolarization-activated cation current (I_h)-positive neurons. Interestingly, Dumont and Williams[17] reported that noradrenaline depolarized I_h-positive neurons in the vBNST. Taken together, these findings suggest that NAergic signals from the NTS and glutamatergic signals from the PBN may converge on the I_h-positive vBNST neurons to induce aversive responses.

Dumont and Williams[17] also reported that NA enhanced inhibitory inputs to the I_h-negative vBNST neurons that project to the ventral tegmental area (VTA). Additionally, Jennings et al[18] reported that optogenetic activation of the GABAergic neurons projecting from the vBNST to the VTA induced a preference for the place associated with photostimulation. These findings suggest that increased release of NA in the vBNST may suppress the neuronal pathway from the vBNST to the VTA and thereby inhibit the preference for, or induce avoidance of, the place associated with exposure to aversive stimuli.

Fendt et al[9] reported that clonidine, an α_2-adrenoceptor agonist, suppressed the intra-BNST NA release evoked by TMT and blocked TMT-induced postentiation of fear-related behaviors. Furthermore, we reported that clonidine applied to the vBNST inhibited pain-evoked NA release and suppressed pain-induced avoidance behaviors in a CPA test.[4] Intra-vBNST injection of timolol, a β-adrenoceptor antagonist, also suppressed pain-induced avoidance behaviors.[3] Additionally, intra-vBNST injection of a β-adrenoceptor agonist per se induced avoidance responses.[3] These findings suggest that enhanced NAergic neurotransmission in the vBNST may drive avoidance behaviors, that is, escape from hot and cold environments, and thereby may play an important role in behavioral thermoregulatory responses. Further behavioral studies are necessary to elucidate the involvement of intra-vBNST NAergic neurotransmission in the induction of negative emotions by hot and cold environments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA REPOSITORY

We have made our data publicly available through directly submitting as the Supporting Information.

APPROVAL OF THE RESEARCH PROTOCOL BY AN INSTITUTIONAL REVIEWER BOARD

n/a.

INFORMED CONSENT

n/a.

REGISTRY AND THE REGISTRATION NO. OF THE STUDY/TRIAL

n/a.

ANIMAL STUDIES

All experiments were performed with the approval of the Institutional Animal Care and Use Committee at Hokkaido University.

AUTHOR CONTRIBUTION

SM and MM are involved in the conception and design of the experiments. SM and HN performed the experiments and statistical analyses, and wrote the manuscript. MM finalized the manuscript. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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