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Acid-induced hyperalgesia and anxio-depressive comorbidity in rats

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HIGHLIGHTS

- Repetitive intramuscular injections of pH 4 saline cause chronic widespread hyperalgesia.
- Comorbid anxiety-like behavior exists in the acid-induced pain model.
- Comorbid depressive-like behavior exists in the acid-induced pain model.

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ABSTRACT

Fibromyalgia is a prevalent disorder characterized by chronic widespread pain (CWP) and complex comorbid symptoms. A CWP model is developed through repeated unilateral intramuscular injections of acid saline resulting in bilateral mechanical hyperalgesia in rats. The present study aims to evaluate whether both anxious and depressive comorbidities exist in this acid-induced pain model, similarly to patients with CWP syndromes. The anxiety-like behaviors were evaluated using the open field and elevated plus maze tests, and depression-like behaviors were measured by the forced swimming, sucrose consumption, and sucrose preference tests. The pain group receiving acidic saline displayed significantly lower paw withdrawal thresholds for 4 weeks than animals in the vehicle group after repetitive intramuscular injections. The pain group showed a significantly shorter duration of exploring the central zone of the open field and the open arms of the elevated plus maze compared to the vehicle group. The pain group had a significantly lower preference for and consumption of the hedonic sucrose. Moreover, rats with chronic pain showed significantly longer immobility than the vehicle group in the forced swimming test. The results indicate that psychiatric behaviors are exacerbated in the CWP model. This study provides evidence for the validity of the acid-induced pain model analogous to patients with CWP syndromes.

1. Introduction

Fibromyalgia is a chronic widespread pain (CWP) syndrome of unknown etiology characterized by widespread and long-lasting musculoskeletal pain. It affects 2% of the adult population in the United States [1]. CWP syndromes are associated with significant disability and medical costs. A considerable portion of fibromyalgia patients present with symptoms of mood disorders, particularly for anxiety and depression [2,3]. The prevalence of anxiety and depression in fibromyalgia patients are 21–64% and 30–80%, respectively [4–7]. Numerous studies also indicate that mood disorders aggravate pain syndromes and vice versa [2,8]. Chronic pain and affective disorders lead to a vicious cycle. Unfortunately, the current treatment for CWP syndromes is still unsatisfactory because of a poor understanding of the mechanisms underlying persistent pain pathways [9,10]. The available animal models play an important role in elucidating the mechanisms of the development of CWP syndromes [10,11].

An animal model with chronic muscle hyperalgesia has been developed using repeated acid injections to the gastrocnemius muscle at two- to five-day intervals to produce a long-lasting, bilateral, mechanical but not thermal hyperalgesia without motor deficits or tissue damage [12]. Morphine and pregabalin are effective to ameliorate hyperalgesia of this CWP model [13,14]. Previous studies have partially validated the acid-induced muscle pain as similar to CWP syndromes. Anxio-depressive comorbidity exists in a considerable portion of fibromyalgia patients [4–7]. Despair occurs in a CWP-like model using an amine depletion drug, reserpine [15]. However, it is unknown whether anxiety and depressive comorbidity co-occurs in the acid-induced CWP model. The present study aimed to assess the phenomena of mechanical hyperalgesia and affective behaviors after repetitive acid injections to

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validate the similarity between this acid-induced pain model and humans with CWP syndromes.

2. Materials and methods

Male Sprague–Dawley rats (10–12 weeks) were kept in a sound-attenuated room (lights on at 06:00–18:00) with food and water provided *ad libitum*. The rats were randomly assigned into a group receiving the vehicle (pH 7.2) or acidic saline (pH 4.0). The Institutional Animal Care and Use Committee of National Cheng Kung University reviewed and approved the experimental procedures. All experiments complied with the guidelines for the ethical use of animals of the US National Institutes of Health.

2.1. Induction of muscle pain

The method used to induce muscle-mediated chronic pain was performed as previously described [12]. All rats were briefly anesthetized with vaporized isoflurane (3% to 5%). After the skin covering was shaved, the left gastrocnemius muscle was injected with 100 μl neutral saline (pH 7.2) in the control group or 100 μl acidic saline (pH 4.0) in the experimental pain group on days 1 and 6. The pH of the acidic saline was adjusted with an MES acidic solution to pH 4.0 ± 0.1.

