[Short Communication]

Chronic Inflammatory Pain Induces Maladaptation to Stress in Mice

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Abstract: The aim of the present study was to create an animal model of the maladaptation to stress by chronic pain. We found that a single exposure to restraint stress induced a decrease in head-dipping behavior of mice in the hole-board test. This emotional stress response was not observed in mice that had been exposed to repeated restraint stress for 14 days, which confirmed the development of stress adaptation. In contrast, mice that were subjected to chronic inflammatory pain did not develop this stress adaptation. These results indicate that chronic inflammatory pain impairs the ability to adapt to stress.

Key words: pain, stress adaptation, mice

INTRODUCTION

Clinical evidence indicates that chronic pain induces psychiatric disorders and reduces quality of life.[1, 2] It has been estimated that over 50% of patients who suffer from chronic pain also exhibit clinically diagnosable symptoms of depression including adjustment disorder.[3] Additional reviews have confirmed that adjustment disorders are the most common mental disorder.[4] Based on reports of the comorbidity between chronic pain and mood disorder in human patients, it is possible that there may be a close relation between these disease states. It has become clear that painful signals (nociceptive information) evoked at the periphery are transmitted via various circuits to multiple cerebral cortices where pain signals are processed and perceived. Consequently, pain has a somatosensory-discriminative aspect and an affective-cognitive aspect that are processed in different but correlated brain structures in the ascending circuits.[5, 6] These reports suggest that painful stimulation may affect the response to stress.

The ability to adapt to stress is an important defensive function of a living body, and impairment of this ability may contribute to some stress-related disorders. Thus, the identification of brain mechanisms that contribute to stress adaptation could help pave the way for new therapeutic strategies for stress-related psychiatric disorders. A series of behavioral experiments has demonstrated that repeated exposure to the same type of stress stimuli diminishes acute stress responses.[7–9] We can also create stress-adaptive models by repeatedly exposing rats to restraint stress.[10] In addition, more recently, to further characterize models of stress adaptation, we created stress-adaptive models in mice, as described below.[11] A single exposure to restraint stress for 60 min produced a decrease in the number and duration of head-dipping behaviors of mice in the hole-board test, and these acute emotional responses were recovered by exposure to repeated restraint stress for 60 min/day for 7 or 14 days, but not 3 days. However, mice that had been exposed to repeated restraint stress for 240 min/day for 7 or 14 days continued to show a decrease in head-dipping behavior in the hole-board test. Thus, these animal models may be useful for investigating the mechanisms of stress adaptation.

To clarify the relationship between chronic pain and adjustment disorder, the present study was undertaken to investigate whether chronic inflammatory pain could induce stress-maladaptive behaviors in mice.

MATERIALS AND METHODS

The present studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the Committee on the Care and Use of Laboratory Animals of the International University of Health and Welfare.

Male ICR mice (Japan SLC, Inc., Shizuoka, Japan) weighing 25–30 g were housed at a room temperature of 23±1℃ with a 12-h light-dark cycle (light on 7：00 a.m. to 7：00 p.m.). Food and water were available *ad libitum*. All experiments were carried out in the light phase of the cycle.

A persistent inflammatory pain model was produced by unilateral intraplantar injection of complete Freund's adjuvant (CFA; *Mycobacterium tuberculosis*; Sigma, St. Louis, MO, USA) in a volume of 50 μl into the plantar surface of the right hind paw (ipsilateral side) of mice under anesthesia with sodium pentobarbital (70 mg/kg, i.p.).[12] Control mice were given saline into the plantar surface of the right hind paw.

To assess the sensitivity to thermal stimulation, mice were tested using a 51±0.5℃ warm-plate (Muromachi

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Kikai Co., Ltd., Tokyo, Japan) as the nociceptive stimulus, and the latency to either tapping or licking of the right hind paw or an attempt to escape by jumping was taken as the endpoint. To prevent tissue damage, mice that showed no response within 60 s (cut-off time) were removed from the warm-plate and assigned a score of 60 s.

