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ORIGINAL RESEARCH

Effect of Acute Brief Social Isolation on Visceral Pain

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Objective: The sensitivity of somatic pain could be affected by social isolation; however, few studies have examined the impact of social isolation on visceral pain. In the present study, the effect of acute brief social isolation on visceral pain response was investigated.

Methods: Adult male rats were either reared individually or grouped for 2 hours or 24 hours. Colorectal distention (CRD)-induced abdominal withdrawal reflex (AWR) score and pain threshold were used to assess visceral pain sensitivity. The amount of fecal bolus was used to determine the stress level.

Results: Acute brief isolation rearing for 2 hours significantly increased AWR score and reduced visceral pain threshold in rats when compared to group rearing. Similarly, acute isolation for 24 hours resulted in visceral hypersensitivity, as indicated by an increase in the AWR score and a decrease in the visceral pain threshold. Furthermore, the amount of fecal bolus in acute isolation rearing (2 or 24 hours) rats was considerably higher than in the control group rearing rats.

Conclusion: Acute short-term social isolation enhances visceral pain sensitivity, which could be related to an increase in stress levels.

Keywords: social isolation, visceral pain, stress

Introduction

Social contact is an essential need of social animals, much like eating and sleeping. Appropriate social interaction has numerous advantages, including the acquisition of partners, cooperative defence, and the upbringing of the next generation.[1] Lack of social connection or social support, on the other hand, has a variety of negative consequences for social individuals' health, including depression,[2] cognitive impairment,[3] and even life cycle shortening.[4] Given the rising prevalence of social isolation in today's society,[5] it's critical to investigate and examine the behavioral changes that occur when an animal is isolated from its social environment, as well as the mechanisms that cause them.

Previous studies have found that social isolation induces aberrant pain perception in rodents. When compared to group-housed rats, the pain sensitivity of isolation housed rats' feet to noxious heat and electric shock is dramatically altered.[6,7] Social isolation rearing decreases mechanical pain sensitivity in mice in a chronic inflammatory condition caused by complete Freund's adjuvant (CFA).[8] In addition, the endogenous opioid and serotonin (5-HT) systems are involved in the changes in somatic pain sensitivity caused by social isolation.[9,10] The impact of social isolation on pain perception has primarily been studied in the context of somatic pain. However, few studies have examined the impact of social isolation on visceral pain. In the present study, we aimed to clarify the effect of social isolation on visceral pain sensitivity. By measuring visceral pain sensitivity in rats, we found that acute short-term social isolation increases visceral pain response.

Materials and Methods

Animal

Adult male Sprague Dawley rats weighing 220g ~ 250g were used. They were collected from the experimental animal center of Hangzhou Medical College. The temperature of the housing room is kept at 22 ± 2 °C with a 12 hours light-dark cycle. All experimental procedures were approved by the animal ethics committee of the Affiliated People's

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Hospital of Hangzhou Medical College and followed the Guide for the care and use of laboratory animals (National Institutes of Health, 1996).

Acute Brief Isolation Rearing

Five rats were housed in each group for at least one month after they were obtained. Partial cage rats were randomly allocated and housed individually for 2 or 24 hours. The rats in the remaining cages were still housed in groups. Figure 1 depicts the experimental paradigm. The sensitivity to visceral pain was tested before and after social isolation rearing.

Visceral Pain Measurement

Colorectal distention (CRD)-induced abdominal withdrawal reflex (AWR) score and pain threshold were used to assess visceral pain sensitivity.[11] Briefly, the rats were fasting for 12 hours and placed in a self-made transparent box for 15 minutes. A balloon was rapidly inflated to a desired pressure (20, 40, 60, or 80 mmHg) in the descending colorectal region for 20 seconds, followed by a 2-minute rest. The AWR was given a score of: 0: no response; 1: brief head movement with no obvious body movement; 2: abdominal muscle contraction; 3: abdominal lifting; 4: abdominal arching. A stimulus intensity that elicited an AWR score of 3 was established as the visceral pain threshold. Starting at 10 mmHg, CRD was applied in 10 mmHg increments. All of the measurements were carried out in a blind manner.

