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Depressive-like history alters persistent pain behavior in rats: Opposite contribution of frontal cortex and amygdala implied

Wei-Jing Qi, Wei Wang, Ning Wang, Jin-Yan Wang, and Fei Luo

Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing, China

Abstract

Numerous studies have shown that pain perception is strongly influenced by depression. However, very few studies have examined whether pain perception is altered in the remission period of depression, and what role the fronto-limbic circuits may play in the behavioral changes associated with remission. Using an unpredictable chronic mild stress (UCMS) animal model of depression, the present study investigated pain-related behaviors in rats with prior exposure to a UCMS stimulus. The γ -aminobutyric acid (GABA)_A receptor agonist muscimol was microinjected bilaterally into the basolateral amygdala (BLA) and the medial prefrontal cortex (mPFC) to examine the modulation of pain by these brain regions in the recovery state. Rats with a depression-like history displayed increased ongoing pain behavior in the formalin test, although their thermal pain thresholds were unchanged. Intra-BLA muscimol during the recovery phase dramatically decreased formalin-induced pain behavior and also significantly increased rats' sucrose preference. By contrast, in the mPFC, muscimol produced the opposite effect, suggesting different, perhaps opposing, roles of the BLA and mPFC in mediating the influence of prior UCMS exposure on pain perception. Taken together, these results demonstrated that a depressive experience may cause long-term alterations in limbic circuit excitability and thus lead to long-lasting changes in pain perception.

Keywords

amygdala; depression; medial prefrontal cortex; pain

The comorbidity of depression and pain is a common phenomenon in clinical settings (Leo, 2005). Their association may be related to their common neuroanatomical pathways and neurobiological substrates (Adler & Gattaz, 1993; Katona et al., 2005). Individuals with depression have previously been shown to show enhanced spontaneous pain (Campbell, Clauw, & Keefe, 2003; Wilson, Mikail, D'Eon, & Minns, 2001) and reduced evoked pain (Bär et al., 2005, 2006; Lautenbacher & Krieg, 1994). It has also been previously observed, with both an unpredictable chronic mild stress (UCMS) animal model (Shi, Qi, Gao, Wang, & Luo, 2010; Shi, Wang, & Luo, 2010) and an olfactory bulbectomy model (Wang, Qi, Xu,

Wang, & Luo, 2010), that rats with depressive-like behaviors expressed more pain behaviors to formalin injection but less sensitivity to noxious thermal and mechanical stimuli than did healthy controls. The differential influence of depression-like behavior on formalin-induced ongoing pain versus thermal-stimulus-evoked pain might be related to the differing emotional involvement between the two or to changes in the motivational processes related to depression (Apkarian, Hashmi, & Baliki, 2011; Lautenbacher, Sternal, Schreiber, & Krieg, 1999; Willner, 1995).

Although a large number of studies have shown a strong association between pain and the presence of a depressed state, few experiments have examined pain perception in currently healthy subjects with a history of depression. There is evidence that depression history can increase the risk for many physical and mental disorders. For example, the chances of developing Alzheimer's disease are increased in persons who have been diagnosed with depression (Speck et al., 1995), and persons with a lifetime history of depression have expressed higher interpersonal sensitivity than those without depression (Sakado et al., 1999). Importantly, Zautra et al. (2007) found that rheumatoid arthritis patients with a history of two or more episodes of major depression reported more spontaneous pain. Hence, given that pain is a multidimensional phenomenon that is composed of both sensory and affective components, a history of depression could have a long-lasting influence on subsequent pain perception.

The brain areas involved in the formation and development of depression overlap with the "pain matrix," a dynamic network of cortical and subcortical brain regions (including the prefrontal cortex and amygdala) activated by painful stimulation (Derbyshire, 2000; Drevets et al., 1992; Neugebauer, Galhardo, Maione, & Mackey, 2009; Sheline et al., 2009; Somborski & Bingel, 2010). Moreover, patients suffering from depression or chronic pain have been shown to exhibit dysfunction of the medial prefrontal cortex (mPFC) and amygdala (Ji et al., 2010; Neugebauer et al., 2009; Siegle, Thompson, Carter, Steinhauer, & Thase, 2007). Amygdala hypermetabolism (Drevets, 1999) and/or mPFC deactivation (Drevets, 2000a) have been observed in patients with major depressive disorder, and an amygdala-mPFC imbalance is thought to mediate the sensory and affective abnormalities caused by depression. Despite the ample literature on regional brain functions in depression, there have been few studies that have examined functional changes in the amygdala and mPFC after an episode of depression.

