ISSN: 1813-4890

Transfer of pain and changes of brain substances in mice co-housed with spared nerve injured mice

Xiaoqing Chen [#], Yaorong Yang [#], Xuanyue Ma, Lei Shi *

JNU-HKUST Joint Laboratory for Neuroscience and Innovative Drug Research, College of Pharmacy, Jinan University, Guangzhou 510632, Guangdong, China

[#] These authors contributed equally to this study.

* Corresponding author: Lei Shi, Ph.D., Professor (E-mail: t_shilei@jnu.edu.cn or sophielshi80@gmail.com)

Abstract

Empathy is a social ability to recognize feelings, thoughts and intentions of others. Positive empathy such as empathy in pain is not only an important response to comfort the one who are suffering from pain but also helpful for us to connect with society. However, the mechanisms of empathy are not fully understood. In this study, we examined whether co-housing with spared nerve injury (SNI)-modeled mice, a classical neuropathic pain model, could induce pain behavior and brain chemical alterations in normal mice. We found that the companion mice began to exhibit mechanical allodynia from 7 days of co-housing with SNI mice. To figure out the biochemical mechanisms of pain empathy, pain-related brain chemical substances of normal mice including neurotransmitters and neuropeptides were examined, which revealed no obvious changes in levels of 5-HT, oxytocin, enkephalin and β-endorphin but increased dopamine level and decreased ACTH level in the brains of the companion mice co-housed with SNI mice. In summary, this study found that the normal mice co-reared with SNI mice developed pain empathy with increased dopamine level and decreased ACTH level in the brain.

Keywords

Empathy; Pain; Spared nerve injury; Neuropeptide; Neurotransmitter.

1. Introduction

Empathy is a social ability to recognize feelings, thoughts and intentions of others, as well as to respond to the mental states of others with appropriate emotions[1]. Empathy includes positive empathy and negative empathy. Positive empathy is a state of empathy in which we admire others when we see their accomplishment or sorry for others when we see them fail. On the other hand, negative empathy occurs when we are gloat for others' suffering or jealous for others' success. So far, the positive empathy state is an attracting field for people to investigate, such as empathy in pain or fear.

Normal mice could perceive pain from their social partners who are suffering inflammatory pain, and subsequently exhibit lowered mechanical pain threshold[2]. Studied found that both experiencing pain and perceiving another's pain could activate the insula cortex and anterior cingulate cortex[3]. Moreover, mice display fear and empathic behaviors after seeing their peers suffering from foot shocks even without experiencing shocks themselves[1, 2]. However, whether social interaction with SNI mice can affect mechanical pain of normal mice have been rarely explored. It has been shown that social touch to mice increases the excitability of neurons in the midbrain periaqueductal gray (PAG), a brain region that modulates pain[4-7]. Activation of PAG also leads to increased excitability of oxytocin neurons in paraventricular hypothalamus

ISSN: 1813-4890

(PVH), which is highly related to social behavior[8]. Surprisingly, neurochemicals like oxytocin, vasopressin and testosterone have been found to play an important role in modulating empathic responses of pain[9]. The hippocampus, amygdala, nucleus accumbens, and ventral tegmental area are all connected to PVH via synaptic connections. Thus, oxytocin has the ability, to a certain extent, to regulate the activity of related brain regions and neurotransmitters like dopamine may modulate empathy behavior[10].

In the present study, we investigated the impact of social interaction on mechanical pain sensitivity of normal mice when co-cage reared with SNI mice. Besides, to figure out the biochemical mechanism of pain empathy, brain substances of normal mice including neurotransmitters and neuropeptides were examined. We found that normal mice co-cage rearing with SNI mice had a lowered mechanical pain threshold and these mice also had altered chemicals in their brains.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice were purchased from SPF (Beijing) Biotechnology Cod., Ltd and housed four per cage with *ad-lib* access to food and water at 25 ± 1℃, with 50–60% humidity in a 12 h light/dark cycle with light on from 8 a.m. to 8 p.m. Male mice of 13 weeks old and weighed 24 ± 1 g were used in this study. All procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Advisory Committee at Jinan University. Measures have been taken to minimize the number of animals and their suffering and the study did not have human endpoints. No exclusion criteria were pre-determined in our tests and no animals were excluded in our experiments.

