

## Research Article

# *Lycium barbarum* polysaccharide-glycoprotein preventative treatment ameliorates aversive stimuli-induced depression

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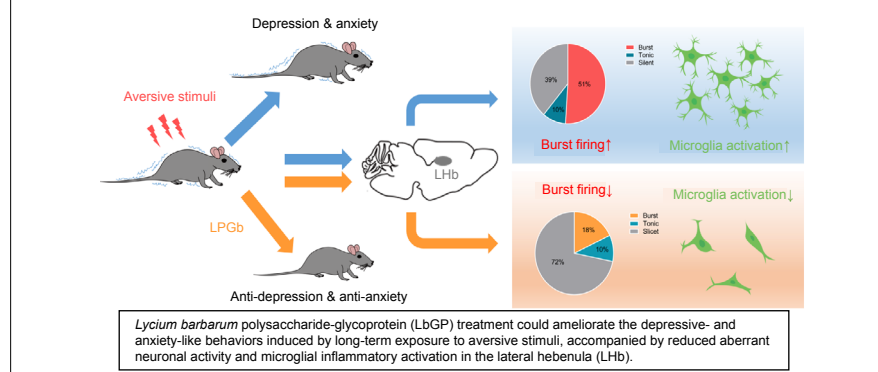
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**Graphical Abstract** *LbGP treatment ameliorates aversive stimuli-induced depression, accompanied by reduced aberrant neuronal activity and microglial activation in the LHb*



## Abstract

Previous studies have shown that *Lycium barbarum* polysaccharide, the main active component of *Lycium barbarum*, exhibits anti-inflammatory and antioxidant effects in treating neurological diseases. However, the therapeutic action of *Lycium barbarum* polysaccharide on depression has not been studied. In this investigation, we established mouse models of depression using aversive stimuli including exposure to fox urine, air puff and foot shock and physical restraint. Concurrently, we administered 5 mg/kg per day *Lycium barbarum* polysaccharide-glycoprotein to each mouse intragastrically for the 28 days. Our results showed that long-term exposure to aversive stimuli significantly enhanced depressive-like behavior evaluated by the sucrose preference test and the forced swimming test and increased anxiety-like behaviors evaluated using the open field test. In addition, aversive stimuli-induced depressed mice exhibited aberrant neuronal activity in the lateral habenula. Importantly, concurrent *Lycium barbarum* polysaccharide-glycoprotein treatment significantly reduced these changes. These findings suggest that *Lycium barbarum* polysaccharide-glycoprotein is a potential preventative intervention for depression and may act by preventing aberrant neuronal activity and microglial activation in the lateral habenula. The study was approved by the Jinan University Institutional Animal Care and Use Committee (approval No. 20170301003) on March 1, 2017.

**Key Words:** aversive stimuli; behaviors; depression; immune response; inflammation; lateral habenula; *Lycium barbarum* polysaccharide; mice; microglia; neuron

Chinese Library Classification No. R453; R741; R284

## Introduction

Depression is the most common class of mental disorders, affecting approximately 16% of the world's population, and is associated with high suicide risk (Lépine and Briley, 2011).

It causes compromising clinical symptoms including impaired sociability, anhedonia, behavioral despair and anxiety (Krishnan and Nestler, 2010). Despite significant progress, most current medical therapies for depression remain

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## Research Article

ineffective in many patients and often cause intolerable side effects (Caraci et al., 2018). Thus, there is an urgent need to understand the underlying mechanisms of depression and find novel strategies to improve current therapy.

