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Plenary Article

Possible involvement of activated locus coeruleus–noradrenergic neurons in pain-related sleep disorders

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HIGHLIGHTS

- Activation of LC noradrenergic neurons increases in wakefulness with decreased NREM.
- Neuropathic pain increases the activity of LC–PFC noradrenergic neurons.
- Activation of LC–PFC noradrenergic neurons facilitates sleep-to-wake transitions.

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ABSTRACT

The locus coeruleus (LC) is a noradrenergic brainstem structure that is considered to play a role in promoting arousal. To further clarify the role of LC noradrenergic neurons, we performed an optogenetic assay by injecting AAV-channelrhodopsin-2 (ChR2) into the LC of cre-tyrosine hydrolase (TH) mice. We found here that the specific activation of LC noradrenergic neurons produced a significant increase in wakefulness and a significant decrease in non-rapid eye movement (NREM) sleep during photostimulation. On the other hand, neuropathic pain is believed to significantly interfere with sleep, and inadequate sleep may contribute to the stressful negative consequences of living with pain. In the present study, sciatic nerve ligation, which produced significant thermal hyperalgesia, significantly increased the levels of noradrenaline released in the prefrontal cortex (PFC) by the weak electrical stimulation of neurons in the LC. Under these conditions, the systemic administration of adrenaline α and β inhibitor cocktail at 7 days after sciatic nerve ligation restored the increased wakefulness and decreased NREM sleep to normal levels. These results suggest that neuropathic pain may accelerate neurons in the LC, and its overactivation may be, at least in part, associated with sleep disturbance under neuropathic pain.

1. Introduction

The locus coeruleus (LC) is a noradrenergic brainstem structure that is thought to play a major role in promoting arousal [1]. LC neurons fire during wakefulness, decrease firing during non-rapid eye movement (NREM) sleep, and are virtually silent during rapid eye movement (REM) sleep [2]. It has been reported that the level of mRNA for norepinephrine transporter (NET) was increased in rats that were deprived of REM sleep for 3 days or longer [3].

Neuropathic pain can be very difficult to treat, and the presence of comorbidities, such as poor sleep and mood disorders, can greatly affect the outcome. Clinical studies have shown that most patients experience problems with sleep after they develop chronic pain [4]. In our previous study, we demonstrated that neuropathic pain accelerated the activity of dorsal raphe nucleus (DRN)-serotonergic neurons, implying that the activation of DRN neurons may play a role in sleep dysregulation under a neuropathic pain-like state [5]. However, the neurophysiological mechanism by which neuropathic pain affects sleep-arousal patterns remains unclear. Therefore, in this study, we investigated the role of the LC-prefrontal cortex (PFC) noradrenergic system in sleep dysregulation in mice with neuropathic pain.

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2. Methods

2.1. Ethics statement

All experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at Hoshi University.

2.2. Animals

Male C57BL/6J mice (8 weeks old; Tokyo Laboratory Animals Science, Tokyo, Japan) and tyrosine hydroxylase (TH)-cre transgenic mice (B6. Cg-Tg(Th-cre) 1Tmd/J) (Jackson Laboratory) were used for this study. TH is the rate-limiting enzyme in the synthesis of noradrenaline and dopamine. Animals were housed in a room maintained at 23 ± 1 °C with a 12 h light–dark cycle. Food and water were available *ad libitum*.

2.3. Virus preparation

We purchased AAV-FLEX-rev-ChR2 (H134R)-mCherry from Addgene (Plasmid 18916). The recombinant AAV vectors were serotyped with AAV5 coat proteins and packaged by a viral vector core, which was provided by Drs. Ryosuke Matsui and Dai Watanabe (University of Kyoto). The final viral concentration was 2×10^{13} particles/mL. Aliquots of virus were stored at −80 °C before stereotaxic injection. AAV viruses were injected in the bilateral LC (from the bregma: antero-posterior, −5.4 mm; mediolateral, ±1.28 mm; and dorsal, −3.6 mm) using a microsyringe pump (0.25 μL/min, 4 min).

