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Chronic pain enhances excitability of corticotropin-releasing factor-expressing neurons in the oval part of the bed nucleus of the stria terminalis

Ryoko Uchida[1], Yasutaka Mukai[2,5,9], Taiju Amano[1], Kenji Sakimura[3], Keiichi Itoi[4], Akihiro Yamanaka[2,6,7,8] and Masabumi Minami[1*]

Abstract

We previously reported that enhanced corticotropin-releasing factor (CRF) signaling in the bed nucleus of the stria terminalis (BNST) caused the aversive responses during acute pain and suppressed the brain reward system during chronic pain. However, it remains to be examined whether chronic pain alters the excitability of CRF neurons in the BNST. In this study we investigated the chronic pain-induced changes in excitability of CRF-expressing neurons in the oval part of the BNST (ovBNST^{CRF} neurons) by whole-cell patch-clamp electrophysiology. CRF-Cre; Ai14 mice were used to visualize CRF neurons by tdTomato. Electrophysiological recordings from brain slices prepared from a mouse model of neuropathic pain revealed that rheobase and firing threshold were significantly decreased in the chronic pain group compared with the sham-operated control group. Firing rate of the chronic pain group was higher than that of the control group. These data indicate that chronic pain elevated neuronal excitability of ovBNST^{CRF} neurons.

Keywords Bed nucleus of the stria terminalis, Chronic pain, Corticotropin-releasing factor, Neuronal excitability, Negative emotion

*Correspondence:
Masabumi Minami
mminami@pharm.hokudai.ac.jp
[1]Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan
[2]Department of Neuroscience II, Research Institute of Environmental Medicine, Nagoya University, Nagoya 464-8601, Aichi, Japan
[3]Department of Animal Model Development, Brain Research Institute, Niigata University, Niigata 951-8585, Japan
[4]Department of Nursing, Tohoku Fukushi University, Sendai 981-8522, Japan
[5]Department of Cellular Pharmacology, Graduate School of Medicine, Hokkaido University, Sapporo 060-8638, Japan
[6]Chinese Institute for Brain Research, Beijing 102206, China
[7]Division of Brain Sciences, Institute for Advanced Medical Research, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan
[8]National Institute for Physiological Sciences, National Institutes of Natural Sciences, Okazaki 444-8585, Aichi, Japan
[9]Postdoctoral Research Fellow, Japan Society for the Promotion of Science, Tokyo 102-0083, Japan

Introduction

Pain-induced aversive responses are important for the physiological role of pain as a biological warning system. However, chronic pain induces maladaptive emotional states, which often lead to psychiatric disorders, such as depression and anxiety disorders. Therefore, it is important to elucidate the neural mechanisms of chronic pain-induced maladaptive emotional states. We have reported that enhanced release of corticotropin-releasing factor (CRF) in the anterolateral part of bed nucleus of the stria terminalis (BNST) is involved in acute pain-induced aversive responses [1], and that sustained enhancement of CRF signaling in the anterolateral BNST during chronic pain suppresses the brain reward system, which may lead to depression-like states [2]. However, it remains to be examined whether chronic pain alters the excitability of CRF neurons in the BNST. Thus, in this study we investigated the chronic pain-induced changes in excitability of CRF-expressing neurons in the oval part of the BNST (ovBNST^{CRF} neurons), where CRF neurons are densely located, by whole-cell patch-clamp electrophysiology using brain slices prepared from a mouse model of neuropathic pain.

Materials and methods

CRF-Cre [3]; Ai14 mice on C57BL/6J background were used to visualize ovBNST^{CRF} neurons. In this study, we followed the Allen Mouse Brain Atlas for the anatomical terminology of the subnuclei within the BNST [4]. Immunohistological analysis using an antibody for PKCδ, which specifically localize in the oval part within the BNST (ovBNST) [5], was conducted to confirm the localization of CRF-expressing neurons in the ovBNST (Fig. 1A). A mouse model of neuropathic pain (spared nerve injury model, SNI) was prepared by ligating then cutting the tibial and common peroneal nerves on the left side [6] under anesthesia with isoflurane (induction, 3.0%; maintenance, 2.0%). The von Frey test was performed 1 day before and 1, 2, 3, and 4 weeks after the surgery to confirm the induction of chronic pain. (Fig. 1B). Four to five weeks after the surgery, mice were sacrificed and the brain slices including the BNST were prepared for whole-cell patch-clamp recordings from ovBNST^{CRF} neurons. Resting membrane potential, membrane resistance, tau, and rheobase were measured. The action potential threshold was defined as the membrane potential at which the derivative of the voltage (dV/dt) exceeded 10 mV/ms. The detailed materials and methods were described in the additional information 1. Data indicate means ± SEM. Statistical analyses were conducted using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA). Two-tailed unpaired *t* test and two-way repeated measures ANOVA were used to analyze the data as shown in the figure legend. Differences with $P<0.05$ were considered significant.