2.2. Conditions of rearing

Mice littermates that were housed in four (with established former social relationship) were chosen for the experiments. For each cage, two mice were arbitrarily chosen as the normal companionship mouse, and the other two mice were arbitrarily chosen as an SNI-modeled mouse or a sham mouse. After the surgery, An SNI-modeled mouse or a sham mouse was co-reared with a normal companionship mouse for 23 days.

2.3. Spared nerve injury (SNI) and sham surgery

SNI model was established as described previously [11]. Mice were anesthetized by intraperitoneal injection of 1.25% tribromoethanol (Sigma, T48402). The skin of the left hind limb was incised for a 0.5 cm incision and the muscle layers close to the femur were separated to expose the sural, the tibial, and the common peroneal nerves, which are the three branches of the sciatic nerve. For SNI-modeled mice, the tibial and common peroneal nerves were lifted by an L-shaped glass capillary pipette and then ligated with surgical sutures. The tibial and common peroneal nerves were sectioned distal to the ligation while the sural nerve remained intact. Great care was taken to avoid any damage to the sural nerve. After that, the muscle and skin were sutured in two layers using surgical sutures respectively. Finally, the mice were placed on a heating pad until they woke up and then placed in the appropriate cage as described on the method of conditions of rearing. For sham mice, we performed the same surgical procedure but without ligated and sectioned the tibial and common peroneal nerves.

2.4. Von Frey test

Von Frey test was used to evaluate mechanical withdrawal threshold of mice at Day 1, 4, 7, 11, 14, and 21 after SNI or sham surgery. Mice were placed individually on a wire mesh grid and restricted in an upside-down glass beaker (70 cm diameter × 90 cm height) to acclimatize 40

ISSN: 1813-4890

min before the tests. The von Frey filaments (North Coast Medical Inc., 12775-99) of 0.07 g were first applied to stimulate the lateral plantar surface of each hind paws and held for 6–8 s with the filament bent slightly. The strength of the next filament was increased when the animal had no response, or decreased when the animal had positive response (licking, shaking or withdrawal of the hind paw suddenly) according to a published up-down paradigm [12]. After the first time of positive response, four more tests were performed. The mechanical paw withdrawal threshold was calculated by the following formula: 50% g threshold $=10^{(X_f + k\delta)}$, where X_f = the log(force) value of the final von Frey filament applied, the k value is a constant related to the up-down response pattern of the individual test mouse, which is provided by the statistics table in Appendix 1 of the method paper by Chaplan *et al* [12], and δ = the average interval between log(force) values of filaments applied in the last five tests [13, 14].

2.5. Enzyme-Linked Immunosorbent Assay (ELISA)

On the 23rd day after SNI or sham surgery, the brains of mice were collected. The tissue was weighed and 1 × PBS was added to prepare for an 10% homogenates. The supernatant was taken by centrifugation for ELISA.

ELISA Kits (ELSBIO) were used to detect the levels of dopamine (CK-E20298M), 5-HT (CK-E20435M), oxytocin (CK-E20200M), enkephalin (CK-E93902M), β-Endorphin (CK-E20413M), and adrenocorticotropic hormone (ACTH, CK-E20424M).

To detect the levels of dopamine, the antibody-coated strip plates were equilibrated at room temperature for 20 min before used firstly. Secondly, 50 μl standard substance with concentration gradients of 0, 7.5, 15, 30, 60, 120 pg/mL and samples were added to the wells. Thirdly, 100 μl HRP-conjugate detection reagents were added to each well and incubated at 37°C for 60 min using electric thermostatic incubator (Jing Hong, DNP-9052). Next, plates were washed by Wash Solution for 5 times. After that, 50 μl Chromogen Solution A and 50 μl Chromogen Solution B were added to each well. After incubated at 37°C for 15 min in the dark, 50 μl Stop Solution was added and the Optical Density (O.D.) at 450 nm was read by a microtiter plate reader (Rayto, RT-6100). The standard curve was established with the concentration of standard substance as the abscissa and the corresponding O.D value as the ordinate and calculate the concentration values of samples according to the standard curve.