A considerable amount of evidence indicates that inflammation plays an important role in the pathophysiology of depression. Previous studies using postmortem brain samples from suicide victims with depression showed elevated gene expression of proinflammatory cytokines (Miller et al., 2009; Drago et al., 2015). Moreover, administration of inflammatory cytokines (for example, interferon- $\alpha$ ) or bacterial endotoxin lipopolysaccharide to nondepressed individuals causes inflammatory responses and depression symptoms (Capuron et al., 2002; Zhang et al., 2016; Guan et al., 2020). Conventional antidepressant medications, including selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, act by increasing monoamines and thereby ameliorate lipopolysaccharide-induced depressive-like behavior and proinflammatory response (O'Connor et al., 2009; Ohgi et al., 2013; Yao et al., 2015). Together, these studies indicate that inflammation is correlated with depressive-like behavior and the use of anti-inflammatory drugs could alleviate depressive symptoms.

*Lycium barbarum* is a commonly used Chinese herbal medicine; its main effective component is *Lycium barbarum* polysaccharide (LBP) (Uasuf et al., 2020; Zhou et al., 2020). LBP has been reported to possess a variety of beneficial anticancer, immunomodulatory, gut microbiota, and antiaging effects (Li et al., 2007; Chang and So, 2008; Tang et al., 2012; Jiang et al., 2019; Feng et al., 2020; Pop et al., 2020; Zhao et al., 2020; Zhu et al., 2020). Recent studies have found that LBP exhibits neuroprotective activity in various central nervous system diseases via its anti-inflammatory and antioxidant properties (Chen et al., 2014; Wang et al., 2014; Bie et al., 2015; Starzak et al., 2020; Yang et al., 2020). However, the effects and potential mechanisms of LBP on depression remain poorly understood. We speculated that LBP may ameliorate depression symptoms via anti-inflammatory effects.

The lateral habenula (LHb) is an extremely conserved brain region across mammalian species and plays a key role in the regulation of depressive-like behaviors (Proulx et al., 2014). Accumulated data have shown that long-term exposure to aversive stimuli (AS) can increase depressive-like behaviors by increasing aberrant neuronal activity in the LHb (Yang et al., 2018; Huang et al., 2019) and this increased microglial inflammatory activation (Guan et al., 2020). Conversely, inhibition of the LHb alleviates depressive-like behaviors (Proulx et al., 2014; Huang et al., 2019). However, whether LBP can ameliorate depressive symptoms and reduce aberrant neuronal activity and microglial inflammation in the LHb still needs to be determined.

We designed this study to investigate the effects and mechanism underlying the antidepressive effects of LBP-glycoprotein (LbGP), a complex of immunoactive components of LBP, using behavioral, electrophysiological and biochemical analyses.

## Materials and Methods

### Animals

Experimental approval was obtained from the Jinan University Institutional Animal Care and Use Committee (approval No. 20170301003) on March 1, 2017. Adult (6–8 weeks old) male C57BL/6 mice (Guangdong Medical Laboratory Animals Center, China; license No. SCXK (Yue) 2018-0002) were used in the present study. All mice were housed under a 12-hour light/dark cycle with food and water available. For depressive-like behavior establishment, animals were randomly allocated to AS ( $n = 10$  for all behavior test) and control ( $n = 10$  for all behavior test) groups. For the LbGP treatment test, mice were

exposed to long-term AS ( $n = 20$ ) and randomly assigned to two groups that received either saline (AS + saline group,  $n = 10$  for SPT and FST test;  $n = 9$  for OFT test) or LbGP (AS + LbGP group,  $n = 10$  for SPT and FST test;  $n = 9$  for OFT test). The experimenters were blind to the assignments of the mice to the experimental groups and the order of outcome testing across both days was fully counterbalanced.

### Treatments and experimental schedule

Animals in AS + saline group were exposed to AS and received saline (0.5 mL) by intragastric administration daily. Mice in AS + LbGP group were also exposed to AS and received LbGP (0.5 mL, 5 mg/kg; Tianren Bio-engineering Co., Ltd., Zhongning, China) by intragastric administration each day. The length of therapy for all groups was 28 days. Behavioral tests and electrophysiological recordings were conducted on day 29 and 30, respectively.

