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Role of Opioid System in Empathy-like Behaviours in Rats

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Original Article

Abstract

Background: Empathy is defined as the ability to simulate the mental states of others. Recent studies have demonstrated empathy-like behaviors in other animals including rats and mice. The objective of the current study was to evaluate the effect of acute administration of morphine and naloxone on cognition and nociception changes following observing conspecifics undergoing nociceptive stimulus.

Methods: Adult male Wistar rats were used (n = 8 for each group). One cagemate received formalin injection into the hindpaw five times within a nine-day period and the other cagemate observed the pain while being pretreated with saline, morphine, or naloxone [10 mg/kg, intraperitoneal (i.p.)]. Pain behaviors, anxiety-like behaviour, locomotion, balance and muscle strength were evaluated in the observer animals.

Findings: Observing a cagemate in pain increased anxiety-like behavior and reduced thermal pain threshold in the observer animals. Administration of morphine reversed these effects and naloxone did not affect the responses.

Conclusion: Results of the current study reveal an important role for opioid receptors (ORs) in empathy for pain, so that activation of this system dampens the empathy-like responses.

Keywords: Morphine; Empathy; Rats; Anxiety

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Introduction

Empathy is defined as the ability to simulate the feelings of the others, so that emotional responses are evoked in the observer.[1-3] Though there is debate over the presence of such trait in the lower level animals such as rats, studies have demonstrated that rats demonstrate helping behavior in response to observation of pain in a conspecific, which is considered as a sign of empathy-like behavior in these animals.[4-6]

It has already been demonstrated that observing another person in pain leads to the activation of brain regions responsible for processing of affective component of pain in humans. Human imaging studies have unravelled an important role for the anterior cingulate cortex (ACC) and insular cortex in the empathy process.[7] Animal studies have also demonstrated that rats help each other while seeing a conspecific in pain and it is considered as a prosocial behavior attributed to empathy.[2,5,8] Langford et al. demonstrated that observation of pain in another rat led to the alterations in the sensory modulation of pain and a reduced threshold to noxious stimuli was observed in the rats that observed other rats in pain.[8] Furthermore, it has been demonstrated that observing pain in a conspecific leads to anxiety-like behavior and alterations in cognitive function of the animals.[9]

Opioidergic system is one of the most important regulators of pain in every animal. Its activation during pain experience leads to a dampened response to the noxious stimuli and inhibition of this system exacerbates nociceptive responses.[10] Both sensory and affective aspects of pain are modulated by Opioidergic system and many brain regions are responsible for regulation of this system. One of the most important brain regions that activates the opioid pathway is prefrontal cortex, which is activated in both first-hand experience of pain and following observation of pain in a conspecific.[10]

In the current study, we first evaluated the effect of observing pain on the thermal pain thresholds and anxiety-like behaviors in the animals[11] and then evaluated the effect of pretreatment with either morphine or naloxone before observation of pain on the nociception, anxiety-like behaviors, and motor and balance function of the animals. Results of the current study would provide new insights into the role of opioid receptors (ORs) in empathy for pain in the rats.

Methods

In this Experimental study, adult male Wistar rats were used (weighing 250-270 g, n = 8 for each group). All the procedures were approved by the Ethics Committee of Kerman University of Medical Sciences, Kerman, Iran (Code: EC/KNRC/98-62.). Maximum effort was made to minimize harm to the animals. They were kept in cages of two (one considered as the observer and the other as the one that would undergo painful stimuli) with access to food and water ad libitum. Light and dark cycle was kept as standard protocols (12/12 light/dark cycle) and room temperature was kept at 25 ± 2 ˚C. All procedures were performed from 8 AM to 4 PM.