To detect the levels of 5-HT, we performed the same procedure except for adding 50 μl standard substance with different concentration gradients of 0, 15, 30, 60, 120, 240 ng/mL for manufacture standard curve.

To detect the levels of oxytocin, we performed the same procedure except for adding 50 μl standard substance with different concentration gradients of 0, 1.5, 3, 6, 12, 24 pg/mL for manufacture standard curve.

To detect the levels of enkephalin, we performed the same procedure except for adding 50 μl standard substance with different concentration gradients of 0, 25, 50, 100, 200, 400 pg/mL for manufacture standard curve.

To detect the levels of β-Endorphin, we performed the same procedure except for adding 50 μl standard substance with different concentration gradients of 0, 3, 6, 12, 24, 48 pg/mL for manufacture standard curve.

To detect the levels of adrenocorticotropic hormone (ACTH), we performed the same procedure except for adding 50 μl standard substance with different concentration gradients of 0, 5, 10, 20, 40, 80 pg/mL for manufacture standard curve.

2.6. Statistical analysis

Sample size chosen for Von Frey test [15, 16] and ELISA [17, 18] was established by published studies. GraphPad Prism 8.4.2 was used to analyze the data. The paw withdrawal threshold curves among two groups and the means of paw withdrawal threshold were compared by Mann

ISSN: 1813-4890

Whitney test. The pain-related brain chemical substance levels between two groups were analyzed using Two-tailed unpaired *t* test. All data are expressed as means ± s.e.m, and $p < 0.05$ is regarded as statistically significant difference. During experiments and analysis, the investigators were blinded to experimental group.

3. Results

3.1. SNI mice or sham mice were co-cage reared with a normal companionship mouse.

SNI model was established in mice by surgery on the left hind limbs to induce mechanical allodynia. After surgery, the SNI-modeled mice were paired with a previous cage mate as a company (co-rearing condition) and the sham mice were also paired with a previous cage mate and served as the control group (Fig. 1A). At Day 1, 4, 7, 11, 14, and 21 after surgery, the mechanical withdrawal thresholds of the left hind paws (the ipsilateral side of SNI position) and the right hind paws (the contralateral side of SNI position) were evaluated using the von Frey test. At Day 23, the brain tissues of mice were collected for ELISA tests (Fig. 1A).

We measured mechanical withdrawal threshold on both hind paws of SNI or sham mice co-reared with companion mice respectively at Day 7, 11, 14, 21 after co-rearing. Comparing to the sham group, the SNI group developed gradually severe mechanical allodynia on the ipsilateral paws from Day 1 to 21 after surgery, showing marked difference on the overall time curve of pain threshold, suggesting that the SNI model was established successfully (Fig. 2B).