To investigate the changes in general emotional behaviors, mice were tested using an automatic hole-board apparatus (model ST-1; Muromachi Kikai). The apparatus (model ST-1; Muromachi Kikai) consisted of a gray wooden box (50×50×50 cm) with four equidistant holes 3 cm in diameter in the floor.[13] An infrared beam sensor was installed on the wall to detect the number and duration of head-dipping behaviors. The distances mice travelled in the hole-board were recorded by an overhead digital video camera; the heads of the mice were painted yellow and the digital video camera followed their center of gravity. Data from the digital video camera were collected through a custom-designed interface (DV-Track; Muromachi Kikai) as a reflection signal. Head-dipping behaviors were double-checked via both an infrared beam sensor and the overhead digital video camera. Thus, head-dipping behavior was counted only when both the head intercepted the infrared beam and the head was detected at the hole by the digital video camera. All of the data were analyzed and stored in a personal computer using analytical software (Comp ACT HBS; Muromachi Kikai).

The data are presented as the mean with SEM. Statistical analyses were performed using one-way ANOVA with the Bonferroni/Dunnett multiple comparison test.

In the present study, we investigated the association between chronic pain and emotionality under two different protocols, as follows. 2-week pain model: Mice were either exposed to repeated restraint stress for 60 min/day by being inserted into a syringe (50 ml) (stressed group) or left in their home cage (non-stressed group) for 14 days beginning the day after they were injected with CFA (DAY0). To assess the sensitivity to thermal stimulation, mice were tested using a 51±0.5℃ warm-plate before and 1, 7, and 14 days after CFA injection. Immediately after the final exposure to restraint stress, the emotionality of mice was estimated using the automatic hole-board apparatus. In addition, to investigate the effect of exposure to acute restraint stress on emotionality, mice were exposed to a single restraint stress for 60 min or left in their home cage at 1 day after CFA injection. Immediately after exposure to restraint stress, the emotionality of mice was estimated by the hole-board test. 4-week pain model: Mice were exposed to repeated restraint stress or left in their home cage for 60 min/day for 14 days beginning 15 days after CFA injection. To assess the sensitivity to thermal stimulation, mice were tested using a 51±0.5℃ warm-plate before and 1, 7, 14, 21, and 28 days after CFA injection. Immediately after the final exposure to restraint stress, the emotionality of mice was estimated using the automatic hole-board apparatus. In addition, to investigate the effect of exposure to acute restraint stress on emotionality, mice were exposed to a single restraint stress for 60 min or left in their home cage at 15 days after CFA injection. Immediately after exposure to restraint stress, the emotionality of mice was estimated by the hole-board test.

The group names of mice in this paper are abbreviated as follows; Saline-treated and non-stressed group: Sal-NS, Saline-treated and stressed group: Sal-S, CFA-treated and non-stressed group: CFA-NS, CFA-treated and stressed group: CFA-S.

RESULTS

In the 2-week pain model, unilateral intraplantar injection of a CFA solution into the mouse hind paw caused a significant decrease in the latency of paw withdrawal on the 51±0.5℃ warm-plate in mice regardless of exposure to restraint stress (Fig. 1A; Day7: $p<0.001$ Sal-NS vs. CFA-NS, $p<0.001$ Sal-NS vs. CFA-S, Day14: $p<0.05$ Sal-NS vs. CFA-NS, $p<0.01$ Sal-NS vs. CFA-S). Similarly, in the 4-week pain model, the latency of paw withdrawal was decreased in CFA-treated mice regardless of exposure to stress (Fig. 1B; Day28: $p<0.05$ Sal-NS vs. CFA-NS, $p<0.01$ Sal-NS vs. CFA-S). In these conditions, we investigated the association between chronic pain and the ability to adapt to stress.

In the 2-week pain model, a single exposure to restraint stress for 60 min produced a decrease in the number and duration of head-dip in mice that had been injected with saline or CFA into the plantar surface of the right hind paw in the hole-board test (Fig. 2A; $p<0.001$ Sal-NS vs. Sal-S, $p<0.01$ CFA-NS vs. CFA-S, Fig. 3B; $p<0.001$ Sal-NS vs. Sal-S, $p<0.01$ CFA-NS vs. CFA-S), and the decreased number and duration of head-dip were not observed in mice exposed by repeated restraint stress for 60 min/day for 14 days (Fig. 2C, D). In the 4-week pain model, a single exposure to restraint stress produced a decrease in the number and duration of head-dip in mice that had been injected with saline or CFA (Fig. 3A; $p<0.001$ Sal-NS vs. Sal-S, $p<0.001$ CFA-NS vs. CFA-S, Fig. 3B; $p<0.01$ Sal-NS vs. Sal-S, $p<0.001$ CFA-NS vs. CFA-S). In addition, the decreased number and duration of head-dip of saline-treated mice disappeared by exposure to repeated restraint stress for 14 days (Fig. 3C, D). On the other hand, in the mice that were injected with CFA a decrease in the number and duration of head-dip were observed (Fig. 3C; $p<0.01$ CFA-NS vs. CFA-S, Fig. 3D; $p<0.01$ CFA-NS vs. CFA-S). In addition, the injection of CFA induced an increase in the number and duration of head-dip in non stress-mice at day 15 (Fig. 3A; $p<0.05$ Sal-NS vs. CFA-NS, Fig. 3B; $p<0.01$ Sal-NS vs. CFA-NS).

