Tonic electrical stimulation of the locus coeruleus enhances cortical sensory-evoked responses via noradrenaline α1 and β receptors

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Short running title: Locus coeruleus impacts sensory response

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**Abstract**

The locus coeruleus (LC) neurons send extensive projections to the somatosensory cortex and release noradrenaline (NA) at synaptic terminals, which is thought to regulate the activation of sensory-related cells by acting on three types of receptors (α1, α2 and β). Although previous studies have examined the effects of LC stimulation on single-unit sensory neurons, their impact on somatosensory evoked potentials (SEPs) and their temporal variations, as well as the specific roles of NA receptors remain unclear. Herein, we investigated how SEPs are modulated by tonic LC stimulation at physiological frequencies (0.1, 1 and 4 Hz) and identified the receptors involved in these changes. Forepaw stimulation-induced amplitudes in SEP were enhanced in response to 1 Hz stimulation of the LC but not in response to 0.1 and 4 Hz stimulation. Interestingly, the enhancement of SEPs after LC stimulation persisted for tens of minutes following the cessation of stimulation. Optical imaging using a voltage-sensitive dye showed an increase in the depolarizing response in the somatosensory cortex after 1 Hz stimulation. Prazosin (α1 receptor antagonist) and propranolol (β receptor antagonist) inhibited SEP enhancement following 1 Hz LC stimulation, whereas yohimbine (α2 receptor antagonist) had no effect. This suggests that the observed enhancement in SEP observed is primarily mediated by the activation of cortical excitatory α1 and β receptors. These findings provide insight into the impact of the NA system on sensory information processing as well as the pathophysiology of sensory disorders related to the disruption of the NA system.

Keywords: norepinephrine, somatosensory cortex, long-term potentiation

Introduction

The locus coeruleus (LC) is a small nucleus located in the pons of the brainstem. Neurons in the LC have axonal projections to a wide range of brain regions and release the neurotransmitter noradrenaline (NA, also known as norepinephrine), which acts on α1, α2, and β NA-receptors (Mather *et al.*, 2016). In higher brain regions involving the prefrontal and anterior cingulate cortices, the released NA mediates higher cognitive processes, such as attention, memory, and learning (Holland *et al.*, 2021). Furthermore, the LC-NA system modulates the activity of sensory neurons in the downstream signaling of these higher cortical areas (Grzanna & Molliver, 1980; Jones & Yang, 1985; McBurney-Lin *et al.*, 2019).

To determine the somatosensory modulatory effects of the LC-NA system, animal studies have primarily investigated the changes in sensory-evoked discharges during local NA administration or LC stimulation. These studies report an inverted U-shaped relationship between sensory-evoked response and tonic LC activation, whereby the cellular response to sensory afferent input is enhanced with increasing LC activity, while excessive activation can lead to diminished responses (Devilbiss & Waterhouse, 2000; 2004; Aston-Jones & Cohen, 2005; McBurney-Lin *et al.*, 2019). Although previous research has explored this relationship using single-unit recordings (Devilbiss & Waterhouse, 2004; Devilbiss *et al.*, 2006), no studies have verified this relationship using somatosensory evoked potentials (SEPs), an indicator of somatosensory pathway activity using electroencephalography (Cruccu *et al.*, 2008; Jackson & Bolger, 2014).

Additionally, released NA at synaptic terminals have been shown to induce long-term changes in glutamatergic synapses in various brain regions, including the hippocampus and sensory cortical areas (Heginbotham & Dunwiddie, 1991; Brocher *et al.*, 1992). Although unclear, this suggests a potential temporal variation in the relationship between sensory-evoked response and LC activation.

Since the characteristic spontaneous firing rate of the LC is 0.1–5 Hz (Akaike, 1982; Berridge & Foote, 1991), stimuli within this frequency range are often used in studies (Devilbiss & Waterhouse, 2004; Devilbiss *et al.*, 2006). Using this as a reference, the present study applied various frequencies (0.1, 1 and 4 Hz) of tonic electrical stimulation to the LC of anesthetized rats to examine their effects on SEPs. Moreover, voltage-sensitive dye (VSD) imaging, which is suitable for measuring the spatiotemporal dynamics of cortical activity (Grinvald & Hildesheim, 2004), was used for the spatial representation of sensory responses. Finaly, pharmacological antagonists were used to evaluate the involvement of cortical NA receptors in changes in SEPs.