Figure 1

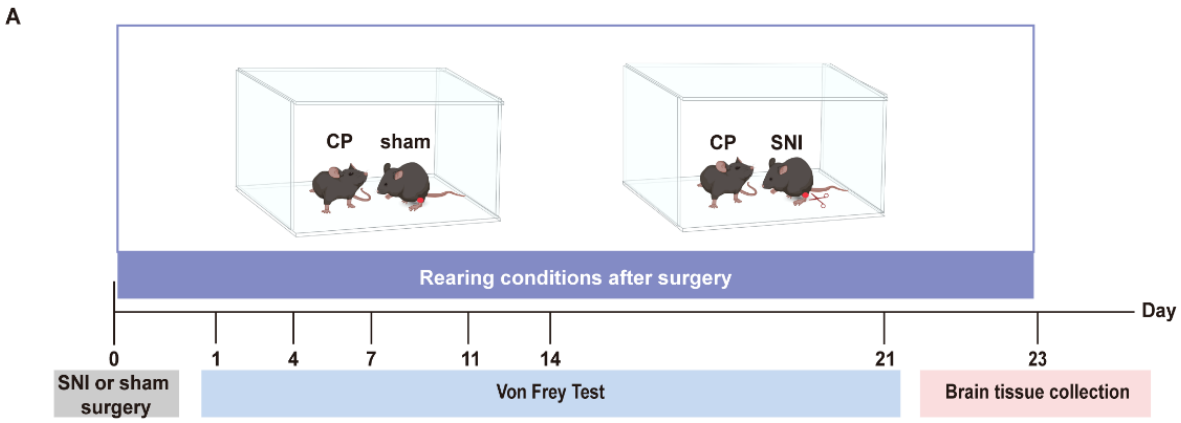

Figure 1. Experimental procedure.

(A) Schematic of rearing conditions after SNI or sham surgery and the experimental timeline. The two rearing conditions are: a sham mouse was pair-reared with a companion (CP) mouse (a sex- and age-matched normal mouse without surgery); an SNI mouse was pair-reared with a CP mouse. Mechanical pain threshold was evaluated by von Frey test at Day 1, 4, 7, 11, 14, and 21 after surgery, and brain tissues were collected at Day 23 for ELISA assays.

ISSN: 1813-4890

Figure 2

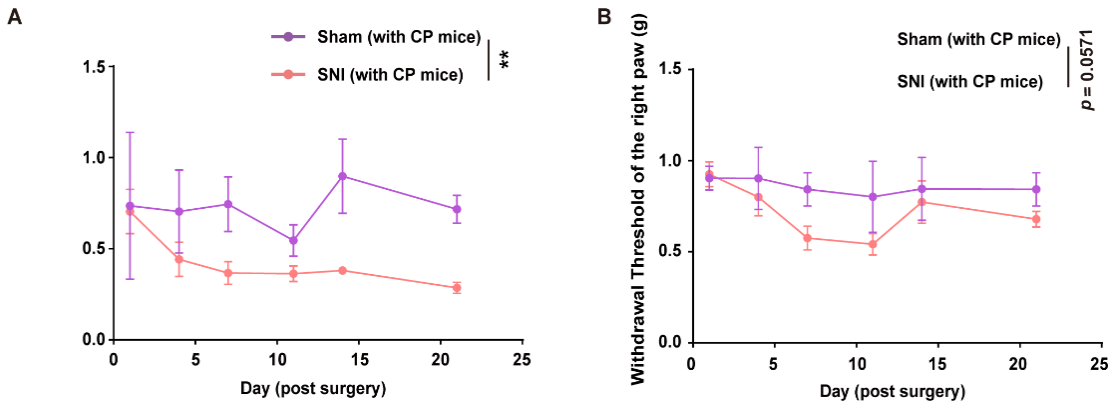

Figure 2. SNI model was established.

(A) Time course changes of withdrawal thresholds of the left (the ipsilateral side of SNI) paws of sham mice co-reared with CP mice (purple) and SNI mice co-reared with CP mice (red) at Day 1, 4, 7, 11, 14, and 21 after surgery. Mann Whitney test, sham (with CP mice) versus SNI (with CP mice), $^{**}p = 0.0043$.

(B) Time course changes of withdrawal thresholds of the right (the contralateral side of SNI) paws. Mann Whitney test, sham (with CP mice) versus SNI (with CP mice), $p = 0.0571$.

Sham (with CP mice), $n = 3$ mice; SNI (with CP mice), $n = 8$ mice. Data are shown as means ± s.e.m.