Observation of pain in the cagemate: Animals were kept in shared cages in pairs 30 days prior to the experiments, so that they could be considered as cagemate and were brought to the laboratory environment each day, so that they got accustomed to that place. On the first day of experiments, one animal was chosen as the pain subject (pain) and the other one was considered as the observer. An injection of formalin (50 µl, 2%) into the plantar surface of the hindpaw[12] was made with an insulin syringe and after injection, the pain animal was placed inside the cage beside the observer. This procedure was repeated the following nine days with formalin injection every other day, so that a total of five injections were made for each animal and then it was placed beside the observer cagemate. Pretreatment with saline, morphine, or naloxone [10 mg/kg, intraperitoneal (i.p.)] was performed 30 minutes prior to the observation of pain in the cagemate, so each observer rat received five IP injections during this period. One day after the last injection of either formalin or saline, the behavioral assays were performed. The following behavioral assays were performed, respectively. Observing rats receiving saline were used as the control group.

Open field test (OFT): This assay evaluates the locomotion of the animals and anxiety-like behaviors. An opaque Plexiglass box [90 × 90 × 45 (height) cm] was used in OFT procedure. All of the animals' behaviors were recorded using EthoVision software (Noldus Technology, Netherlands). Animals were placed in the middle of the field and

the following parameters were recorded by the software for each rat during a five-minute interval: total distance moved (TDM) (cm), velocity, and the number of rearing and grooming.[13]

Wire grip: This test assays the muscle strength and balance in the animal. Each rat was placed on the apparatus with two forepaws grasping to a steel wire and suspending in the air (80 cm long, 7 mm diameter). Latency to fall was recorded for each animal using a stop watch. Three consecutive trials were made for each rat with five-minute inter-trial duration.[14]

Rotarod: An accelerating rotarod was used in the current paradigm. Rotarod started at a speed of 10 round per minute (RPM) and reached a maximum of 60 RPM. Three trials were made for each rat with five-minute inter-trial duration and each trial lasted a maximum of five minutes. Total duration of maintaining balance on the rotarod was recorded for each rat.[13]

Hot plate: A hot plate device (LE710 model, Lsi LETICA, Spain) was used in this procedure. The apparatus was made of a plate with a diameter of 19 cm and a Plexiglass wall of 30 cm height. The plate temperature was set at 52.0 ± 0.5 ˚C and the reaction time after placing the animal on the plate was recorded for each animal. Reactions were defined as licking hindpaw or jumping and the cut-off time was 45 seconds to avoid tissue damage.[15]

Tail flick: This paradigm assays the nociceptive response at the spinal level. The animal was restrained in a restrainer cage with tail hanging free. Each animal was given a 30-minute adaptation time. The lower five centimeters of tail was put under a burning light and the time since emitting light and withdrawing tail was recorded for each animal as the reaction time in this paradigm.[16]

SPSS software (version 20, IBM Corporation, Armonk, NY, USA) was used for the current study. Paired t-test and one-way analysis of variance (ANOVA) were used to compare different groups. $P < 0.05$ was considered statistically significant. All the data were presented as mean ± standard error of the mean (SEM).

Results

OFT: There was no significant difference in the speed of the animals in the OFT paradigm (Figure 1, A). TDM was significantly altered in the saline group in comparison to the control group ($P < 0.05$) (Figure 1, B). No significant difference was observed in the morphine and naloxone groups in comparison to control ($P > 0.05$).