2.2. Von Frey filament testing

The rats were placed in a Lucite cubicle on an elevated metal grid allowing for the stimulation of the plantar surface of the paw. The grid hole diameter was 3 mm, and the center distance between two consecutive holes was 5 mm. The rats were placed on the platform to adapt to the apparatus for 5–10 min prior to the experimental measurement. Von Frey filaments of varying bending forces (2, 4, 6, 8, 10, 15, and 26 g) were applied to the plantar surface of the paw to assess the withdrawal response. A "response" to the stimuli was defined as an abrupt lifting of the foot upon application of the von Frey filaments. Each trial contained five von Frey stimulations, with an inter-stimulus interval of 5–6 min to reduce possible stimulus habituation. The von Frey testing forces were performed in an ascending sequence. The paw withdrawal threshold was defined as the lowest force that elicited ≥3 withdrawals in five consecutive stimulations.

The von Frey nylon hairs were calibrated both prior to and throughout the duration of the study to ensure that consistent bending forces were routinely applied. The withdrawal threshold of the ipsilateral left hindpaw was measured first, followed by the contralateral right hindpaw. The following times were tested (Fig. 1): before injection 1 (D1), 24 h after injection 1 (D2), before injection 2 (D6), 24 h after injection 2 (D7), and weekly after injection 2 over a four-week testing period (D13, D20, D27, and D34).

2.3. Body weight assessment

Body weight was assessed using a commercial scale at particular time points similar to those described for von Frey filament testing (Fig. 1).

2.4. Behavioral tests

Five behavioral tests were used to evaluate the anxiety- and depression-like behaviors of the rats [16,17]: open field, elevated plus maze, forced swimming, sucrose consumption, and sucrose preference. The open field and elevated plus maze tests were used to reveal possible signs of psychomotor disturbances that were characteristic of anxiety in an open environment. The forced swimming test was used to assess the duration of immobility, which was the experimental analog of a depressed mood; i.e., despair. The sucrose consumption test was a measure of the 'hedonic' state of an animal, or the ability to experience pleasure. Its impairment (anhedonia or decreased sensitivity to reward) was a fundamental feature of clinical depression. The sucrose preference test was performed to measure the hedonistic state of the experimental animals.

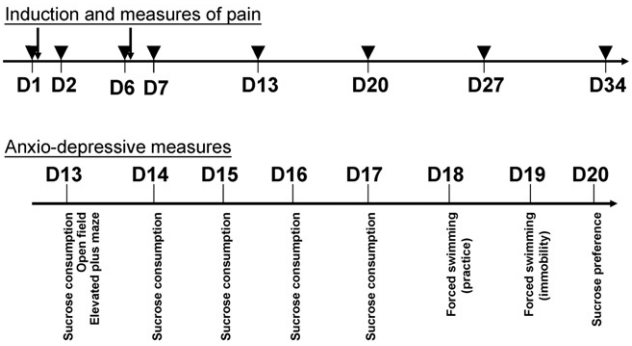

Fig. 1. Experimental procedure of the induction of chronic pain (upper panel) and anxio-depressive behavioral assessment (lower panel). The body weight and paw withdrawal threshold (▼) were measured at particular times (D1, D2, D6, D7, D13, D20, D27, D34). Vehicle (pH 7.2) or acidic (pH 4.0) saline was injected at D1 and D6 (arrows). Measures of anxiety- and depression-like behaviors were performed in particular times. The sucrose consumption test was performed for 5 days (D13–D17). The open field and elevated plus maze tests were performed at D13. Immobility of the forced swimming test was measured at D19. The sucrose preference test was performed at D20. In the first experiment, anxio-depressive behavioral measures contained the sucrose consumption, open field, elevated plus maze, and forced swimming tests. The second experiment used the sucrose preference test.

