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MICRO REPORT

Role of noradrenergic transmission within the ventral bed nucleus of the stria terminalis in nicotine withdrawal-induced aversive behavior

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Abstract

Aim: Cessation of smoking induces nicotine withdrawal symptoms such as anxiety, depression, and dysphoria, which could lead to smoking relapse. In the present study, we examined the role of noradrenergic transmission within the ventral bed nucleus of the stria terminalis (vBNST) on nicotine withdrawal-induced aversive behavior.

Methods: Nicotine dependence in rats was established by subcutaneous implantation with a nicotine-filled osmotic minipump on day 1. Nicotine withdrawal was precipitated by administration of the nicotine receptor antagonist, mecamylamine (3.0 mg/kg, s.c.), on day 15. Nicotine withdrawal-induced intra-vBNST noradrenaline release and aversive behavior were examined by in vivo microdialysis and a conditioned place aversion (CPA) test, respectively.

Results: Intra-vBNST noradrenaline release was significantly increased during nicotine withdrawal. Nicotine withdrawal induced aversive behavior, which was attenuated by intra-vBNST injection of the β-adrenoceptor antagonist, timolol.

Conclusions: These results suggest that enhanced noradrenergic transmission via β-adrenoceptors in the vBNST plays a crucial role in nicotine withdrawal-induced aversive behavior.

KEYWORDS
bed nucleus of the stria terminalis, nicotine withdrawal, noradrenaline

1 | INTRODUCTION

Cessation of smoking induces nicotine withdrawal symptoms including anxiety, depression, and dysphoria, which can lead to smoking relapse.[1] Therefore, it is necessary to clarify the neural mechanisms underlying nicotine withdrawal-induced negative emotions. The bed nucleus of the stria terminalis (BNST) has been implicated in the regulation of negative emotional states, such as fear, anxiety, and aversion.[2,3] The BNST receives a dense projection of noradrenergic fibers from the A1/A2 cell groups.[4] We reported previously that noradrenergic transmission in the ventral part of the BNST (vBNST) plays a crucial role in pain-induced aversion.[5] In the present study, to clarify the role of intra-vBNST noradrenergic transmission in nicotine withdrawal-induced aversion, nicotine withdrawal-induced intra-vBNST noradrenaline release and aversive behavior were examined using in vivo microdialysis and a conditioned place aversion (CPA) test, respectively.

2 | MATERIALS AND METHODS

One hundred male Sprague–Dawley rats weighing 190–250 g (Japan SLC, Hamamatsu, Japan) were used. All rats were maintained at a constant ambient temperature (23 ± 1°C) under a 12-hour light (light on at 07:00)/dark (light off at 19:00) cycle with food and water available ad libitum. All experiments were performed with the approval of the Hokkaido University Institutional Animal Care and Use Committee.

Nicotine dependence in rats was established in rats by subcutaneous implantation with a nicotine-filled osmotic minipump (2ML2 for microdialysis experiments, 2ML4 for behavioral experiments, Durect Corporation, Cupertino, CA, USA). The pumps were filled with (−)-nicotine tartrate (Cayman Chemical Company, Ann Arbor, MI, USA) dissolved in saline and subcutaneously implanted into the rats under anesthesia with 2.5% isoflurane. Nicotine was delivered at 13.7 mg/kg/d as nicotine tartrate (4.8 mg/kg/d as a nicotine base). The dose of nicotine was determined based on the previous study by Brynildsen et al.[6]

