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Short communication

Involvement of the bed nucleus of the stria terminalis in the negative affective component of visceral and somatic pain in rats

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Abstract

Using a conditioned place paradigm, we examined the involvement of the bed nucleus of the stria terminalis (BST) in the negative affective component of visceral and somatic pain induced by intraperitoneal acetic acid and intraplantar formalin injections, respectively, in rats. Bilateral BST lesions suppressed both the acetic acid- and formalin-induced conditioned place aversion, suggesting the crucial role of the BST in the negative affective component of visceral and somatic pain.

Keywords: Aversion; Bed nucleus of the stria terminalis (BST); Emotion; Extended amygdala; Nociceptive behavior; Pain

Pain is a complex experience composed of sensory and affective components. The neural systems underlying the sensory component of pain have been studied extensively, while those underlying its negative affective component are only beginning to emerge. Recently, some behavioral studies examined the neural circuits and mechanisms underlying the negative affective component of pain. Using a conditioned place paradigm, Johansen et al. demonstrated the involvement of the anterior cingulate cortex (ACC) in the conditioned place aversion (CPA) induced by the intraplantar (i.pl.) injection of formalin [13]. We previously revealed that the central (CeA) and basolateral (BLA) nuclei of the amygdala are differently involved in i.pl. formalin- and intraperitoneal (i.p.) acetic acid-induced CPA [22]. Moreover, Han and Neugebauer showed that the CeA plays an important role in noxious stimulus-evoked vocalizations, which is related to the aversive emotion in the rat arthritic pain model [11].

The bed nucleus of the stria terminalis (BST) is a forebrain structure that is one of the key neural substrates regulating stress responses and negative affective states, such as anxiety, fear and aversion [3,4,7,15,20,21,24]. Anatomical studies have shown that the BST, as well as the limbic regions, such as the ventromedial hypothalamus and amygdale, receives direct and indirect nociceptive inputs from the spinal dorsal horn [2,9]. However, no direct evidence exists for the involvement of this brain region in the negative affective component of pain. Therefore, we used a conditioned place paradigm to examine the effect of bilateral excitotoxic lesions of the BST on the negative affective component of visceral and somatic pain in rats.

Male Sprague–Dawley rats (180–240 g; Japan SLC, Hamamatsu, Japan) were housed individually under a 12-h light–dark cycle (lights on at 08:00 h) with free access to food and water. The experiments were carried out during the light portion of the light–dark cycle. All experiments were conducted in accordance with the ethical guidelines of the institutions involved and the guidelines of the Japanese Pharmacological Society.

Bilateral excitotoxic lesions of the BST were made as previously described [22] with slight modifications. Briefly, under sodium pentobarbital anesthesia (50 mg/kg, i.p.), 0.1 M ibotenic acid (Sigma, St. Louis, MO, USA) dissolved in phosphate-buffered saline (PBS) was bilaterally infused into the BST (AP: −0.3 mm; ML: ±1.6 mm; DV: −7.0 mm from the bregma) [17] in a volume of 0.3 or 0.4 μl/side over 3 min via stainless steel injection cannulae (33 gauge, o.d. 0.2 mm). Sham-operated rats received the identical surgical treatment, except they received an injection of PBS alone. After the injection, the rats were

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returned to their cages and left for at least 1 week before the experiments.

The CPA test was conducted as previously described [22]. A shuttle box composed of two equal-sized compartments (30 cm × 30 cm × 30 cm each) with distinct tactile and visual cues (one compartment was black with a smooth floor and the other was white with a textured floor) under dim illumination (25 ± 5 lx at the center of the box) was used for a four consecutive day experimental procedure. On days 1 (habituation session) and 2 (preconditioning session), the rats freely explored the two compartments for 900 s, and the time spent in each compartment over 900 s was measured automatically (KN-80; Natsume Seisakusho, Tokyo, Japan). No significant difference was observed between the time spent in the black (473 ± 16.6 s, $n = 27$) and white (428 ± 16.7 s, $n = 27$) compartments, indicating the absence of a significant bias in the preference of a compartment before conditioning. In this study, we used a bias-like protocol to nullify the initial preference of each rat, as discussed previously [23]. This type of protocol was successfully used to examine the CPA induced by opioid withdrawal [19] and visceral and somatic pain [22]. We designated the compartment in which the rat spent longer (>450 s) on day 2 (preconditioning session) as the pain-paired compartment for each animal. The rats that spent more than 80% (>720 s) of the total time in one side on day 2, or that showed a difference of more than 200 s in the time spent in one side between days 1 and 2 were eliminated from the following experiments. On day 3, place conditioning was performed as follows: in the vehicle control session (conducted between 09:00 h and 13:00 h), each rat was given an i.p. injection (1 ml) or an i.pl. injection (100 μl into the left hind paw) of saline, and then confined in the non-pain-paired compartment for 1 h. After at least 4 h, in the conditioning session (conducted between 14:00 h and 18:00 h), the rat was given an i.p. injection of 2% acetic acid (1 ml) or an i.pl. injection of 2% formalin (100 μl into the right hind paw), and then confined in the pain-paired compartment for 1 h. On day 4, in the test session, each rat was allowed to explore the two compartments freely, and the time spent in each compartment over 900 s was recorded. The CPA scores were calculated by subtracting the time spent in the pain-paired compartment in the preconditioning session from that in the test session.