2.4.1. Elevated plus maze test

The elevated plus maze consisted of black polypropylene plastic that was elevated 68 cm above the floor. Each maze arm extended 45 cm from the junction area, which measured 13 × 13 cm. The open and closed arms were 13 cm wide, and the closed maze arms had walls extending 25 cm from the junction area. During testing, each rat was placed in the central square facing an enclosed arm and allowed to explore the maze for 10 min. The frequency of entering the open arms, the duration of time spent in the open arms, and the total movement in the elevated plus maze were analyzed. Arm entries were defined as the placement of all four paws within an arm of an elevated maze. Either the low frequency of entering the open arms or the short duration spent in the open arms is considered a validation of anxiety [18].

2.4.2. Open field test

The open field test box was composed of black acrylic plastic that formed a 99 × 99 cm square with a wall height of 45 cm. The box was divided into nine equal squares measuring 33 × 33 cm. Each rat was placed in the center zone of an open field at the beginning and allowed to explore the maze for 10 min. The frequency of crossing the central zone, the duration in the center area, and the total movement in the open field were analyzed. A low frequency of crossing the central zone or a short duration of time spent within the central zone is considered a validation of anxiety [19].

2.4.3. Sucrose consumption test

In the sucrose consumption test, each rat was placed in a test cage identical to its home cage. Consumption of a 20% sucrose solution was recorded for 15 min. The sucrose intake was measured by the volume consumed at the end of the test. Prior to each testing, the rats were not deprived of food or water. In this study, the fluid intake over 5 days was analyzed in the two groups of rats. Decreased sucrose intake, i.e., anhedonia, is a validated index of a depression-like state in animals [16,20].

2.4.4. Forced swimming test

The forced swimming test apparatus was a plastic cylinder (47 cm height, 38 cm inside diameter) containing 38 cm of water at 25 ± 1 °C. The forced swimming test consisted of two phases. In the initial

15-min habituation session of the first day, which was excluded from the data analysis, the rats were individually forced to swim in a plastic cylinder. After a period of vigorous swimming, all of the rats reduced their movements to only those necessary to maintain their head above the water level, with no other displacement. The 5-min test sessions began 24 h later. The duration of immobilization was measured. After the test, the rats were removed and dried with a towel before being returned to their home cages. Increased immobility in the forced swimming test is indicative of depression-like behavior [21].

2.4.5. Sucrose preference test

In the sucrose preference test, animals were exposed to both the test solution (20% sucrose) and drinking water for a period of 1 h following 23 h of food and water deprivation. Preference (%) for sucrose over water (calculated as [sucrose intake / total fluid intake] × 100%) or sucrose consumption are the most reliable hedonic indexes in rats [16].

2.5. Experimental procedure

All rats were placed in the recording room 1 week prior to the experiment for adaptation. The rats were placed in a Lucite cubicle on an elevated metal grid allowing stimulation of the plantar surface of the hindpaw for at least 20 min to allow them to become acclimatized to the test environment. This was performed for 3 days before the von Frey filament testing. Two intramuscular injections of pH 7.2 or 4.0 saline were performed at D1 and D6. The body weight and paw withdrawal threshold were measured at particular time points (Fig. 1). Time points of experiments were before the 1st injection (D1), 24 h after the 1st injection (D2), before the 2nd injection (D6), 24 h after the 2nd injection (D7), and weekly after the 2nd injection for 4 weeks (D13, D20, D27, and D34), respectively. All behavioral evaluations were performed at 14:00–17:00 to minimize the circadian influences.

Two experiments were performed in the present study. In the first experiment, the sucrose consumption, open field, and elevated plus maze tests was designed originally. In the progress of the first experiment, the forced swimming test was suggested to evaluate despair characteristic of the depression-like behavior. Thus, the forced swimming test was added at the late phase of the experiment. Because the forced swimming test is a stressful measure [16], it was performed after the other behavioral measures to reduce possible interference. A detailed flowchart of the first experiment is depicted in Fig. 1. The sucrose consumption, open field, and elevated plus maze tests were performed one week after the 2nd intramuscular injection (D13) in sequence. One day later, the sucrose consumption test was performed daily for 4 days (D14–D17). Subsequently, the forced swimming test was conducted. In the forced swimming test, a 15-min habituation was performed on the first day (D18). A 5-min test was carried out the next day (D19) to measure the duration of immobilization.

After the completion of the first experiment, the sucrose preference test was recommended to ascertain the anhedonic response. The present study carried out the sucrose preference test at D20 in the two groups as the second experiment.

