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Research Paper

# Why depression and pain often coexist and mutually reinforce: Role of the lateral habenula

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ABSTRACT

The interrelation of depression and pain is increasingly coming under scrutiny. Although the lateral habenula (LHb) is widely implicated in the pathogenesis of depression and pain, its role in the interaction of depression and pain remains unknown. Thus, the aim of current study was to investigate the role of LHb in rat depression-pain comorbidity. Single extracellular firing recording and immunofluorescence methods were used to compare firing rates and c-Fos expression of the LHb neurons in normal and model rats. Following subcutaneous injection of formalin into the hind paw to simulate natural pain, we assessed pain behavior in rats subjected to the chronic, unpredictable mild stress procedure (CUMS, a model of depression). Pain sensitivity in the model rats was increased over that of controls. These rats showed a significant increase in the firing activity of LHb neurons compared with normal rats. Significantly, about 73% of neurons with high discharge frequency in LHb of model rats were pain-activated neurons (PANs), and the firing rates of PANs were inhibited by intraperitoneal injection of a tricyclic antidepressant, clomipramine. Immunofluorescence showed that the percentage of c-Fos positive cells in LHb was significantly increased in rats receiving CUMS alone, rats receiving pain stimulation alone, and rats receiving both CUMS and pain stimulation, but especially the last. The interaction effect was inhibited by injection of clomipramine. The LHb lesion can improve both depression-like behavior and pain sensitivity in depression model rats with pain. These suggest that hyperactivity of the LHb neurons contributes to depression-pain comorbidity in rats.

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## 1. Introduction

The development of depression is closely associated with pain symptoms, and each disorder may exacerbate the other under conditions of comorbidity (Li, 2015; Doan et al., 2015). The dysfunction of several important brain nuclei is central to the onset of depression and pain (Thompson et al., 2015; Shabel et al., 2014). However, their actions and roles in the underlying mechanisms of pain-depression comorbidity remain largely unknown.

The lateral habenular nucleus (LHb) of the thalamus is one of the brain regions involved in modulating emotion and pain (Shelton et al., 2012a, 2012b; Shabel et al., 2014; Li et al., 2013). It has been shown that the metabolic activity of the LHb is increased in a variety of depression models (Shumake et al., 2003). The inhibition of the LHb can improve symptoms in both patients and rats with major depression (Yang et al., 2008; Sartorius et al., 2010; Kiening and Sartorius, 2013). Furthermore, animal research has demonstrated that an antagonist of the substance-P receptor can produce antidepressive effects by inhibiting LHb activity (Yang et al., 2014). Therefore, the LHb is thought to play an important role in the pathogenesis of major depression and is a target of clinical treatment of depression (Sartorius et al., 2010; Kiening and Sartorius, 2013). It is generally accepted that the dysfunction of monoamine neurotransmitters, especially serotonin (5-HT) is related to pathogenesis of depression (Middlemiss et al., 2002). The largest population of 5-HT cells is located in the dorsal raphe nucleus (DRN) (McLean et al., 2007). The LHb is a key brain region of regulating the 5-HT activity in the DRN (Zhao et al., 2015) and itself reciprocally receives input from the DRN (Wang and Aghajanian, 1977). After lesion of the LHb, depressive behavior was improved as the result of an increase in the 5-HT level in the DRN (Yang et al., 2008). A recent study has found that the firing rate is remarkably lower in the DRN of the Wistar WKY rat, a model of depression, than in the DRNs of normal Wistar or Sprague Dawley rats (Bruzos-Cidón et al., 2014). However, the question of whether firing rate in the LHb neurons is also changed in depressed rats has not been addressed.

Ample studies proved that the LHb is involved in regulating many functions, including stress, pain, sleep, cognition, reward, and emotion

*Abbreviations:* LHb, lateral habenula; CUMS, chronic, unpredictable mild stress procedure; PANs, pain-activated neurons; 5-HT, serotonin; DRN, dorsal raphe nucleus; FST, forced swimming test; SPT, sucrose preference test.

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(Zhao and Rusak, 2005;Hikosaka, 2010; Shen et al., 2012; Han et al., 2014; Luo et al., 2015). While the dysfunction is associated with the onset of depression, and pain is especially associated with the depression that has recently attracted increasing attention (Li, 2015; Qi et al., 2013). Clinical epidemiological investigation has shown that 34%–66% of patients with depression also have some degree of pain symptoms (Regier et al., 1984). Tricyclic antidepressants have an excellent therapeutic effect on pain (Gilron et al., 2015; Megat et al., 2015). The pain symptoms can also be ameliorated in patients treated with a placebo (Colloca et al., 2013). This illustrates that emotion and pain can affect each other. Moreover, some brain areas activated in depressive patients are specifically pain-related (Bär et al., 2007), suggesting that pain and depression might share common neurobiological mechanisms. The LHb is also a key player in pain modulation (Shelton et al., 2012a, 2012b). There are a large number of pain-activated neurons (PANs) in the LHb, which respond to peripheral noxious stimulation with increased firing rates. Moreover, excitation of LHb neurons has been shown to decrease the pain threshold (Zhao and Wang, 1995). Consistent with this, the analgesic effect of some brain regions projecting to the LHb is produced by inhibiting habenular nucleus activity (Wang et al., 1987; Liu and Wang, 1987). Thus, the LHb is believed to play a key role in the regulation of both pain and emotion and may be the important element of the brain mediating clinical pain/depression comorbidity. The present study focused on the role of the LHb in mediating an interaction of pain and depression.

