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Research article

Social interaction with a cagemate in pain increases allogrooming and induces pain hypersensitivity in the observer rats

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ABSTRACT

Empathy, which is a highly cognitive and emotional process, is the ability to share the emotional states of others. Empathy has also been observed in rodents. The empathic sharing of the distressful experience of a conspecific can even motivate altruistic behaviors, which are critical for survival. However, previous studies investigating empathy or prosocial behaviors in rodents mainly employed fearful or other stressful stimuli to elicit emotional changes; whether pain empathy can also motivate prosocial behaviors has yet to be investigated. By using the writhing test, the present study found that cagemate observer (CO) rats, compared with non-cagemate observer (NCO) rats, increased partner-directed grooming (allogrooming) toward conspecifics that had received an intraperitoneal injection of acetic acid during a dyadic social interaction. Following a dyadic social interaction with a demonstrator that received an intraperitoneal injection of acetic acid, the CO rats, compared with NCO rats, exhibited bilateral mechanical pain hypersensitivity and an enhanced acetic acid-induced writhing response. Our results here provided further evidence of pain empathy in rats, suggesting that empathy for pain may motivate prosocial behaviors in rats.

1. Introduction

Empathy is a highly complex cognitive and/or emotional process that involves an ability to understand the emotional state of others [4,14,31], and it plays a fundamental role in the social life of humans [1]. Although empathy, which is a higher order brain function, was presumed to be unique to humans, it has been demonstrated to also exist in other mammals [12,21,23,27,28]. Research has shown that the painful or fearful experience of rodents can be transferred to their familiar conspecifics [2,18,19,21,23,26] (for review see [7,27,29]). For example, the pain-related behaviors of a pair of mice that are cagemates or siblings can be modulated by the stressful experience of their conspecifics when they are socially engaged [6,21]. Our previous work in this area showed that, after social interaction with a demonstrator rat in pain, the cagemate observer (CO) rats showed facilitated spinal nociception, which supports the existence of pain empathy in rats [23].

With the accumulating data, it has been shown that rodents are not only capable of empathic sharing of the emotional state of their partners but also exhibit prosocial behaviors towards their distressed conspecifics [3,5,10,32]. Rats can forego food rewards of their own to prevent their conspecific from being electrically shocked [10] or to lower down their stressed partners that have been hoisted off the floor [32]. The monogamous prairie vole shows increased partner-directed grooming toward familiar conspecifics that have been fear conditioned, and this other-directed, prosocial, consolation behavior is mediated by oxytocin in the anterior cingulate cortex [5]. However, previous studies investigating empathy or prosocial behaviors in rodents mainly employed fearful or other stressful stimuli to elicit emotional changes; whether pain empathy can also motivate prosocial behaviors remained to be investigated.

By using the writhing test, the present study investigated the effects of social interaction with a demonstrator that received intraperitoneal injection of acetic acid on subsequent pain-related behaviors of the observer rats and the allogrooming behaviors directed by the observer rats toward the demonstrator rats.

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2. Materials and methods

2.1. Animals

All experiments were performed on male albino Sprague-Dawley rats (weighing 180–220 g, 8–9 weeks old) obtained from the Laboratory Animal Center of Fourth Military Medical University (FMMU). The animals were housed in groups of 4–6 in plastic boxes (48 × 35 × 25 cm) and maintained under standard conditions (12 h dark/light circle, temperature 22–26 °C, and air humidity 40–60%) with free access to food and water. 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 EC Directive 86/609/EEC and the guidelines set forth by the International Association for the Study of Pain [33]. The animals were housed together for two weeks before the initiation of the experiments. In all experiments, the term "cagemates" refers to rats taken from the same cage, but they were not necessarily siblings; the term "non-cagemates" refers to rats taken from different cages. The animals were habituated to the testing environment for 3 days consecutively before the experiments. Every effort was made to minimize the number and suffering of the animals. For each experiment, a new cohort of rats was used and no rats were subjected to more than one test, so as to exclude interactions among different manipulations.

2.2. Experimental paradigm

Group assignment: The animals were separated into different groups according to the pre-treatments they received. Pre-treatments: Cagemate control (CC), a pair of naïve cagemate rats was put into a transparent plastic box (20 × 20 × 25 cm) to socially interact with each other for 30 min; Cagemate observer (CO), a pair of cagemate rats was put into a transparent plastic box, one rat received an intraperitoneal injection of 0.9% acetic acid (10 ml/kg) (denoted the "cagemate demonstrator") and the other rat (denoted the "cagemate observer") was allowed to interact freely with the cagemate demonstrator rats for 30 min; Non-cagemate control (NCC), a pair of rats drawn from different cages was put into a transparent plastic box to socially interact with each other for 30 min; Non-cagemate observer (NCO), a pair of rats drawn from different cages was put into a transparent plastic box, one rat received an intraperitoneal injection of 0.9% acetic acid (denoted the "non-cagemate demonstrator") and the other rat (denoted the "non-cagemate observer") was allowed to interact freely with the non-cagemate demonstrator rats for 30 min.