DISCUSSION

Selye, who pioneered research on the biological effects of exposure to stress stimuli, noticed that the body adapts to external stressors in terms of a biological pattern that is actually predictable, so that the internal

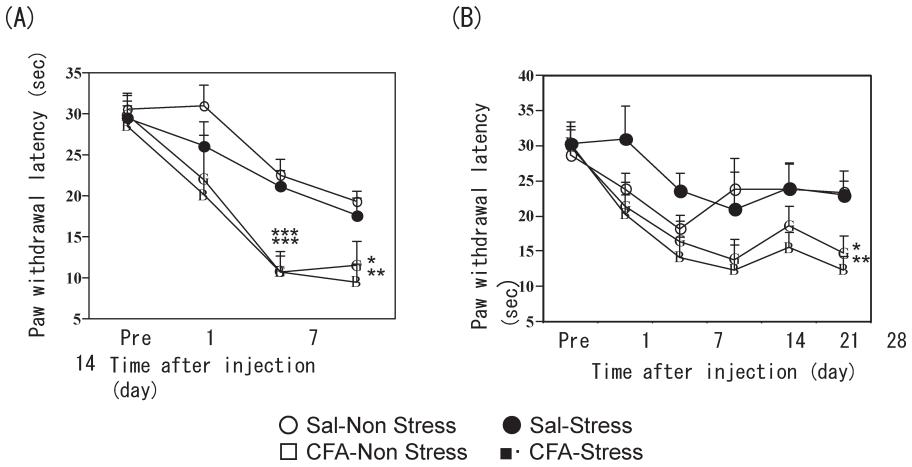

Fig. 1 Time-course changes in the escape response on a warm-plate induced by CFA injection. Mice were placed on a 51±0.5℃ warm-plate and the latency to intense tapping or licking of the hindpaw or jumping was measured. The cut-off time for the warm-plate test was set at 60 s. Mice were tested on days 1–14 (A) or 1–28 (B) after CFA injection. Each point represents the mean with SEM of 8–10 mice. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. SAL-Non stress.