2.6. Statistical analysis

Temporal changes of the paw withdrawal thresholds in all groups were assessed by Friedman repeated measures ANOVA on rank, if appropriate, followed by Dunnett's test. The paw withdrawal thresholds and the frequency used in the elevated plus maze and open field tests between the two groups were compared using the Mann–Whitney rank sum test. The body weight and sucrose intake in the sucrose consumption test were analyzed using two-way repeated measures ANOVA with one factor repetition, if appropriate, followed by post hoc Fisher's least-significant difference (LSD) test. The duration indexes used in the elevated plus maze, open field, and forced swimming tests as well as the preference and consumption in the sucrose preference test were analyzed by Student's *t*-tests. Cohen's effect size for the four behavioral tests was calculated. All statistical analyses were carried out using SigmaPlot statistics. Statistical significance was set at $p < 0.05$.

3. Results

In the first experiment, 106 rats (vehicle $n = 50$, pain $n = 56$) were used. Six rats receiving intramuscular injections of acidic saline failed to exhibit mechanical hyperalgesia. Rats with hyperalgesia ($n = 50$) were used for behavioral assessment. In the second experiment, rats (vehicle $n = 10$, pain $n = 10$) were submitted to the sucrose preference test at D20. In the present study, a hyperalgesia induction rate was 90.9% in the group receiving pH 4.0 saline.

3.1. Paw withdrawal threshold

The changes in the withdrawal thresholds of the bilateral hindpaws of the rats in response to various von Frey filaments are shown in Fig. 2. The paw withdrawal threshold of the vehicle group was very consistent

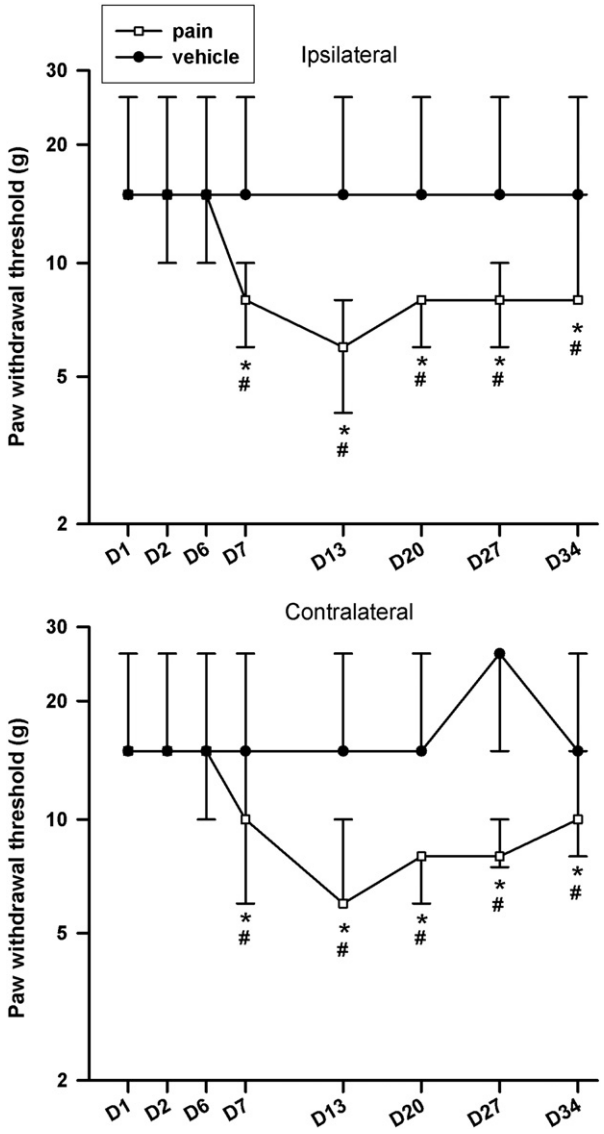

Fig. 2. Changes in bilateral paw withdrawal thresholds of rats that received pH 7.2 (vehicle group) or pH 4.0 (pain group) saline at the eight time points. $^{*}p < 0.05$ vs. vehicle group; $\#p < 0.05$ vs. baseline (D1). Data are presented as the median with the 25th and 75th percentile.

in the bilateral hindlimbs. The threshold was primarily at 15 g throughout the measured period (Fig. 2). The vehicle group showed no difference in the paw withdrawal threshold test. On the other hand, the paw withdrawal threshold of the rats receiving acidic saline had the same level (15 g) as that of the vehicle group before the 2nd injection of acid saline. The paw withdrawal thresholds of the bilateral hindlimbs were significantly reduced to 6 to 8 g (primarily at 8 g) after the 2nd injection of acid saline. This hyperalgesic response lasted for 4 weeks.