Materials & Methods

Animals

Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 240–380 g were used. Rats were housed in cages under a 12 h light/dark cycle with ad libitum food and water. All experiments were conducted in accordance with the Regulations for Animal Experiments at Niigata University of Health and Welfare (Approval No. 02112-02122).

Surgical procedures and electrophysiological recordings

Before surgery, each animal was anesthetized with 3.0% isoflurane and then fixed in a stereotaxic frame (Narishige, Tokyo, Japan). During surgery and experiments, anesthesia was maintained with 1.5–1.7% isoflurane and the body temperature was maintained at 37°C via a rectally inserted feedback heating plate (BWT-100A; Bio Research Center, Nagoya, Japan). A craniotomy was performed to create two circular cranial windows centered on the left forelimb primary somatosensory (S1) area (anteroposterior, AP, 0 mm; mediolateral, ML, 4.5 mm from the bregma) and directly over the left LC (AP, -9.8 mm; ML, 1.2 mm from the bregma) (Paxinos & Watson, 2014), with diameters of approximately 5.0 and 2.0 mm, respectively. A dental acrylic chamber was built around the cranial window of S1 and filled with 0.9% saline.

Local field potentials (LFPs) were recorded using a silver ball electrode (UL-3010; Unique Medical Co., Ltd., Tokyo, Japan) placed on the S1 cortical surface (AP, 0 mm; ML, 4.5–5.0 mm from the bregma). Voltage signals were amplified (150×), band-pass filtered (0.1–500 Hz) using an instrumental amplifier (Model 440; Brownlee Precision, San Jose, CA, USA), and digitally acquired at a sampling rate of 1 kHz using dedicated data acquisition software (Power Lab; AD Instruments, Nagoya, Japan). SEPs were obtained by applying a single electrical pulse to the forepaw (100–500 µA, pulse width 1 ms). Stimulus intensity was set at 50–70% of the parameter value that elicited the maximal response for each rat.

LFPs and SEPs were analyzed using LabChart 8 software (AD Instruments). The amplitude of SEP was defined as the difference between the positive peak (10–25 ms from the onset of the forepaw stimulation) and subsequent negative peak (25–40 ms from the onset of the forepaw stimulation).

LC stimulation and histology

The LC stimulation site was identified by an electrophysiological index, using similar procedures to those described in previous studies (Marzo *et al.*, 2014; Nagasaka *et al.*, 2017). A concentric bipolar stimulation electrode (IMB-9002; Intermedical, Portland, ME, USA) was positioned directly over the LC and advanced downward in 400-µm steps, and a stimulus train (100 µA, 100 Hz, 50 pulses, pulse width 100 µs) was applied. When the LC was stimulated, cortical desynchronization (i.e., from large-amplitude slow fluctuations to small-amplitude fast oscillations) was induced. Based on the cortical desynchronization, the electrode that targeted LC was placed at AP -9.8 ± 0.5 mm, ML 1.0 ± 0.4 mm from the bregma, and a depth of 5.8 ± 0.4 mm from the brain surface and was fixed during experiments. In Experiments 1–5 described below, electrical stimulation (150 µA, pulse width 200 µs) was applied to the identified LC for 20 min. The stimulation frequency was set for each experiment as described in each section.

To confirm the stimulus site through histologically, a small electrolytic lesion was made by passing direct current (100 µA, 5 s). At the end of experiment, the animals were deeply anesthetized with pentobarbital (i.p.) and transcranial perfused with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was removed, and coronal sections (30 μm thickness) were cut. Subsequently, Nissl staining using cresyl violet (Sigma-Aldrich, St. Louis, MO, USA) was performed, and images of the stained sections were acquired using a microscope (ECLIPSE Ci-Lplus, Nikon, Tokyo, Japan).

Experiment 1: Effect of LC stimulus frequency on SEPs

In Experiment 1, 11 rats were used to evaluate the effects of tonic electrical stimulation to the LC at three stimulation frequencies (0.1, 1, and 4 Hz) on SEPs. These stimulation frequencies were set to account for the tonic discharge range (0.1–5 Hz) of the LC neurons (Akaike, 1982; Berridge & Foote, 1991). Stimulation of the forepaw was performed at 20 s intervals, with the first 12 trials (4 min) as “baseline,” followed by the addition of tonic stimulation to the LC for 20 min (“during LC stimulation”). After the series of tonic stimulation to the LC was completed, 36 trials (12 min) of forepaw stimulation were performed as “post-LC stimulation”. The percent change from baseline was calculated by taking the average of six SEPs every 2 min and dividing it by the average amplitude of the baseline. To prevent simultaneous pulses to the forepaw and LC, the interval between stimuli was delayed 100–330 ms for each animal.

