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·Original Article·

The effect of depression on the thermal nociceptive thresholds in rats with spontaneous pain

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Abstract: Objective Recently, there has been growing interest in the interaction between depressive disorders and pain. The purpose of this study was to examine whether depression would lead to a decreased sensitivity to noxious stimuli in rats with spontaneous pain. **Methods** The olfactory bulbectomized rats were used as a model of depression. The depression-like behaviors were assessed by open field test and changes in body weight. Formalin solution was injected into the rat hindpaw to produce ongoing pain. Noxious thermal stimuli were applied onto the hindpaw contralateral to formalin injection, and the withdrawal thresholds were measured. **Results** In non-depressive rats, the formalin-treated paw developed hypoalgesia to noxious stimuli while the contralateral paw was not affected. The depressive rats, however, showed a significantly lower sensitivity to noxious thermal stimulus, represented as higher withdrawal thresholds of the contralateral paw, when compared to the non-depressive rats. **Conclusion** These results demonstrate that depression can alleviate the stimulus-evoked pain even in the context of formalin inflammatory pain, consistent with the previous clinical observations that patients suffering from both depression and persistent pain have decreased sensitivities to noxious experimental stimuli.

Keywords: depression; evoked pain; formalin; olfactory bulbectomy; thermal stimulation

1 Introduction

Both depression and pain are debilitating diseases that lead to enormous demands on medical services and compromise the life qualities of patients. In recent years, there has been growing interest in the relationship between depressive disorders and chronic pain[1,2]. In clinical practice, depressed patients exhibit a high degree of pain complaints. It is documented that approximately 65% of patients with depression have reported one or more pain diseases[3]. Croft *et al.* have shown that the prevalence of low back pain in individuals with depressive symptoms is more than twice higher than in those without depression[4]. Furthermore, clinical observations have supported that depression can predict the occurrence of pain and worse pain outcomes[5,6].

To elucidate the relationship between pain and depression, experiments have been done on depressed patients. However, some studies have revealed a decreased sensitivity to experimentally evoked pain in depressive patients[7-10], while a few reports have described an opposite finding[11,12]. Our previous studies using 2 independent animal models (thermal stimulus-evoked pain *vs* formalin injection-induced spontaneous pain) have found that depression inhibits evoked pain but facilitates spontaneous pain[13-15]. Nevertheless, in clinical practice, symptoms of evoked pain tend to be present in company with spontaneous pain in

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patients. For instance, the symptoms of fibromyalgia involve both tenderness in specific body sites and spontaneous pain in limbs and trunk[16].

In order to elucidate the effect of depression on pain sensitivity, here the thermal stimulus-evoked withdrawal behavior was observed in rats with formalin pain under the condition of depression.

2 Materials and methods

2.1 Animals Male Sprague Dawley rats (weighing 200-220 g) were supplied by the Laboratory Animal Center of the Academy of Military Medical Sciences, Beijing, China. Rats were housed individually under a 12:12 h light/dark cycle (light on at 07:00) with access to food and water *ad libitum*. The ambient temperature was set at (22±2) ℃. Animals were acclimated for one week before the experiments, and handled by the experimenter each day. All experimental procedures were approved by the Institutional Review Board for Animal Care and Use of the Chinese Academy of Sciences.

2.2 Experimental design The experimental protocol was depicted in Fig. 1. Two experiments were performed. The first examined the nociceptive sensitivity of both hindpaws to radiant heat stimulation after formalin injection (Fig. 1A). In this experiment, 16 rats were divided into spontaneous pain group (n=9) and control group (n=7). Paw withdrawal latencies (PWLs) were tested before (baseline), 2 h, 1, 3, 5, 7, 10, 14, 21, and 28 d after formalin or normal saline (NS) injection. The injection sides were counterbalanced between left and right hindpaws. The ipsilateral or contralateral side referred to the side with or without formalin/NS injection, respectively.