Spontaneous Visceral Pain Behaviors

Prior to measuring visceral pain sensitivity, video recording was used to observe the spontaneous visceral pain behaviors of rats in a resting state in the cage for 20 minutes. Abdominal licking, abdominal stretching, squashing of the lower abdomen, and abdominal retractions are considered as visceral pain behaviors.[12,13]

Fecal Bolus Collection

The rats were freely available to food and water. The number of fecal bolus deposited in the cage was counted for 2 hours.[14] The number of fecal bolus deposited in the cage of group rearing rats was monitored via video recording.

Statistical Analysis

All statistical analyses and graphics were carried out by Origin Pro (2021) software. The data were presented as mean ± SD. Differences between the two groups were compared using Student's *t*-test. For repeated measurements (AWR score), data were analyzed by two-way repeated-measures ANOVA. Post hoc Bonferroni's multiple comparisons were used if there was a significant difference. Statistical significance was defined as a $P < 0.05$.

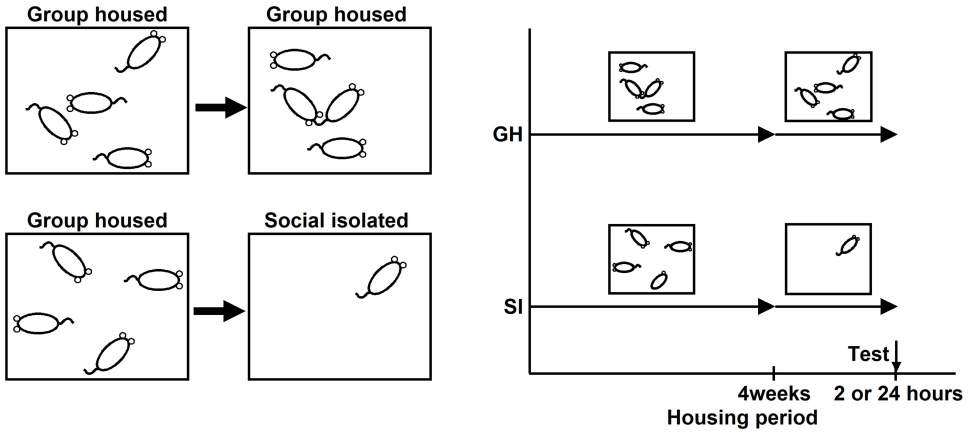

Figure 1 Schematic diagrams of the experimental protocols for experiments.
Abbreviations: GH, group housing; SI, social isolation.

Result

Acute 2h Social Isolation

Acute 2h Isolation Increased Visceral Pain Caused by CRD Stimulation

To investigate the effect of social isolation on visceral pain, rats were reared individually for 2 hours, and then their visceral pain sensitivity was assessed. No significant difference in baseline visceral pain sensitivity was identified between group rearing rats and isolated rearing rats. CRD-induced AWR score and pain threshold were comparable among the two groups before social isolation (Figure 2A and B). However, as compared to group rearing rats, 2 hours of isolation rearing increased the CRD-induced AWR score and decreased the pain threshold (Figure 2E and F), implying that 2 hours of acute solation rearing causes visceral hypersensitivity in rats.

Acute Social Isolation for 2 Hours Had No Effect on Visceral Pain Behaviors

In addition to the assessment of CRD-induced visceral pain, we also detected spontaneous visceral pain in rats within 2 hours of isolation. There was no significant difference in the number of spontaneous pain behaviors between group rearing rats and isolated rearing rats before and after social isolation (Figure 2C and G), suggesting that the 2 hours of isolation had no influence on the spontaneous visceral pain.

