Long-term social isolation increases visceral pain in rats

Xuelong Zhou\textsuperscript{a}, Xiaolu Zhou\textsuperscript{b}, Chenjing Zhang\textsuperscript{b,*}

\textsuperscript{a} Department of Anesthesiology and Perioperative Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China
\textsuperscript{b} Department of Gastroenterology, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou, China

ARTICLE INFO

Keywords:
Social isolation
Visceral pain
Stress

ABSTRACT

Long-term social isolation can alter the sensitivity to somatic pain, however, its impact on visceral pain sensitivity remains unknown. The purpose of this study is to investigate the effect of long-term social isolation on visceral pain sensitivity in rats. 4-week social isolation rearing significantly increased CRD-induced AWR score and decreased pain threshold as compared to group rearing. There was no significant difference in the number of spontaneous visceral pain behaviors between group and isolation rearing rats. The number of fecal bolus produced by isolation rearing rats was comparable to that produced by group rearing rats. Consistent with 4-week isolation, CRD-induced AWR score in 8-week isolation rearing rats was higher than that in group rearing rats. While, pain threshold was lower in 8-week isolation rearing rats than in group rearing rats. There was no significant difference in the number of spontaneous visceral pain behaviors and fecal bolus between 8-week isolation and group rearing rats. These results indicated that long-term social isolation induces visceral hypersensitivity in rats, but this effect is unrelated to an increase in stress levels.

1. Introduction

Social interaction is a crucial characteristic of social animals. Good social interaction brings many benefits: cooperative defense, finding spouses, raising the next generation, and so on [1]. Social dysfunction or social isolation, on the other hand, has a variety of negative health consequences. Learning and memory impairment [2], mental illnesses [3], increased cancer prevalence [4], and even mortality [5] are all linked to long-term social isolation. Nowadays, active or passive social isolation is becoming increasingly widespread [6]. Therefore, research into the behavioral changes brought by social isolation, as well as the molecular mechanisms that underlie them, is required. The findings will give theoretical foundations for the prevention and treatment of diseases associated with social isolation.

Previous studies have reported that long-term social isolation alters the sensitivity of somatic pain in animals. Long-term social isolation, for example, increased paw withdrawal latency in response to thermal, mechanical, and electrical shock stimulation in rats [7, 8, 9]. Morphine's analgesic efficacy on somatic pain was also enhanced in rodents following a long period of social isolation [7]. Furthermore, the endogenous opioid and serotonin systems are involved in the alternations of somatic pain caused by long-term social isolation [10, 11]. However, few studies have examined the impact of long-term social isolation on visceral pain. In the current study, by isolating rats for 4 or 8 weeks, we found that long-term isolation increases visceral pain sensitivity.

2. Materials and methods

2.1. Experimental animal

Adult male Sprague Dawley rats weighing 220 g–250 g were used in this study. All of the rats had free access to food and water. Animal housing room was kept with a temperature at 22 ± 2 °C, a humidity between 40% and 60%, and a 12-hour light-dark cycle. All experimental procedures were approved by the animal ethics committee of Zhejiang Provincial People's Hospital, and according to the guide for the care and use of laboratory animals (National Institutes of Health, 1996).

2.2. Long-term social isolation

After being obtained, the rats were housed in groups (four rats in each group) for four weeks. At the beginning of the fifth week, partial groups were randomly chosen for isolation rearing. The rats from the chosen group were kept individually. The remaining group rats were still housed in groups. Experimental protocol was depicted in Figure 1. Visceral pain tests were performed before (baseline) and after social isolation. The
body weight was comparable between group rearing rats and isolated rearing rats after isolation.

2.3. Visceral pain measurement

Visceral pain sensitivity was measured using the colorectal distention (CRD)-induced abdominal withdrawal reflex (AWR) score and pain threshold [12]. Briefly, the rats were fasting for 12 h and placed in a self-made transparent box for 15 min. Under isoflurane anaesthesia, rats were given a balloon insertion into the descending colon. They were placed in a transparent plastic box for 15 min after waking up. The balloon was rapidly inflated to a desired pressure (20, 40, 60, or 80 mmHg) for 20 s at a 2-minute interval. AWR score was defined as follows: 0, no behavioral response; 1, brief head movement; 2, abdominal muscle contraction; 3, abdominal lifting; 4, abdominal arching. The threshold of pain was defined as AWR score of 3. CRD was applied in 10 mmHg increments from baseline of 10 mmHg. All tests were carried out in a blind manner.

2.4. Spontaneous visceral pain behaviors

Before CRD-induced visceral pain sensitivity measurement, spontaneous visceral pain behaviors of resting rats were observed by video recording for 20 min. Visceral pain behaviors include: 1, abdomen licking; 2, abdominal stretching; 3, squashing of the lower abdomen, and 4, abdominal retractions [13, 14].