Intramuscular administration of acidic saline produced long-lasting and significant decreases in the paw withdrawal threshold (ipsilateral, χ^2 (7) = 250.9, $p < 0.001$; contralateral, χ^2 (7) = 239.3, $p < 0.001$). A significant decrease in the paw withdrawal threshold occurred at D7, D13, D20, D27, and D34. There was also a significant difference between the two rat groups at the same time points.

3.2. Body weight

The body weight progressively increased from 370 to 430 g over time in the two rat groups ($F(7,684) = 149.629$, $p < 0.001$, power = 1.0). The body weights did not statistically differ in the two groups.

3.3. Elevated plus maze test

Rats with chronic pain ($n = 50$) showed significantly shorter durations of staying in the open arms ($t(98) = 2.355$, $p = 0.021$, power = 0.554) and lower frequencies of entering the open arms ($T = 2909.5$, $p = 0.007$) of an elevated plus maze compared to the vehicle group ($n = 50$) (Fig. 3A,B). The effect sizes of the duration of time spent in an open arm and the frequency of entering an open arm in the elevated plus maze test were 0.471 and 0.652, respectively. Approximately 60% of the pain group showed a remarkably lower duration and frequency of entering the open arms than the vehicle group (Table 1). The total movement during the elevated plus maze test was not significantly different in the two groups (vehicle, 20.1 ± 1.7 m; acid, 19.3 ± 1.5 m; $t(98) = 0.348$, $p = 0.73$).

Table 1
The rat number in the pain group beyond the range (mean ± 99% confidence interval) of the 5 behavioral tests of the vehicle group.

Behavior	Increase	Decrease
Elevated plus maze test ($n = 50$)		
OA—duration	2 (4%)	29 (58%)
OA—frequency	3 (6%)	31 (62%)
Open field test ($n = 50$)		
CZ—duration	2 (4%)	28 (56%)
CZ—frequency	9 (18%)	28 (56%)
Sucrose consumption test ($n = 36$)		
Sucrose intake (D13)	13 (36%)	15 (42%)
Sucrose intake (D14)	10 (28%)	17 (47%)
Sucrose intake (D15)	8 (22%)	22 (61%)
Sucrose intake (D16)	9 (25%)	17 (47%)
Sucrose intake (D17)	7 (19%)	23 (64%)
Sucrose preference test ($n = 10$)		
Preference	0 (0%)	6 (60%)
Sucrose intake	0 (0%)	8 (80%)
Forced swimming test ($n = 37$)		
Immobility	15 (41%)	5 (14%)

Abbreviations: OA, open arm; CZ, central zone.

3.4. Open field test

Rats with chronic pain ($n = 50$) showed significantly shorter durations of staying in the central zone ($t(98) = 2.083$, $p = 0.04$, power = 0.426) of an open field than the vehicle group ($n = 50$) (Fig. 3C,D). The effect sizes of the duration staying at the center zone and the frequency entering the center area in the open field test were 0.417 and 0.321, respectively. Of the pain group, 56% showed a remarkably lower duration and frequency of entering the center zone