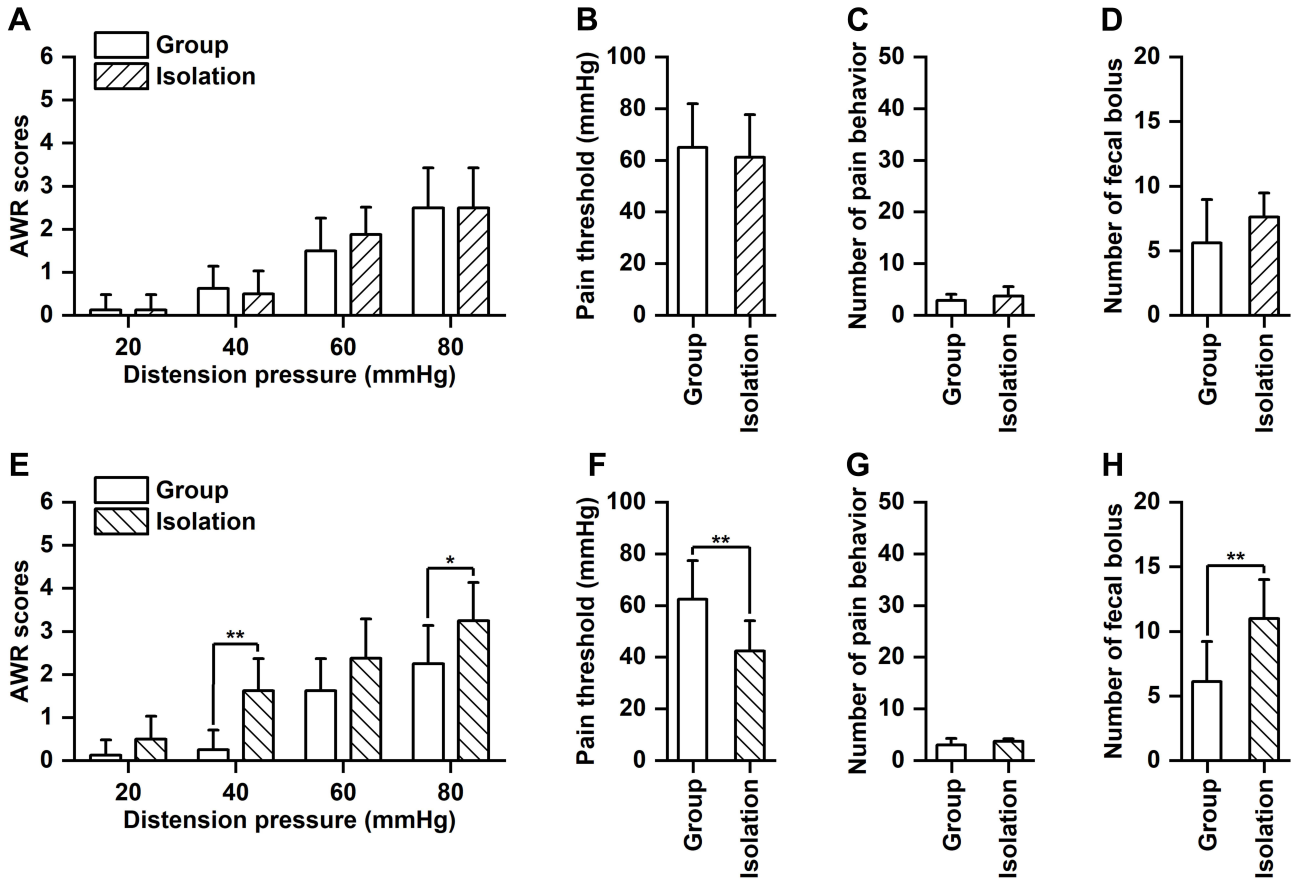

Figure 2 Effect of 2-hour acute isolation on visceral pain. (**A** and **B**) Baseline of CRD-induced AWR score (**A**) and pain threshold (**B**) in group and isolation rearing rats, n = 8. (**C** and **D**) Baseline of spontaneous visceral pain (**C**) and the number of fecal boluses (**D**) in group and isolation rearing rats, n = 8. (**E**) CRD-induced AWR score was increased in 2-hour isolation rearing rats as compared to the control group rearing rats, $^{*}P < 0.05$, $^{**}P < 0.01$, n = 8. (**F**) CRD-induced pain threshold was decreased in 2-hour isolation rearing rats as compared to the group rearing rats. $^{*}P < 0.05$, $^{**}P < 0.01$, n = 8. (**G**) Number of spontaneous visceral pain behaviors was comparable between 2-hour isolation rearing rats and group rearing rats. n = 8. (**H**) Number of fecal boluses was increased in 2-hour isolation rearing rats as compared to the group rearing rats, $^{**}P < 0.01$, n = 8.

Abbreviations: CRD, colorectal distention; AWR, abdominal withdrawal reflex.

The Number of Fecal Bolus Was Increased After a 2-Hour Period of Social Isolation

The defecation frequency of rats reflects their stress level.