Therefore, the present study aimed to explore the influence of a depressive-like history on subsequent pain perception in evoked and persistent pain paradigms, as well as the roles of the basolateral amygdala (BLA) and the mPFC in mediating such behavioral changes. A UCMS model was employed to produce depressive-like behavior in rats. In the recovery phase, when depressive-like behaviors had disappeared, we measured acute thermal thresholds (evoked pain) and the time spent paw licking in the formalin test (persistent pain). The effects of inhibiting BLA and mPFC activity through intracortical microinjection of the γ -aminobutyric acid (GABA)_A receptor agonist, muscimol, on these behavioral measures were assessed.

Materials and methods

Animals

One hundred and twenty male Wistar rats (purchased from the Academy of Military Medical Science, Beijing, China) weighing 160–180 g at the beginning of the experiments were used in this study. The rats were housed individually and given food and water ad libitum. The colony was maintained at 22 ± 2 °C with a standard 12-hr light-dark cycle (lights on at 7:00 a.m.). The animals were allowed to habituate to the environment for 1 week before being subjected to the behavioral experiments, and were handled daily by the experimenter. Testing was performed during the light cycle. Adequate measures were taken to minimize pain or discomfort. Experiments were carried out in accordance with the *Guide for the care and use of laboratory animals* (National Institutes of Health, 1996). The research protocol was approved by the institutional review board of the authors' academic institute.

UCMS procedure

The rats in the UCMS groups were exposed to 6 weeks of unpredictable chronic mild stressors. The schedule of stress-or administration is shown in Table 1. The stress stimuli were unpredictable in their nature, duration, and frequency. Stress procedures included, in a pseudorandom order: paired housing, cold room, hot room, novel odor, overnight illumination, group housing, soiled bedding, strobe light, cage tilt, restricted food access, empty water bottle, water deprivation, and food deprivation (Shi, Wang, & Luo, 2010).

Sucrose preference tests were executed before UCMS testing (baseline) and weekly during the UCMS testing period. At the beginning of the experiment, the animals were first trained to drink a 1% sucrose solution by exposing them to sucrose solution instead of water for 48 hr. The rats then received a series of sucrose preference tests, preceded by 22 hr of food and water deprivation. The sucrose preference tests were conducted every Thursday (2:00–3:00 p.m.) during the experimental period (weeks 0–10), when each animal was supplied simultaneously with two bottles, one containing sucrose solution (1%) and the other containing only water. Each testing session lasted approximately 1 hr (drinking water or sucrose solution). The preference for sucrose consumption was calculated according to the following formula: Percentage sucrose preference = (sucrose solution consumption/[sucrose solution consumption + water consumption]) $\times$ 100.

Pain tests

The apparatus and testing procedure for thermally evoked pain and hyperalgesia were the same as described by Wang et al. (2010). The animals were placed in a Plexiglas chamber on a glass floor, beneath which a radiant heat apparatus (100-W projector lamp) was situated. A beam of light was shone through a hole (4 mm in diameter) and focused on the plantar surface of the left hind paw. Paw withdrawal latency (PWL) was defined as the length of time between the light onset and the paw lift. The intensity of light was adjusted to produce a baseline PWL of approximately 10 s, with a maximum cutoff time of 22 s to prevent tissue damage. Four trials were performed with an intertrial interval of 5 min or less. The latencies recorded for the last three trials were averaged to obtain a mean latency that was taken as the threshold of the thermally evoked pain.

Thermal hyperalgesia was measured using a chronic inflammatory pain model in which 100 μ l of complete Freund's adjuvant (CFA) solution was subcutaneously injected into the rat's left hind paw. The thermal thresholds were assessed before (baseline) and 1, 3, 7, 14, 21, and 28 days after CFA injection.

The formalin pain test was conducted in a quiet room. The room was maintained at a steady temperature in the range of 21–23 °C. The animals were placed in a plastic test chamber (25 $\times$ 25 $\times$ 30 cm) for at least 30 min to allow them to acclimatize to the environment prior to testing. The rats were then manually restrained and given a subcutaneous injection of 50 μ l of 5% formalin into the plantar surface of the left hind paw. Nociceptive behaviors were recorded for 60 min following the injection using a PNT-902N digital CCD camera that was mounted on the lab floor below the transparent chamber floor, and recorded via an 18–28 DVR Board V4 with video recording software, HY-R2008. The pain intensity was determined by measuring the amount of time the rats spent licking the injected paw during each 5-min observation period after injection. The formalin-induced nociceptive behaviors were scored by a rater who was blind to the treatment.

Surgery and intracortical microinjection

Cannula implantation was performed 1 week before the UCMS/control procedure. The animals were deeply anesthetized with sodium pentobarbital (50 mg/kg ip). Supplementary doses of anesthetics were given when necessary. Thirty-gauge bilateral stainless steel infusion guide cannulae were stereotactically implanted into the BLA (2.8 mm posterior to Bregma, 4.5 mm lateral to the midline, and 8.5 mm below the skull surface) or the mPFC (3.2 mm anterior to Bregma, 0.8 mm lateral to the midline, and 3.5 mm below the skull surface). The guide cannulae were fixed to the skull with three stainless steel screws and dental cement. Each cannula was kept patent with a sterile obturator until the time of drug administration. After receiving penicillin (16,000 U per rat im), the animals were given 1 week to recover from the surgery.

