Animal Study

NLRP3-mediated Neuroinflammation Exacerbates Incisional Hyperalgesia and Prolongs Recovery After Surgery in Chronic Stressed Rats

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Background: Postoperative pain management has increasingly become a public health problem worldwide. Psychological factors can be considered as independent risk factors for the intensity of postoperative pain and the occurrence of postoperative chronic pain.

Objectives: As stress events could facilitate NLR family pyrin domain-containing 3 (NLRP3) inflammasome activation in the central nervous system, we aimed to explore the role of perioperative NLRP3-mediated neuroinflammation in the exacerbation of incisional hyperalgesia in stressed rats.

Study Design: Experimental trial in rats.

Setting: Department of Anesthesiology, Shanghai, China.

Methods: All animal experimental procedures were approved by the Animal Care and Use Committee of Shanghai Jiaotong University School of Medicine. This study was conducted in rat models of chronic restraint stress and hind paw incision model. Serum corticosterone level measurement and emotion-related behavioral tests were used to confirm that chronic restraint stress can cause depression-like behavior in rats. Pain behavior after surgery was assessed by withdrawal response to von Frey filament application. Immunofluorescence staining and the Western blot test were used to evaluate the protein level of NLRP3, IL-1β, C-fos in the basolateral amygdala (BLA) and GluN2B-containing N-methyl-D-aspartate (NMDA) receptors (GluN2B) expression in the central nucleus of the amygdala (CeA), respectively. Intra-BLA cannulation and microinjection of an NLRP3 specific inhibitor——MCC950 (0.5 μL, 2 μg/μL) were applied to the stressed rats for 4 days perioperatively to explore whether the stress-induced postoperative hyperalgesia and GluN2B expression in CeA can be altered.

Results: The results showed that chronic restraint stress exposure led to depressive behavior in rats. Moreover, chronic restraint stress exposure increased NLRP3 and interleukin 1 beta (IL-1β) expression in the basolateral amygdala (BLA), as well as exacerbated postoperative hyperalgesia and prolonged the recovery time of postoperative pain. Meanwhile, GluN2B expression in the CeA of the stressed group was higher than that of the control incision group. Inhibition of NLRP3 reversed the exacerbation of postoperative hyperalgesia by stress exposure, and down-regulated GluN2B expression in the CeA.

Limitations: The upstream mechanism by which NLRP3 is elevated in stressed rats was not explored.

Conclusion: These findings suggest that chronic restraint stress may influence postoperative hyperalgesia and NLRP3-mediated neuroinflammation, which may in turn contribute to stress-induced postoperative pain exacerbation.

Key words: Postoperative pain, NLRP3, chronic restraint stress, amygdala, neuroinflammation, rat, incision, depression

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More than 320 million people undergo surgery each year worldwide, which results in a considerable number of patients with chronic postsurgical pain (CPSP) (1). The occurrence of CPSP leads to physical discomfort, mental distress, and reduced quality of life for patients, and is also a main reason for the opioid crisis. The best way to decrease postoperative opioid use is to effectively manage postoperative pain (2,3). Therefore, it is critical to explore the mechanism that may influence postoperative pain sensation (4). The results of previous clinical studies suggest that emotional states such as anxiety and depression can increase pain perception and the risk of CPSP (5,6). However, the underlying mechanism remains largely unknown.

Long-term stress events are the main reasons for psychological disorders. A previous study showed that chronic forced swimming induced depressive behavior in rats due to stress. The neurons in the basolateral amygdala (BLA) appeared to be sensitized and the BLA to central nucleus of the amygdala (CeA) synaptic transmission efficiency was enhanced, resulting in the facilitation of neuropathic pain and overexpression of GluN2B-containing N-methyl-D-aspartate (NMDA) receptors (GluN2B) in the CeA of rats with spared nerve injury (7). The role of postsynaptic GluN2B receptor in the CeA has also been proven to be essential in pain-related information processing in animal models of arthritis pain (8,9).

Innate immunity of the brain regions related to emotional regulation are activated in depression, and the expression of NLR family pyrin domain-containing 3 (NLRP3) and interleukin 1 beta (IL-1β) are increased (10). Also, the depression symptoms of experimental mice are significantly improved after treatment with an NLRP3 inhibitor (11). NLRP3 is one of the most widely studied nucleotide-binding oligomerization domain-like receptor (NOD)proteins; it is expressed in the central nervous system and can be activated by a series of danger signals (12).