Six rats used in Experiment 1 underwent a single session, while the remaining five rats underwent multiple stimulation sessions to evaluate frequency-specific changes. The stimulation intensity to the paw was adjusted so that the SEP amplitude was at the baseline level from the first session, and the interval between each LC stimulation session was at least 80 min.

Experiment 2: SEP changes with non-paired tonic LC stimulation

In Experiment 2, using five rats, the LC tonic stimulation was separated from the forepaw stimuli to evaluate whether their simultaneous application was necessary for somatosensory response changes. SEPs were obtained before and after tonic LC stimulation at 1 Hz. For comparisons among conditions, we calculated the average amplitude of 12 trials with 20-s intervals.

Experiment 3: Voltage sensitive dye imaging for forepaw sensory response

In Experiment 3, using five rats, we performed VSD imaging as described previously (Nagasaka *et al.*, 2017; Dezawa *et al.*, 2021). During the experiment, the head of each rat was rotated by 25–27° to ensure that the camera axis was perpendicular to the S1 surface. The 0.9% saline solution in the dental acrylic chamber was replaced with RH1691 (3.0 mg/ml in 0.9% saline; Optical Imaging, Rehovot, Israel) and stained for 2 h while shaded from room light. Thereafter, the unbound dye was removed, and the cortical surface was washed with saline for 10 min. The saline-filled chamber was then covered with a glass coverslip. For imaging, the VSD was excited with 632 nm light from an LED illumination system (X-cite Xylis; Excelitas Technologies, Salem, MA, USA). The excitation light was reflected using a 650 nm dichroic mirror and focused onto the cortical surface using a camera lens (Plan Apo 2x; Nikon). Fluorescence was collected via a long-pass filter (>665 nm), focused onto the sensor of a high-speed CMOS camera (MiCAM03-N256; BrainVision, Tokyo, Japan), and sampled at 500 Hz. This CMOS camera had a detector with 128 × 128 pixels and a field of view of approximately 5 × 5 mm. In each trial, a single pulse electrical stimulation (100–500 µA, pulse width 1.0 ms) was applied to forepaw, and 16 consecutive trials with 10-s intervals were performed. Data acquisition was performed before (baseline) and immediately after 1 Hz LC stimulus for 20 min (post-LC stimulation), and was completed by 45 min after the end of the LC stimulus.

The ratio of the relative fluorescent change and the reference fluorescence (ΔF/F) was calculated by taking the reference (F) as the signal 16 ms before forepaw stimulation. For the analysis of sensory-evoked responses, the mean response in the 9 × 9 binned area around the peak value during 50 ms from the forepaw stimulation onset; the activation area was defined as the region that exceeds 60% of the mean peak amplitude during 50 ms from the stimulation onset. The analysis was conducted using offline BV\_ana software (BrainVision).

Experiment 4: Effect of a glutamate receptor antagonist

In Experiment 4, using three rats, the effect of tonic LC stimulation on glutamatergic signaling evoked by stimulation of the forepaw was evaluated. We administered a 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 250 µM; Sigma-Aldrich), which is an antagonist of non-*N*-methyl-d-aspartate glutamate receptors, onto the cortical surface (Kunori *et al.*, 2014). After obtaining baseline and post-LC stimulation SEPs following the procedures described in Experiment 2, CNQX was applied to the dental acrylic chamber above S1 for 20 min and then washed with saline. After treatment, SEPs were obtained as “post-CNQX” estimates. A recovery experiment was performed in which the exposed cortical surface was rinsed continuously with saline for 30 min. SEPs were obtained as “post-wash” estimates.

