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Social interaction with a cagemate in pain facilitates subsequent spinal nociception via activation of the medial prefrontal cortex in rats

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ABSTRACT

Empathy for the pain experience of others can lead to the activation of pain-related brain areas and can even induce aberrant responses to pain in human observers. Recent evidence shows this high-level emotional and cognitive process also exists in lower animals; however, the mechanisms underlying this phenomenon remain unknown. In the present study we found that, after social interaction with a rat that had received subcutaneous injection of bee venom (BV), only the cagemate observer (CO) but not the noncagemate observer (NCO) showed bilateral mechanical hypersensitivity and an enhanced paw flinch reflex following BV injection. Moreover, neuronal activities labeled by c-Fos immunoreactivity in the spinal dorsal horn of CO rats were also significantly increased relative to the control 1 hour after BV injection. A stress-related response can be excluded because serum corticosterone concentration following social interaction with demonstrator rats in pain was not changed in CO rats relative to NCO and isolated control rats. Anxiety can also be excluded because anxiety-like behaviors could be seen in both the CO and NCO rats tested in the open-field test. Finally, bilateral lesions of the medial prefrontal cortex eliminated the enhancement of the BV-induced paw flinch reflex in CO rats, but bilateral lesions of either the amygdala or the entorhinal cortex failed. Together, we have provided another line of evidence for the existence of familiarity-dependent empathy for pain in rats and have demonstrated that the medial prefrontal cortex plays a critical role in processing the empathy-related enhancement of spinal nociception.

1. Introduction

Pain, a complex experience comprising sensory discrimination, affective motivation, and cognitive evaluation [7,49], may be modulated by emotional and cognitive factors [7,62,63]. Empathy is a necessary prerequisite for prosocial behaviors and has been recently defined as "an integrated affective response stemming from the perception of another's emotional state or condition similar to what the other person is feeling or would be expected to feel in the given situation" [19]. Empathy has also been believed to be a brain functional adaptive process resulting from evolution and social experiences [6,17–20,36]. Experimental human studies have demonstrated that empathy for pain may influence the way an individual feels pain themselves, and lead to heightened pain perception [25,26,41]. Moreover, spouses with partners in chronic pain are more vulnerable to distress [37,58]. These results suggest the existence of a modulation of pain by empathy in the brain. It has been generally accepted that brain areas that can be most commonly activated by pain are the somatosensory cortices (S1 and S2), anterior cingulate cortex (ACC), insula, prefrontal cortex (PFC), thalamus, and cerebellum [3,7]. Within the pain network, the S1 and S2 are believed to encode sensory discrimination of pain [3,7,8], while the ACC and insula are involved in encoding affective-motivational aspects of pain [3,7,51,52]. It is interesting to find that the ACC and anterior insula (AI) can consistently be activated, whereas the S1/S2 are less activated by the vicarious experience of pain [6,20,30,32,59], suggesting a key role for the ACC/AI in processing empathy for pain.

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Although empathy in animals was noted in the middle of the last century [14,56], attention has not been paid by contemporary neuroscientists until this century because empathy was generally believed to be unique to humans [43,45]. In 2006, Mogil and colleagues [33] provided the first line of experimental evidence showing the occurrence of empathetic responses in mice socially interacting with each other in pain. Briefly, the pain-related response was enhanced only in a pair of cagemates or siblings who simultaneously received intraperitoneal (i.p.) injection of acetic acid. However, this pain response enhancement was not seen either in a pair of strangers or in an isolated mouse suffering from pain. More recently, empathically motivated helping behavior has also been seen in rats [4,5]. A free rat is able to help trapped conspecifics by releasing them from a restrainer, and this prosocial behavior is largely dependent upon social experiences from birth regardless of genetic background [4,5]. However, genetic background cannot be excluded because it was shown to be associated with empathy for fear [13]. The objectives of the current study were to determine: (1) whether empathy for pain can be produced in an observer rat after 30 minutes' social interaction with a demonstrator in pain; (2) whether the neuronal activities in the spinal dorsal horn can be modulated by empathy for pain; (3) whether the function of the hypothalamic-pituitary-adrenocortical (HPA) axis is changed by pain-related empathetic responses; and (4) whether the ACC and other brain regions are important for the mediation of pain empathy and its modulation of spinal nociception.

2. Methods

2.1. Animals

All experiments were conducted on male albino Sprague-Dawley rats (weighing 180–220 g, 8–9 weeks old) purchased from the Laboratory Animal Center of Fourth Military Medical University (FMMU), Xi'an, Shaanxi Province, P.R. China. The animals were housed in groups of 4–6 and maintained under standard conditions (12 hours dark/light cycles, temperature 22–26°C, air humidity 40–60%) with food and water available ad libitum. The experimental protocols were approved by the Institutional Animal Care and Use Committee of FMMU and animals were maintained and cared for in line with the guidelines set forth by the International Association for the Study of Pain [67]. Every effort was made to minimize the number of animals used and their suffering. The rats were housed together for at least 2 weeks before the initiation of the experiments. In all experiments the term "cagemates" refers to rats drawn from the same cage and are not necessarily siblings; "noncagemates" refers to rats drawn from different cages. For each experiment, a new cohort of rats was used and no rats were subjected to more than one test.