The second experiment investigated the effect of depression on thermal-evoked pain behaviors in formalin-injected rats (Fig. 1B). In this experiment, 18 rats were randomly assigned to olfactory bulbectomy (OB) group (n=11) and SHAM group (n=7), receiving OB or sham surgery, respectively. PWLs of rats before surgery were determined in the 2 groups as the baseline. Then rats underwent bilateral OB or sham operation. Open field tests were employed to

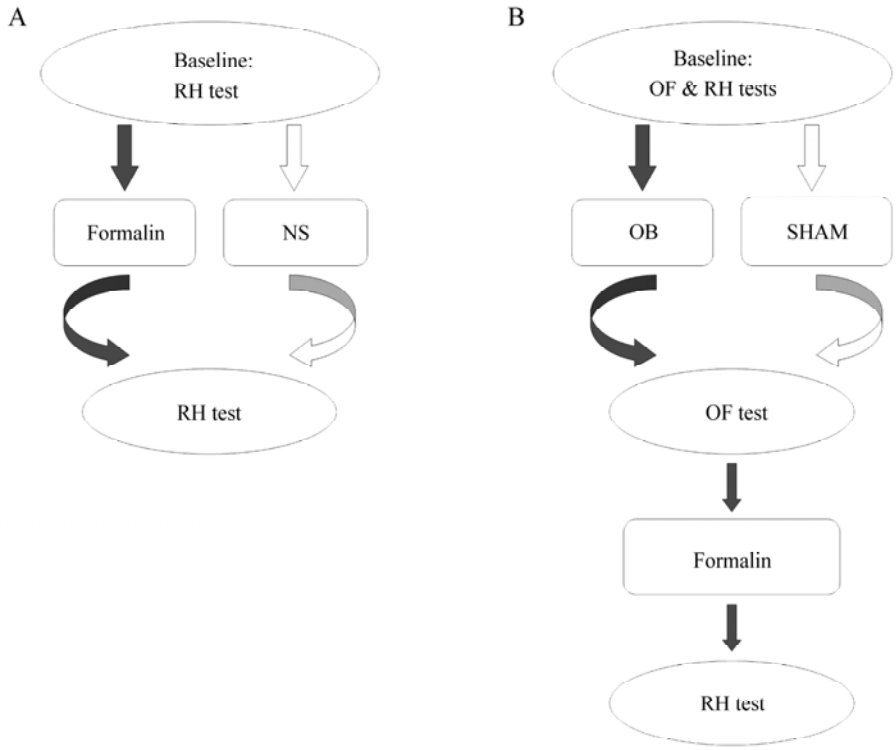

Fig. 1 Schematic diagrams of the experimental protocols for experiments 1 (A) and 2 (B). NS: normal saline; OB: olfactory bulbectomy; OF: open field; RH: radiant heat.

assess the depressive state before (baseline) and 2 weeks after surgery. Formalin was injected following open field test. PWLs of the contralateral hindpaw were measured 20 min, 1, 2, 4 and 24 h after formalin injection.

2.3 Surgery Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and fixed on a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). A longitudinal incision was made in the midline to expose the skull. Bilateral 2-mm diameter holes were drilled 8 mm rostral to the bregma and 2 mm lateral to the midline separately. The bilateral olfactory bulbs were aspirated from the holes with a blunt hypodermic needle and a vacuum pump. The cavities were plugged with gel foam (Coltene whaledent, Switzerland) to reduce haemorrhage. Special care was taken to avoid damaging the frontal cortex. Penicillin powder was sprinkled on the wound prior to suture. Sham-operated rats were subjected to the same procedure described above, except that no brain tissues were removed. A period of 2 weeks was allowed for recovery and development of depressive behaviors. At the end of the experiment, surgical treatment was verified histologically.

2.4 Pain test In the spontaneous pain group, 50 μL 5% formalin was injected into the plantar surface of one hindpaw. Controls were given the same volume of NS.

The thermal pain thresholds were assessed using radiant heat stimulation[17]. Rats were placed into a Plexiglas chamber with a glass floor for at least 30 min to accommodate to the environment. Then a radiant thermal stimulus was applied from underneath the floor onto the plantar surface of the contralateral or ipsilateral hindpaw. The interval between light onset and paw lift was recorded and defined as PWL. Four trials were conducted with at least 5-min intervals. In view of considerable variability in the first latency measurement, the average PWL of the last 3 trials was taken as the threshold of thermal-evoked pain.