[15,16] To determine the effect of acute isolation on stress levels, we collected fecal bolus during the 2 hours of isolation rearing. The baseline number of fecal bolus was comparable between isolation rearing rats and group rearing rats (Figure 2D). However, the number of fecal bolus was significantly increased in 2 hours of social isolation rearing rats than in group rearing rats (Figure 2H), indicating that the stress level of rats was considerably increased after 2 hours of acute social isolation.

Acute 24h Social Isolation

Acute 24h Isolation Increased CRD Stimulation-Induced Visceral Pain

Because a 24-hour isolation time is also regarded as an acute social isolation period,[1] we investigated the effect of acute 24-hour social isolation on visceral pain sensitivity in rats further. Between isolation and group rearing rats, there was no significant difference in baseline visceral pain sensitivity caused by CRD (Figure 3A and B). However, as compared to group rearing rats, 24 hours of acute isolation significantly increased CRD-induced AWR score and reduced visceral pain threshold (Figure 3E and F), suggesting that isolation rearing for 24 hours produces visceral hypersensitivity in rats.

Acute 24h Isolation Did Not Affect Spontaneous Visceral Pain

The findings of the spontaneous visceral pain behaviours test revealed that there was no significant difference in baseline between rats reared in groups and rats reared in isolation (Figure 3C). In isolation rearing rats, the number of spontaneous