The GABA_A receptor agonist, muscimol (0.5 μ g dissolved in 0.5 μ l of normal saline), was bilaterally microinfused into the BLA or the mPFC (0.25 μ l for each side). The control group animals received the same volume of saline. The infusion volume and drug concentration (1 μ g/ μ l) used in this study were chosen according to those used in previous studies (Edeline, Hars, Hennevin, & Cotillon, 2002; Holt & Maren, 1999; Wilensky, Schafe, & LeDoux, 2000).

The injection needle (a thin dental needle with a 0.3-mm outer diameter) was introduced through the guide cannula such that its terminal protruded 1 mm below the guide cannula. The displacement of an air bubble inside a polyethylene catheter (PE-10) connecting the syringe needle to the intracerebral needle was followed to monitor the microinjection. The solutions were slowly microinjected into the BLA or the mPFC over a 1-min period. Then the needle was held in place for another 1 min to maximize diffusion away from the tip of the needle.

Sucrose preference and formalin tests were carried out 1 hr after the microinjection (for details see the experimental design). Previous studies have suggested that it usually takes

approximately 30 min for muscimol to become effective and that the effects persist for up to 6 hr after microinjection (Ghazanfar, Krupa, & Nicolelis, 2001; Jia, Xie, Xiao, & Tang, 2004; Monconduit, Lopez-Avila, Molat, Chalus, & Villanueva, 2006).

Experimental design

This study consisted of two stages. In the first stage (Experiment 1), we investigated the influence of a pre-existing history of depression-like phenotype on animals' pain behavior (evoked vs. persistent pain). In the second stage (Experiment 2), we explored the neuropharmacological mechanisms underlying the effects of muscimol on evoked and persistent pain. The experimental protocol is illustrated in Figure 1.

Experiment 1—Thirty-two rats were randomly divided between the UCMS group ($n = 16$) and the control group ($n = 16$). The two groups were kept in separate rooms in order to allow independent manipulation of their environments. The rats in the UCMS group were subjected to 6 weeks of mild, unpredictable stressors, while there were no manipulations for the control group. Four weeks after the UCMS/control exposure, when the sucrose intake of the UCMS group returned to the control level, both groups were further divided into two subgroups for delivery of radiant heat stimulation (acute thermal-evoked pain and hyperalgesia, $n = 8$) or formalin injection (persistent pain, $n = 8$), respectively. The body weights and sucrose preferences of all rats were measured weekly during the experimental period (weeks 0–10).

Experiment 2—The GABA_A agonist, muscimol, was microinjected into the BLA (Experiment 2a, $n = 45$) or the mPFC (Experiment 2b, $n = 43$). For both experiments, the rats were randomly divided into the UCMS and control groups. The UCMS procedure and tests were the same as those used in Experiment 1. The microinjections were performed at the 11th experimental week. The UCMS and control groups were further divided into the following subgroups for intracranial microinjections of muscimol (Mu) or normal saline (NS), respectively: UCMS/Mu group ($n = 13/11$ for 2a/2b respectively), UCMS/NS group ($n = 12$), Control/Mu group ($n = 12$), and Control/NS group ($n = 8$). For all groups, the sucrose preference test and formalin test were conducted on two different days (the first and the fourth day of week 11, respectively). Muscimol/saline was microinjected 1 hr before the behavioral tests.

Histology

The locations of the guide cannulae were verified histologically after the behavioral testing was complete. The animals were deeply anesthetized intraperitoneally with pentobarbital sodium (60 mg/kg) and perfused transcardially with 0.9% saline followed by 4% formalin in phosphate buffer (PB; pH 7.4). The brains were removed and sunk in 20%, then 30% sucrose dissolved in 0.1 M PB. Frozen coronal sections (30 μ m) were cut with a cryostat and mounted on gelatin-coated glass slides. The slides were stained with hematoxylin and eosin and then viewed under a light microscope to determine the location of each infusion needle track in the BLA or mPFC, based on the stereotaxic atlas of Paxinos and Watson (2007). Rats with cannula placements that were not in the target areas were excluded from the analysis.

Statistical analysis

Statistica 5.1 and GraphPad prism 5.0 were used to analyze the data and to produce the graphs, respectively. Body weight gain was calculated as the percentage increase week over week. Data involving two factors were analyzed using multifactor ANOVAs and Duncan's test was employed as a post hoc test. Sucrose preference in the recovery phase was analyzed using one-way ANOVAs followed by Tukey's post hoc test. Student's *t*-test was used for pair-wise group comparisons. The data are presented as means $\pm$ standard errors of the mean. The statistical significance level was set at $p < .05$.