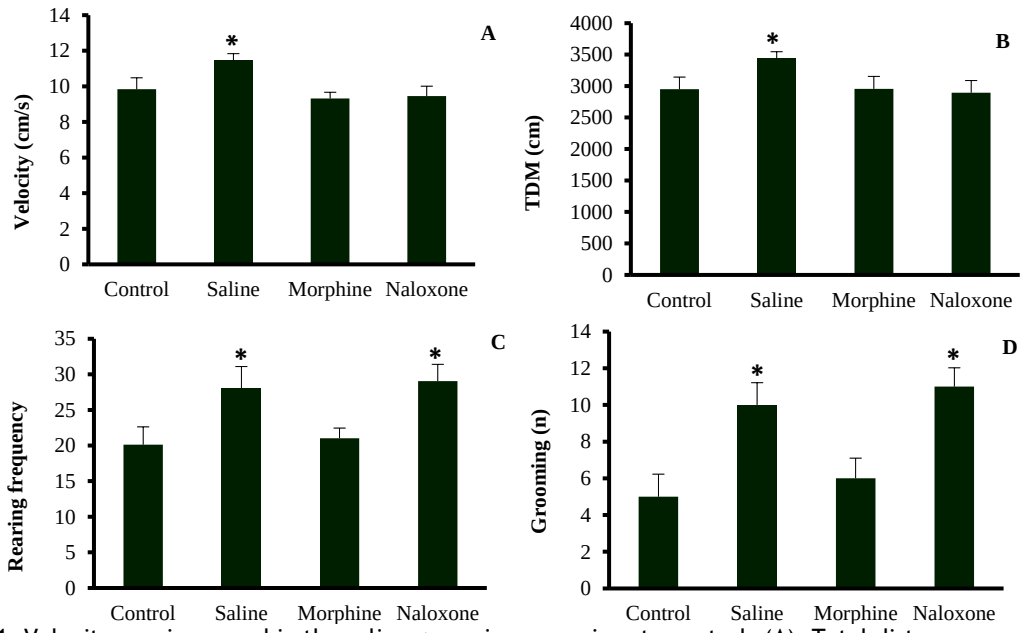

Figure 1. Velocity was increased in the saline group in comparison to controls (A); Total distance moved (TDM) was increased in comparison to control group (B); Rearing was increased in the saline and naloxone group in comparison to control, revealing an increased anxiety-like behavior (C); Grooming was increased in the saline and naloxone group, demonstrating a higher level of anxiety in these two groups (D)
*P < 0.05 in comparison to control group

The number of rearing and grooming was significantly increased in the observer group following observation of a cagemate in pain which indicates an increased anxiety-like behavior in these animals. No such difference was found for morphine group, which demonstrates an anxiety-alleviating effect for this opioid agonist (Figure 1, C and D).

Wire grip and rotarod: Time on rod was not altered in the study groups (P > 0.05) (Figure 2). Time to fall was not altered in the groups in comparison to control, implicating that observing a cagemate in pain had no effect on motor strength and balance in the animals (P > 0.05) (Figure 3).

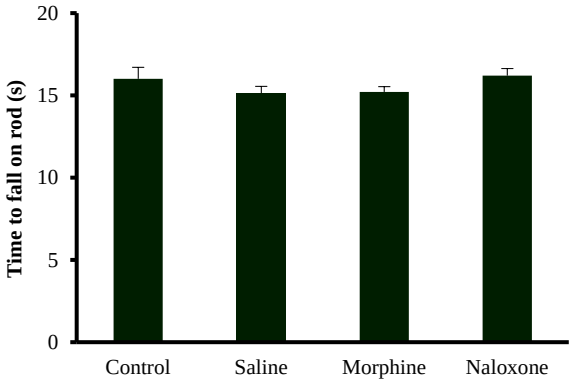

Figure 2. Time to fall was not significantly different among study groups

Hot plate and tail flick: Latency to show a nociceptive response to hot plate was significantly reduced in the saline group and morphine reversed this effect, while naloxone did not reverse the response (P < 0.05, ANOVA) (Figure 4, A).

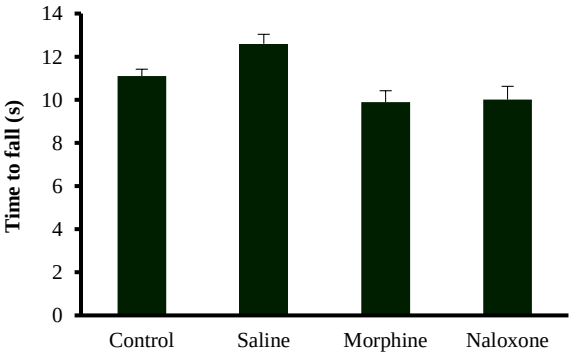

Figure 3. Muscle strength was not different among study groups

Latency to withdraw tail in the tail flick assay revealed that observing a cagemate in pain reduced the nociceptive threshold at the spinal level and pretreatment with morphine reversed this effect of empathy for pain (Figure 4, B).