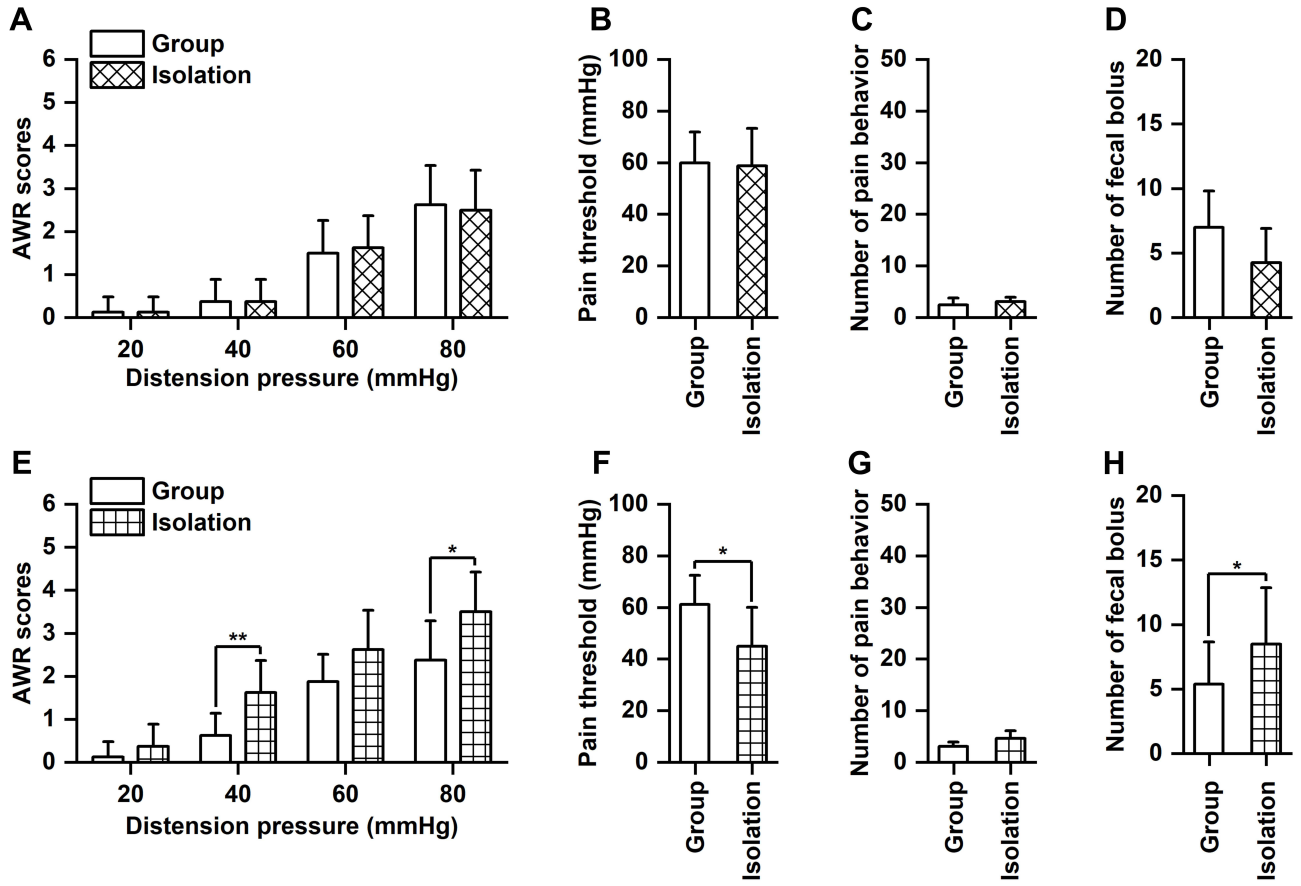

Figure 3 Effect of 24-hour acute isolation on visceral pain. (**A** and **B**) Baseline of CRD-induced AWR score (**A**) and pain threshold (**B**) in group and isolation rearing rats, n = 8. (**C** and **D**) Baseline of spontaneous visceral pain (**C**) and the number of fecal boluses (**D**) in group and isolation rearing rats, n = 8. (**E**) CRD-induced AWR score was increased in 24-hour isolation rearing rats as compared to the control group rearing rats, $^{*}P < 0.05$, $^{**}P < 0.01$, n = 8. (**F**) CRD-induced pain threshold was decreased in 24-hours isolation rearing rats as compared to the group rearing rats. $^{*}P < 0.05$, n = 8. (**G**) the Number of spontaneous visceral pain behaviors was comparable between 24-hour isolation rearing rats and group rearing rats. n = 8. (**H**) Number of fecal boluses was increased in 24-hour isolation rearing rats as compared to the group rearing rats, $^{*}P < 0.05$, n = 8.

Abbreviations: CRD, colorectal distention; AWR, abdominal withdrawal reflex.

visceral pain behaviours was comparable to that of group rearing rats (Figure 3G). These findings imply that a period of acute isolation of 24 hours had no noticeable impact on spontaneous visceral pain.