Results

Induction of and recovery from depressive-like behaviors

As shown in Figure 2A, the rats subjected to UCMS exhibited a significant reduction in sucrose consumption after 2 weeks of chronic stress and the reduced consumption was maintained throughout the remainder of the 6-week-long UCMS procedure. The UCMS-exposed rats showed partial recovery 1 week later (seventh experimental week), and recovered to the control level by 2 weeks post-UCMS (eighth experimental week), indicating that the stress-induced depressive-like behaviors had subsided: A two-way ANOVA showed a Group $\times$ Time interaction, $F(10, 300) = 4.34, p < .001$; a group effect, $F(1, 30) = 36.60, p < .001$; and a time effect, $F(10, 300) = 7.18, p < .001$.

Stressed rats had a significantly lower mean body weight than control rats from the end of the second experimental week through the end of the experiment: A two-way ANOVA found a Group $\times$ Time interaction, $F(10, 300) = 40.27, p < .001$; a group effect, $F(1, 30) = 146.68, p < .001$; and a time effect, $F(10, 300) = 833.23, p < .001$ (Figure 2B). Notably, weight gain analysis revealed that, relative to the control group, the UCMS rats lost more weight during weeks 1, 3, and 5, during stress exposure, and then gained more weight following the UCMS treatment period in experimental weeks 8 and 9: A two-way ANOVA showed a Group $\times$ Time interaction, $F(9, 270) = 8.66, p < .001$; a group effect, $F(1, 30) = 50.02, p < .001$; and a time effect, $F(9, 270) = 153.74, p < .001$ (Figure 2C). Thus the rats showed a gradual recovery after exposure to the environmental stressor ceased.

Changes in nociceptive behaviors after chronic stress

Acute thermal pain tests were performed at weeks 0 (baseline), 6 (depression phase), and 11 (recovery phase). As shown in Figure 3A, UCMS exposure produced significant increases in PWL (*t*-test, 14.15 ± 0.36 s vs. 10.00 ± 0.49 s, $p < .001$), suggesting a lower pain sensitivity in the stress-exposed rats compared with controls. With the recovery from depressive-like behaviors, the rats in the UCMS group showed the same level of pain threshold as the control group (*t*-test, 10.42 ± 0.43 s vs. 10.45 ± 0.40 s, $p = .96$), suggesting that the prior chronic stress experience did not influence the subsequent pain sensitivity of rats to acute thermal stimulation. Thermally evoked hyperalgesia assessment in CFA-injected rats showed results consistent with these acute thermal pain results. PWLs and paw thickness were examined at days 1, 3, 7, 14, 21, and 28 after CFA injection. Thermal thresholds (Figure 3B1) and paw swelling (Figure 3B2) did not differ between rats with a prior depression experience versus rats without a prior depression-like experience.

In the formalin test, a typical biphasic pattern of licking behavior was observed following subcutaneous injection of formalin into the hind paw (Figure 3C1). Compared with controls, the rats with prior exposure to UCMS showed a significant augmentation of licking behavior throughout the 1-hr observation period: A two-way ANOVA showed a Group $\times$ Time interaction, $F(11, 154) = 1.98, p < .05$; a group effect, $F(1, 14) = 15.84, p < .01$; and a time effect, $F(11, 154) = 13.59, p < .001$. The cumulative licking time increased in previously stressed rats during Phase I (0–5 min, t -test, 56.06 ± 2.79 s vs. 41.96 ± 4.20 s, $p < .05$), the interphase period (5–15 min, 22.77 ± 2.77 s vs. 13.98 ± 2.01 s, $p < .05$), and Phase II (15–60 min, 407.6 ± 36.67 s vs. 211.1 ± 35.91 s, $p < .01$; Figure 3C2). These results indicated that previous exposure to chronic stress may have led to an amplification of subsequent persistent pain, even after the depressive-like behavior had disappeared.

Neuropharmacological mechanisms underlying the modulation of persistent pain behaviors by depressive-like experience

We further explored the mechanisms by which the experience of depression affected pain perception by examining the effects of intra-BLA or mPFC microinjection of the GABA_A agonist, muscimol, on sucrose consumption and formalin-induced pain behavior (Figures 4 and 5, and Tables 2 and 3). As illustrated in Figure 4A, GABA_A receptor agonism in the BLA robustly increased sucrose preference in the UCMS/Mu subgroup after recovery from depressive-like behavioral impairments, as confirmed by a one-way ANOVA, $F(3, 44) = 3.24, p < .05$, and a post hoc test, $88.66 \pm 4.25\%$ for UCMS/NS versus $95.08 \pm 5.54\%$ for UCMS/Mu, $p < .05$. Meanwhile, microinjection of muscimol into the BLA dramatically reduced the rats' responses to formalin pain (UCMS/Mu group; Figure 4B and Table 2), as represented by decreased paw-licking time compared with saline-injected, stressed rats (UCMS/NS group): A two-way ANOVA showed a Group $\times$ Time interaction, $F(33, 451) = 2.57, p < .001$; a group effect, $F(3, 41) = 11.93, p < .001$; and a time effect, $F(11, 451) = 28.75, p < .001$. A detailed post hoc comparison between the subgroups across time points is presented in Table 3. These results suggest that inhibition of BLA can suppress the augmented pain perception normally observed in rats that have been subjected to chronic moderate stress.