3.2. Paired rearing with SNI mice leads to mechanical allodynia in companion mice.

To investigated whether the companion mice induced pain empathy when co-rearing with SNI mice. We measured mechanical withdrawal threshold on both hind paws of companion mice co-reared with SNI or sham mice respectively at Day 7, 11, 14, 21 after co-rearing. Over the whole time course from Day 7 to 21, the pain thresholds of the left paws of companion mice were remarkably decreased by co-rearing with SNI mice, as compared to those with sham mice (Fig. 4A, B). Interestingly, the pain thresholds of the right paws of companion mice only began to display a reduction of pain threshold at Day 21, and the allodynia was milder than the left paws (Fig. 4C, D). Thus, even though the companion mice did not receive any surgery, they developed specific pain patterns by exactly mirroring the different severity in the ipsilateral and contralateral hind paws of their SNI cage mates, suggesting that the pain empathy could be precisely presented in different body parts.

ISSN: 1813-4890

Figure 3

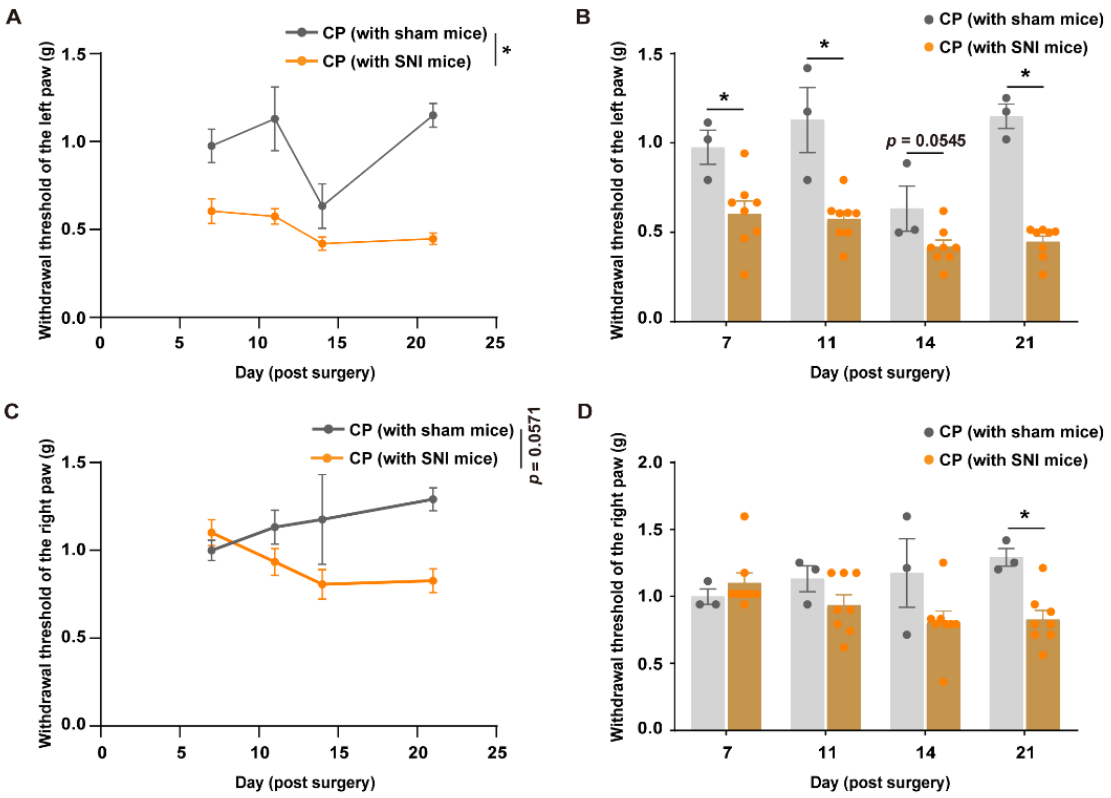

Figure 3. Paired rearing with SNI mice leads to mechanical allodynia in companion mice.

(A) Time course changes of withdrawal thresholds of the left paws of companion mice co-reared with sham mice (grey) or SNI mice (orange) at Day 7, 11, 14, and 21 after surgery. Mann Whitney test, *p = 0.0286.

(B) Withdrawal thresholds of the left paws of CP mice co-reared with sham mice or SNI mice at Day 7, 11, 14 and 21 after surgery. Mann Whitney test, *p = 0.0242 at Day 7, *p = 0.0182 at Day 11, p = 0.0545 at Day 14, *p = 0.0121 at Day 21.