The NLRP3 inflammasome contains 3 main effectors: NLRP3, apoptosis-related speckle-like protein (ASC) and pro-caspase-1. After activation, NLRP3 interacts with ASC to activate pro-caspase-1 into active caspase-1, which ultimately promotes the maturation and release of IL-1β. IL-1β is one of the most common proinflammatory cytokines, which initiates various signaling pathways and drives inflammation, affecting the functional activities of neurons (13). In vitro experiments have shown that proinflammatory cytokines can sensitize neurons (14). However, NLRP3-mediated neuroinflammation in the BLA region induced by chronic stress has not yet been explored. This study aimed to verify the impact of chronic stress exposure on the threshold of postoperative pain in rats, and the GluN2B expression of CeA region. Furthermore, c-fos (a marker of neuron activation[15]), NLRP3 and IL-1β expression in the BLA region were explored in each rat group. We also applied MCC950, a specific inhibitor of NLRP3, to the BLA region in order to observe whether the stress-induced postoperative hyperalgesia and GluN2B expression in CeA can be altered.

Methods

Animals

Male Sprague-Dawley rats, weighing 220 g to 280 g, were purchased from SLAC Laboratory Animal (Shanghai, China). The chronic stressed rats were housed in separate cages, while the control rats were housed 3 rats to a cage. Both groups had free access to food and water. The room temperature was maintained at 22°C - 24°C, under a 12 h/12 h light–dark cycle. All animal experimental procedures were approved by the Animal Care and Use Committee of our university.

Chronic Restraint Stress Procedure

For the establishment of this model, rats were subjected to chronic-restraint stress in self-made plastic cylinders, in which some degree of movement was allowed (such as head grooming with the forelimbs, but not with the hind limbs), and the rats were prevented from turning around or escaping (16). The restraint duration for each rat was 6 hours per day for 21 consecutive days. The stressed rats were individually housed, with free access to food and water, except during the restraint period each day.

Postoperative Pain Animal Model

We chose the hind paw incision model as a representative of postoperative pain in humans (17). Rats were briefly anesthetized with isoflurane for 3 minutes as previously described (18), and an incision was made on the left hind paw of each rat with a length of one cm through the skin and fascia of the plantar. The skin was cut 0.2 cm from the proximal edge of the heel as a starting point and extended distally. The tendon was lifted and cut longitudinally, with the origin of the tendon and the insertion site intact. Then, the skin and subcutaneous tissue were sutured with 5.0 silk thread. After the operation was completed, the rats were al-

lowed to recover in a cage with sterile bedding. The control rats were anesthetized only as a sham operation with no surgical incision on the hind paw. Withdrawal responses were measured using von Frey filaments before surgery and for 15 days postsurgery according to the recovery time of each experiment.

Behavioral Test

Sucrose Preference Test

Animals were individually housed and habituated to 2 bottles of water for 2 days, followed by 2 bottles of 2% sucrose for 2 days. Animals were then water-deprived for 24 hours and exposed to one bottle of 2% sucrose and one bottle of water for 2 hours in the dark phase. Bottle positions were switched after one hour (for a 2 hour test). Total consumption of each fluid was measured and sucrose preference was defined as the average sucrose consumption ratio during the first 2 hours. Sucrose consumption ratio was calculated by dividing the total consumption of sucrose by the total consumption of both water and sucrose (19). The sucrose preference test was performed before grouping (rats were excluded if any neurological deficits were observed) and the day after chronic restraint stress.

Forced Swim Test

Rats were individually placed in a plexiglass container for 6 minutes (diameter: 20 cm; water depth: 30 cm). The water temperature was maintained at 25°C ± 2°C. At the end of each test, rats were removed and thoroughly dried. Each session was videotaped, and the relative amount of time for which a rat was immobile was recorded by an expert observer who was blind to the grouping of the rats (20).

Open Field Test

The open field test was used to evaluate the motor function and anxiety-like behavior of the rats in this study. Briefly, the animal was placed in a bright open-field experimental device (track lighting 50 lux). The size of the open-field experimental device was reported in a previous study (21). The open field apparatus (100 cm X 100 cm X 50 cm) had a video tracking system above it to record the behavior of each rat. The apparatus was divided into 5 X 5 squares, and the inner 9 squares represented the central area with a size of 60 cm X 60 cm. Each rat was placed in one corner of the apparatus, facing the wall, and was allowed to explore freely for 10 minutes. The total movement distance and the percentage of time in the center grid were recorded and analyzed with an EthoVision XT 8.5 system (Noldus Information Technology, Wageningen, the Netherlands).