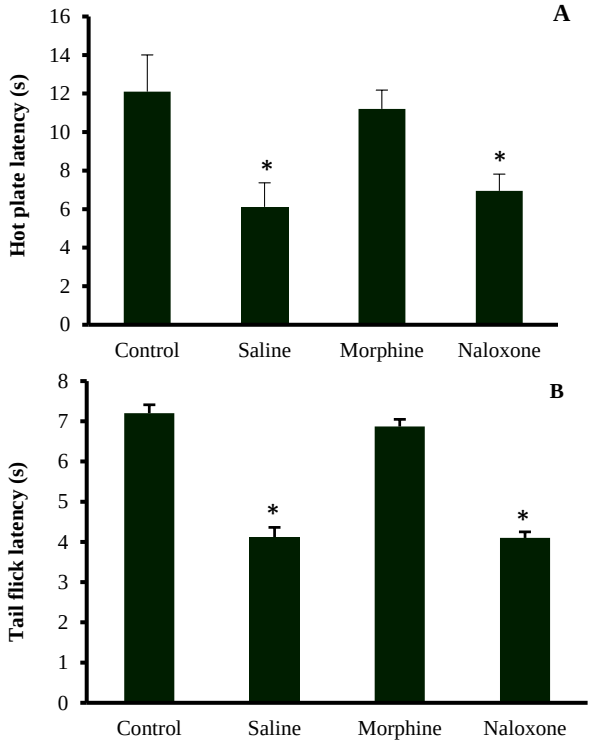

Figure 4. Thermal pain threshold in hot plate and tail flick assays was significantly decreased in the saline and naloxone group, revealing a hyperalgesic effect of observing a cagemate in pain; pretreatment with morphine reversed this effect (A and B)
*P < 0.05 in comparison to control group

Discussion

The current study was performed to evaluate the effect of observing a cagemate on nociception, anxiety-like behavior, locomotion, and muscle strength in a rat model of empathy. The effect of pretreatment with agonist or antagonist of ORs was evaluated on these changes. Observing a cagemate in pain increased anxiety-like behavior and reduced thermal pain threshold in the observer animals. Administration of morphine reversed these effects and naloxone did not affect the responses. These findings confirmed the hyperalgesic effect of observing other conspecific in pain and a modulating role was revealed for opioid system in empathy-like responses in the rats.

Empathy is defined as the capability to

understand and simulate the feelings of others. It is recently demonstrated that both affective and sensory aspects of pain are altered in the rat model of empathy used in the current study and this model of rat empathy for pain have been validated.[10] Langford et al.[8] and mogil[17] were the first to discuss empathy in rats. Their frontier works demonstrated that observation of pain in a conspecific led to an increased response to noxious stimuli, even from a different modality. Rutgen et al. demonstrated that placebo analgesia led to a reduced perception of other people's pain in the observer human subjects, thus demonstrating a significant role for opioid system in empathy in humans.[11] Findings of the current study also confirm this finding with broader implication for further researches; since administration of opioid agonist and antagonists to human subjects might confront ethical issues, animal studies provide an opportunity for the study of empathy in animals and evaluating the role of opioidergic system.[10] Activation of brain regions involved in opioid regulation including prefrontal cortex and periaqueductal gray (PAG) matter following observation of another person in pain as revealed by functional magnetic resonance imaging (fMRI) implies that even observation of pain in other people activates the brain regions responsible for opioid regulation, thus justifying the effect of administration of morphine on empathy-induced changes in nociception.[11,18] Rutgen et al. used placebo analgesia to evaluate the role of ORs in human subjects, since placebo analgesia leads to the release of endorphins which activate the μORs.[11] In the current study, morphine as the agonist of μ receptors was used and the same findings were obtained in the animal model of empathy.