Results

Electrophysiological recordings were carried out from ovBNST^{CRF} neurons (sham: $n=39$ cells from 7 mice, SNI: $n=39$ cells from 8 mice) labeled by tdTomato. Although resting membrane potential (RMP) was indistinguishable between the SNI and sham groups (Fig. 1C), membrane resistance (Fig. 1D) and tau (Fig. 1E) were significantly increased in the SNI group. In the I-V curve, inward rectifying current was observed at higher membrane potentials in both the SNI and sham groups. Negative membrane current was smaller in the SNI group at lower membrane potentials (Fig. 1F). These data suggest that chronic pain altered intrinsic electrophysiological properties of ovBNST^{CRF} neurons. Next the neuronal excitability was examined in SNI and sham groups. Rheobase (Fig. 1G) and firing threshold (Fig. 1H) were significantly lower in the SNI group compared with the sham group. The number of action potentials evoked by +10 pA step current (500-ms duration) across the range of 0-120 pA was measured in 31 and 33 cells of the sham and SNI groups, respectively. The firing rate of the SNI group was higher than that of the sham group (Fig. 1I). These data indicate that chronic pain elevated neuronal excitability of ovBNST^{CRF} neurons.

Discussion

We previously reported that sustained enhancement of CRF signaling within the BNST during chronic pain suppresses the dopaminergic neurons in the ventral tegmental area [2]. However, it remains to be examined whether chronic pain alters the neuronal excitability of CRF neurons in the BNST. In this study, we utilized CRF-Cre; Ai14 mice to visualize CRF-expressing neurons in the brain slices prepared from the mouse model of neuropathic pain and examined chronic pain-induced changes in excitability of ovBNST^{CRF} neurons. The results showed that chronic pain elevated neuronal excitability of ovBNST^{CRF} neurons.

Alcohol withdrawal, which is known to cause increased anxiety-like behavior [7], has been shown to increase excitability of a subpopulation of putative local CRF-expressing neurons in the BNST [8]. Hu et al. reported that chronic variable mild stress (CVMS) induced anxiety- and depression-like behaviors and increased neuronal excitability of ovBNST^{CRF} neurons and that intra-ovBNST injection of R121919, a CRFR1-selective antagonist, ameliorated the CVMS-induced anxiety- and depression-like behaviors [9]. These findings suggest that enhanced neuronal excitability of ovBNST^{CRF} neurons induces anxiety- and depression-like behaviors under the pathological conditions. Hu et al. also reported that