Interestingly, bilateral microinjection of muscimol into the mPFC produced effects that were opposite to those observed with intra-BLA muscimol, that is, a decrease in sucrose consumption and an increase in the nociceptive response in the formalin test. UCMS/Mu animals given intra-mPFC muscimol exhibited a weaker sucrose preference, as shown by a one-way ANOVA, $F(3, 42) = 4.69, p < .01$, and a post hoc test, $91.85 \pm 8.71\%$ for UCMS/NS versus $77.37 \pm 17.65\%$ for UCMS/Mu, $p < .01$ (Figure 5A). The UCMS/Mu rats also exhibited stronger paw licking responses than the rats in the UCMS/NS group, particularly at the 5-, 25-, 35-, and 40-min post-formalin injection time points (Figure 5B and Tables 2 and 3): A two-way ANOVA found a Group $\times$ Time interaction, $F(33, 429) = 2.35, p < .001$; a group effect, $F(3, 39) = 20.71, p < .001$; and a time effect, $F(11, 429) = 42.73, p < .001$. These findings suggested that the mPFC and the BLA may play opposing roles in the modulation of depressive-like and pain behaviors following long-term pre-exposure to stress in rats.

Histologically verified placement of cannulae

A schematic of coronal rat brain sections showing the sites of cannula placement is presented in Figure 6. One rat was excluded due to improper cannula placement. All rats included in our data analysis had histologically verified placement of cannulae in the BLA or the mPFC.

Discussion

In the present study, we investigated the effects of past depressive-like experience on subsequent pain-related behaviors in rats and the neuropharmacological mechanisms underlying these effects. The results showed that a depressive-like history led to increased persistent pain behavior in the formalin test, but no changes in thermal pain thresholds, compared with animals with no depressive-like experience. GABAergic agonism in the BLA and the mPFC produced opposite effects (alleviation vs. aggravation, respectively) on depressive-like behavior (sucrose consumption) and formalin-induced persistent pain, demonstrating distinct roles of the BLA and the mPFC in mediating the influence of prior depressive-like history on pain perception. These findings may shed light on our understanding of the reciprocal relations between depression and pain.

Differential modulation of persistent versus stimulus-evoked pain by a history of depression-like behavior

There is substantial literature concerning the complicated relations between depressive disorders and pain. The occurrence of pain complaints in patients suffering from major depression has been shown to be significantly higher than in the general population (Corruble & Guelfi, 2000). Animal studies have also provided evidence that rats with depressive-like behaviors exhibit amplified pain responses to intraplantar formalin injection (Shi, Qi et al., 2010; Wang et al., 2010). However, there is a paucity of research related to changes in pain perception after a history of depression. The present study extended prior research with respect to elucidating the connection between depression and pain in the remitted state of depression. We found that rats with a history of depression-like behavior showed more intense responses in the formalin pain test than controls that were never depressive.

According to their “scar hypothesis of depression,” Lewinsohn, Steinmetz, Larson, and Franklin (1981) proposed that depression may leave an enduring residual deficit that makes one more sensitive to future affective disturbances in a manner analogous to the way a cut or incision leaves a physical scar (Rohde, Lewinsohn, & Seeley, 1990). Individuals who have been depressed in the past may acquire such a “scar” during or after a depressive episode that predisposes them to suffer from mood disorders in the future. Indeed, as formalin-induced licking is considered to be a reflection of mostly emotional reactions to pain (Nakagawa et al., 2003; Vaccarino, Clemmons, Mader, & Magnusson, 1997), it can be inferred that the residual effects of depression can intensify these behaviors by influencing the affective dimension of pain. Tennen, Affleck, and Zautra (2006) and Zautra et al. (2007) found that rheumatoid arthritis patients with multiple episodes of depression consistently had greater self-reported pain as well as physically evaluated pain than those with either one

episode or no depression history, suggesting an enduring scar-like vulnerability to pain in the former. In addition, the memory of patients with emotional problems can be selectively biased toward the recall of negative events (Edwards, Pearce, Collett, & Pugh, 1992). Thus, it is possible that remembering past unpleasant experiences (e.g., stress exposure) may augment suffering from pain in the present.