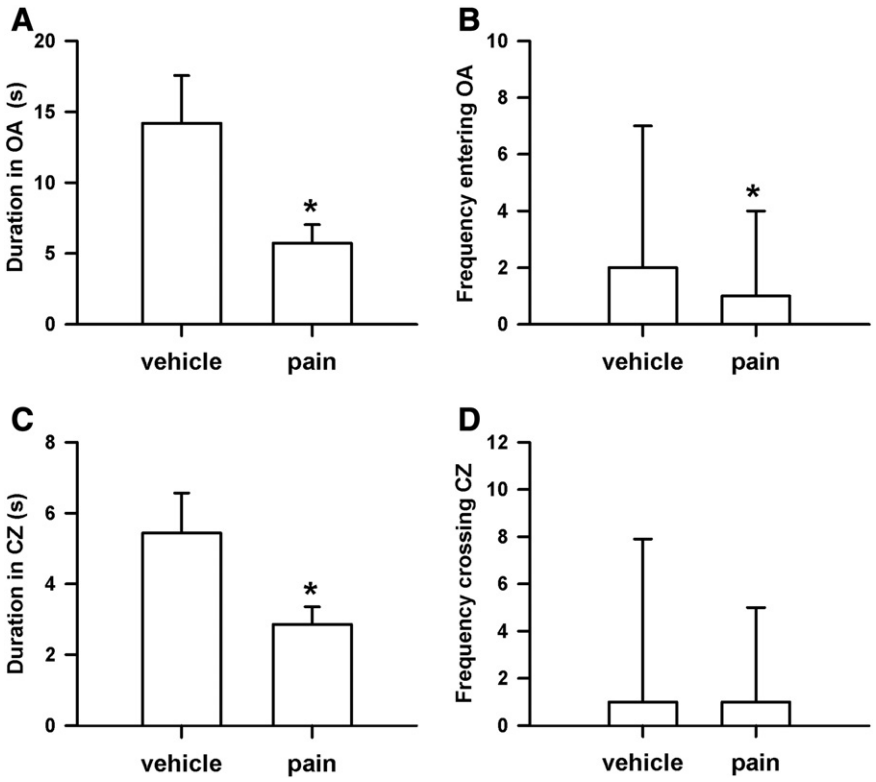

Fig. 3. (A) The duration of time spent in the open arms (OAs) and (B) the frequency of entering OAs of an elevated plus maze were compared between the pain and vehicle groups. (C) The duration of staying in the central zone (CZ) and (D) the frequency of crossing the CZ of an open field were compared between the pain and vehicle groups. The data are presented as the mean ± standard error for the duration in the OAs or CZ and the median + 90th percentile for the frequency of entering the OAs or CZ. *$p < 0.05$.

compared to the vehicle group (Table 1). The total movement during the open field test was not significantly different in the two groups (vehicle, 36.9 ± 2.3 m; acid, 35.7 ± 2.2 m; $t(98) = 0.398$, $p = 0.69$).

3.5. Sucrose consumption test

In the present study, 36 rats with chronic pain and 38 control rats were analyzed because some rats only had a record of the first-day sucrose intakes. No behavioral impediment was observed in either group. The fluid intake over 5 days progressively increased as time passed, but with different trends in the two groups (Fig. 4A). Significant differences were found in the factors of time ($F(4,288) = 91.819$, $p < 0.001$, power = 1.0) and the interaction between treatment and time ($F(4,288) = 6.132$, $p < 0.001$, power = 0.973) during the sucrose consumption test. The pain group showed significantly lower fluid consumption on D15 ($p = 0.041$) and D17 ($p = 0.006$) compared to the vehicle group. The effect sizes of the sucrose consumption test from the first to the 5th days were 0.107, 0.081, 0.493, 0.275, and 0.611, respectively. More than 60% of the pain group showed remarkably lower sucrose intake on D15 and D17 compared to the vehicle group (Table 1).

3.6. Forced swimming test

In the first experiment, the forced swimming test was performed in the chronic pain group ($n = 37$) and control group ($n = 37$). Immobility during the forced swimming test showed a significant difference ($t(72) = 2.23$, $p = 0.029$, power = 0.493) in the two groups (Fig. 4B). The effect size of immobility in the forced swimming test was 0.519. Of the pain group, 41% showed longer immobility compared to the vehicle group (Table 1). No behavioral impediment was observed in either group during the 2-day test.

3.7. Sucrose preference test

The pain group (43.7 ± 7.2%) showed a significantly lower preference for sucrose compared to the vehicle group (67.3 ± 4.9%) ($t(18) = 2.698$, $p = 0.015$, power = 0.662). The pain group (5.9 ± 0.8 ml) had significantly lower sucrose intake compared to the vehicle group (12.9 ± 1.7 ml) ($t(18) = 3.810$, $p = 0.001$, power = 0.949). The effect sizes of the preference and sucrose intake in the sucrose preference test were 1.207 and 1.704, respectively. More than 60% of the rats with chronic pain showed remarkably lower intake and preference to hedonic sucrose compared to the vehicle group (Table 1).