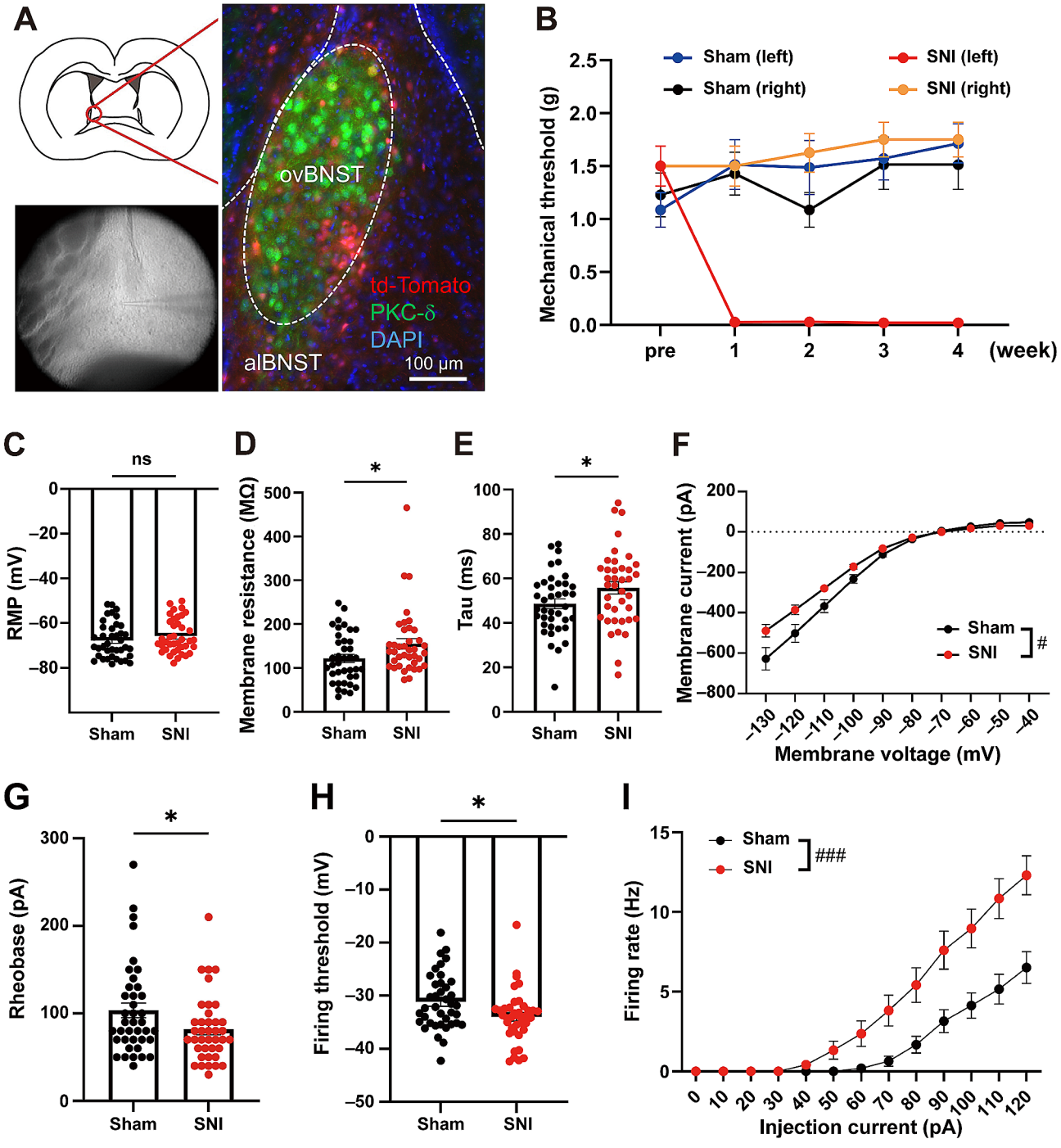

Fig. 1 Chronic pain enhances excitability of CRF neurons in the ovBNST. A, Electrophysiological recordings from ovBNST^{CRF} neurons labeled by tdTomato. Immunohistological analysis using an antibody for PKCδ, which specifically localize in the ovBNST, was conducted to confirm the localization of tdTomato-positive CRF-expressing neurons in the ovBNST. B, Time courses of pain thresholds in the SNI ($n=8$) and sham ($n=7$) groups. C-F, Intrinsic electrophysiological properties of ovBNST^{CRF} neurons: RMP (C; sham: -67.77 ± 1.26 mV vs. SNI: -65.80 ± 1.21 mV, $t_{76}=1.129$, $P=0.2625$), membrane resistance (D; sham: 121.7 ± 9.1 MΩ vs. SNI: 155.5 ± 11.8 MΩ, $t_{76}=2.271$, $P=0.026$), tau (E; sham: 48.60 ± 2.18 ms vs. SNI: 55.76 ± 2.76 ms, $t_{76}=2.037$, $P=0.0451$), and I-V curve (F; interaction, $F_{(9,\,684)}=5.441$, $P<0.0001$, group: $F_{(1,\,76)}=4.884$, $P=0.0301$, membrane voltage: $F_{(9,\,684)}=309.3$, $P<0.0001$). G-I, Neuronal excitability of ovBNST^{CRF} neurons: rheobase (G; sham: 103.6 ± 8.4 pA vs. SNI: 81.8 ± 6.0 pA, $t_{76}=2.105$, $P=0.0386$), firing threshold (I; sham: -31.16 ± 0.84 mV vs. SNI: -33.97 ± 0.79 mV, $t_{76}=2.443$, $P=0.0169$), and firing rate (J; interaction: $F_{(12,\,744)}=9.411$, $P<0.0001$, group: $F_{(1,\,62)}=12.36$, $P=0.0008$, injection current: $F_{(12,\,744)}=82.09$, $P<0.0001$). Data are expressed as means ± standard error of the mean. $^{ns}P>0.05$, $^{*}P<0.05$ (unpaired *t*-test), $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ (two-way repeated measures ANOVA)

increased excitability of ovBNST^{CRF} neurons was caused by potentiation of miniature excitatory postsynaptic currents and inhibition of M-currents [9]. A similar mechanism may be involved in the enhanced excitability of ovBNST^{CRF} neurons during neuropathic pain. In addition to the BNST-intrinsic neurons, CRF-expressing central amygdala (CeA) neurons send their axons to the BNST. Asok et al. reported that optogenetic inhibition of a CRF pathway from the CeA to the BNST disrupted sustained fear [10]. Furthermore, Rouwette et al. [11] and our group [2] demonstrated that CRF mRNA expression was elevated both in the BNST and CeA of neuropathic pain model rats. These findings suggest the involvement of not only BNST-intrinsic but also CeA-derived CRF nerve terminals in the enhanced CRF signaling within the BNST during neuropathic pain.

The results of this study, together with our previous studies showing that enhanced CRF signaling in the BNST caused the aversive responses in acute pain [1] and suppressed the brain reward system in chronic pain [2], suggest that chronic pain induces negative emotional states by increasing neuronal excitability of ovBNST^{CRF} neurons.

Abbreviations

BNST	Bed nucleus of the stria terminalis
CeA	Central amygdala
CRF	Corticotropin-releasing factor
CVMS	Chronic variable mild stress
ovBNST	Oval part of the bed nucleus of the stria terminalis
RMP	Resting membrane potential
SNI	Spared nerve injury

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13041-024-01094-6.

Supplementary Material 1

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Author contributions
RU, YM, TA, KS, KI, AY and MM designed the experiments and prepared the manuscript. RU performed the experiments. RU, YM, TA, AY and MM analyzed the data. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All experiments were approved by the Institutional Animal Care and Use Committees of the Research Institute of Environmental Medicine, Nagoya University, Japan (approval #19232 and #19268). All efforts were made to reduce the number of animals used and to minimize the pain and suffering of animals.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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