Experiment 5: Effect of an NA receptor antagonist

In Experiment 5, using 19 rats, we examined the effects of each NA receptor on the SEPs altered by tonic LC stimulation. For the application of an antagonist, saline in the dental acrylic chamber was replaced with a NA receptor antagonist. The antagonists were prazosin (an α1 receptor antagonist, 1 mM; Sigma-Aldrich), yohimbine (an α2 receptor antagonist, 1 mM; Fujifilm Wako Pure Chemicals, Osaka, Japan), and propranolol (a β receptor antagonist, 1 mM; Sigma-Aldrich) (Schiemann et al., 2015); saline (0.9% NaCl) was used as a control. After 20 min following the application of the antagonist, the unbound pharmacological agent was removed, and tonic LC stimulation at 1 Hz was applied for 20 min. The average of SEPs from 12 forepaw stimulations was obtained before antagonist application (baseline) and after the end of LC stimulation (post-LC stimulation). The interval between forepaw stimulations was 20 s.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). For Experiment 1, data are presented as the median and interquartile ranges owing to repeated measurements. Therefore, statistical significance was assessed using the nonparametric Kruskal–Wallis test with Dunn’s post hoc tests. For all other experiments, data are presented as the mean ± standard error of the mean (SEM), and data were analyzed using paired t-test or one-way analysis of variance (ANOVA) with repeated measures, followed by Tukey’s multiple comparisons tests.

Results

Based on cortical desynchronization produced by burst electrical stimulation (100 Hz, 50 pulses), the LC was identified. The mapping of the electrode positions that caused desynchronization is shown in Figure 1A. Desynchronization persisted for 4–5 s after burst stimulation and then returned to the initial state (Figure 1A, left, lower trace). When displaced 400 μm from the position where this desynchronization was observed, the stimulus failed to produce desynchronization (Figure 1A, left, upper trace). Electrode tips were confirmed using Nissl-stained sections (Figure 1B). The site of stimulation inducing cortical desynchronization was localized to the LC.

Effects of tonic LC stimulation on the SEPs

After identifying LC, the effect of electrical stimulation on SEP was examined (Figure 2A-C, Experiment 1). Time series data for the amplitude of SEPs at baseline, during, and after tonic LC stimulation under stimulation at 0.1, 1, and 4 Hz to the LC are shown in Figure 2D, E, and F, respectively. The amplitudes of SEPs (% baseline) increased after the onset of tonic LC stimulation at 1 Hz and showed a sustained increase even after the end of the stimulation (Figure 2E). No change in amplitudes was observed for 0.1 or 4 Hz (Figure 2D and F). In two rats, we examined how long the enhancement in SEP following 1 Hz stimulation persisted after the end of stimulation and observed a return to baseline values after approximately 80 min (data not shown). SEP amplitudes recorded 18–20 min after the onset of tonic LC stimulation (shown as “b” in Figure 2D–F) differed significantly among the three conditions (Kruskal-Wallis statistic = 12.01, p < 0.001). As evaluated using Dunn's multiple comparisons test, SEP amplitudes were significantly higher for 1 Hz stimulation (n = 7, 218%; interquartile range 162 to 234%) than for 0.1 Hz (n = 6, 96%; interquartile range 63% to 105%, p = 0.010) or 4 Hz (n = 6, 83%; interquartile range 66% to 111%, p = 0.008) (Figure 2G). Furthermore, when different stimulation frequencies were randomly administered within individuals, the increase in SEP amplitudes was greater for 1 Hz stimulation than for other conditions (Figure 2H).

To examine whether the combination of 1 Hz LC stimulation and forepaw stimulation was essential for causing the increased SEP amplitude, we performed an experiment in which the forepaw stimulation was omitted during the tonic LC stimulation (Experiment 2). A comparison between baseline and post-LC stimulation showed a significant increase in amplitudes after stimulation (baseline, 0.27 ± 0.064 mV; post-LC stimulation, 0.451 ± 0.069 mV, *t*(8) = 4.825, p = 0.009, paired *t*-test, Figure 2I), suggesting that a similar effect is obtained without combining the two stimuli. In Experiment 2, the increase for “post-LC stimulation” was 168% (interquartile range 141% to 256%) relative to baseline, which was similar to the “post-LC stimulation” increases of 173% (interquartile range 153% to 283%) in Experiment 1.

VSD imaging of the sensory response modulated by 1 Hz LC stimulation.