## 2. Methods and materials

### 2.1. Animals

The animal experiments were reviewed and approved by the Committee for Animal Care in Research at Jilin University and were in compliance with the Chinese Law for the care and use of laboratory animals. Two hundred and one adult, male Wistar rats weighing 180–240 g each were used for the electrophysiological recordings and immunofluorescence experiments. The animals were kept in plastic cages under normal laboratory conditions (room temperature 22 ± 2 °C, 12-h light-dark cycle, lights on at 7 a.m.) with free access to food and water. Rats were randomly divided into two groups: a control group and a chronic, unpredictable mild stress (CUMS) group.

### 2.2. The chronic unpredictable mild stress procedure

The chronic unpredictable mild stress (CUMS) procedure was a slight modification of that described by Roth and Katz (Roth and Katz, 1981). Briefly, rats were subjected to one or two different stressors per day randomly selected from seven possible stressors, for 21 days. The stressors were: (i) a 24-h period in which the cage was tilted by 45°, (ii) a 40-h period of food deprivation, (iii) a 5-min period of swimming in cold water at 4 °C, (iv) a 24-h period of water deprivation, (v) a 5-min period of heat stress at 45 °C, (vi) reversal of the light/dark cycle, and (vii) a 1-min application of a clip to the tail.

### 2.3. Forced swimming test

Depression-like behavior in the animals was assessed by the widely used forced swimming test (FST). Briefly, the experimental session consisted of two trials: a conditioning trial and a test trial. The conditioning trial was for acclimation purposes and no data were collected; rats were gently placed individually into the swim apparatus, which was a vertical, transparent Plexiglas cylinder of 18 cm internal diameter × 50 cm high filled with water at 25 ± 1 °C and without possibility of escape, for 15 min (pretest). Twenty-four hours after the first exposure, a test trial was carried out in which the rats were placed in the swim apparatus for 5 min (test). During the test session, behavior was recorded and assessed by a blinded observer who was unaware of the experiment. After each forced swim test, the water was decanted and the cylinder was cleaned, dried, and refilled with fresh water to avoid the influence of alarm substances. The individual data were summarized as time spent immobile, climbing, or swimming. The increase in immobile time and the decrease in climbing time relative to controls were mathematically converted into a measure of depressive symptoms.

### 2.4. Sucrose preference test

The sucrose preference test (SPT) was performed after administration of CUMS. At the start of the experiment, the animals were habituated to 1% sucrose solution (*w*/*v*) by exposing them to two bottles of sucrose solution for 72 h. Afterwards, the 1% sucrose solution was replaced with water for 24 h. Lastly, rats were given two bottles containing 1% sucrose solution and water, respectively. Consumption of sucrose solution and water were recorded after a 24-h period of exposure to the choice condition. The percent preference for sucrose consumption was calculated according to the following formula: percentage sucrose preference = (sucrose solution consumption/total liquid intake) × 100.

### 2.5. Electrophysiological procedures

Prior to surgery, rats were anesthetized with 20% urethane (i.p., 1.2 g/kg) and then placed in a stereotaxic apparatus. An extracellular recording electrode (impedance 8–15 MΩ) filled with 0.5 M NaCl and 2% Pontamine Sky Blue was lowered into the LHb (3.3–4.16 mm posterior to bregma, 0.3–1.0 mm lateral to midline, and 4.2–4.6 mm ventral to dura) by a hydraulic drive and stepping motor (PC-5 N; Narishige, Tokyo, Japan). The signal from the electrode was amplified and filtered by a microelectrode amplifier (ME2-8301; Nihon Kohden, Tokyo, Japan) and monitored continuously on an oscilloscope (VC-10; Nihon Kohden, Tokyo, Japan). The potentials of the LHb neurons were collected and analyzed using a data acquisition system (ML-112; ADI, Sydney, Australia). When a discriminated single neuron was detected and showed stable firing for a period of 5–10 min, a 5-s tail pinch was applied, and the neuron was classified as a PAN if firing rate increased by 20% or more.

After completing the electrophysiological studies, the final tip positions of the recording electrodes were determined by Pontamine Sky Blue staining (electrophorese at 5 μA for 10 min). Recording sites were easily identifiable as blue dots. Data from locations found to be outside the LHb were excluded from the analysis.

### 2.6. Immunofluorescence

Rats from all six groups (control, control + pain, CUMS, CUMS + pain, CUMS + pain + drug, and CUMS + pain + saline; each $n = 4$–6) were anesthetized with urethane (i.p., 1.2 g/kg). The brains were removed, frozen, and cut into 10-μm-thick coronal sections using a freezing microtome (LEICA CM1950, Nussloch Germany). Sections were blocked in phosphate-buffered saline (PBS) containing 10% normal goat serum and 2% Triton-100 in PBS for 1 h on a shaker at room temperature. Sections were then incubated overnight at 4 °C with primary antibodies (1:200, rabbit anti–c-Fos polyclonal antibody, bs-0469R, BEIJING BIOSYNTHESIS BIOTECHNOLOGY, Beijing, China). After washing with PBS three times for 5 min each, sections were incubated for 1 h at room temperature with secondary antibodies (1:200, rabbit IgG [H + L] polyclonal secondary antibody for immunofluorescence, A10520, Life Technologies Corporation, Pockford, USA). Sections were then rinsed three times in PBS for 5 min each and were then stained for 8 min with DAPI (C1005, Beyotime, Shanghai, China) to visualize nuclei. Sections were then rinsed in PBS three times for 5 min each, mounted on glass slides, and cover-slipped with 75% (*v*/*v*) glycerol in 0.1 M PBS. The results were photographed with an epifluorescence microscope equipped with a digital camera (Olympus BX51, Olympus U-RFL-T, Tokyo, Japan).

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