2.5 Body weight gain Body weight was measured before surgery (baseline) and on each day for 2 weeks following surgery. The weight gain was determined as the percentage of weight change to the baseline, using the following formula: Body weight gain = (postsurgery weight – baseline weight)/ baseline weight × 100%. This index can be used as a measurement of depressive behaviors.

2.6 Open field test The open field test was employed to analyze the locomotion and exploration related to depression. Briefly, rats were placed in an iron circular base (180 cm in diameter) with 50-cm high walls. The floors and walls were painted black and the apparatus was illuminated by a 40 W bulb positioned in a corner of the room. Animals were placed individually into the center, facing the same position of the wall in all the tests. Each rat was allowed to explore for 5 min. The distance traveled during the test was tracked with a computer-based system Etho Vision (Noldus Information Technology, Wageningen, the Netherlands). The rearing behaviors were recorded by the observer.

2.7 Data analysis Data were presented as means±SEM. Statistical comparisons and graphs were made by Statistica 5.1 and GraphPad prism 5.0 software. Data involving 2 or 3 factors were analyzed by multifactor analysis of variance (ANOVA) followed by Duncan's *post hoc* test. $P < 0.05$ was indicated as significantly different.

3 Results

3.1 Changes in the nociceptive thresholds following formalin injection The results of Experiment 1 showed that the formalin injection produced significant changes in the PWLs, compared with the NS group [three-way ANOVA, $F(1, 252) = 9.511$, $P < 0.001$, Fig. 2]. *Post hoc* analysis revealed significantly longer PWLs of the formalin-injected paw ($Formalin_{ips}$), as compared with the NS-injected paw (NS_{ips}), at 2 h and days 1, 3 and 5 after formalin injection ($P < 0.01$). These results suggest that the formalin-treated paw developed hypoalgesic responses to the noxious thermal stimuli.

Although the main effect of injection site did not reach statistical significance [side effect: $F(1, 252)=1.694$, $P=0.204$], the interaction between treatment and injection site was significant [treatment×side: $F(1, 252)=4.807$, $P=0.037$]. Remarkable differences in PWLs were found between $Formalin_{ips}$ and $Formalin_{con}$ sides at days 1 and 3 after injection (*post hoc* test, $P < 0.001$), and between NS_{ips} and NS_{con} sides at 2 h after injection (*post hoc* test, $P < 0.01$). Interestingly, no difference was observed between formalin and NS groups in the contralateral paw response ($Formalin_{con}$ *vs* NS_{con}, $P > 0.05$). These

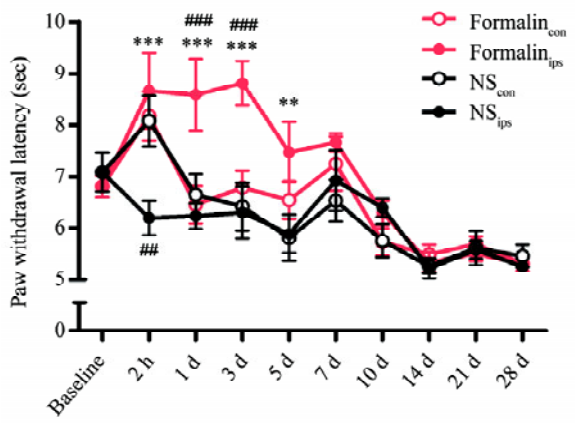

Fig. 2 Changes in the nociceptive thresholds following formalin injection. The paw withdrawal latencies (PWLs) in response to the radiant heat stimulation were recorded before (baseline), 2 h, 1, 3, 5, 7, 10, 14, 21, and 28 d after formalin or normal saline (NS) injection. The PWLs of formalin-injected paws (Formalin$_{ips}$) were significantly longer than that of NS-injected paws (NS$_{ips}$) at 2 h and days 1, 3 and 5 following injection. No significant difference was observed in the PWLs of contralateral paws between formalin and NS groups. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* NS$_{ips}$. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs* the contralateral response in the corresponding group.