2.5. Fecal bolus collection

The rats were freely available to food and water. Two hours before and after social isolation, the fecal bolus was cleared. The amount of fecal bolus deposited in the cage was collected within 2 h at the begin and end of social isolation [15]. Video recording was used to track the number of fecal bolus in group rearing rats. The number of fecal bolus did not change significantly with age or weight.

2.6. Statistical analysis

All statistical analyses were carried out on original pro (2021) software. Data were presented as mean ± SD. Comparisons between two groups were made using Student’s t-test. Repeated measurement data were analyzed by two-way repeated-measures ANOVA. If there is significant difference, Post hoc Bonferroni’s multiple comparison was used. \( P < 0.05 \) was considered as statistically significant.

3. Result

3.1. 4-week social isolation

3.1.1. 4-week isolation increased visceral pain caused by CRD stimulation

Coudereau et al. found that long-term social isolation required at least 4 weeks to produce alterations in somatic pain threshold of rodents [16]. To investigate the effect of long-term social isolation on visceral pain sensitivity in rats, we therefore chose 4 weeks to induce a long-term isolation. The body weight was comparable between group rearing rats and isolated rearing rats after isolation. Our results showed that the baseline AWR score and pain threshold were equivalent across group and 4-week isolation rearing rats. However, when compared to group rearing or isolation rearing baseline, 4-week isolation rearing significantly increased CRD-induced AWR score and decreased pain threshold (Figure 2A and 2B), suggesting that 4-week long-term isolation enhances visceral pain sensitivity.

3.1.2. 4-week isolation had no effect on spontaneous visceral pain

In addition to CRD stimulation-induced visceral pain measurements, spontaneous visceral pain behaviors at resting state were evaluated. The baseline number of spontaneous visceral pain behaviors in both group and isolation rearing rats was comparable before isolation. Similarly, no significant difference in the number of spontaneous visceral pain behaviors was found between group and isolation rearing rats after isolation (Figure 2C), suggesting that 4-week long-term isolation did not affect the spontaneous visceral pain of rats.

3.1.3. 4-week isolation did not increase the output of fecal bolus

To assess stress levels, we measured the amount of fecal bolus output in rats [17, 18]. There was no significant difference in the baseline number of fecal bolus between group and isolation rearing rats. After 4-week isolation, the number of fecal bolus was still equivalent between group and isolation rearing rats (Figure 2D). Meanwhile, the number of fecal bolus did not change significantly with age. These findings revealed that rats’ stress levels were unaffected by a 4-week isolation.

3.2. 8-week social isolation

3.2.1. 8-week isolation increased CRD stimulation-induced visceral pain

In addition to 4 weeks, 8-week isolation rearing were also considered as a long-term social isolation [1, 7, 19]. To further confirm the effect of long-term isolation on visceral pain sensitivity, we performed another 8 weeks’ isolation rearing experiment in the current study. The body weight was comparable between group rearing rats and isolated rearing rats after isolation. Baseline AWR score and pain threshold caused by CRD stimulation were comparable between group and isolation rearing rats. However, as compared to group rearing or isolation rearing baseline, 8-week isolation significantly increased the AWR score and reduced pain threshold (Figure 3A and 3B), suggesting that 8-week isolation produces visceral hypersensitivity.

3.2.2. 8-week isolation had no effect on spontaneous visceral pain

The number of spontaneous pain behaviors in both group and isolation rearing rats was identical before isolation. There was also no apparent difference in the number of spontaneous pain behaviors between the group and isolation rearing rats after an 8-week isolation (Figure 3C), suggesting that isolation for 8 weeks had no obvious effect on the spontaneous visceral pain behaviors of rats.

3.2.3. 8-week isolation did not increase fecal bolus output

The number of fecal bolus did not change significantly with age. In isolation rearing rats, the baseline number of fecal bolus was comparable to group rearing rats before isolation. There was no significant difference in the number of fecal bolus between the group and isolation rearing rats after an 8-week isolation (Figure 3D), suggesting that the 8-week isolation did not cause a noticeable change in stress levels.
4. Discussion

In the present study, we investigated the effect of long-term social isolation on visceral pain sensitivity. The primary findings were: long-term social isolation rearing for 4 or 8 weeks significantly increased CRD-induced AWR score and reduced pain threshold. However, no difference in spontaneous visceral pain or defecation frequency between group and 4- or 8-week isolation rearing rats. These findings indicate that long-term social isolation causes visceral hyperalgesia in rats.