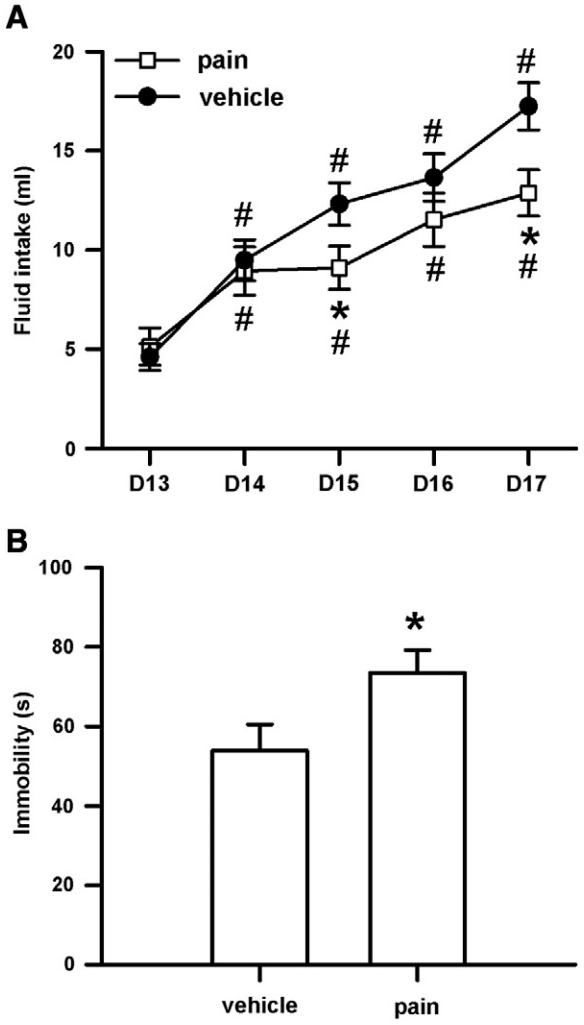

Fig. 4. (A) Changes in fluid consumption during the 5-day sucrose consumption test (D13–D17) in the two groups. The data are presented with the mean ± standard error. *$p < 0.05$ vs. vehicle group; #$p < 0.05$ vs. baseline. (B) Comparison of immobility of the forced swimming test in the two groups. *$p < 0.05$.

4. Discussion

The major findings of this study are as follow: 1) Repeated intramuscular injections of acidic saline elicited bilateral, long-lasting mechanical hyperalgesia. 2) Rats with chronic pain displayed anxiety-like behavior in terms of the elevated plus maze and open field tests. 3) Rats with chronic pain showed anhedonic response (lower sucrose preference and lower sucrose consumption) and despair mood (longer immobility in the forced swimming test).

Rats with chronic pain showed a propensity to develop anxiety- and depression-like behaviors in terms of five anxiety- and depressive-like behavioral tests. A considerable portion of fibromyalgia patients are accompanied by the comorbidity of anxiety (21% to 64%) or depression (30% to 80%) [4–7]. In the present study, anxiety-like behaviors occurred in 56% to 62% while depression-like behaviors occurred in 41% to 80% of the pain group, which shows a similar prevalence to psychiatric comorbidity in fibromyalgia. The body weights of all rat groups were consistently increased with time and were not significantly different in the two groups. Hyperalgesia may not induce a disturbance of appetite. There was no difference in the total movement during the measurement period of the open field and elevated plus maze tests. In the same model, rats that received low-pH saline did not differ from those that received normal saline on the motor assessment of the rota-rod treadmill test [12]. Moreover, there was normal swimming or exploring behavior with no motor impairment during the depressive behavioral tests. These results suggest that the anxio-depressive behaviors are related to the pain status of the animals.