(C) Time course changes of withdrawal thresholds of the right paws. Mann Whitney test, p = 0.0571.

(D) Withdrawal thresholds of the right paws of CP mice co-reared with sham mice or SNI mice at Day 7, 11, 14 and 21 after surgery. Mann Whitney test, p = 0.3394 at Day 7, p = 0.0788 at Day 11, p = 0.4970 at Day 14, *p = 0.0182 at Day 21.

CP (with sham mice), n = 3 mice; CP (with SNI mice), n = 8 mice. Data are shown as means ± s.e.m, two-tailed unpaired t test was used to analyze the data.

3.3. Paired rearing with SNI mice alters the brain levels of dopamine and ACTH in the companion mice.

Next, we examined whether co-rearing with SNI mice leads to alterations of pain-related brain chemical substances. We found that the whole brain level of dopamine was higher in the companion mice co-reared with SNI mice than those with sham mice (Fig. 5A). On the contrary, the brain level of ACTH was decreased after co-rearing with SNI mice (Fig. 5F). Apart from these two chemicals, the brain levels of 5-HT, oxytocin, enkephalin and β-endorphin were not changed by co-rearing with SNI mice (Fig. 5B–E). These results suggest that the altered levels of dopamine and ACTH may contribute to the neurobiology that underlies the pain empathy of mice toward their SNI partners.

ISSN: 1813-4890

Figure 4

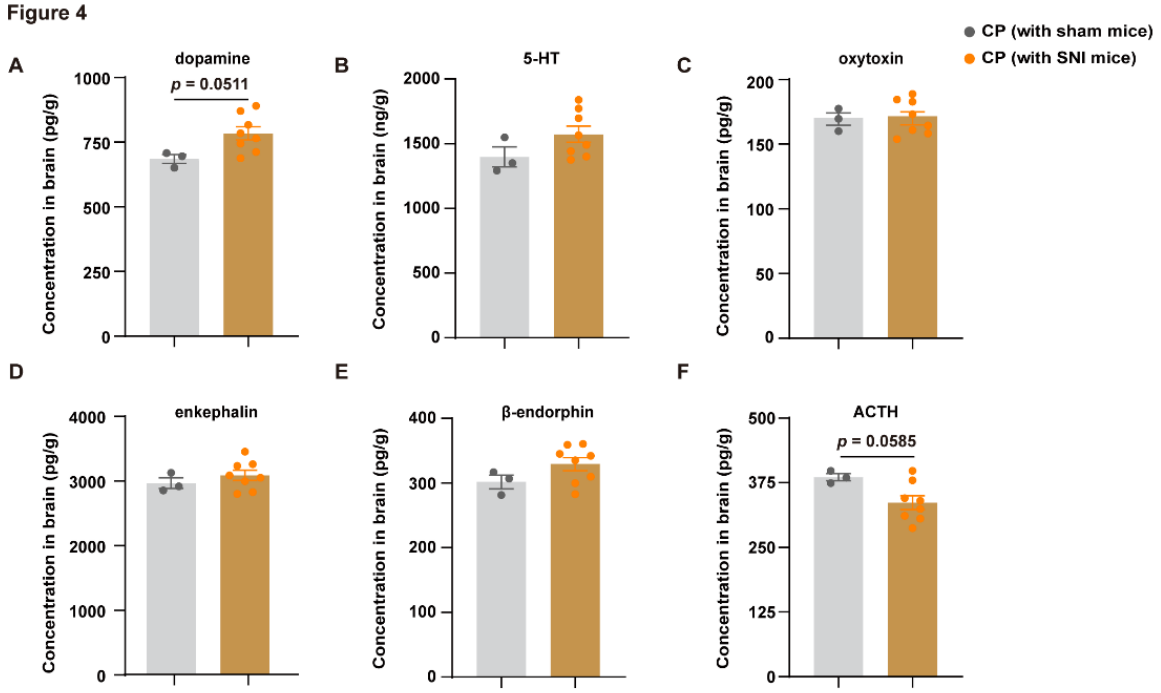

Figure 4. Paired rearing with SNI mice alters the brain levels of dopamine and ACTH in the companion mice