### Long-term AS exposure model establishment

Depression model was established through long-term AS exposure as previously described (Huang et al., 2017). The exposure to fox urine, air puff and foot shock and physical restraint were performed daily for 28 days.

### Foot shock exposure

Animals were placed in an acrylic box (25 cm  $\times$  25 cm  $\times$  40 cm) equipped with a metal mesh floor. The inter-trial interval ranged from 15–30 seconds, randomly selected for each trial. To set up a depression mouse model, electric foot shocks (20 times/day, 0.6–1 mA, 500 ms; Yihong Technology Co., Ltd., Wuhan, China) were randomly transmitted. In the control group, mice were positioned in an identical acrylic box, but did not receive any stimulation.

### Air puff exposure

Animals were positioned in a cage, air ejectors (20 times/day) were randomly activated, and the inter-trial intervals ranged from 10–15 seconds.

### Fox urine exposure

Animals were set in a diaphanous plastic container (25 cm  $\times$  10 cm  $\times$  10 cm, with holes) containing four cotton balls soaked in red fox urine for 30 minutes per day. In the control group, mice were set in their own cages and 2 mL water was used instead of fox urine.

### Physical restraint

Animals were imprisoned for 2 hours/day in a plastic restrainer (Corning Inc., Corning, NY, USA). After constraint, the mice were placed back in the cage.

### Behavioral tests

All behavioral tests were performed after 28-day AS exposure as previously described (Huang et al., 2017) and were conducted during the light phase (7 a.m.–7 p.m.). The researchers were blinded to the experimental group during the scoring.

### Sucrose preference test

Mice were examined for preference for a 2% sucrose solution (Sigma-Aldrich, St. Louis, MO, USA) using a two-bottle choice process. Each animal was housed individually for the 2 days. Mice were provided with two water bottles, one containing 2% sucrose solution and the other tap water. The sucrose and water intake by each animal was measured every 24 hours. To avoid the preference of position, the positions of the two bottles were exchanged every 24 hours. The sucrose preference was calculated as the ratio of sucrose intake to total fluid intake and values were converted to percentages.

### Forced swim test

Mice were individually placed in a cylinder (20 cm in diameter,

27 cm in height) containing water at 23–25°C and swam for 6 minutes. The depth of water was set to prevent animals from touching the bottom of the cylinder with their hind limbs. Animal behavior was video-tracked (Canon Corporation, Tokyo, Japan) from the side. The time during which mice remained immobile was quantified by two independent researchers in a blinded manner.

### Open field test

Locomotion was counted in an open field test box (50 cm × 50 cm × 40 cm; Noldus Information Technology, Netherlands). Animals were positioned in the center of a plastic box, were permitted to freely explore for 15 minutes, and were video-recorded using an infrared camera positioned above the box. Motor activity and the time in center during the last 10 minutes were calculated by Ethovision XT software (Noldus Information).

### Immunocytochemistry and image analysis

Mice were anesthetized (Avertin, 13 µL/g, intraperitoneal injection; Sigma Chemical Co., St. Louis, MO, USA) and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde in phosphate-buffered saline. Brains were removed and placed in a test tube. Ionized calcium binding adaptor molecule 1 (Iba-1) is a specific marker for microglia and inflammation (Flores et al., 2018; Nie et al., 2018). For Iba-1 labeling, slices of brain containing the Lhb were washed five to six times with phosphate-buffered saline, and then slices were put in 0.1 M phosphate-buffered saline containing 10% normal goat serum (Vector Laboratories, Burlingame, CA, USA) and 0.3% Triton X-100 (Cat# T8787; Sigma-Aldrich) for 1 hour, before incubation in primary antibody against Iba-1 (rabbit; 1:1000; Cat# 019-19741, Fujifilm, Kanagawa, Japan) for 36 hours at 4°C. The slices were then incubated with the DyLight 488 goat-anti-rabbit IgG (1:400; Cat# 115-546-046; Jackson ImmunoResearch, West Grove, PA, USA) at room temperature for 6 hours. Finally, all slices were washed with 0.1 M phosphate-buffered saline and covered with anti-fading aqueous fixation medium. Slices were imaged using a Zeiss 700 confocal microscope (Zeiss, Oberkochen, Germany) at 20× magnification. For three-dimensional reconstruction from the microscopic images, optical sections were acquired at 0.2-µm intervals. To determine the number of Iba-1<sup>+</sup> cells per field, the center of the Lhb was placed under a Zeiss Axioimager Z2 microscope in 12 Iba-1-immunostained sections from three animals (four sections/animal) from each group used in this study, and the number of Iba-1<sup>+</sup> cells was counted in a 150 × 80 µm area in each section. The soma diameter of Iba-1<sup>+</sup> cells was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and the average diameter was calculated. The number of primary microglial processes was determined visually as the number of primary processes emanating from the cell body.