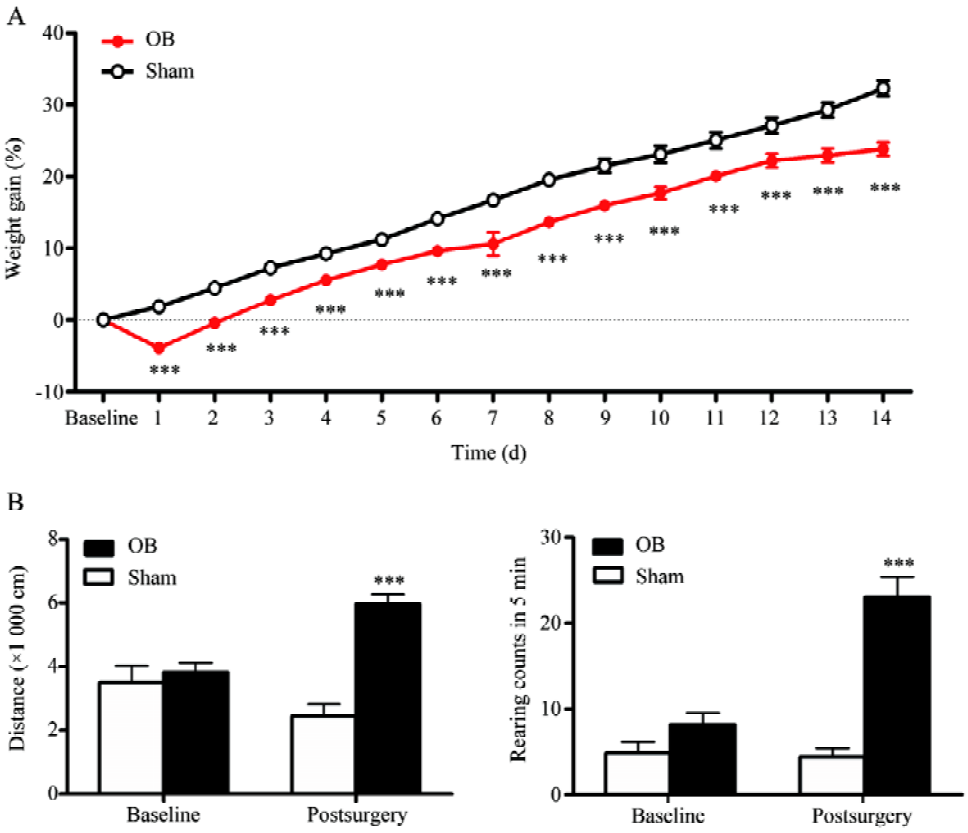

Fig. 3 Olfactory bulbectomized rat model of depression. A: Changes in body weight gain. Body weight gain was significantly reduced in olfactory bulbectomy (OB) group, compared with the control rats, throughout the 2-week recovery period. B: Open field test. The OB rats exhibited a significantly higher level of locomotor activity (left panel) and more rearing behaviors (right panel) than the sham controls. $^{***}P < 0.001$ *vs* the sham group post surgery.