During the pre-treatments, the dyadic social interaction was videotaped, and then the time of the licking or grooming directed by the observer rats toward the demonstrator rats (allogrooming) was recorded by an experimenter blinded to the group assignment. Rats from the CC, CO, NCC and NCO groups were subjected to two parts of experiments in isolation immediately after the 30 min social interaction: Part 1, the mechanical and thermal pain sensitivity of rats from CC, CO, NCC and NCO groups were measured in random order and were compared to baseline pain sensitivity that was measured before pre-treatment; and Part 2, rats from CC, CO, NCC and NCO groups received an intraperitoneal injection of 0.9% acetic acid in isolation and the number of writhes was counted for each 5 min block for 30 min.

2.3. Writhing test

For the writhing test, the rats received an intraperitoneal injection of 0.9% acetic acid (10 ml/kg), and then were transferred to a transparent plastic box with a transparent glass floor. The number of writhes, a behavior of characteristic lengthwise stretches of the torso with a concomitant concave arching of the back, was counted for each 5 min block over 30 min [15,20]. Animals that received intraperitoneal injection of acetic acid but did not show any writhing behaviors, which may be caused by misplaced injection, were excluded from the experiments.

2.4. Measurement of paw withdrawal mechanical threshold

As for measurement of mechanical pain sensitivity, the rat was placed in a transparent plastic box (20 × 20 × 25 cm) with a metal mesh floor in isolation, and a series of Von Frey filaments with different bending forces (58.8, 78.4, 98, 117.6, 156.8, 176.4, 196 and 245 mN) was applied to the center of the hindpaw bilaterally with 10 s block, and 10 repetitions were done for each side. The forces able to elicit more than 50% paw withdrawal reflexes were considered as the paw withdrawal mechanical threshold (PWMT) [8].

2.5. Measurement of paw withdrawal thermal latency

As for measurement of thermal pain sensitivity, the rat was put into a transparent plastic box (20 × 20 × 25 cm) with a transparent glass floor in isolation. The heat stimulation was generated from a radiant heat stimulator and applied to the center of the plantar surface of the hindpaw with 10-min intervals for the same side and 5 min intervals for different sides with five repetitions on each side. Paw withdrawal latency was measured, and the final three values were averaged to give the mean paw withdrawal thermal latency (PWTL) [8]. A cut-off of 30 s was chosen to prevent potential tissue damage.

2.6. Statistical analysis

All data were expressed as the mean ± SEM. One-way analysis of variance (ANOVA) (followed by post hoc Fisher's LSD – least significance difference) and two-way ANOVA repeated measures with Bonferroni post hoc tests were used to analyze mean differences when necessary. Data were first tested for normal distribution and then subjected to ANOVA only if the data conformed to a normal distribution. $P < 0.05$ was considered to be of statistical significance.

3. Results

3.1. Cagemate observer rats exhibited more allogrooming towards the demonstrator rats in pain

To examine the social behaviors of rats under different social contexts, the present study measured the time of grooming directed by the observer rats toward demonstrators rats (allogrooming). When a pair of naïve rats was housed together, whether they were cagemates or non-cagemates, one rat initiated allogrooming behavior towards its partner. The time of allogrooming initiated by CC (n = 12) rats was longer than of NCC (n = 16) rats (30.58 ± 6.4 vs. 17.44 ± 2.52); however, no statistical significance was detected. When the demonstrator rats were injected with acetic acid, the CO (n = 10) rats and NCO (n = 10) rats both spent more time on allogrooming the distressed partner than the CC rats and NCC rats ($F_{(3,44)} = 8.794$, $P < 0.001$; $P < 0.01$ for CO vs CC and NCO vs NCC; one-way ANOVA with LSD post hoc test). Moreover, when the dyadic rats were familiar cagemates, the time of allogrooming towards the distressed partner was longer than when they were non-cagemates ($P < 0.05$, one-way ANOVA with LSD post hoc test) (Fig. 1).

3.2. Social interaction with a distressed cagemate induces mechanical hypersensitivity and enhances acetic acid-induced writhing behaviors in the CO rats

Following the 30 min social interaction, the PWMT and PWTL of CC (n = 11) rats remained relatively unchanged compared to that measured before the social interaction. After social interaction with a stressed cagemate for 30 min, the bilateral PWMT of CO (n = 14) rats