Assessment of Postoperative Hyperalgesia

Pain behavior after surgery was assessed by paw withdrawal response to von Frey filament application. Briefly, unrestrained rats were placed beneath a clear plastic chamber (21 cm X 27 cm X 15 cm) on an elevated mesh floor (grid 12 mm X 12 mm). Withdrawal responses to mechanical stimulation were determined using calibrated von Frey filaments applied from underneath the cage to the skin 2 mm adjacent to the wound and the same area on the noninjured hind paw of the control rats. We set the cutoff value at 15 g, and the first filament in the series that evoked at least 3 responses (from the 5 applications) was regarded as the threshold. If there was no response to the cutoff force, then 15 g was recorded. Paw withdrawal latency was assessed before surgery and once daily during the 15 days postoperation (22).

Intra-BLA Cannulation and Microinjection

Rats were anesthetized with 1% pentobarbital sodium (40 mg/kg) and intra-BLA cannulation was performed under a stereotaxic apparatus. A guide cannula was implanted dorsal to the right BLA (anteroposterior: -2.8 mm; mediolateral: 5 mm right to midline; dorsoventral: 7 mm from the skull). After disinfection with 75% alcohol, the skin was cut along the sagittal line and the periosteum was scraped with a bone scraper. The right BLA position was marked under the stereotaxic apparatus. The guide cannula was embedded in the corresponding position. Finally, self-curing denture powder was used to seal the surrounding area of the borehole. The skin was disinfected again and sutured. Each rat was individually housed and allowed to recover for one week after the operation.

For drug injection, rats were briefly anesthetized with isoflurane, the microinjection needle was inserted one mm beyond the guide cannula, and 0.5 μL of drug (MCC950 or sterile water as vehicle) was pumped slowly for one minute. After the injection, the needle was withdrawn slowly to avoid backflow. MCC950 or corresponding vehicle was administered on day 0, and days 1-3 postoperation. The von Frey test was conducted one hour after each administration.

Serum Corticosterone Measurement

Venous blood was collected from the orbital vein after anesthesia with isoflurane (n = 10 per group for

the control group and stressed group) at 10:00 AM -11:00 AM. The serum was separated by centrifugation at 3500 g for 15 minutes at 4°C, and stored at -80°C for later use. Rat corticosterone ELISA kit (Nanjing Jiancheng, China) was used to measure the level of serum corticosterone.

Immunofluorescence Staining

At 24 hours after hind paw incision, rats in the control incision group and stressed incision group were anesthetized by pentobarbital overdose (100 mg/kg, i.p. [intraperitoneal injection]) and transcardially perfused with saline and 4% paraformaldehyde. Rats were then decapitated, and their brains were removed, post-fixed and dehydrated. Five μm thick sections were cut coronally at the level of the BLA region. The brain sections were permeabilized with 0.3% Triton X-100 for 20 minutes and blocked using goat serum for 30 minutes, then incubated with primary antibodies for C-fos (Cell Signaling Technology, Danvers, MA), NeuN (Cell Signaling Technology, Danvers, MA) and NLRP3 (Servicebio, Wuhan, Hubei, China) overnight at 4°C. Sections were then incubated with Alexa Fluor 488 (Abcam, Cambridge, UK) or Alexa Fluor 594 (Abcam, Cambridge, UK) for one hour at room temperature. Thereafter, sections were mounted with a drop of anti-fade mounting medium and stored in the dark for observation with a fluorescence microscope.

Western Blot

Brain tissues were lysed in RIPA solution (Sigma-Aldrich, Shanghai, China). Samples were applied to sodium dodecyl sulfate polyacrylamide gels, and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% skimmed milk for one hour at room temperature and incubated at 4°C overnight with primary antibodies of GluN2B (Cell Signaling Technology, Danvers, MA), NLRP3 (Abcam, Cambridge, UK), IL-1β (Abcam, Cambridge, UK), and α-Tubulin (Abcam, Cambridge, UK). The GluN2B and IL-1β primary antibodies were diluted at a concentration of 1:1000, NLRP3 primary antibody was diluted at a concentration of 1:500, and α-Tubulin was diluted at 1:10000. Blots were then incubated with peroxidase-conjugated secondary antibodies and visualized with the electrochemiluminescence procedure. The results were analyzed with ImageJ software (public domain).

Statistical Analysis

SPSS 23.0 software (IBM Corporation, Armonk, NY) was used for statistical analysis and GraphPad Prism 8.0.1 (San Diego, CA) was used for preparing graphs. All data are expressed as mean ± standard deviation. Unpaired Student's t test was used for comparing 2 groups. One-way analysis of variance (ANOVA) or 2-way repeated measures ANOVA, followed by post hoc Tukey's test were used for multiple comparisons of normally distributed data. Non-normally distributed data were analyzed with the Kruskal-Wallis nonparametric analysis. A P value < 0.05 was considered as statistically significant.