### Brain slices preparation and electrophysiological recordings

All experiments were performed as previously reported (Huang et al., 2017; Lin et al., 2018). In brief, animals were anesthetized with isoflurane (RWD, Shenzhen, China) and a coronal slice (250 mm thick) of Lhb was cut with a vibratome under ice-cold artificial cerebrospinal fluid (in mM: 119 NaCl; 2.5 KCl, 1 NaH<sub>2</sub>PO<sub>4</sub>, 11 glucose, 26.2 NaHCO<sub>3</sub>, 2.5 CaCl<sub>2</sub>, and 1.3 MgCl<sub>2</sub>, at pH 7.4, 290 mOsm). Brain slices were incubated in artificial cerebrospinal fluid for 1 hour at room temperature. After incubation, individual slices containing the Lhb were placed into a recording chamber immersed in oxygenated artificial cerebrospinal fluid. To record the firing pattern of Lhb neurons, the electrodes were filled with a K<sup>+</sup>-based solution (in mM) 135 KMeSO<sub>4</sub>, 10 KCl, 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10 Na<sub>2</sub>-phosphocreatine, 4 MgATP, 0.3 Na<sub>3</sub>GTP at 290 mOsm, adjusted to pH 7.4 with KOH. A depolarizing current was conducted from a membrane

potential of –70 mV. In the current clamp mode (*I* = 0 pA), spontaneous firing was measured in the electrode. Lhb neurons were classified as silent, tonic, or burst according to their spontaneous patterns of firing (Lépine and Briley, 2011; Huang et al., 2017; Yang et al., 2018). A Multiclamp 700B amplifier (Molecular Devices, San Jose, CA, USA) was used for recording. Clampfit 10.0 software (Molecular Devices) was used for offline data analysis.

### Statistical analysis

All data were calculated using GraphPad Prism 7 software (GraphPad Software Inc., San Diego, CA, USA) by experimenters blind to the experimental conditions. Statistical significance was set at *P* < 0.05. Data are expressed the mean ± standard error of mean (SEM). Student's *t*-test was used to compare the differences between groups. One-way analysis of variance uses Šidák's multiple comparison tests to quantify the number of Iba-1<sup>+</sup> cells, their diameters and number of processes. The chi-square test is used to calculate the percentage of burst firing cells.