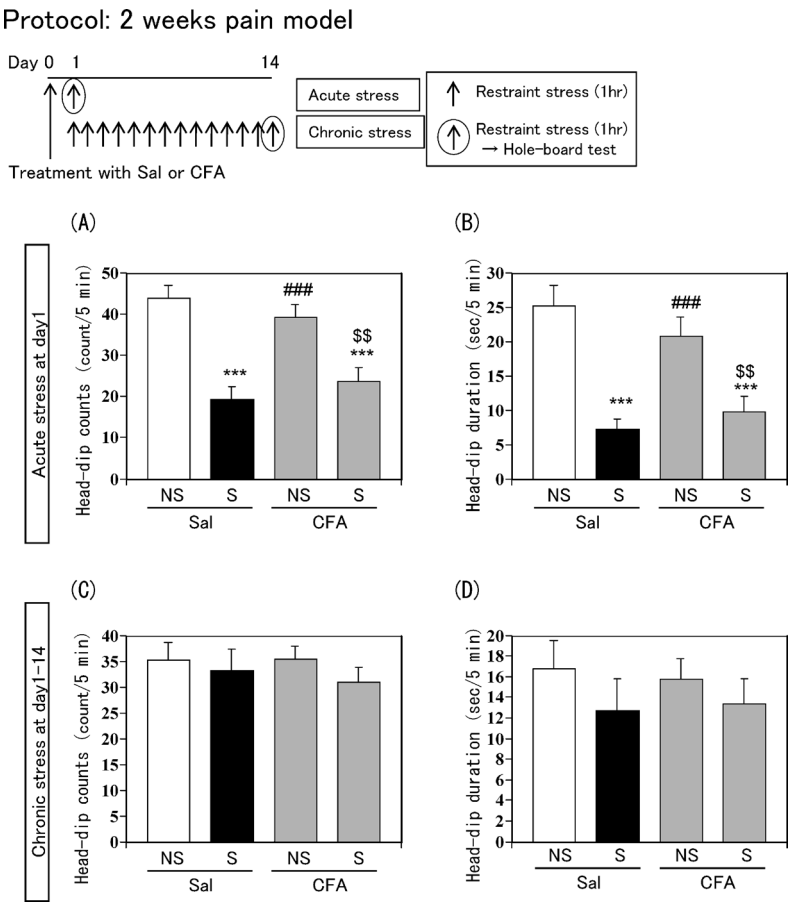

Fig. 2 Effect of chronic pain (14 days) on the behavioral responses of mice to restraint stress in the hole-board test. The acute exposure to restraint stress (60 min) reduced the head-dip counts (A) and duration (B) on the hole-board in control mice and CFA-treated mice. On the other hand, chronic exposure to restraint stress (60 min/day) for 14 days failed to change the head-dip counts (C) or duration (D) on the hole-board in control mice and CFA-treated mice. Each column represents the mean with SEM of 8–10 mice. $^{***}p<0.001$ vs. SAL-Non Stress, $^{###}p<0.001$ vs. SAL-Stress, $^{\$\$}p<0.01$ vs. CFA-Non Stress.

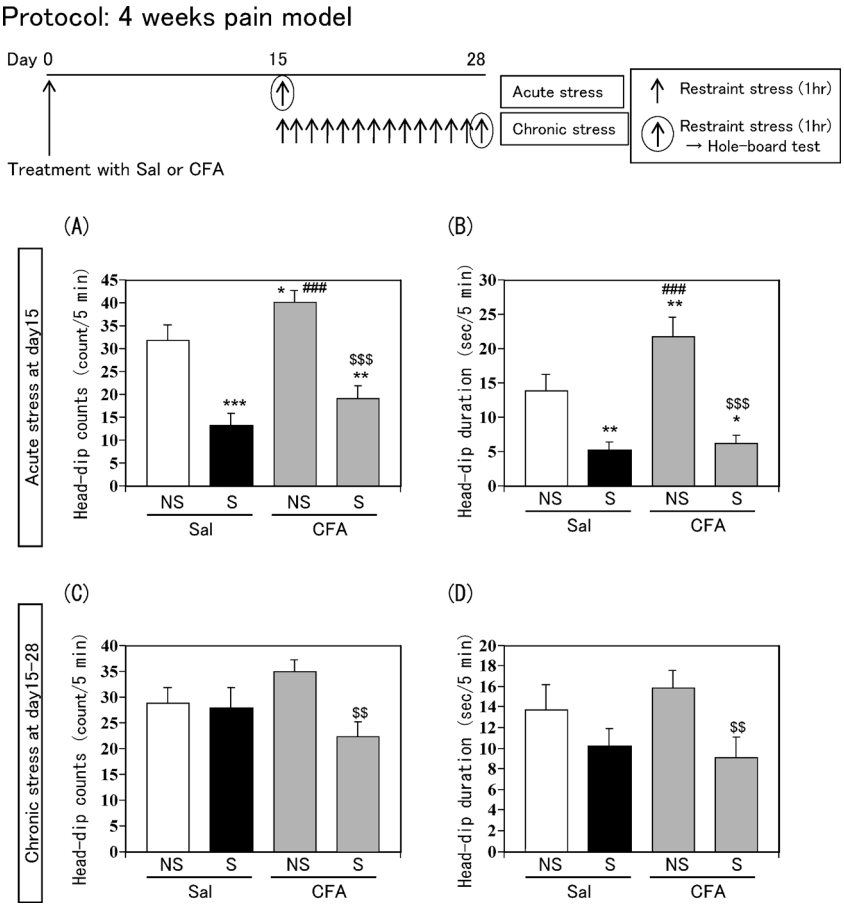

Fig. 3 Effect of chronic pain (28 days) on the behavioral responses of mice to restraint stress in the hole-board test. The acute exposure to restraint stress (60 min) decreased the head-dip counts (A) and duration (B) on the hole-board in control mice and CFA-teated mice. Chronic exposure to restraint stress (60 min/day) for 14 days failed to change the head-dip counts (C) or duration (D) on the hole-board in control mice. On the other hand, significant decreses in the head-dip counts (C) and duration (D) were observed in CFA-tretaed mice. Each column represents the mean with SEM of 8–10 mice. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. SAL-Non Stress, $^{\#\#\#}p<0.001$ vs. SAL-Stress, $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$ vs. CFA-Non Stress.

balance, or homeostasis, is restored and maintained.[14, 15] The ability to adapt to stress is an important defensive function of a living body, and disturbance of this adaptability to stress may be related, at least in part, to the pathophysiology of stress-related psychiatric disorders. As mentioned above, a relationship between abnormal emotionality, including adjustment disorder, and pain has been proposed. Some functional changes in brain regions related to emotionality must occur in association with chronic pain. However, little is known about whether chronic pain is the cause of stress maladaptation. Therefore, in the present study, we investigated the association between chronic pain and the ability to adapt to stress.