Results

Influence of chronic restraint stress on weight gain, serum corticosterone, and emotion-related behavioral change

As described before, chronic stress might influence weight gain, therefore, we evaluated body weight every week. Rats were weighed on the day before restraint stress, and subsequently weighed on days 7, 14, and 21 poststress. The weight gain of each rat was calculated by subtracting the initial body weight from each time point that the body weight was measured. The weight gain decreased in stressed rats from day 7 to day 21 (Fig. 1A). Chronic stress also elevated serum corticosterone level (Fig. 1B). Rats displayed significant depressive-like behavior in the sucrose preference test and forced swimming test, which manifested as a decreased sucrose preference percentage (Fig. 2A) and prolonged immobility in the forced swimming test (Fig. 2C). No locomotor change or anxiety-like behavior was observed in the open field test since the total travel distance was not significantly different between the groups and the percentage of time spent in the center area was also not statistically different (Fig. 2B). The emotion-related behavioral tests showed that chronic restraint stress led to depression behavior in rats, but not anxiety.

Exacerbation of Postoperative Pain by Chronic Restraint Stress

The von Frey test was conducted preoperation and once per day until postoperative day 15. After 21 days of restraint stress exposure, paw withdrawal latency to a von Frey filament was statistically decreased. Meanwhile, the recovery time was prolonged in the stressed rats. In the control incision group, paw withdrawal latency returned to normal compared to that of the

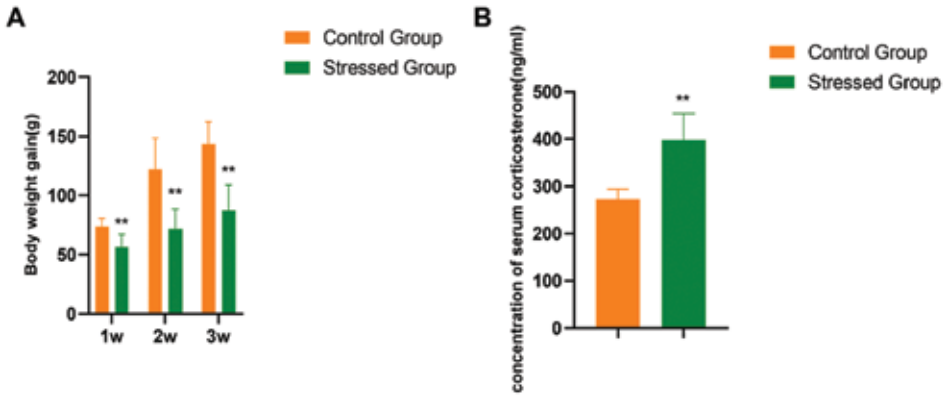

Fig. 1. *The influence of chronic stress on weight gain and concentration of serum corticosterone. A) Body weight gain was significantly lower in the stressed group at the poststress time points (1 week, 2 weeks and 3 weeks). B) Serum corticosterone level was significantly up-regulated in the stressed group. **P < 0.01, n = 10 per group.*

stressed group on postoperative day 3. The recovery time for the stressed incision group was 10 days, with a trend of pain chronicity until postoperative day 15 (Fig. 3).

Chronic Restraint Stress Enhanced the Up-regulation of GluN2B Expression Induced by Incisional Surgery

GluN2B receptors are known to play an indispensable role in central pain processing and chronic pain modulation (7,22-24). Down-regulation of GluN2B protein expression in the amygdala can also inhibit reserpine-induced pain and depression in mice. Therefore, we detected GluN2B in CeA of each group (9). There was no difference between the control and chronic restraint stress groups. However, chronic restraint stress remarkably enhanced the up-regulation of GluN2B expression induced by incision (Fig. 4). The results were consistent with the behavioral results in the acute pain phase of postoperative day one (Fig. 3).

Up-regulation of NLRP3, IL-1β and C-fos Expression in the BLA

After 21 days of restraint stress, the expression of NLRP3 was elevated as detected by Western blot, but incision alone did not influence the expression of NLRP3 protein (Figs. 5A,B). Inflammatory cytokine, IL-1β, which is a product of NLRP3 inflammasome activation, was also significantly increased (Figs. 5C,D). The immunofluorescence test for C-fos, which is considered as a marker for neuronal activation and NLRP3 expression, showed a significant elevation in the stressed incision group compared to the control incision group (Figs. 5E,F).