2.4. Immunohistochemistry

TH-Cre transgenic mice on day 14 after the injection of AAV were deeply anesthetized and perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). After perfusion, the brain was removed and thick coronal blocks of the brainstem including the LC were rapidly dissected and fixed in 4% paraformaldehyde for 2 h. They were then permeated with 20% sucrose in 0.1 M PBS for 1 day and 30% sucrose in 0.1 M PBS for 2 days with agitation. The brain sections were stored at −30 °C. Transverse sections were cut with a cryostat (Leica CM1510; Leica Microsystems, Heidelberg, Germany) at a thickness of 8 μm and thaw-mounted on poly-L-lysine-coated glass slides. Brain sections that included the LC were incubated in blocking solution, 3% normal goat serum (NGS; Vector Laboratories, Inc., USA) in 0.01 M PBS, for 1 h at room temperature, and then incubated for 48 h at 4 °C with primary antibodies diluted in 3% NGS: anti-TH (mouse monoclonal, ImmunoStar, Inc., Hudson, WI, USA). The antibody was then rinsed with PBS and incubated with an appropriate secondary antibody conjugated with Alexa™ 488 (Molecular Probes, Inc.) for 2 h at room temperature. The fluorescence of immunolabeling was detected using a light microscope (BX-61, Olympus, Inc., Tokyo, Japan) and digitized images.

2.5. Photostimulation

An 8 mm unilateral cannula (EIM-330; Eicom) was surgically implanted at least 1 day before the experiment. The cannula was placed above the LC and fixed to the skull with quick self-curing acrylic resin. An optical fiber (50 μm diameter; Lucir) was placed inside the cannula at least 2 h before the stimulation experiments. The light source was a 473 nm blue laser (Lucir; COME2-LB473 model, Japan) that was controlled by an electronic stimulator (Nihon Kohden, Japan) to generate tonic light pulses: interval (323 ms), duration (10 ms), main interval (1 s), train (3). The power output was measured at the tip of the fiber with a light meter (NOVA, Ophir, Japan) when the laser was activated in continuous mode.

2.6. In vivo microdialysis and high-performance liquid chromatography

A microdialysis probe (D-1-6-01; Eicom, Japan) was inserted directly into the PFC (from the bregma: anteroposterior, +1.5 mm; mediolateral, +0.5 mm; dorsoventral, −3.7 mm) according to an atlas of the mouse brain. The probe was fixed to the skull with quick self-curing acrylic resin. The probes were perfused continuously (1 μL/min) with artificial cerebrospinal fluid: 0.9 mM $MgCl_2$, 147.0 mM NaCl, 4.0 mM KCl, and 1.2 nM $CaCl_2$. Outflow fractions were collected, and mice were subjected to photostimulation or electrostimulation for 15 min. Dialysis samples were collected for 1 h after stimulation and analyzed by high-performance liquid chromatography with electrochemical detection (HTEC-500; Eicom). Noradrenaline was separated by column chromatography and identified and quantified by the use of a standard (Sigma–Aldrich, Inc., USA).

2.7. Electroencephalogram and electromyogram recordings

Electroencephalogram (EEG) and electromyogram (EMG) electrodes were implanted for polysomnographic recordings (Pinnacle Technology, Inc., USA). For monitoring EEG signals, two stainless-steel EEG recording screws were positioned 1 mm anterior to the bregma or lambda, both 1.5 mm lateral to the midline. Stainless-steel, Teflon-coated wires were placed bilaterally into both trapezius muscles to monitor EMG activity. We began to monitor the sleep/wake state 2 days after placement of the EEG recording screws. EEG/EMG signals were amplified, filtered (EEG, 0.5–30 Hz; EMG, 20–200 Hz), and digitized at a sampling rate of 128 Hz. The data were subjected to an analysis by SLEEPSIGN software (Kissei Comtec, Japan). Vigilance was automatically classified off-line into three stages using 5-s epochs: wakefulness, rapid eye movement (REM) and non-REM (NREM) sleep. For each epoch, the EEG power density in the δ (0.75–4.0 Hz) and θ waves (6.25–9.0 Hz) and the integrated EMG value were displayed on a PC monitor. For each 5-s epoch, the vigilance state was determined to be either wakefulness (high EMG and low EEG amplitude and high theta activity concomitant with highest EMG values), NREM sleep (low EMG and high EEG amplitude, high delta activity) or REM sleep (low EMG and low EEG amplitude, high theta activity), and the scores were manually entered into a PC using a keyboard. EEG and EMG activities were measured 7 days after sciatic nerve ligation.

2.8. Neuropathic pain model

Mice were anesthetized with 3% isoflurane. A partial sciatic nerve ligation model was produced as described previously [6]. Briefly, the sciatic nerve on the right side (ipsilateral side) was ligated by tying a 8–0 silk suture around approximately one-half its diameter under a light microscope (SD30, Olympus, Tokyo, Japan). In sham-operated mice, the nerve was only exposed without ligation.

2.9. Measurement of thermal thresholds

The right plantar surface of mice was subjected to a well-focused, radiant heat light source (model 33 Analgesia Meter; IITC/Life Science Instruments, Woodland Hills, CA) to assess its sensitivity to thermal stimulation. Quick movements of the hind paw away from the stimulus, regardless of licking of the hind paw, were considered to be a withdrawal response.