In contrast to the enhanced pain behavior induced by formalin injection, the thermally evoked pain thresholds in rats with depressive-like history were not different from those of normal rats. One possible explanation for this dissociation is that stimulus-evoked pain and persistent pain may be processed by different brain mechanisms (Apkarian et al., 2011; Buffington, Hanlon, & McKeown, 2005). The processing of formalin-induced pain may involve more supraspinal modulation of nociceptive transmission than stimulus-evoked pain. The latter, being a nociceptive pain that was processed primarily by the spinal ascending pathway, is associated with activation of A δ and C fibers that result in a transient, well-localized pain that does not require long-term changes in spinal nociceptive neurons (Woolf & Costigan, 1999). Even in the presence of CFA hyperalgesia, the thermally evoked flexor reflex may not involve a supraspinal mechanism, in that both spinal transection and inactivation of the rostral ventromedial medulla (RVM) do not prevent primary thermal hyperalgesia after CFA (Urban & Gebhart, 1999). In formalin-induced pain, however, the biphasic behaviors are believed to be mediated by direct chemical activation of nociceptors in the early phase and ongoing nociceptor activity coupled with central sensitization in the late phase (Shibata, Ohkubo, Takahashi, & Inoki, 1989; Vaccarino & Melzack, 1992). The finding that inactivation of the hypothalamus, reticular nuclei, or cingulum bundle by lidocaine produced analgesia in the formalin test but not in the foot-flick test strongly supported the involvement of supraspinal sites in formalin-induced pain (Tasker, Choinière, Libman, & Melzack, 1987; Vaccarino & Melzack, 1989). According to Vaccarino and Melzack (1989), the responses in the formalin test represented an integration of motivational and affective behaviors so as to protect the injured area. Therefore, unlike stimulus-induced pain that is brief and avoidable, the formalin-induced pain has a long duration and is unavoidable, and may involve more emotional responses. As mentioned above, the emergence of behavioral and neurophysiological alterations caused by bouts of depression may be triggered by subsequent negative experiences, such that they become evident with elevated stress (Rohde et al., 1990). Thus, such alterations may affect behavior under unavoidable, chronic stress conditions, but not as a result of exposure to brief, avoidable stressors such as stimulus-evoked pain.

Mechanisms underlying the effects of depression history on persistent pain behavior

An imbalance between the amygdala and mPFC interactions could underlie, at least in part, the comorbidity of depression and pain (Drevets, Price, & Furey, 2008; Ji et al., 2010; Neugebauer et al., 2009; Price & Drevets, 2010). A growing body of evidence documents a close structural and functional association between the amygdala and the mPFC (Ji et al., 2010; Likhtik, Pelletier, Paz, & Paré, 2005; Pape & Pare, 2010; Quirk, Likhtik, Pelletier, & Paré, 2003; Rosenkranz & Grace, 2001). In the present study, we explored the functional modulation of formalin-induced pain behaviors by both of these brain areas during the period of recovery from depressive-like behavior in rats and found that inhibition of

activation within the BLA and mPFC had opposite (inhibitory vs. facilitatory) effects on the pain responses.

Dysregulation of emotional processes is one of the core clinical features of major depressive disorder (Goodman, New, Triebwasser, Collins, & Siever, 2010; Gotlib & Joormann, 2010). A number of studies have demonstrated the involvement of emotional brain circuits, including the fronto-temporo-limbic brain areas, in the pathophysiology of depression (Davidson, Pizzagalli, Nitschke, & Putnam, 2002; Mayberg, 1997; Pizzagalli et al., 2004). Drevets and colleagues (Drevets et al., 1992; 1997) have reported amygdala hyperactivity and mPFC hypoactivity in depressed patients. Meanwhile, changes in fronto-limbic activity have been described in both clinical and experimentally induced pain conditions (Apkarian et al., 2011; Ji et al., 2010). For example, in an animal model of arthritis pain, Ji et al. (2010) found hyperactivity in the amygdala accompanied by hypoactivity in the mPFC. Given that the brain regions involved in depression and pain processing are highly overlapping (Giesecke et al., 2005; Rome & Rome, 2000), there may be reciprocal reinforcing effects between depressive symptoms and pain perception through their common substrates, particularly in the limbic system (Duric & McCarson, 2006).