## Results

### LbGP preventative treatment ameliorates AS-induced depressive-like behaviors

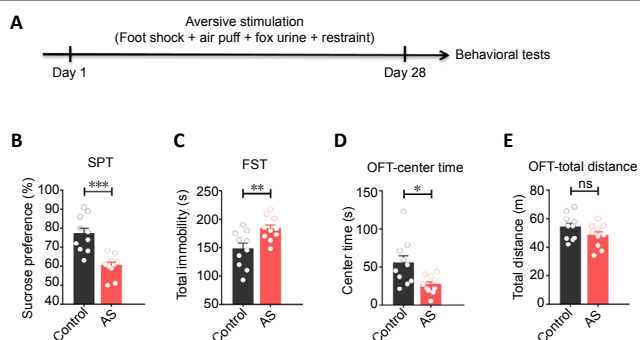
Previous studies have found that long-term exposure to AS can enhance depressive-like behaviors in mice (Yang et al., 2018; Huang et al., 2019). In line with these studies, we first exposed mice to 28 days of AS. This was followed by a series of behavioral tests on the mice (**Figure 1A**). To examine depressive-like behaviors, we used two well-validated depression assays, the sucrose preference test and the forced swimming test. Compared with control mice, mice exposed to long-term AS exhibited less sucrose preference (*P* = 0.0002; **Figure 1B**), indicating an increased level of the anhedonia phenotype. Furthermore, mice that received long-term exposure to AS also exhibited significantly longer duration of immobility in the forced swimming test, indicating an increased level of despair phenotype (*P* = 0.0096; **Figure 1C**). Given that symptoms of depression are often accompanied by increased anxiety (Smagin et al., 2017; Huang et al., 2019), we also tested anxiety-like behaviors. Mice that suffered long-term AS also showed a significant increase in anxiety-like behaviors tested by the open field test (*P* = 0.0139; **Figure 1D**). In contrast, long-term exposure to AS did not change locomotor activities (*P* = 0.1487; **Figure 1E**), indicating that the enhanced depressive and anxiety-like behaviors were not due to aberrant locomotion.

We examined whether LbGP treatment exerts preventative effects on the depressive-like behaviors generated by long-term AS exposure. During the 28 days of exposure to AS (**Figure 2A**), LbGP was administered daily by gavage at a concentration of 5 mg/kg, the most effective concentration previously reported (Xing et al., 2016). We found that LbGP preventative treatment significantly decreased the anhedonia and behavioral despair examined by the sucrose preference test and forced swimming test, respectively (*P* = 0.0043, *P* = 0.0002; **Figure 2B** and **C**). LbGP preventative treatment also significantly decreased the anxiety-like behaviors (*P* = 0.0449; **Figure 2D**) without affecting the locomotor activities of the mice (*P* = 0.4263; **Figure 2E**). The above results suggest that LbGP preventative treatment could ameliorate the depressive and anxiety-like behaviors induced generated by long-term exposure to AS.

### LbGP preventative treatment rescues AS-induced aberrant neuronal activity in the Lhb

Recent studies indicate that exposure to AS can increase depressive and anxiety-like behaviors by increasing aberrant neuronal activity in the Lhb (Proulx et al., 2014; Yang et al., 2018). We next performed whole-cell recordings from

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**Figure 1 | Long-term exposure to AS induces depressive-like behaviors.**

(A) Schematic of the experimental design. Male C57BL/6 mice were first exposed to 28 days of AS (foot shock, 20 times/day; air puff, 20 times/day; fox urine, 30 min/day; physical restraint, 2–4 h/day), followed by behavioral evaluations. (B) Reduced sucrose preferences of mice after exposure to long-term AS ( $n = 10$  animals/group,  $P = 0.0002$ ). (C) Increased immobility duration in mice after exposure to long-term AS ( $n = 10$  animals/group,  $P = 0.0096$ ). (D) Decreased time spent in center during the OFT in mice after exposure to long-term AS ( $n = 10$  animals/group,  $P = 0.0139$ ). (E) Total distance tested in the OFT was not significantly changed in mice after exposure to long-term AS ( $n = 10$  animals/group,  $P = 0.1487$ ). Data are expressed the mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (Student's  $t$ -test). AS: Aversive stimuli; ns: no significant difference; OFT: open field test.