The role of BLA neuroinflammation in pain perception and GluN2B expression in CeA

In order to explore the role of NLRP3-mediated neuroinflammation in pain perception, we injected MCC950, a specific inhibitor of NLRP3, into the right BLA region through a cannula (before the operation, and postoperative day one to postoperative day 3). The dose of MCC950 was calculated based on the IC50 of NLRP3 and the dose used in a previous study (25). The MCC950 injection alleviated postoperative pain in the acute postoperative phase. Meanwhile, the recovery time of the drug injection group was also shortened. In the vehicle injection group, the recovery time of postoperative hyperalgesia was 10 days, while MCC950 shortened the recovery time to day 7 after surgery (Fig. 6). The expression of GluN2B induced by incision in CeA was also lowered by MCC950 injection (Figs. 7A,B).

Discussion

The 2 main findings of the present study were as follows: First, chronic restraint stress not only exacerbated postoperative hyperalgesia in rats but also prolonged the recovery time of postoperation pain. Second, chronic restraint stress up-regulated NLRP3 and IL-1β expression in the BLA region, as well as elevated GluN2B expression induced by incisional pain. NLRP3 inflammasome inhibitor MCC950 alleviated postoperative hyperalgesia and promoted recovery after surgery. These results indicated that chronic restraint stress may influence postoperative pain sensation via central inflammation mediated by NLRP3 inflammasome activation.

The opioid crisis is currently a serious public health problem. A total of 70,630 deaths from drug overdose