Next, VSD imaging of the forepaw S1 area was performed before and after 1 Hz LC stimulation (Figure 3A, Experiment 3). Figure 3B and C show the spatiotemporal patterns and response intensities of forepaw stimulation before LC stimulation (baseline; upper panels) and after 1 Hz tonic stimulation to the LC (post-LC stimulation; lower panels). At baseline, depolarizing neuronal responses (indicated by warm colors) occurred approximately 10 ms after the forepaw stimulation and then propagated in a rostral direction. After LC stimulation, these latencies and directions were similar; however, the fluorescence intensity showed increased activity relative to baseline (Figure 3C). VSD signals were significantly higher post-LC stimulation than at baseline (n = 5, baseline, 0.32 ± 0.10%; post-LC stimulation, 0.42 ± 0.13%, *t*(8) = 2.968, p = 0.041, paired *t*-test). Enlargement of the activation zone was observed in several individuals; however, the difference was not statistically significant (in kilo-pixels: baseline, 3.85 ± 1.05; post-LC stimulation, 4.24 ± 0.98, paired *-*test, p = 0.484).

Effects of blocking glutamatergic inputs on the LC-mediated SEP

To confirm whether the alterations in SEPs in response to tonic LC stimulation were due to glutamate input, we used the receptor antagonist CNQX (Experiment 4). When CNQX was administered, the increase in SEP amplitude induced by 1 Hz LC stimulation almost completely disappeared (Figure 4A and B). The recovery of the response was confirmed after a 30 min wash of CNQX. These results suggest that the neural response modulated by tonic stimulation to the LC is consistent with glutamatergic transmission.

Effects of blocking NA inputs on the LC-mediated SEP

To determine if blocking NA receptors impairs the enhancement of SEP, each receptor antagonist was administered prior to 1 Hz tonic stimulation of the LC (Experiment 5, Figure 5A). Figure 5B shows the changes in SEP after LC stimulation for each antagonist administration. The SEP amplitudes after LC stimulation differed significantly across the conditions (control, n = 5, 345 ± 68%; prazosin, n = 5, 116 ± 20%; yohimbine, n = 5, 202 ± 43%; propranolol, n = 4, 109 ± 14%; *F*(3, 15) = 6.256, p = 0.006, one-way ANOVA). SEPs after LC stimulation were significantly lower in the prazosin and propranolol groups than in the control group (vs. prazosin, p = 0.009; vs. propranolol, p = 0.011, Tukey’s post hoc test). Yohimbine administration resulted in a moderate enhancement of SEP; however, SEP did not differ significantly from that in the control group (p = 0.132). These results indicate that the action of NA receptors, such as α1 and β, is required for the enhancement of SEP after 1 Hz LC stimulation.

Discussion

In the present study, 1 Hz tonic electrical stimulation of the LC for 20 min increased the forepaw-sensory response. Interestingly, the enhancement of SEP was maintained for tens of minutes after the interruption of LC stimulation, indicating possible long-term plasticity in the somatosensory pathways. Furthermore, pharmacological validation demonstrated that the enhancement of SEP after 1 Hz LC stimulation is likely mediated through cortical α1 and β receptors, whereas α2 receptors do not appear to play a significant role. The present study suggests that the long-term effects of LC activation are reflected in sensory pathways via specific cortical receptors.

To elucidate the impact of the LC-NA system on somatosensory pathways, we focused on how LC stimulation modulated cortical SEPs in this study. SEPs are widely used to assess somatosensory pathways in healthy subjects and patients with sensory-related neurological conditions (Florence *et al.*, 2004; Ishii *et al.*, 2021; Sasaki *et al.*, 2022). Unlike unit activity recordings, LFPs have been correlated with blood oxygenation level-dependent signals detected by magnetic resonance imaging (MRI) (Ogawa *et al.*, 1990; Ogawa *et al.*, 1993; Logothetis *et al.*, 2001). Therefore, SEPs not only shed light on the mechanisms underlying NA-mediated pathway modulation, but also provide valuable insights for the interpretation of MRI and SEP-based studies in healthy individuals and patients with NA dysfunction.

In previous studies of LC-mediated effects on thalamic VPM and barrel cortical responses, single-unit recordings in awake rats were obtained for a range of tonic LC stimulation frequencies that mimicked physiological firing rates (≤5 Hz), revealing region- or cell type-specific regulatory effects (Devilbiss & Waterhouse, 2004; Devilbiss *et al.*, 2006). Specifically, thalamic VPM nuclei showed efficient whisker-evoked responses in response to 0.5–1 Hz LC stimulation, while barrel cortical neurons showed the highest sensitivity at 0.5 Hz stimulation. Our SEP results likely reflect facilitation in both thalamic and cortical areas, which are embedded in sensory pathways. These findings align with those of previous whisker sensation experiments.