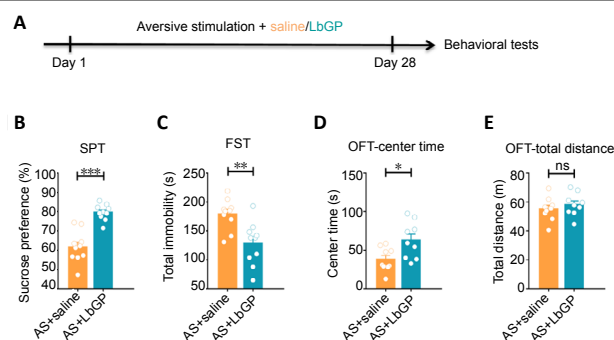
LHb neurons in slices to measure their neuronal excitability and firing patterns (Figure 3A). We found that long-term exposure to AS significantly increased the action potentials evoked by current pulses in LHb neurons ( $P < 0.0001$ ; Figure 3B), indicating increased neuronal excitability in the LHb. Furthermore, both the percentage of burst firing cells in the LHb and the frequency of firing were significantly increased in mice that had been exposed to long-term AS ( $P < 0.0001$  or  $P = 0.0034$ , respectively; Figure 3C). The above results suggest that long-term exposure to AS enhanced depressive-like behaviors and these were accompanied by increased aberrant neuronal activity in the LHb.

To probe the possible cellular mechanisms underlying the antidepressant effect of LbGP, we measured the neuronal excitability and firing patterns of LHb neurons (Figure 4A). We found that LbGP preventative treatment significantly decreased the action potentials evoked by current pulses ( $P = 0.0139$ ; Figure 4B) and the percentage of burst firing in LHb neurons ( $P = 0.0435$ ,  $P = 0.0026$ ; Figure 4C), indicating that preventative treatment of LbGP could not only improve depressive symptoms but also reduce the aberrant neuronal activity induced by long-term exposure to AS.

### LbGP preventative treatment suppresses microglial activation in the LHb

Given that microglia are implicated in the modulation of neuronal activity under conditions of physiology or pathology (York et al., 2018) and that microglial activation is observed in both patients with depression and depressive animal models (Setiawan et al., 2015; Zhang et al., 2017; Leonard, 2018), we tested whether long-term exposure to AS could lead to microglial activation in the LHb. We found that long-term exposure to AS significantly increased the cell diameter ( $P < 0.0001$ ), the number of cells ( $P < 0.0001$ ) and the number of processes ( $P < 0.0001$ ) of Iba-1<sup>+</sup> cells in the LHb (Figure 5A–D), indicating that long-term exposure to AS enhanced microglial activation in the LHb.

Previous studies have found that LBP played potent anti-inflammatory and antioxidant roles in the treatment of various diseases (Chen et al., 2014; Wang et al., 2014; Bie et al., 2015). We next tested whether preventative treatment with LbGP could attenuate AS-induced microglial activation in the LHb. Interestingly, we found that the cell number (control vs. AS + LbGP,  $P = 0.0137$ ; AS + saline vs. AS + LbGP,  $P < 0.0001$ ),