(A–F) The brain levels of dopamine (A, $p = 0.0511$), 5-HT (B, $p = 0.1581$), oxytocin (C, $p = 0.8722$), enkephalin (D, $p = 0.4066$), β-endorphins (E, $p = 0.1603$) and adrenocorticotrophic hormone (ACTH; F, $p = 0.0585$), in CP mice co-reared with sham mice or SNI mice for 23 days post-surgery.

CP (with sham mice), $n = 3$ mice; CP (with SNI mice), $n = 8$ mice. Data are means ± s.e.m. Two-tailed unpaired t test was used to analyze the data.

4. Conclusion

Co-housing with a familiar SNI mouse increases mechanical pain in normal mice.

Co-housing with a familiar SNI mouse alteres brain dopamine and ACTH levels in normal mice.

5. Discussion

In the current study, we showed that the normal mice developed pain empathy to SNI mice when co-reared with them. This finding was consistent with previous studies that bystander mice develop allodynia when shared the same house with mice suffered inflammatory pain or withdrawal from morphine or alcohol [19]. We showed that the dopamine level in the brain of the companion mice was increased when co-reared with SNI mice, which may reinforce the idea that dopamine affects empathy-related behaviors [1]. However, how dopamine affects empathy is still under explored. As a stress-related brain hormone, ACTH was decreased in the brain of companion mice when co-reared with SNI mice. Previous studies showed that stress and empathy could modulate pain sensitivity in humans and rodents [20, 21]. However, the mechanism between pain empathy and stress still awaits further investigation.

In summary, this study found that the normal mice co-reared with SNI mice developed pain empathy with increased dopamine level and decreased ACTH level in the brain, which shed light on studies of the interactive regulation of social-emotional states and pain.

ISSN: 1813-4890

Acknowledgements

This work was supported in part by the STI2030-Major Projects (2022ZD0214400), the National Natural Science Foundation of China (82071535), the National Key R&D Program of China (2022YFA1104900), the Key-Area Research and Development Program of Guangdong Province (2019B030335001), the Guangdong Basic and Applied Basic Research Foundation (2022B151513007, 2023B1515040015).

References

[1] S. Keum, H.S. Shin, Neural Basis of Observational Fear Learning: A Potential Model of Affective Empathy, Neuron, 104 (2019) 78-86.

[2] M.L. Smith, N. Asada, R.C. Malenka, Anterior cingulate inputs to nucleus accumbens control the social transfer of pain and analgesia, Science, 371 (2021) 153-159.

[3] F.B.M. de Waal, S.D. Preston, Mammalian empathy: behavioural manifestations and neural basis, Nat Rev Neurosci, 18 (2017) 498-509.

[4] J. Huang, Z. Zhang, E. Gambeta, L. Chen, G.W. Zamponi, An orbitofrontal cortex to midbrain projection modulates hypersensitivity after peripheral nerve injury, Cell Rep, 35 (2021) 109033.

[5] J. Huang, V.M. Gadotti, L. Chen, I.A. Souza, S. Huang, D. Wang, C. Ramakrishnan, K. Deisseroth, Z. Zhang, G.W. Zamponi, A neuronal circuit for activating descending modulation of neuropathic pain, Nat Neurosci, 22 (2019) 1659-1668.

[6] J.S. Tang, C.L. Qu, F.Q. Huo, The thalamic nucleus submedius and ventrolateral orbital cortex are involved in nociceptive modulation: a novel pain modulation pathway, Prog Neurobiol, 89 (2009) 383-389.

[7] Z. Hu, Y. Mu, L. Huang, Y. Hu, Z. Chen, Y. Yang, X. Huang, Y. Fu, Y. Xi, S. Lin, Q. Tao, F. Xu, K.F. So, C. Ren, A visual circuit related to the periaqueductal gray area for the antinociceptive effects of bright light treatment, Neuron, 110 (2022) 1712-1727 e1717.