2.2. Experiment 1: Effects of social interaction with a conspecific in pain on subsequent pain-related responses of observer rats

To assess the effect of social interaction, rats were previously housed in the same cages (cagemate) or different cages (noncagemate) before any experiments. The bee venom (BV) model was produced by subcutaneous (s.c.) injection of a solution containing 0.2 mg BV (Sigma, St. Louis, MO, USA) dissolved in 50 μL physiological saline into the left hind paw [11,12,34]. BV injection in rats can induce long-term persistent spontaneous pain-related behaviors (at least 1 hour) and pain hypersensitivity (72–96 hours) [11,12,34], and the spinally mediated paw flinch reflex was evaluated in the present study [11,38,66]. In this part of study, rats were divided into 4 groups (isolated control [Iso], cagemate control [CC], cagemate observer [CO] and noncagemate observer [NCO]) according to different pretreatments. The pretreatment conditions included: (1) Iso: naive rats were housed in isolation in a transparent plastic box (the apparatus that was used for measurement of the flinch reflex, described later) for 30 minutes; (2) CC: a pair of cagemate rats interacted within a transparent plastic testing box for 30 minutes, but neither of them had any irritant treatments; (3) CO: a rat was allowed to interact freely within a transparent plastic testing box for 30 minutes with a cagemate demonstrator in pain produced by s.c. BV injection; (4) NCO: a rat was allowed to interact freely within a transparent plastic testing box for 30 minutes with a noncagemate demonstrator in pain produced by s.c. BV injection. After pretreatment, rats from each of these 4 groups (Iso, CC, CO, and NCO) were immediately subjected to the following experimental manipulations in isolation: (1) mechanical and thermal pain sensitivity were measured in random order and were compared with baseline pain sensitivity that was measured before pretreatment (n = 6–7 rats per group); (2) each rat received BV injection and the paw flinch reflex was counted for 1 hour in the same box that had been used for the 30-minute housing or social interaction (n = 9–11 per group). To exclude interactions among different manipulations, a rat was only used for one condition in the experiments.

For the measurement of mechanical sensitivity, a transparent plastic box (20 × 20 × 25 cm) with a metal mesh floor was employed for the measurement of the paw withdrawal mechanical threshold (PWMT). Von Frey monofilaments (with bending forces of 58.8, 78.4, 98, 117.6, 137.2, 156.8, 176.4, 196, and 245 mN) were applied perpendicularly to the bilateral hind paws of rats at 10-second intervals and with 10 repetitions on each side. The bending force able to elicit 50% paw withdrawal reflexes was considered as the threshold (PWMT, mN).

For the measurement of thermal sensitivity, a transparent plastic box (20 × 20 × 25 cm) with a transparent glass floor was used for the measurement of paw withdrawal thermal latency (PWTL). The heat stimulation was generated from a TC-1 radiant heat stimulator (new generation of RTY-3, Bobang Technologies of Chemical Industry, P.R. China) and applied to the center of the hind paw at 10-minute intervals for the same side and 5-minute intervals for different sides, with 5 repetitions on each side. The duration from the beginning of the heat stimulation to the occurrence of the paw withdrawal reflex was determined as the PWTL and the final 3 values were averaged to give the mean PWTL. A cutoff of 30 seconds was adopted to avoid tissue damage.

For the measurement of persistent spontaneous nociception, a transparent plastic testing box (30 × 30 × 30 cm) with a transparent glass floor was placed on a supporting frame 30 cm above the mirror-surfaced experimental table. The number of paw flinches in rats receiving s.c. injection of BV was counted for each 5-minute time block for 60 minutes.

The details regarding measurement of changes in pain sensitivity and persistent spontaneous nociception, and the procedures used for calculations based on these measurements, can be found in our previous publications [11,12].

Spinal dorsal horn nociceptive neuronal activities can be measured by immunocytochemical labeling of c-Fos, a biomarker of neuronal activities encoded by the immediate early gene c-fos [28]. For this analysis, rats were divided into 3 groups (Iso, CO, and NCO). After pretreatment, each group of rats received an s.c. BV injection. Our previous research has shown that the expression of c-Fos in the spinal dorsal horn reaches a peak at 2 hours following BV injection [42] and hence 1 hour was chosen as the time point in this study to avoid the plateau effect that may make it difficult to distinguish potential changes in c-Fos expression among the different groups.

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