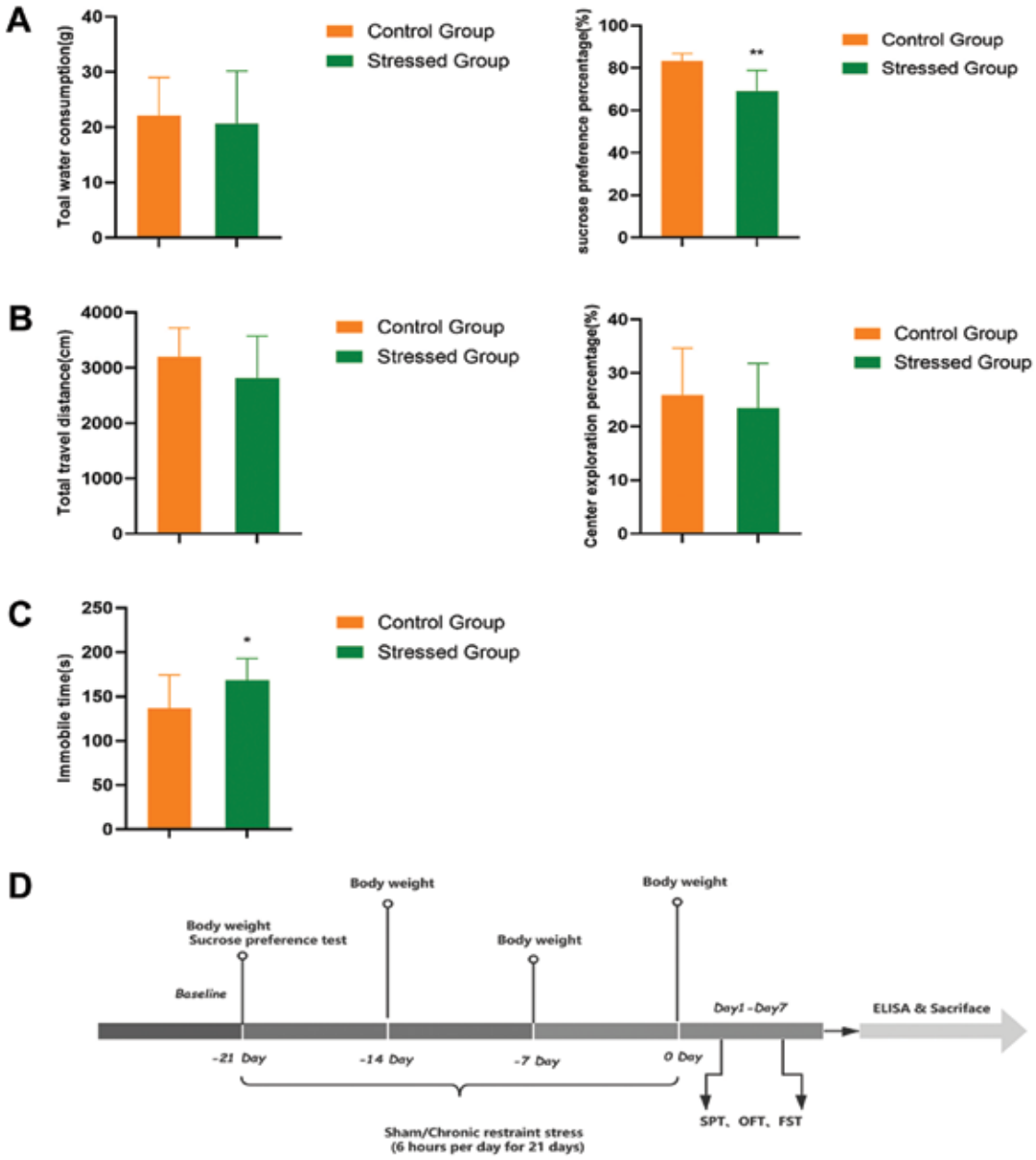

Fig. 2. *Chronic restraint stress induced depressive behavioral change in rats. A. Chronic stress-reduced sucrose intake in rats, represented by reduced sucrose consumption percentage in the stressed group, while the total water intake was not significantly different between the groups. B. Open-field test results of the control group and stressed group, including total travel distance and percentage time spent in the center grids. C. Forced swimming test of the control group and stressed group. The total immobile time during the last 4 minutes was calculated and compared between the groups. D. The flow chart of the emotion-related behavioral tests. *P < 0.05, **P< 0.01, n = 10 per group.*

occurred in the United States in 2019, and about half of them involved the overdose of synthetic opioids (26). From 2013 to 2019, the fatality rate of synthetic opioids increased by 1,040% (26). Inappropriate opioid prescriptions after surgery is one of the most important risk factors for the opioid crisis (27).

With the aging of the population the number of operations each year is also increasing and postopera-

tive pain is one of the most common symptoms after surgery. Poor control of acute postoperative pain is not only a contributor to opioid abuse, but it is also an important risk factor for the development of chronic postsurgical pain. In recent years, researchers have been advocating the application of a multimodal nonopioid pain management strategy, which is shown to be associated with better management of postoperative pain and a lower rate of opioid prescriptions and misuse (28). Of note, postoperative pain perception has obvious individual specificity, which proves that there are many factors affecting postoperative pain sensation. Only by targeted treatment of certain risk groups can postoperative pain be better managed. The results of this study show that chronic stress might enhance postoperative hyperalgesia in the acute phase after surgery and that perioperative administration of MCC950 mitigated surgical incision-induced hyperalgesia, as well as promoted recovery; it also shows that early intervention may improve the prognosis after surgery.

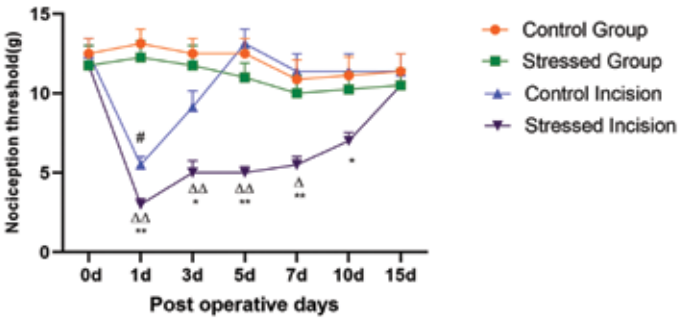

Fig. 3. *Chronic restraint stress exacerbated postoperative nociception and prolonged the recovery time after surgery.*
#$P < 0.01$ compared with the control group, **$P < 0.01$,*$P < 0.05$, compared with the control incision group, ΔΔ$P < 0.01$, Δ$P < 0.05$, compared with the stressed group. n = 8 per group.

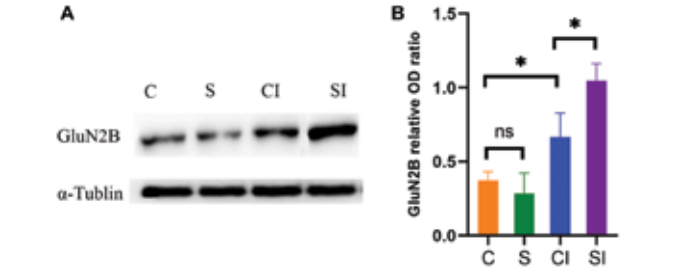

Fig. 4. *Chronic stress enhanced surgical incision-induced GluN2B expression in CeA. A. Surgical incision in the left hind paw (lanes 3 and 4) elevated the levels of GluN2B expression in right CeA. However, chronic stress alone (lane 2) did not influence the expression of GluN2B in CeA. B. Quantification of Western blot showed that hind paw incision significantly up-regulated GluN2B expression, especially in the chronic stressed group.*
C = control group, S = stressed group, CI = control incision group, SI = stressed incision group. *P < 0.05 compared with the CI group; ns = no significance, n = 3 per group.