For in vivo microdialysis, the animals were implanted with a nicotine- or saline-filled osmotic minipump on day 1. A microdialysis guide cannula (o.d., 0.5 mm; AG-8; Eicom, Kyoto, Japan) was implanted unilaterally 1.0 mm above the vBNST under anesthesia with 2.5% isoflurane on day 6–8. The animals were individually housed in cages for a recovery period of 6–8 days and were handled for 1–2 min/d for 3 consecutive days before in vivo microdialysis experiments. In vivo microdialysis was conducted in non-anesthetized freely moving rats on day 15 as described previously.[5,7] Briefly, a microdialysis probe (dialysis membrane: 50 kDa molecular weight cutoff polyethylene membrane; length, 1.0 mm; o.d., 0.22 mm; FX-I-8-01; Eicom) was inserted through the guide cannula and continuously perfused with Ringer's solution at a flow rate of 1 μL/min. After the extracellular noradrenaline level had been stabilized, 11 samples of 15-minutes dialysates were collected. The first 3 of these served as baseline samples. Immediately after collecting the last baseline sample, chronic nicotine- or saline-treated animals were injected with mecamylamine (3.0 mg/kg, s.c.; Sigma-Aldrich). The dose of mecamylamine was determined based on the previous study by Pipkin et al.[8] The dialysate samples were separated by HPLC, and noradrenaline contents were measured using an electrochemical detector (HTEC-500; Eicom). Data are expressed as percentages of baseline, which were calculated as the average of the 3 baseline samples. The criteria to exclude data from statistical analyses were baseline values <0.025 pg noradrenaline/sample (n = 2), unstable baseline level (defined as >30% difference among the three baseline samples, n = 3), or a bursting increase in serotonin level likely due to microhemorrhages (n = 2). The areas under the curve (AUC) for the noradrenaline levels measured from 0 to 120 minutes were calculated.

The CPA test was conducted as described previously.[5,9] We used a shuttle box composed of two equal-sized compartments (30 × 30 × 30 cm) with distinct tactile and visual cues, which were separated by a removable partition (Muromachi Kikai, Tokyo, Japan). The CPA chambers were set in sound-attenuating boxes equipped with a ventilating fan. On day 13 (habituation) and day 14 (pre-conditioning test), the rats freely explored the two compartments for 900 s; the time spent in each compartment during the exploration period was measured using infrared sensors (Supermex; Muromachi Kikai) positioned on the top cover of each compartment. Rats that spent >80% (>720 s) of the total time (900 s) in one compartment on day 14 (n = 3) or showed a difference of >200 s in the time spent in one compartment between days 13 and 14 (n = 1) were excluded from subsequent procedures. We used a bias-like protocol.[10] Specifically, the compartment in which each rat spent more time on day 14 (pre-conditioning test) was designated as each animal's withdrawal-paired compartment. Conditioning was conducted on days 15 and 16 as follows. In the vehicle control session, rats were administered saline (s.c.) and immediately confined to the non-withdrawal-paired compartment for 1 hour. After 6 hours, each rat received bilateral intra-vBNST injections of timolol (10 nmol/side; Sigma-Aldrich) or vehicle (PBS) using the injection cannula at a volume of 0.5 μL/side at a rate of 0.5 μL/min. The dose of timolol was determined to be 10 nmol/side because we previously showed that timolol significantly attenuated pain-induced CPA at a dose of 10 nmol/side, but not 1 nmol/side.[5] Five minutes after injection, in the conditioning session, the rats were injected with mecamylamine (3.0 mg/kg, s.c.) or saline, and then confined to the withdrawal-paired compartment for 1 hour. On day 17 (post-conditioning test), rats were allowed to freely explore the two compartments for 900 s, and the time spent in each compartment was measured. The CPA scores were calculated by subtracting the time spent in the withdrawal-paired compartment during the post-conditioning test from the time spent in the same compartment during the pre-conditioning test. In a pilot experiment in which the CPA test was conducted during the light period (daytime), nicotine withdrawal did not induce CPA at least under the conditions of this experiment (Figure S1). Because another preliminary experiment showed that nicotine withdrawal induced CPA during the dark period (nighttime), we carried out pre- and post-conditioning tests, conditioning in the non-withdrawal-paired compartment, and conditioning in the withdrawal-paired compartment during 02:00–04:30, 20:00–24:00, and 02:00-06:00, respectively.

Histological analysis was performed as described previously.[9] Data from rats with misplaced microdialysis probes (n = 7) or misplaced intra-vBNST injections (n = 13) were excluded from the statistical analyses. Statistical analyses were performed using GraphPad Prism version 9.00 (GraphPad Software). Data were expressed as means ± SEM. One-way repeated-measures analysis of variance (ANOVA), two-way repeated-measures ANOVA followed by Sidak's multiple comparison test, and the unpaired *t*-test was used for statistical analysis of the in vivo microdialysis data. The paired *t*-test and one-way ANOVA followed by Sidak's multiple comparison test were used for statistical analysis of the CPA test data. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