Previous researchers have reported that depressed individuals tend to feel more pain than their nondepressed peers (Campbell et al., 2003; Shi, Wang, & Luo, 2010). In the present study, the formalin-induced pain in stress-exposed rats was significantly more intense than that in control rats not exposed to stress, despite the disappearance of overt depressive symptoms (i.e., restoration of sucrose consumption to baseline levels). This enduring difference in pain response might be due to a depression-associated abnormality in amygdala-mPFC activity that persists even after recovery from a depressive episode. We explored this possibility using a GABAergic agonist to selectively inhibit the BLA or the mPFC, and found that both brain areas maintained a modulatory influence on depressive-like behavior (sucrose consumption) and response to formalin pain in the recovery state. Given that inhibition of the BLA or the mPFC did not affect formalin pain in our control animals, as seen in previous studies (Gao, Ren, Zhang, & Zhao, 2004; Rea, Roche, & Finn, 2011; Wang, Ren, Zhang, & Zhao, 2005), it is reasonable to deduce that the behavioral changes observed in our post-stress-exposure animals reflected functional changes in the BLA and the mPFC that had resulted from their previous depressive-like experience. Furthermore, the opposite effects of inhibiting the BLA versus the mPFC on sucrose preference and formalin pain responses observed in this study are consistent with the established roles of these brain areas during an ongoing depressive episode (Drevets, 1999; Drevets et al., 1997, 2002). The present findings extend prior research suggesting that a prior depressive experience can induce long-term alterations in the excitability of limbic circuits, resulting in enduring functional abnormalities in the amygdala-mPFC circuits (Drevets, 2000b; Drevets et al., 1992, 2002; Holthoff et al., 2004). These long-lasting functional changes in brain circuits may render one more susceptible to stressful events, elevate the risk of relapse, and lead to more severe problems in the future.

Conclusion

In conclusion, the main finding of this study was that, compared with never-depressed control subjects, rats with a prior depressive-like experience exhibited a facilitated response to formalin-induced persistent pain while in the recovery phase. Our findings further suggest that this effect on pain perception may be mediated by enduring functional abnormalities in the amygdala-mPFC circuits.

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Figure 1.

Experimental protocol. All rats were subjected to 6 weeks of unpredictable chronic mild stress (UCMS)/control procedures and 4 weeks of recovery. Two experiments were carried out. In Experiment 1, the effects of depression-like history on thermally evoked pain (using both acute and chronic pain models) versus persistent pain (formalin test) were examined. In Experiment 2, the influence of basolateral amygdala (BLA)/medial prefrontal cortex (mPFC) activity on depressive-like behavior and formalin-induced pain in the recovery period of depression was investigated. CFA = complete Freund's adjuvant; F = formalin test; Mu = muscimol; NS = saline; RH = radiant heat test; SPT = sucrose preference test.

Figure 2.

Induction of and recovery from depressive-like behaviors. Sucrose consumption and body weights were measured weekly before, during, and after the unpredictable chronic mild stress (UCMS)/control procedures. (A) Sucrose preference test. Two weeks following chronic stress exposure, there was a significant reduction in sucrose consumption that was maintained throughout the 6-week-long UCMS procedure. Stressed rats showed a gradual increase in sucrose consumption through the seventh experimental week, which recovered to normal levels by the end of the eighth experimental week, indicating that the UCMS-exposed rats had recovered from depressive-like behavior. (B) Body weight. A significant decrease in mean body weight was observed in the UCMS group relative to the control group over the 10-week experiment. (C) Body weight gain. Compared with control rats,

stressed rats lost more weight during the UCMS period (weeks 1–6) and gained more weight after (weeks 7–8). Data are presented as means $\pm$ SEM. $n = 16$. The baselines refer to the sucrose preference tests before the UCMS treatment. The gray rectangles mark the period of UCMS exposure, and the dotted frames mark the recovery period from depressive-like behavior. * $p < .05$. ** $p < .01$. *** $p < .001$.

Figure 3.

Changes in nociceptive behaviors after unpredictable chronic mild stress (UCMS) exposure. (A) Acute thermal pain test. The UCMS group exhibited significantly higher paw withdrawal latencies (PWLs) during the UCMS exposure than did the control group. No difference was observed between the UCMS and the control groups at baseline (i.e., before the UCMS procedure) and in the recovery phases. (B1–B2) Complete Freund's adjuvant (CFA)-induced thermal hyperalgesia. Pain thresholds and paw swelling (examined at days 1, 3, 7, 14, 21, and 28 after CFA inoculation) were similar between CFA-injected UCMS rats and CFA-injected nonstressed rats. (C1–C2) The persistent pain induced by formalin injection. Rats previously subjected to UCMS exhibited significantly increased licking behavior in Phase I (0–5 min) and Phase II (15–60 min) as well as in the interphase (5–15 min) following formalin injection. Data are presented as means $\pm$ SEM. $n = 8$. * $p < .05$. ** $p < .01$. *** $p < .001$.

Figure 4.

Effects of muscimol (Mu) microinjection into bilateral basolateral amygdala (BLA). (A) In rats previously exposed to unpredictable chronic mild stress (UCMS), intra-BLA microinjections of muscimol (UCMS/Mu), but not saline (UCMS/NS), increased sucrose consumption. Baseline refers to the sucrose consumption measured at the end of week 10. (B) Formalin pain behavior. Microinjection of muscimol into the BLA dramatically decreased the formalin-induced pain responses in UCMS rats (UCMS/Mu) compared with the UCMS/NS group. Data are presented as mean $\pm$ SEM. $n = 8-13$ per group. $*p < .05$ versus UCMS/NS group. $**p < .01$ versus UCMS/NS group. $***p < .001$ vs. UCMS/NS group. $\#p < .05$ versus control/NS group. $###p < .001$ versus control/NS group.