Long-term social isolation leads to a variety of behavioral and mental health issues. Animals exposed to long-term social isolation exhibited excessive aggressive behavior [20], anxiety [21], and depression [3]. In addition, in long-term isolated rodents, aberrant pain perception was induced [22]. The thermal pain threshold induced by hot plate stimulation and the mechanical pain threshold caused by von Frey filament stimulation were both enhanced after four weeks of isolation rearing [16, 23, 24]. Similarly, 8 weeks of social isolation resulted in analgesic effects, as evidenced by a lower thermal pain threshold triggered by radiant heat stimulation [19]. Furthermore, long-term social isolation increased morphine’s analgesic effect [7, 23]. In the current study, we found that long-term social isolation (4 or 8 weeks) enhanced visceral pain sensitivity, which differed from the effect of long-term social engagement on somatic pain.

Not only was the visceral pain generated by external stimulation (CRD stimulation) measured in our study, but also the spontaneous visceral pain behavior in the resting state. According to our findings, long-term social isolation rearing had no effect on the number of spontaneous visceral pain behaviors. This could be because physical visceral stimulus is insufficient to elicit the effect of long-term social isolation on spontaneous visceral pain. The impact of long-term social isolation on spontaneous visceral pain will be assessed in the future using an ongoing spontaneous pain paradigm, such as formalin model [14].

Visceral pain is exacerbated by stress. Many visceral pain models, such as water avoidance and mother-infant separation, are established by inducing high levels of stress [25, 26]. However, when evaluating defecation frequency, we found no evidence that long-term social isolation greatly increased stress levels in rats. Acute social isolation for 2 or 24 h dramatically increased the amount of fecal bolus, according to our unpublished results. These findings suggest that during long-term social isolation, stress levels may gradually fall from an early peak to a late low level. The visceral hypersensitivity generated by long-term social isolation may not be due to stress. Visceral pain is also affected by gender. Compared to male rats, female rats have a stronger visceral pain response after long-term social isolation (our unpublished data).

Although there is no uniform standard to define the time window of acute and chronic social isolation, a high degree of stress can be used to distinguish the two. Acute social isolation is more likely to activate the stress system [27], while chronic long-term social isolation has an impact on other systems, such as the central nervous system [1]. Long-term social isolation has been shown to cause abnormally high activity of nucleus accumbens neurons in mice, resulting in sexual behavior defects and anxiety-like behaviors [28]. More research is needed to see if the visceral pain hypersensitivity caused by long-term social isolation is mediated by abnormalities in the central nervous system. More research is needed to investigate whether the visceral hypersensitivity caused by long-term social isolation is mediated by abnormalities in the central nervous system.

Figure 2. Effect of 4-week isolation on visceral pain. A, CRD-induced AWR score was increased after 4-week isolation rearing, *P < 0.05, n = 8. B, CRD-induced pain threshold was decreased after 4-week isolation rearing, *P < 0.05, n = 8. C, Number of spontaneous visceral pain behaviors after 4-week isolation rearing, n = 8. D, Number of fecal boluses after 4-week isolation rearing, n = 8.
In conclusion, the findings revealed that chronic long-term social isolation increases visceral pain. We provide preclinical evidence that long-term social isolation has an influence on visceral pain sensitivity.

Declarations

Author contribution statement

Chenjing Zhang, Xuelong Zhou: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Xiaolu Zhou: Performed the experiments; Analyzed and interpreted the data.

Funding statement

This work was supported by Young Talent Program of the First affiliated hospital of Nanjing medical university (YNRCQN034).