3 | RESULTS

There was no significant difference in the basal noradrenaline level at the first three time points between the groups chronically treated with nicotine (0.672 ± 0.143 pg/15 μL, n = 13) and saline (0.640 ± 0.119 pg/15 μL, n = 11). Administration of mecamylamine significantly increased extracellular noradrenaline levels within the vBNST in chronic nicotine-treated rats ($F_{1.645,\ 19.74}$ = 4.276, P = 0.035, n = 13, one-way repeated-measures ANOVA), but not in chronic saline-treated rats ($F_{1.694,\ 16.94}$ = 1.370, P = 0.277, n = 11, one-way repeated-measures ANOVA) (Figure 1A). Two-way repeated-measures ANOVA revealed a significant effect between the nicotine group and the saline group (nicotine: $F_{1,\ 22}$ = 5.767, P = 0.0252; time: $F_{10,\ 220}$ = 3.425, P = 0.0003; interaction: $F_{10,\ 220}$ = 3.215, P = 0.0007). Sidak's multiple comparison test revealed that intra-vBNST noradrenaline release was significantly increased between 75 and 105 minutes (75–90 minutes: P = 0.0038, 90–105 minutes: P = 0.0094) in the chronic nicotine-treated group, compared to the chronic saline-treated group. The AUCs also revealed a significant increase in noradrenaline release in the chronic nicotine-treated group (Figure 1B, t_{22} = 2.401, P = 0.0252, unpaired t-test).

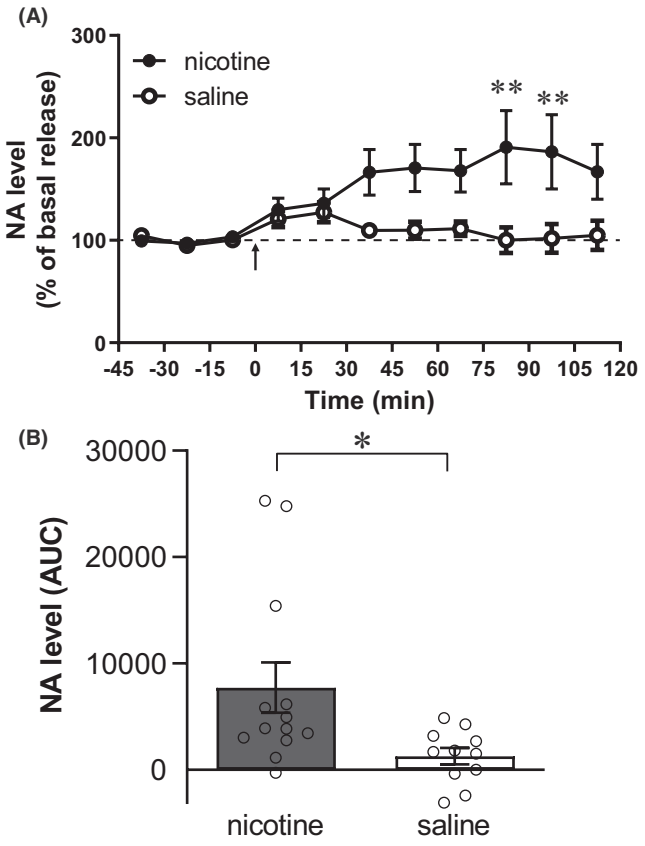

FIGURE 1 Nicotine withdrawal elevated the noradrenaline (NA) level in the vBNST. (A) Time course of changes in NA level in the vBNST of rats chronically treated with nicotine (n = 13) or saline (n = 11). The rats received subcutaneous injection of mecamylamine at time 0 (arrow). $^{**}P < 0.01$, nicotine vs. saline (Sidak's multiple comparison test). (B) Areas under the curve (AUCs) of intra-vBNST NA levels measured from 0 to 120 min. Circles indicate individual data points. Data are shown as means ± SEM. $^{*}P < 0.05$ (unpaired t-test)