Figure 5.

Effects of muscimol (Mu) microinjection into the bilateral medial prefrontal cortex (mPFC). (A) Sucrose preference test. In rats previously exposed to unpredictable chronic mild stress (UCMS), intra-mPFC microinjections of Mu (UCMS/Mu), but not saline (UCMS/NS), decreased sucrose consumption. Baseline refers to the sucrose consumption measured at the end of week 10. (B) Formalin pain behavior. The UCMS/Mu group showed a significant increase in the formalin-induced behavior compared with the UCMS/NS group. Data are presented as mean $\pm$ SEM. $n = 8-12$. $*p < .05$ versus UCMS/NS group. $**p < .01$ versus UCMS/NS group. $***p < .001$ versus UCMS/NS group. $\#p < .05$ versus control/NS group. $##p < .01$ versus control/NS group. $###p < .001$ versus control/NS group.

Figure 6.

Schematic drawings of the injection sites. Cannula placements within the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) shown in coronal sections modified from the atlas of Paxinos and Watson (2007) for mPFC and BLA. The injection sites are marked by solid black circles.

Table 1**Unpredictable Chronic Mild Stress Procedure**

Type of stress	Schedule
Food deprivation	Monday 9:00 a.m. to Tuesday 5:00 p.m.; Wednesday 4:00 p.m. to Thursday 2:00 p.m.
Restricted access to food	Tuesday 5:00–8:00 p.m.
Hot room	Monday 9:00–9:30 a.m.; Friday 4:00–4:30 p.m.
Soiled bedding	Thursday 5:00 p.m. to Friday 9:00 a.m.; Sunday 5:00 p.m. to Monday 9:00 a.m.
Group housing	Monday 5:00 p.m. to Tuesday 9:00 a.m.
Overnight illumination	Monday 5:00 p.m. to Tuesday 9:00 a.m.; Saturday 5:00 p.m. to Sunday 9:00 a.m.
Strobe light	Tuesday 9:00 a.m. to 5:00 p.m.; Friday 9:00 a.m. to 4:00 p.m.
Cold room	Wednesday 10:00–10:30 a.m.; Sunday 10:00–10:30 a.m.
Cage tilt	Wednesday 5:00 p.m. to Thursday 9:00 a.m.; Saturday 9:00 a.m. to 5:00 p.m.
Novel odor	Wednesday 5:00 p.m. to Thursday 9:00 a.m.; Saturday 5:00 p.m. to Sunday 9:00 a.m.
Paired housing	Friday 5:00 p.m. to Saturday 9:00 a.m.
Water deprivation	Friday 5:00 p.m. to Sunday 9:00 a.m.; Wednesday 4:00 p.m. to Thursday 2:00 p.m.
Empty water bottle	Sunday 9:00–10:00 a.m.

Table 2

ANOVAs for Formalin Pain Behaviors following Intracerebral Microinjections

Effect	<i>df</i>	<i>MS</i>	<i>F</i>-value	<i>p</i>-value
Basolateral amygdala microinjection				
Treatment	3	15642	11.93	<.0001
Time	11	7049.02	28.75	<.0001
Treatment × Time	33	631.01	2.57	<.0001
Medial prefrontal cortex microinjection				
Treatment	3	24700.38	20.71	<.0001
Time	11	13263.22	42.73	<.0001
Treatment × Time	33	729	2.35	<.0001

Table 3

Post Hoc Inter-Group Comparisons of Formalin Pain Behaviors

Comparison (<i>p</i> -values)	Phase I	Phase II (min)							
		20	25	30	35	40	45	50	
Basolateral amygdala microinjection									
UCMS/Mu vs. UCMS/NS	<.001	<.001	<.001	<.001	<.001	.005	.001	.03	
UCMS/Mu vs. Control/Mu	<.001	<i>ns</i>	.05	<i>ns</i>	.03	.04	.02	<i>ns</i>	
UCMS/NS vs. Control/NS	<.001	<i>ns</i>	.04	<i>ns</i>	.05	<i>ns</i>	<i>ns</i>	<i>ns</i>	
Control/Mu vs. Control/NS	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	
Medial prefrontal cortex microinjection									
UCMS/Mu vs. UCMS/NS	<.001	<i>ns</i>	.004	<i>ns</i>	.006	.015	<i>ns</i>	<i>ns</i>	
UCMS/Mu vs. Control/Mu	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.004	
UCMS/NS vs. Control/NS	0.003	<.001	.002	.04	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	
Control/Mu vs. Control/NS	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	

Note. Mu = muscimol; NS = saline; UCMS = unpredictable chronic mild stress.