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest’s statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] C.R. Lee, A. Chen, K.M. Tye, The neural circuitry of social homeostasis: consequences of acute versus chronic social isolation, Cell 184 (2021) 1500–1516.
[2] M.P. Paulus, G.B. Varty, M.A. Geyer, The genetic liability to stress and postweaning isolation have a competitive influence on behavioral organization in rats, Physiol. Behav. 68 (2000) 389–394.
[3] X.C. Ma, D. Jiang, W.H. Jiang, et al., Social isolation-induced aggression potentiates anxiety and depressive-like behavior in male mice subjected to unpredictable chronic mild stress, PLoS One 6 (2011), e20955.
[4] C.H. Kroenke, L.D. Kubzansky, E.S. Schernhammer, et al., Social networks, social support, and survival after breast cancer diagnosis, J. Clin. Oncol. 24 (2006) 1105–1111.
[5] A. Steptoe, A. Shankar, P. Demakakos, et al., Social isolation, loneliness, and all-cause mortality in older men and women, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 5797-5801.
[6] T.I. Almeida, J.F. Rego, A.C.G. Teixeira, et al., Social isolation and its impact on child and adolescent development: a systematic review, Rev. Paul. Pediatr. 40 (2021), e2020385.
[7] M.W. Adler, C. Mauron, R. Samanin, et al., Morphine analgesia in grouped and isolated rats, Psychopharmacologia 41 (1975) 11–14.
[8] N. Horiguchi, Y. Ago, S. Hasebe, et al., Isolation rearing reduces mechanical allodynia in a mouse model of chronic inflammatory pain, Pharmacol. Biochem. Behav. 113 (2013) 46–52.
[9] T.A. Kosten, M.J. Miserendino, J.C. Bombace, et al., Sex-selective effects of neonatal isolation on fear conditioning and foot shock sensitivity, Behav. Brain Res. 157 (2005) 235–244.
[10] N. Horiguchi, Y. Ago, K. Asada, et al., Involvement of spinal 5-HT1A receptors in isolation rearing-induced hypalgesia in mice, Psychopharmacology (Berl) 227 (2013) 251–261.
[11] A.M. Konecka, I. Sroczynska, Stressors and pain sensitivity in CPW mice. Role of opioid peptides, Arch. Int. Physiol. Biochem. 98 (1990) 245–252.
[12] T.J. Nies, G.F. Gebhart, Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudofunctional reflexes in the rat, Brain Res. 450 (1988) 153–160.
[13] J.M.A. Laidl, L. Martinez-Caro, E. Garcia-Nicas, et al., A new model of visceral pain and referred hyperalgesia in the mouse, Pain 92 (2001) 335–342.
[14] M. Miampamba, S. Chery-Crozé, F. Gorry, et al., Inflammation of the colonic wall induced by formalin as a model of acute visceral pain, Pain 57 (1994) 327–334.
[15] R.T. Han, H. Lee, J. Lee, et al., Brief isolation changes nociceptive behaviors and compromises drug tests in mice, pain, In Pract. 16 (2016) 749–757.

[16] J.F. Coudereau, C. Monier, J.M. Bourre, et al., Effect of isolation on pain threshold and on different effects of morphine, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 21 (1997) 997–1018.

[17] B. Bonaz, Y. Tache, Water-avoidance stress-induced c-fos expression in the rat brain and stimulation of fecal output: role of corticotropin-releasing factor, Brain Res. 641 (1994) 21–28.

[18] K. Miyata, T. Kamato, A. Nishida, et al., Role of the serotonin3 receptor in stress-induced defecation, J. Pharmacol. Exp. Therapeut. 261 (1992) 297–303.

[19] S. Poggi-Allegra, A. Oliverio, Social isolation: effects on pain threshold and stress-induced analgesia, Pharmacol. Biochem. Behav. 19 (1983) 679–681.

[20] G.B. Varty, S.B. Powell, V. Lehmann-Masten, et al., Isolation rearing of mice induces deficits in prepulse inhibition of the startle response, Behav. Brain Res. 169 (2006) 162–167.

[21] S. Cuesta, A. Funes, A.M. Pacchioni, Social isolation in male rats during adolescence inhibits the wnt/beta-catenin pathway in the prefrontal cortex and enhances anxiety and cocaine-induced plasticity in adulthood, Neurosci. Bull. 36 (2020) 611–624.

[22] M. Larasche, G. Gourcerol, M. Million, et al., Repeated psychological stress-induced alterations of visceral sensitivity and colonic motor functions in mice: influence of surgery and postoperative single housing on visceromotor responses, Stress 13 (2010) 343–354.

[23] W. Kostowski, A. Gzlonkowski, W. Rewderski, et al., Morphine action in grouped and isolated rats and mice, Psychopharmacology (Berl) 53 (1977) 191–193.

[24] G. Tuboly, G. Benedek, G. Horvath, Selective disturbance of pain sensitivity after social isolation, Physiol. Behav. 96 (2009) 18–22.

[25] F. Barreau, L. Ferrier, J. Fioramonti, et al., New insights in the etiology and pathophysiology of irritable bowel syndrome: contribution of neonatal stress models, Pediatr. Res. 62 (2007) 240–245.

[26] I. Schwetz, S. Bradesi, J.A. McRoberts, et al., Delayed stress-induced colonic hypersensitivity in male Wistar rats: role of neurokinin-1 and corticotropin-releasing factor-1 receptors, Am. J. Physiol. Gastrointest. Liver Physiol. 286 (2004) G683–691.

[27] J.H. Taylor, A.C. Mustoe, J.A. French, Behavioral responses to social separation stressor change across development and are dynamically related to HPA activity in marmosets, Am. J. Primatol. 76 (2014) 239–248.

[28] M. Barrot, D.L. Wallace, C.A. Bolanos, et al., Regulation of anxiety and initiation of sexual behavior by CREB in the nucleus accumbens, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 8357–8362.