Subcutaneous injection of mecamylamine induced CPA in the chronic nicotine-treated rats: the time spent in the withdrawal-paired compartment during post-conditioning test (post) was decreased significantly compared to that during the pre-conditioning test (pre) (Figure 2A, pre: 512.1 ± 15.2 s vs. post: 438.5 ± 16.5 s, t_{13} = 3.699, P = 0.0027, n = 14, paired t-test). No significant difference was observed in the time spent in the withdrawal-paired compartment between pre-conditioning and post-conditioning tests in the group injected subcutaneously with saline instead of mecamylamine (pre: 472.9 ± 20.6 s vs. post: 467.6 ± 22.7 s, t_{13} = 0.3347, P = 0.7432, n = 14, paired t-test). Bilateral intra-vBNST injection of timolol suppressed nicotine withdrawal-induced CPA: no significant difference was observed in the time spent in the withdrawal-paired compartment between pre-conditioning and post-conditioning tests (pre: 478.1 ± 15.6 s vs. post: 462.2 ± 14.3 s, t_{16} = 1.543, P = 0.1423, n = 17, paired t-test). Figure 2B shows that the CPA score of the withdrawal group without intra-vBNST injection of timolol (73.6 ± 19.9 s) was significantly higher than those of the non-withdrawal group (5.3 ± 15.8 s, t_{42} = 3.060, P = 0.0077, Sidak's multiple comparison test) and the withdrawal group with the injection of timolol into the bilateral vBNST (15.9 ± 10.3 s, t_{42} = 2.705, P = 0.0196, Sidak's multiple comparison test).

4 | DISCUSSION

In the present study, in vivo microdialysis revealed that extracellular noradrenaline levels were elevated in the vBNST during nicotine withdrawal, which was precipitated by subcutaneous injection of mecamylamine into chronically nicotine-treated rats. Subcutaneous injection of mecamylamine into chronically saline-treated rats did not increase intra-vBNST noradrenaline levels, so mecamylamine alone did not evoke intra-vBNST noradrenaline release. Additionally, intra-vBNST injection of timolol suppressed nicotine withdrawal-induced CPA. These results suggest that enhanced noradrenergic transmission via β-adrenoceptors within the vBNST plays an important role in nicotine withdrawal-induced aversive behavior. Delfs et al. reported that microinjection of β-antagonists or an α_2-agonist into the BNST markedly attenuated opiate withdrawal-induced CPA in rats.[11] Zhao et al. recently reported that acute mild-restraint stress increased intra-vBNST noradrenaline levels in ethanol withdrawal rats but not in ethanol-naive control rats.[12] They also found that ethanol withdrawal rats, but not controls, exhibited anxiety-like behavior in the elevated plus-maze test after exposure to acute mild-restraint stress, suggesting the possible involvement of intra-vBNST noradrenergic transmission in stress-induced anxiety in ethanol withdrawal rats. These findings, including those obtained in the present study, suggest that noradrenergic transmission in the vBNST plays a critical role in the induction of negative emotions such as aversion and anxiety by withdrawal from various drugs of abuse. Delfs et al.