[8] H. Yu, W. Miao, E. Ji, S. Huang, S. Jin, X. Zhu, M.Z. Liu, Y.G. Sun, F. Xu, X. Yu, Social touch-like tactile stimulation activates a tachykinin 1-oxytocin pathway to promote social interactions, Neuron, 110 (2022) 1051-1067 e1057.

[9] S. Keum, H.S. Shin, Genetic factors associated with empathy in humans and mice, Neuropharmacology, 159 (2019) 107514.

[10] H.J. Rutherford, S.K. Williams, S. Moy, L.C. Mayes, J.M. Johns, Disruption of maternal parenting circuitry by addictive process: rewiring of reward and stress systems, Front Psychiatry, 2 (2011) 37.

[11] I. Decosterd, C.J. Woolf, Spared nerve injury: an animal model of persistent peripheral neuropathic pain, Pain, 87 (2000) 149-158.

[12] S.R. Chaplan, F.W. Bach, J.W. Pogrel, J.M. Chung, T.L. Yaksh, Quantitative assessment of tactile allodynia in the rat paw, Journal of Neuroscience Methods, 53 (1994) 55-63.

[13] S.L. Christensen, R.B. Hansen, M.A. Storm, J. Olesen, T.F. Hansen, M. Ossipov, J.M.G. Izarzugaza, F. Porreca, D.M. Kristensen, Von Frey testing revisited: Provision of an online algorithm for improved accuracy of 50% thresholds, Eur J Pain, 24 (2020) 783-790.

[14] W.J. Dixon, Efficient analysis of experimental observations, Annu Rev Pharmacol Toxicol, 20 (1980) 441-462.

[15] K. Sandor, S. Krishnan, N.M. Agalave, E. Krock, J.V. Salcido, T. Fernandez-Zafra, P.E. Khoonsari, C.I. Svensson, K. Kultima, Spinal injection of newly identified cerebellin-1 and cerebellin-2 peptides induce mechanical hypersensitivity in mice, Neuropeptides, 69 (2018) 53-59.

[16] F. Guida, M. Iannotta, G. Misso, F. Ricciardi, S. Boccella, V. Tirino, M. Falco, V. Desiderio, R. Infantino, G. Pieretti, V. de Novellis, G. Papaccio, L. Luongo, M. Caraglia, S. Maione, Long-term neuropathic pain behaviors correlate with synaptic plasticity and limbic circuit alteration: a comparative observational study in mice, Pain, 163 (2022) 1590-1602.

ISSN: 1813-4890

[17] V.E. Mulgrave, A.A. Alsayegh, A. Jaldi, D.T. Omire-Mayor, N. James, O. Ntekim, E. Walters, E.O. Akala, J.S. Allard, Exercise modulates APOE expression in brain cortex of female APOE3 and APOE4 targeted replacement mice, Neuropeptides, 97 (2023) 102307.

[18] N. Xu, X.-H. Tang, W. Pan, Z.-M. Xie, G.-F. Zhang, M.-H. Ji, J.-J. Yang, M.-T. Zhou, Z.-Q. Zhou, Spared Nerve Injury Increases the Expression of Microglia M1 Markers in the Prefrontal Cortex of Rats and Provokes Depression-Like Behaviors, Frontiers in Neuroscience, 11 (2017).

[19] M.L. Smith, C.M. Hostetler, M.M. Heinricher, A.E. Ryabinin, Social transfer of pain in mice, Sci Adv, 2 (2016) e1600855.

[20] L.J. Martin, A.H. Tuttle, J.S. Mogil, The interaction between pain and social behavior in humans and rodents, Curr Top Behav Neurosci, 20 (2014) 233-250.

[21] L. Tomova, J. Majdandzic, A. Hummer, C. Windischberger, M. Heinrichs, C. Lamm, Increased neural responses to empathy for pain might explain how acute stress increases prosociality, Soc Cogn Affect Neurosci, 12 (2017) 401-408.