A series of previous studies have proposed an inverted U-shaped regulatory mechanism underlying performance-related sensory processing that depends on the tonic activity of LC neurons and NA concentration at synaptic terminal regions (Devilbiss & Waterhouse, 2000; 2004; Devilbiss *et al.*, 2006; Manella *et al.*, 2017; McBurney-Lin *et al.*, 2019; Poe *et al.*, 2020). In vitro studies have demonstrated that moderate concentrations of NA enhance glutamate-evoked discharges in the barrel cortex via α1 receptors, whereas higher concentrations can suppress these responses via α2 receptors (Devilbiss & Waterhouse, 2000). It is likely that 1 Hz LC stimulation increased SEP by inducing an optimal NA concentration to activate α1 receptors, while 4 Hz stimulation induced a higher concentration of NA, thereby activating α2 receptors in addition to α1, explaining the lack of change in SEP. These results further support the idea that different receptors were activated under different levels of LC activity and affect sensory pathways. However, given the positive linear relationship between the NA concentration at synaptic terminals and LC activity level (Devoto *et al.*, 2005; Devilbiss *et al.*, 2006), the excitability increase should be shifted to a decrease during 20 min of 1 Hz LC stimulation; however, this was not observed. This may be due to the inhibition of additional NA release via α2 receptors and the interactions of NA receptors with each other or with receptors for other transmitters (Mather *et al.*, 2016). Based on these considerations, we suggest the importance of combining microdialysis, which can estimate local NA concentrations, and electrophysiological recordings to interpret the results of this study.

β receptors enhance excitatory synaptic transmission in the cortex (Salgado *et al.*, 2016). This is consistent with the results of the present study, in which the blockade of β-receptors with propranolol resulted in a lack of enhancement of SEP after LC stimulation. On the other hand, the activation of β receptors also promotes an intracellular influx of Ca2+ and increased intracellular concentration of cyclic adenosine monophosphate (Marzo *et al.*, 2009), thereby inducing synaptic long-term potentiation (LTP) (Neuman & Harley, 1983; Almaguer-Melian *et al.*, 2005). Thus, β receptors may be involved in long-term changes rather than immediate effects on SEP. Although we were unable to link NA receptors to long-term changes in SEP in this experiment, future studies should investigate expression levels of proteins associated with synaptic plasticity and the use of inhibitors of NMDA receptors, which contribute to LTP (Luscher & Malenka, 2012). Moreover, the functional significance of the long-term changes in the somatosensory pathways induced by the LC stimulation is not well understood. Our next step is to investigate how the enhancement of SEPs induced by LC stimulation affects sensory perception and behavioral outcomes in awake animals.

Consistent with the SEP results, VSD imaging showed an increase in the somatosensory response after 1-Hz LC stimulation. However, some VSD data showed increased activity not only in S1 but also in M1 and M2 regions. These results for adjacent areas may reflect increased somatosensory activity because S1 activity from somatosensory stimulation propagates to the higher motor cortex via the primary somatosensory cortex (Manita *et al.*, 2015; Kunori & Takashima, 2016). Stimulation of the LC or a resulting increase in the NA concentration may cause a dynamic functional shift in cortical or subcortical neural networks (Zerbi *et al.*, 2019).

Conclusion

The present study demonstrated that LC stimulation increased the excitability of somatosensory pathways via cortical NA receptors. The effective stimulation frequency was limited to 1 Hz, with no effects at lower or higher frequencies, suggesting that the stimulation produced NA concentration changes appropriate for receptor activation. Furthermore, these observed effects were sustained, and elucidating the temporal characteristics of the effects of NA will be important in understanding the complexity of the LC-NA system. These findings provide insight into the pathophysiology of sensory deficits associated with Parkinson's disease and chronic pain (Taylor & Westlund, 2017; Vermeiren & De Deyn, 2017), which are associated with a disruption of the NA system.

**Conflicts of Interest**

The authors declare no competing financial interests.

**Data Availability**

All data and code supporting the findings of this studyare available upon request.

**Author Contributions**

Takanobu Suzuki: Conceptualization; formal analysis; investigation. Kazuaki Nagasaka: Conceptualization; formal analysis; funding acquisition; visualization; writing—original draft. Tomofumi Otsuki: Resources. Naofumi Otsuru: Resources; supervision. Hideaki Onishi: Funding acquisition; resources; supervision.

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