**Figure 2 | LbGP preventative treatment decreases AS-induced depressive-like behaviors.**

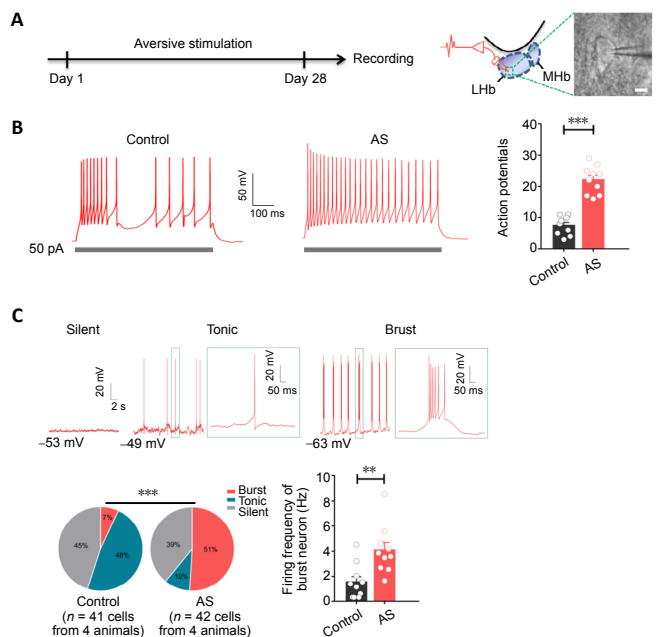
(A) Schematic of the experimental design. LbGP was administered daily by gavage during the 28 days of exposure to AS, followed by behavioral evaluations. (B) LbGP preventative treatment increased the sucrose preference of mice with AS exposure ( $n = 10$  animals/group,  $P < 0.0001$ ). (C) LbGP preventative treatment reduced the duration of immobility of mice with AS exposure ( $n = 10$  animals/group,  $P = 0.0024$ ). (D) LbGP preventative treatment increased the center time of mice with AS exposure tested in the OFT ( $n = 9$  in the AS + saline group;  $n = 9$  in the AS + LbGP group,  $P = 0.0184$ ). (E) The total distances tested in the OFT did not change significantly ( $n = 9$  animals in the AS + saline group;  $n = 9$  animals in the AS + LbGP group,  $P = 0.4310$ ). Data are expressed the mean  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (Student's  $t$ -test). AS: Aversive stimuli; FST: forced swimming test; LbGP: *Lycium barbarum* polysaccharide-glycoprotein; ns: no significant difference; OFT: open field test; SPT: sucrose preference test.

diameter (control vs. AS + LbGP,  $P = 0.0142$ ; AS + saline vs. AS + LbGP,  $P < 0.0001$ ) and the process number (control vs. AS + LbGP,  $P = 0.0978$ ; AS + saline vs. AS + LbGP,  $P < 0.0021$ ) of Iba-1<sup>+</sup> LHb cells were significantly decreased after LbGP treatment (Figure 5A–D). These results suggest that LbGP preventative treatment could also reduce the inflammation caused by long-term exposure to AS.

## Discussion

In the current study, we tested the antidepressive effects of LbGP preventative treatment and probed the underlying mechanisms. Our results showed first that LbGP preventative treatment markedly ameliorated depressive- and anxiety-like behaviors caused by long-term exposure to AS. Second, mice exposed to long-term AS had enhanced aberrant neuronal activity in their LHb, which could be attenuated by LbGP preventative treatment. Finally, mice exposed to long-term AS showed increased microglial activation in the LHb, which could be prevented by simultaneous LbGP treatment. Together, our results demonstrated that LbGP can be a potential preventative intervention for depression that is probably mediated via the restoration of aberrant neuronal activity and microglial activation in the LHb.

In traditional Chinese medicine, *Lycium barbarum* is considered to nourish health by balancing the Qi of Yin and Yang (Xiao et al., 2013). LBP is a complex mixture of highly branched polysaccharides and proteoglycans (Lim, 2012), which exhibits anti-inflammatory and antioxidant effects (Chiu et al., 2009). Recent studies have reported that LBP exerts neuroprotective effects and can improve the symptoms of several neurological disorders, including Alzheimer's disease, Parkinson's disease, ischemic stroke, and retina disorders (Chiu et al., 2009; Chen et al., 2014; Wang et al., 2014; Bie et al., 2015; Xing et al., 2016; Shi et al., 2017). In addition, a large body of evidence suggests that chronic inflammation leads to pathologic changes in depression (Leonard, 2018). Thus, it is reasonable to postulate that LBP may ameliorate depressive symptoms through its anti-inflammatory effect. However, very few studies have been conducted to evaluate the antidepressive effects of LBP. Furthermore, no researchers have tested the effects of LBP on depressive-like behaviors