To measure the acetic acid-induced nociceptive writhing behavior, each rat was given an i.p. injection of 2% acetic acid (1 ml), and then the number of events of typical writhing behavior was counted for each 5-min period over 60 min. In the formalin test, the rats were given an i.pl. injection of 2% formalin (100 μl) into the right hind paw, and then the amount of time the animal spent elevating, licking, shaking or biting the injected paw was measured for each 5-min period over 60 min. Nociception was quantified using the rating scale method by assigning weights to the following categories of nociceptive behaviors: category 0 = weight is evenly distributed among all paws; category 1 = injected paw is elevated; category 2 = injected paw is licked, shaken or bitten. The nociceptive score was calculated for each 5 min (300 s) period using the following formula: nociceptive score = {(time (s) spent elevating the injected paw) × 1 + (time (s) spent licking, shaking or biting the injected paw) × 2}/300 (s).

After the behavioral experiments, histological analyses were performed to check the BST lesions. Briefly, the rats were decapitated and the brain was removed rapidly and frozen in powdered dry ice. Coronal sections (50 μm) were prepared on a cryostat, thaw-mounted onto slides, and stored at −80 °C until staining. The sections were stained with thionin and each section was examined under microscopy (40×). The neuronal cells were clearly retained in the BST and its surroundings in the sham-operated control rats. Lesion boundaries were identified based on neuronal loss and reactive gliosis. Only the animals with excitotoxic lesions restricted within the BST bilaterally were included in the following analyses.

The data are expressed as mean ± S.E.M. Student's *t*-test was used for the statistical analysis of the data from the CPA experiments. Two-way analysis of variance (ANOVA) followed by the Bonferroni's post-hoc test was used for the statistical analysis of the data from the measurements of nociceptive behaviors. Differences with $P < 0.05$ were considered significant.

A schematic representation of the maximum and minimum extent of tissue damage in animals with bilateral excitotoxic lesions of the BST is shown in Fig. 1. The effects of bilateral excitotoxic lesions of the BST on the acetic acid- and formalin-induced CPA were examined (Fig. 2). In the sham-operated control group injected with acetic acid, the time spent in the pain-paired compartment in the test session was 317 ± 26.4 s, which was significantly shorter than that in the preconditioning session (490 ± 23.2 s, $P < 0.001$; Fig. 2A). Conversely, in the lesioned group injected with acetic acid, the time spent in the pain-paired compartment in the test session was 599 ± 67.1 s, which was not

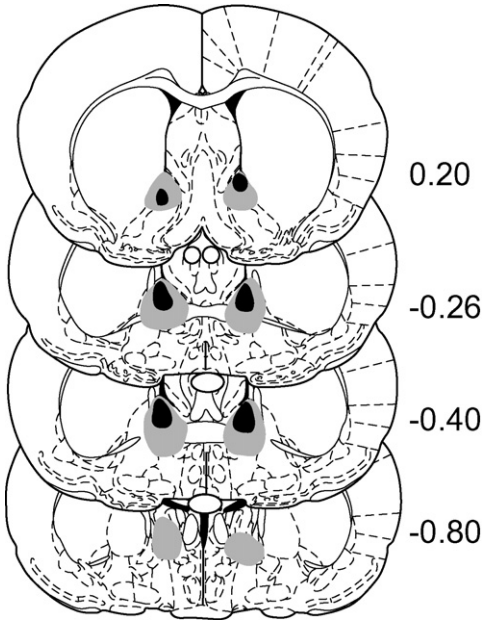

Fig. 1. Schematic representation of the maximum (shaded in grey) and minimum (shaded in black) extent of tissue damage in animals with bilateral excitotoxic lesions of the BST. All the animals had tissue damage within this range. The coronal sections were taken from the atlas of Paxinos and Watson [17]; numbers to the right indicate distance (mm) from the bregma.