Consistent with the findings regarding the nociception, Li et al. demonstrated that social interaction with a cagemate in pain led to the facilitation of nociception at the spinal level.[19] In the current study, observing a cagemate in pain led to the reduction of nociceptive threshold and pretreatment with morphine dampened this hyperalgesic effect, thus providing a modulating role for ORs in the second-hand experience of pain in the animals.

Anxiety-like behaviors were altered following observation of pain in a cagemate, so that an increased anxiety-like behavior was observed in these observers and pretreatment with opioid agonist led to the normalization of anxiety-like behaviors. Konig et al. have demonstrated an altered anxiety profile in rats lacking encephalin OR, thus demonstrating that opioid system is involved in regulation of anxiety behaviors,[20] and this might explain the results of the current study regarding the effect of morphine on anxiety induced by empathy. Furthermore, Nazeri et al. demonstrated an increased anxiety-like behavior in the rats observing pain in their cagemates.[10] Current results are consistent with that finding and in addition, a modulating role for μOR was revealed in empathy-induced anxiety.

In the current study, no significant changes were observed in the balance and muscle strength of the animals following observation of pain in a cagemate. Other studies evaluating the effect of painful stimuli on balance and muscle strength also demonstrated no obvious effect of pain on these two parameters.[10,13]

Conclusion

Results of the current study revealed that observing a cagemate in pain led to impairments in anxiety-like behaviors and locomotion, motor strength and balance, and nociception. Opioid system activation prior to observation of pain in a conspecific reversed the effect of empathy on nociception and anxiety-like behavior. No alteration in motor strength and balance was observed in animal model of empathy and thus, no effect for empathy on these two parameters was found.

Conflict of Interests

The Authors have no conflict of interest.

Acknowledgements

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Authors' Contribution

MN has conceived and designed the concept and road map of the study, searched the literature, collected data and drafted the manuscript. AN has searched the literature, categorized the searched papers and helped design the study and graphs. MSH has critically reviewed the

manuscript, designed the study, and helped in manuscript preparation. MS is the archival author and attests to the integrity of the original data and the analysis reported in this manuscript.

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نقش سیستم اوپیوئیدرژیک در رفتارهای شبه همدردی در موش‌های صحرایی

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مقاله پژوهشی

چکیده

مقدمه: همدردی، به توانایی شبیه‌سازی حالات ذهنی دیگران گفته می‌شود. مطالعات اخیر نشان داده است که حیواناتی از جمله موش و موش صحرایی نیز تا حدی این قابلیت را دارند. هدف از انجام پژوهش حاضر، بررسی تأثیر تجویز حاد مورفین و نالوکسان بر تغییرات شناختی و سیستم درد حیوانات به دنبال مشاهده درد در یک هم‌گونه بود.

روش‌ها: در این مطالعه، از رت‌های نر بالغ نژاد ویستار (۸ سر برای هر گروه) استفاده شد. طی بازه زمانی ۹ روزه، در کف پای یکی از حیوانات هم‌قفس فرمالین تزریق گردید و حیوان مشاهده‌گر با پیش‌درمانی سالین، مورفین و نالوکسان، درد را در هم‌قفس خود مشاهده نمود. رفتارهای درد، شبه اضطرابی، حرکت، تعادل و قدرت عضلانی مورد سنجش قرار گرفت.

یافته‌ها: مشاهده یک هم‌گونه که درد می‌کشد، منجر به افزایش رفتارهای شبه اضطرابی و کاهش آستانه درد حرارتی در حیوان مشاهده‌گر شد. تجویز مورفین این تغییرات را به حالت اولیه برگرداند و نالوکسان تأثیری نداشت.

نتیجه‌گیری: نتایج به دست آمده بیان‌کننده نقش مهم سیستم اپیوئیدرژیک در همدردی می‌باشد و فعال شدن این مسیر از طریق تجویز مورفین، منجر به کاهش رفتارهای شبه همدردی در حیوان مشاهده‌گر می‌شود.

واژگان کلیدی: مورفین، همدردی، موش‌های صحرایی، اضطراب

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