Many clinical studies have revealed that a patient's emotional states can worsen pain perception and increase the risk of chronic pain (29). However, there are few studies on early identification and intervention of psychosocial factors related to the prognosis after surgery (30). The effect of antidepressants in perioperative pain management remains controversial (31). Given that there may be some confounding factors in clinical research, we aimed to use a suitable animal model to evaluate the influence of psychological stress on postoperative hyperalgesia in order to identify a treatment target. Chronic emotional stress is one of the main causes of neuropsychiatric diseases. Numerous studies have confirmed that chronic stress events can lead to biochemical, physical and psychological changes, which can lead to emotional disorders related to stress, including anxiety and depression (32,33). In this study, we used chronic restraint stress as a method to establish a stressed animal model. Serum corticosterone level measurement and emotion-related behavioral tests confirmed that chronic restraint stress can cause depression-like behavior in rats. Thereafter, incision surgery was performed on the left hind paw to explore the effects of postoperative hyperalgesia and recovery time in experimental rats. The results showed that chronic restraint stress exposure not only enhanced acute postoperative hyperalgesia in rats but also prolonged the recovery time of postoperative pain. The animal model used in the present study could also serve as a useful animal model for conducting in-depth research on the mechanism of negative emotions on postoperative pain, and to screen effective intervention drugs.

Perception of pain is controlled by the dual upward activation system and downward regulation system of pain. The latter passes through different limbic structures of the brain, and the functional activities of

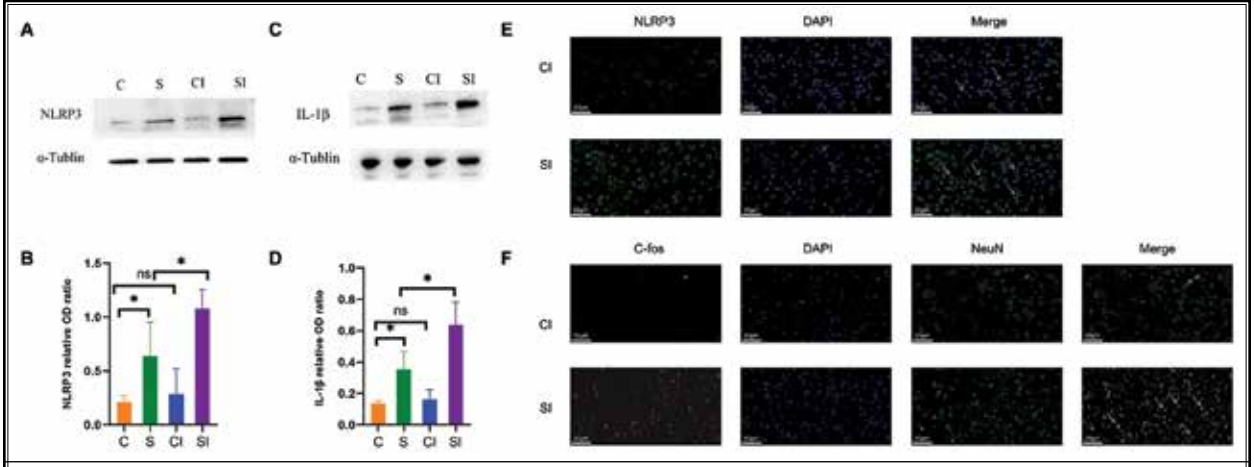

Fig. 5. *Effect of stress and hind paw incision on NLRP3, IL-1β and C-fos expression in the BLA. A. Western blot of NLRP3 expression, α-Tubulin was chosen as a loading control. B. Statistical analysis of NLRP3 protein relative optical density in western blot among the groups (n = 4). C. Western blot of IL-1β expression, α-Tubulin was chosen as a loading control. D. Statistical analysis of IL-1β protein relative optical density in Western blot among the groups (n = 4). E. Immunofluorescence staining of NLRP3 expression (green) in the BLA (n = 3). F. Immunofluorescence staining of C-fos expression (red) in the BLA (n = 3).*
**P* < 0.01, **P* < 0.05; ns = no significance between groups, C = control group, S = stressed group, CI = control incision group, SI = stressed incision group.

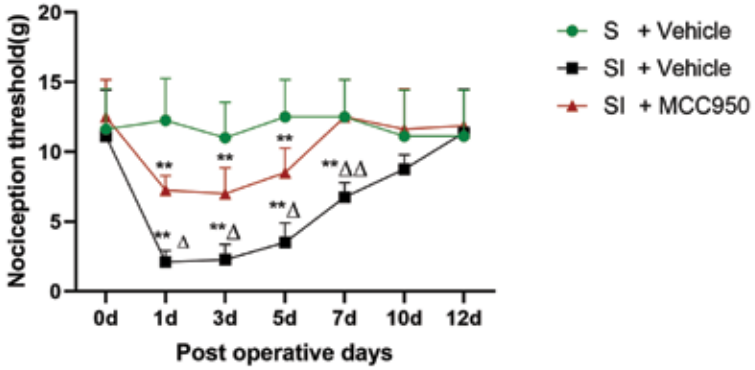

Fig. 6. *MCC950 injection mitigated postoperative nociception and shortened the recovery time after surgery in stressed rats.*
P* < 0.01,P* < 0.05 compared with the S + Vehicle group, ΔΔ*P* < 0.01, Δ*P* < 0.05 compared with the SI+MCC950 group. S = stressed, SI = stressed incision. n = 8 per group.