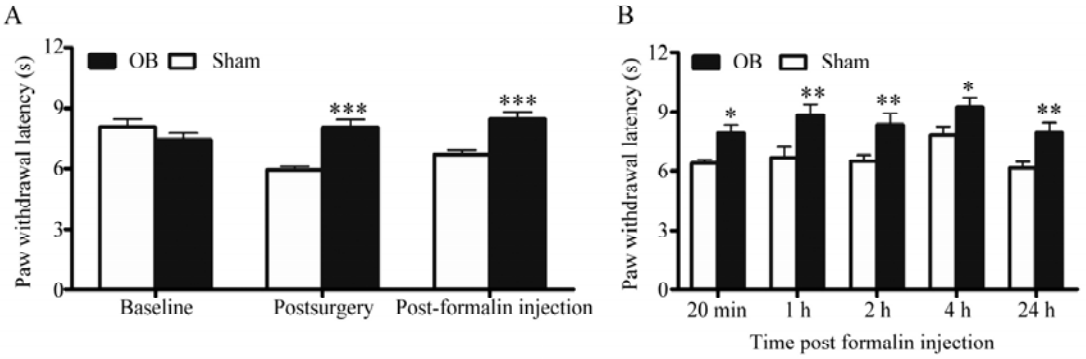

Fig. 4 Effect of depression on the thermal nociceptive thresholds in formalin-injected rats. A: The effects of olfactory bulbectomy (OB) on paw withdrawal latency (PWL). The PWLs in the OB group were significantly longer than those in the sham controls after surgery, both before and after formalin injection. Note that the data in column 'Post-formalin injection' were the mean values of all data sets in B. B: Details of formalin modulation on PWLs. The OB rats displayed significantly prolonged PWLs over the 24-h observation period. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* the sham group.

results suggest that formalin injection did not influence the nociceptive sensitivity of the contralateral paw.

3.2 The effect of depression on the nociceptive thresholds of rats with spontaneous pain The results of Experiment 2 showed the effect of OB-induced depression on the nociceptive thresholds of rats with spontaneous pain. As shown in Fig. 3A, the weight gain of OB group was significantly lower than that of SHAM group throughout the 2-week recovery period [two-way ANOVA, surgery effect: $F(1, 196)=27.341$, $P < 0.001$]. Moreover, the traveling distance of the OB rats in open field test was significantly longer than that of the sham-operated rats [(5957±1031) cm *vs* (2444±1003) cm, $P < 0.001$, Fig. 3B]. Higher rearing counts were also observed in OB rats, compared with control group (23.000±7.950 *vs* 4.429± 2.507, $P < 0.001$). These results indicate that the OB rats have developed depression-like behaviors.

Since formalin injection did not influence the nociceptive thresholds of the contralateral hindpaws (Fig. 2), the contralateral paw was chosen for testing its sensitivity to the noxious thermal stimulation in both OB and SHAM groups. Fig. 4A showed the comparison of PWLs between OB and SHAM groups at the baseline, post-surgery, and post-formalin conditions. As can be seen, the PWLs of the contralateral paw were significantly longer in OB group than in SHAM group, at both post-surgery and post-formalin injection conditions [two-way ANOVA, treatment effect: $F(1, 32)=8.23$, $P= 0.011$; side effect: $F(2, 32)=3.675$, $P=0.037$]. Fig. 4B depicted the time course of PWL changes following formalin injection, and demonstrated a constant decrease of nociceptive sensitivity over the 24-h observation period. These results suggest that the depressive state can influence the sensitivity to noxious stimulation in rats with or without spontaneous pain.

4 Discussion

In the aim of clarifying whether the inhibitory effect of depression on the externally evoked pain can be observed in subjects with ongoing pain, we investigated the effect of depression on the nociceptive thresholds in rats receiving intraplantar injection of formalin. We found that the formalin-treated paw developed hypoalgesic responses to the noxious stimuli at 2 h following formalin injection, while the contralateral paw was largely unaffected. Besides, depression significantly lowered the sensitivity to noxious thermal stimuli, even after the contralateral formalin injection, when compared with non-depressed rats. These results confirm our previous findings, and are consistent with clinical observations that patients suffering from both depression and persistent pain would exhibit decreased sensitivity to noxious experimental stimuli.

Our previous studies using different pain models have demonstrated that depression inhibits evoked pain but fa-

cilitates spontaneous pain[13-15]. One might doubt that the divergent effects of depression on evoked and spontaneous pain may be due to the differences of pain models *per se* (thermal stimulus-evoked pain *vs* formalin injection-induced pain). On the other hand, some might also question that this result can not be adopted to clinical cases since the 2 types of pain may coexist in one patient. Thus, here we employed a mixed pain model, i.e., delivering noxious stimulus to rats with formalin pain, and showed that depression can alleviate evoked pain even when the 2 types of pain co-exist in the same animal. This result, together with our previous findings, supports the suppressive effect of depression on the evoked pain.