Long-lasting, bilateral, mechanical but not thermal hyperalgesia without motor deficits or tissue damage after two acid injections has been reported [12]. Somatic discomfort and visceral hyperalgesia are observed after repetitive acid treatment [22]. Several drugs, such as pregabalin [14], alosetron [22], and morphine [13], are effective for inhibiting the mechanical hyperalgesia of this CWP model. These data support the face and predictive validities of this CWP model as similar to the phenomena observed in patients with fibromyalgia, such as widespread hyperalgesia with no muscle inflammation or pathology and pharmacological effects [9–11]. Acid-induced hyperalgesia in the bilateral hindlimbs was observed and lasted for 4 weeks in 90.9% of the rats receiving acidic saline, which is similar to reports of previous studies [12,13]. The data here support a long-lasting, widespread hyperalgesia induced by unilateral intramuscular acid injection. The rats with chronic pain had a propensity to present anxio-depressive behaviors. The current study provides additional validation of acid-induced muscle pain as a model of CWP syndromes.

Recently, a CWP-like model was proposed using reserpine for the depletion of biogenic amines [15]. Mechanical hyperalgesia lasts for 2 to 3 weeks, which depends on the reserpine dosage. Despair, assessed by the forced swimming test, occurred in the rats that received reserpine. Pregabalin and duloxetine have a short-term effect on hyperalgesic amelioration. In the acid-induced CWP model, mechanical hyperalgesia lasted for 4 weeks or longer. The depressive characteristics of both anhedonia and despair moods were observed in this CWP model. Moreover, the psychomotor disturbance associated with anxiety was observed in this model. Pregabalin ameliorates acid-induced mechanical hyperalgesia [14]. These convergent data may suggest that dysregulation of biogenic amines is related to the development of acid-induced hyperalgesia and affective comorbidity.

Fibromyalgia has been estimated to be around 2% of population, and it mostly occurs in women rather than man [1,23]. The gender dependent phenomenon may indicate a role for sex hormone in the etiology of fibromyalgia syndrome. However, hormone replacement therapy in post-menopause women does not always improve pain symptoms of fibromyalgia [24]. The role of sex hormones in the hyperalgesia of fibromyalgia patients is limited [25]. Previous studies about the acid-induced muscle pain are assessed in male rats [12–14,22]. The present study showed the acid-induced muscle pain in coincidence with anxio-depressive comorbidity in male rats. The results here are unable to be applied in a generalized population. Although the portion of anxio-depressive comorbidity in the pain group of the present study is quite similar to previous reports [4–7], it is interested in understanding the gender effect on acid-induced pain and affective comorbidity.

Five behavioral tests were performed to evaluate anxio-depressive propensity in the acid-induced pain rats. A large sample size was used to assess the statistical power in this study. Effect size of the elevated plus maze test was higher than that of the open field test. The phenomenon of different sensitivities in the two anxiety-like behavioral tests is similar to previous studies [16,20]. The present study evaluated anhedonic responses by the sucrose preference and sucrose consumption tests and despair by the forced swimming test separately. These depressive behavioral tests are validated [16,21,26]. Anhedonia and despair may involve different neural circuits [17]. A clinic questionnaire assesses many characteristics of depression [4,5], but there is no available information in the reports about the anhedonia or despair specifically. Different aspects of depressive symptoms in fibromyalgia may be important to understand possible networks for pain and depressive comorbidity.

In the current study, inconsistent rat number among the behavioral tests existed in the first experiment. It may produce sequential effect on behavioral results or mechanical hyperalgesic levels. To reduce possible interference, the forced swimming test, a quite stressful test, was performed after the completion of the other behavioral tests. In addition, the immobility in the forced swimming test reflects an actively coping strategy to an inescapable situation [21]. However, the immobility in the forced swimming test may be related to not only “behavioral despair” but also “learned helplessness” [27]. The inescapable shock paradigm has been used to evaluate “learned helplessness” in rats [27]. To further characterize the despair mood in the CWP model, the inescapable shock and antidepressant can be used in the future.

In conclusion, repetitive acid injection into the unilateral hindlimb muscle caused a widespread hyperalgesia. The current study provides evidence about the anxio-depressive propensity occurred in the acid-induced chronic muscle pain model.

Disclosure

The authors have no conflicts of interest to disclose.

Acknowledgments

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