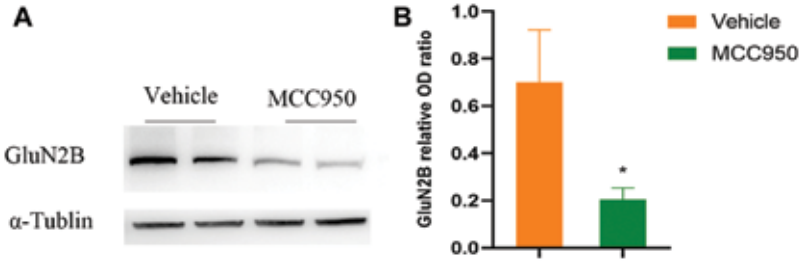

Fig. 7. *MCC950 injection down-regulated GluN2B expression induced by hind paw incision in CeA of chronic stressed rats. A. Western blot of GluN2B expression and α-Tubulin. B. Statistical analysis of relative optical density of GluN2B protein.*
**P* < 0.05 compared with the vehicle group. n = 3 per group.

these regions and brain nuclei are largely affected by emotional states. The amygdala is an important part of the limbic system of the brain, and its role in neuropsychiatric diseases has been confirmed (34,35). Recent evidence shows that depression caused by chronic stress is closely related to the activation of NLRP3 inflammasome (10,36,37). The inflammasome is a complex, which is composed of various proteins. It is assembled by intracytoplasmic pattern recognition receptors and is an important part of the innate immune system.

Under chronic mild stress conditions, mice showed depression-like and anxiety-like behaviors. The serum levels of corticosterone and IL-1β in the mice increased, and the levels of NLRP3 and IL-1β in the hippocampus increased. After treatment with the NLRP3 inhibitor VX-765, the depressive symptoms of experimental mice improved (11). However, the expression of NLRP3 in the BLA region has not yet been explored.

The present study found that after 21 days of chronic restraint stress, the expressions of NLRP3 protein and IL-1β in the BLA region were significantly up-regulated in the stressed group rats. Meanwhile, c-fos protein, which is regarded as a marker for neuronal activation, was also elevated in the stressed group. MCC950, a specific inhibitor of the NLRP3 inflammasome, was perioperatively injected into the BLA, which had a positive effect on postoperative pain outcome. The mechanism by which inhibition of NLRP3 inflammasome in the BLA may influence pain processing remains unclear. The present study attempted to explore this issue. The expression of GluN2B in CeA was significantly up-regulated after hind paw incision, and the level was higher in the stressed group. MCC950 injection alleviated postoperative hyperalgesia and down-regulated GluN2B protein in the stressed incision rats. Increasing evidence have suggested that BLA is not only a storage site for emotion processing but also facilitates synaptic plasticity of other regions, including CeA, which is the main output nucleus in the amygdala to regulate pain-related behaviors (38). Multiple studies have shown that GluN2B-NMDA receptors play a key role in the amygdala synaptic plasticity and the development of chronic pain (8,9). Therefore, we postulated that the role of NLRP3-mediated inflammation in the BLA on pain processing may be due to its influence on synaptic plasticity in CeA via neural circuit mechanism.

The present study has some limitations. First, we did not evaluate the upstream mechanism by which NLRP3 is elevated in stressed rats, which needs to be explored in the future. Second, we aimed to explain the results in a neural circuit mechanism, however, we did not include any neuroimaging or neurophysiology method, so whether NLRP3-mediated neuroinflammation in the BLA directly contributes to the up-regulation of incision-induced GluN2B expression in CeA remains unknown.

Conclusion

The results of this study indicate that NLRP3-mediated neuroinflammation might play an important role in central pain processing in stress-induced exacerbation of postoperative pain. Our results could facilitate further preclinical studies to determine the mechanism of chronic stress on postoperative pain for the purpose of better postoperative pain management and minimizing the use of opioids after surgery, which could eventually improve the outcomes of surgical patients. However, it is undeniable that our current experiment is just exploratory research in the field of basic research; more translational medicine research is still needed for further verification and exploration.

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