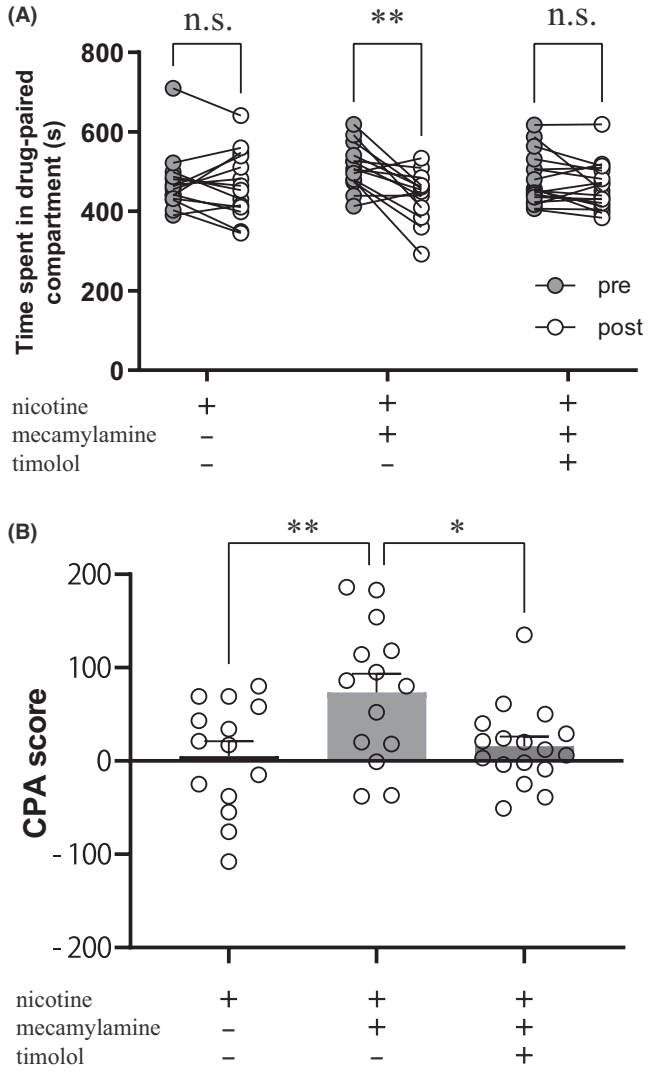

FIGURE 2 Effect of intra-vBNST injection of the β-adrenoceptor antagonist, timolol, on nicotine withdrawal-induced aversive behavior. Nicotine withdrawal was induced by subcutaneous injection of mecamylamine in rats chronically treated with nicotine. The rats received bilateral intra-vBNST injections of timolol (n = 17) or vehicle (n = 14) 5 min before mecamylamine injection. Rats in the non-withdrawal group (n = 14) received subcutaneous injection of saline instead of mecamylamine. (A) Time spent in the withdrawal-paired compartment during the pre-conditioning (pre) and post-conditioning (post) tests. Circles and lines indicate the data from individual animals before and after conditioning. $^{**}P < 0.01$ (paired *t*-test). Not significant (n.s.). (B) CPA scores. Circles indicate individual data points. Data are shown as means ± SEM. $^{**}P < 0.01$, $^{*}P < 0.05$ (Sidak's multiple comparison test)

reported that injection of β-antagonists into the BNST reduced two somatic signs of morphine withdrawal, teeth chattering, and eye twitching,[11] suggesting that the BNST is involved in not only psychological/emotional but also physical changes during withdrawal from drugs of abuse. The effect of intra-vBNST injection of timolol on nicotine withdrawal-induced physical symptoms needs to be examined in the future.

Wilmouth and Spear reported that mecamylamine-precipitated nicotine withdrawal induced anxiety-like behavior in the elevated plus-maze test, suggesting that the nicotine withdrawal-induced aversive behavior observed in the CPA test may be due to an increased aversive effect of nicotine withdrawal.[13] On the other hand, Miyata et al. reported that elevations of reward thresholds in the intracranial self-stimulation paradigm were parallel to the magnitude of place aversion in the CPA test during mecamylamine-precipitated nicotine withdrawal, suggesting that the nicotine withdrawal-induced aversive behavior may result from a decreased rewarding effect of nicotine.[14] Although further studies are needed to address the issue of whether nicotine withdrawal-induced aversive behavior is due to an increased aversive effect or a decreased rewarding effect, approaches that focus on the changes in the activity of the mesolimbic dopaminergic system may help address this issue. Tan et al. demonstrated that optogenetic inhibition of dopaminergic neurons in the ventral tegmental area induced aversive behavior in the real-time/conditioned place aversion test, suggesting that reduction of dopaminergic neuron activity plays an important role in the induction of place aversion.[15] In this context, Natividad et al. reported that nicotine withdrawal decreased extracellular levels of dopamine in the nucleus accumbens in rats.[16]

The current results suggest that enhanced noradrenergic transmission via β-adrenoceptors in the vBNST plays a crucial role in nicotine withdrawal symptoms such as anxiety and dysphoria. Such negative emotions may be closely related to relapse to substance use disorders (SUD), including nicotine dependence, which is a major barrier to effective long-term treatment of these disorders. Further investigation of the roles of noradrenergic transmission within the vBNST in drug withdrawal-induced negative emotions is required to identify the targets for the treatment of SUD.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

MM and SA conceptualized the studies. SA performed experiments and analyzed data. MM and SA wrote the paper.

INFORMED CONSENT

Not applicable.

REGISTRY AND THE REGISTRATION NO. OF THE STUDY/TRIAL

Not applicable.

ANIMAL STUDIES

All animal experiments were approved by the Institutional Animal Care and Use Committee at Hokkaido University.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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