The present study demonstrated that a single exposure to restraint stress for 60 min produced a decrease in head-dipping behavior in naive mice, which had not been exposed to chronic pain, in the hole-board test, and these acute emotional responses were recovered by exposure to repeated restraint stress for 60 min/day for 14 days. These findings are in good agreement with previous reports in rats[7–10] and confirm the development of adaptation to stress. However, mice that were subjected to chronic pain for 4 weeks continued to show a decrease in head-dipping behavior in the hole-board test after chronic exposure to restraint stress for 60 min/day for 14 days. The present study indicates that the injection of CFA induced an increase in the number and duration of head-dip in non stress-mice at day 15, as shown in Fig. 3 A and B. On the other hand, there are no changes in mice under the similar condition, as shown in Fig. 2 C and D. In the previous study, we reported that the typical benzodiazepine anxiolytics diazepam and chlordiazepoxide dose-dependently increased the number and duration of head-dip on the hole-board.[13] Therefore, an increase in head-dipping behavior in the hole-board test may reflect the anxiolytic state of animals. On the other hand, although it has been accepted that the stimulation of 5-HT_{1A} receptor has anxiolytic effect, we reported that 5-HT_{1A} receptor agonists induced a reduction in head-dipping behavior.[16] In addition, Yamada et al. reported

that although lysophosphatidic acid significantly reduced the percentage of time spent in the open arms in an elevated plus maze test, the head-dipping behavior in hole-board test was increased.[17] Taken together, it is difficult to assert that a change in head-dipping behavior in the hole-board test clearly shows the anxiety state. Although additional detailed research is needed, present data indicate the possibility that chronic pain affects the anxiety state. Some research indicates that chronic pain could induce anxiety-like behavior in rodents.[18–20] Other studies failed to find behavioral changes by the chronic pain model.[21] Therefore, experiments that use rodent models to investigate behavior changes evoked by chronic pain have somewhat inconsistent results in terms of the anxiety state.

The key finding of the present study is that mice that were subjected to chronic pain for 4 weeks continued to show a decrease in head-dipping behavior in the hole-board test after chronic exposure to restraint stress. If stress adaptation is developed by chronic stress, it should be observed that the same degree of head-dipping behavior as non-stressed mice and the impaired recovery of the decrease in head-dipping behavior may have resulted from the possibility that chronic pain attenuated the ability to adapt to stress. Therefore, these results suggest that the discontinuation of chronic pain induces stress maladaptation in mice.

Previous studies in stress-adaptive and -maladaptive animals have provided evidence that an increase in brain serotonin (5-HT) signaling may be a key factor in the adaptation to repeated exposure to stress.[8, 9, 22] We also found that 5-HT_{1A} receptor agonists can produce emotional resistance to stress stimuli in mice,[16, 23, 24] suggesting that this receptor type may play a siginificant role in the mechanisms of stress adaptation. This hypothesis is supported by our recent finding that a decrease in the emotional behavior of stress-maladaptive mice was improved by chronic treatment with 5-HT_{1A} receptor agonist.[11] On the other hand, it has been reported that chronic pain induces functional changes in 5-HT receptor in the brain of mice.[25] Those studies also reported that serotonergic antidepressants are effective for treating anxiety induced by chronic pain. Although additional detailed research is needed to elucidate the mechanisms, it is possible that changes in brain 5-HT function induced by chronic pain might disrupt the ability to adapt to stress.

In summary, our findings indicate that chronic inflammatory pain impairs the ability to adapt to stress. In addition, the animal model developed in the present study may be useful for clarifying the mechanisms of the relationship between chronic pain and adjustment disorder, and also for establishing new therapeutic strategies for treating emotional disorders under chronic pain.

Conflict of Interest

The authors have no conflict of interest to disclose with respect to this report.

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