The differential effects of depression on evoked and spontaneous pain have not been clearly elucidated. Several factors may explain this phenomenon, including the dysfunction of neurotransmitter system, abnormal activation of neural network, and limited attention to external circumstance in the state of depression. Alterations in the serotonergic and the noradrenergic neurotransmitter systems are associated with the disorder of depression. It is generally accepted that low levels of serotonin (5-HT) and norepinephrine (NE) are the etiology of depression[18,19]. 5-HT and NE are also important neurotransmitters in the descending pain modulatory pathways. A previous study using the formalin pain model has shown that intrathecal injection with NE antagonist or 5-HT antagonist produces a significantly greater response in phase 1, intermediate period, and phase 2[20]. It is likely that the dysregulation of 5-HT and NE during depression might account for the altered pain sensitivity. Moreover, evidence from functional imaging study has demonstrated that the activated brain areas in depressed patients are also related to the areas for pain processing, including contralateral primary and secondary somatosensory cortices, insula, anterior cingulated cortex, and supplementary motor area[21]. Mialet *et al.* have proposed that impaired attention to environmental stimuli in depressive patients may contribute to the limited attention resources to the external painful stimuli[22]. Nevertheless, further evidence is needed for providing insights into the relationship between depression and pain.

Another important finding of our study is that the formalin-injected paw exhibited a hypoalgesic response to the noxious thermal stimuli, while the contralateral paw showed a relatively stable response. This result is consistent with the findings of Chen *et al.*[23] and Fu *et al.*[24], which showed that the formalin-injured paw displayed mechanical and thermal hypoalgesia in the injection site, whereas no thermal hyperalgesia was found in the contralateral hindpaw. More importantly, we extended this work by setting up a saline control group and enlarging the sample size, making the experiment more reliable. In this way, our results further support the previous findings. It may be argued that the decreased pain sensitivity of the injected paw is probably due to the impairment of the terminal parts of primary sensory fibers and motor fibers induced by formalin. A stable evoked pain response was observed in the contralateral paw, presumably because the central sensitization could not last more than 2 h. For example, Biella *et al.* have demonstrated that the evoked activities of wide dynamic range (WDR) neurons in remote segments are decreased 2 h after formalin injection in the tail[25]. However, there are actually some opposite reports showing that diluted formalin injection can produce hyperalgesia in ipsilateral, contralateral or both paws. For example, Karim F *et al.* reported that both male and female mice exhibited significant ipsilateral thermal hyperalgesia 1-3 h after 2% formalin injection. This discrepancy may be probably due to the differences in formalin concentrations applied or in animal species used in these studies[26].

In summary, our work shows that depression can alleviate evoked pain responses even in the condition of spontaneous pain. This result further provides evidence that the depressive subjects have consistently reduced pain sensitivity, regardless of the existence of ongoing spontaneous pain.

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抑郁状态对伴有自发痛大鼠的热伤害性阈值的调节作用

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摘要：目的 近年来，人们对抑郁症和疼痛之间的相互作用日益关注。本研究通过观察抑郁对福尔马林注射大鼠的伤害性阈值的影响，探讨抑郁能否降低伴有自发痛的大鼠对伤害性刺激的感受性。**方法** 通过嗅球切除术建立大鼠抑郁模型，并采用旷场测试和体重变化来评价抑郁行为。在大鼠后肢足底注射福尔马林溶液诱导持续性疼痛。在福尔马林注射的对侧后肢足底施加伤害性辐射热刺激，测定其抬脚阈值。**结果** 在非抑郁大鼠中，福尔马林注射肢对伤害性刺激表现出痛觉减退，而对侧肢的伤害性阈值则不受影响。然而，与非抑郁大鼠相比，抑郁大鼠对伤害性热辐射刺激的感受性显著降低，表现为对侧后肢抬脚潜伏期延长。**结论** 上述结果表明，在伴有福尔马林炎症痛的条件下，抑郁同样能降低机体对诱发痛的敏感性。该结果与临床上关于同时患有抑郁与慢性痛患者对实验性痛刺激感受性下降的观察结果一致。

关键词： 抑郁；诱发痛；福尔马林；嗅球切除术；热刺激