Acute 24h Isolation Increased the Number of Fecal Bolus

The results revealed that the baseline number of fecal bolus was comparable in both isolation and group rearing rats (Figure 3D). However, isolation rearing for 24 hours significantly increased the number of fecal bolus when compared to group rearing (Figure 3H), indicating that acute 24 hour isolation, equivalent to 2 hours, increased the stress level of rats.

Discussion

In the present study, we investigated the effect of acute social isolation (2 or 24 hours) on visceral pain sensitivity in rats. The main results were as follows: acute isolation increased visceral pain sensitivity in rats after CRD stimulation; acute isolation had no effect on spontaneous visceral pain; the amount of fecal bolus was increased in acute isolation rearing rats.

Many studies have shown that social isolation induces a variety of behavioural abnormalities in animals, including locomotor hyperactivity,[17] learning and memory impairment,[3] and an excessive aggressive defense reaction to external stimulation.[18] Furthermore, a number of research have revealed that social isolation causes aberrant pain sensitivity.[19] Short- and long-term social isolation dramatically reduced rodent pain sensitivity, resulting in a higher thermal and mechanical pain threshold in the animal paws.[8,14] Meanwhile, social isolation increased morphine's analgesic effect.[6] Clinical studies, on the other hand, have indicated that those who are socially isolated are more prone to experience pain, such as muscular and joint pain.[20,21] For the first time, we examined the impact of social isolation on visceral pain sensitivity. In contrast to the effect of social isolation on somatic pain, social isolation increased the sensitivity of rats to visceral pain. Our findings revealed that social isolation has a distinct influence on visceral and somatic pain.

Pain measurement includes external stimuli-induced pain response and spontaneous pain behaviors. In the present study, we found that acute social isolation increased visceral pain sensitivity in rats when pain was detected with CRD stimulation. Acute social isolation, on the other hand, had no effect on spontaneous pain in resting rats. The physiological stimulation in the resting state is insufficient to cause a difference in pain sensitivity between rats raised in isolation and those raised in groups. For future experiments, more powerful stimuli are required. In our unpublished study, we found that spontaneous pain behaviors induced by formalin and capsaicin differed significantly between isolated and grouped rats.

Isolation from others, particularly acute isolation, is a strong source of stress.[22] Models of visceral pain are induced using the acute stress response, such as water avoidance and forced swimming stress.[23] In rodents, these stressors cause significant visceral hypersensitivity. By counting the number of fecal bolus, we found that the stress level of acute social isolation rats was much higher than that of group rearing rats. Acute social isolation-induced visceral hypersensitivity is thought to be mediated by increased stress levels.[24] This supports with our findings that acute social isolation increased visceral pain sensitivity rather than reducing somatic pain sensitivity. In addition to stress, acute social isolation causes an aberrant immunological response. Pro-inflammatory factors are produced more effectively in response to acute stress.[25] One of the key mechanisms for visceral hypersensitivity is the inflammatory response of the digestive tract.[26] At the level of the central nervous system, acute stress can cause brain alterations and defensive actions in response to the threat of an unknown environment.[27] This results in a high level of awareness, which is one of the main causes for the HPA axis activation and stress reaction.

Why was somatic pain reduced but visceral pain enhanced after acute social isolation? Previous studies have reported that acute social isolation-induced decrease in somatic pain sensitivity is related to endogenous opioid and 5-HT systems. Administering naloxone or the serotonin receptor antagonist WAY100635 reduced the analgesic effect of acute social isolation on somatic pain.[9,10] Although we are not well aware of the role of endogenous opioid systems in the different effect of acute social isolation on somatic and visceral pain, the serotonin system may contribute, in part, to these differences. Serotonin has different effects on somatic pain and visceral pain. The threshold of somatic pain was enhanced when serotonin levels were elevated.[9,28] Increased serotonin levels, on the other hand, cause visceral hypersensitivity.[29]

In conclusion, our study found that acute short-term social isolation increases visceral pain sensitivity. Preclinical evidence of the impact of acute social isolation on visceral pain is presented.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no competing interests in this work.

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