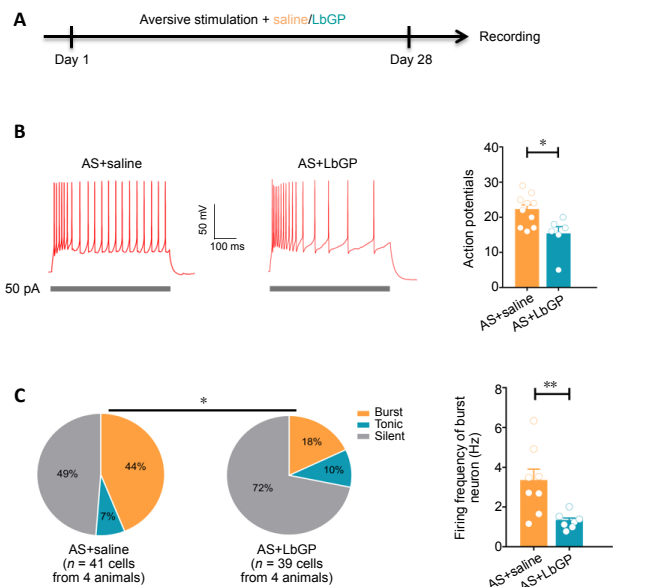


**Figure 3 | Long-term exposure to AS induces aberrant neuronal activity in the LHB.**

(A) Left: Schematic of the experimental design. Right: Representative image of a LHB neuron recording. Scale bar: 30  $\mu$ m. (B) The action potentials evoked by current pulses in brain slices of control and AS groups ( $n = 9$  neurons from three mice in the control group;  $n = 10$  neurons from three mice in the AS group,  $P < 0.0001$ ). (C) Upper: Representative traces of silent, tonic-firing, and burst-firing LHB neurons in control and AS groups ( $n = 41$  neurons from four mice in the control group;  $n = 42$  neurons from four mice in the AS group,  $P < 0.0001$ ). Bottom left: Percentage of the three types of LHB neurons in control and AS groups. Bottom right: Spontaneous firing frequency of burst neuron. Data are expressed the mean  $\pm$  SEM and analyzed by Student's  $t$ -test (B; C-right) or chi-square test (C-middle). \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . AS: Aversive stimuli; LHB: lateral habenula; MHB: medial habenula.

caused by exposure to long-term AS, a well-established animal model of depression (Krishnan and Nestler, 2011). Here, we provide the first line of evidence that preventative treatment using LbGP can reduce the depressive behavior caused by exposure to long-term AS. Interestingly, the anxiety-like behaviors caused by long-term AS were also ameliorated by LbGP preventative treatment, indicating that LbGP can be used as a potential preventative intervention for comorbid depression and anxiety. Indeed, there is a higher percentage of conventional treatment resistance in patients with comorbid depression and anxiety than in patients with either disorder alone (Coplan et al., 2015). Therefore, future clinical research should be aimed at the tolerance to LbGP as well as its potential therapeutic effect, both preventative and post-depression. Although the AS-induced depressive model partly mimics human depressive symptoms, future research should be conducted to validate the antidepressive and anxiolytic effects of LbGP in other models of depression, such as the chronic social defeat stress model and the learned helplessness model (Bianco et al., 2005; Perova et al., 2015).

It is well established that the LHB plays an important role in the pathophysiology of depression (Yang et al., 2018; Huang et al., 2019; Guan et al., 2020). Therefore, we focused on LHB in this study to investigate the potential mechanism underlying the antidepressive effects of LbGP. We first found that exposure of mice to long-term AS increased the action potentials evoked by current pulses and burst firing of LHB neurons. These findings, in line with our previous study (Huang et al., 2019), indicate that increased aberrant neuronal activity in the LHB is correlated with depressive symptoms. We then demonstrated that preventative treatment of LbGP reduced the increase in aberrant neuronal activity induced by long-



**Figure 4 | LbGP preventative treatment rescues AS-induced aberrant neuronal activity of LHB neurons.**

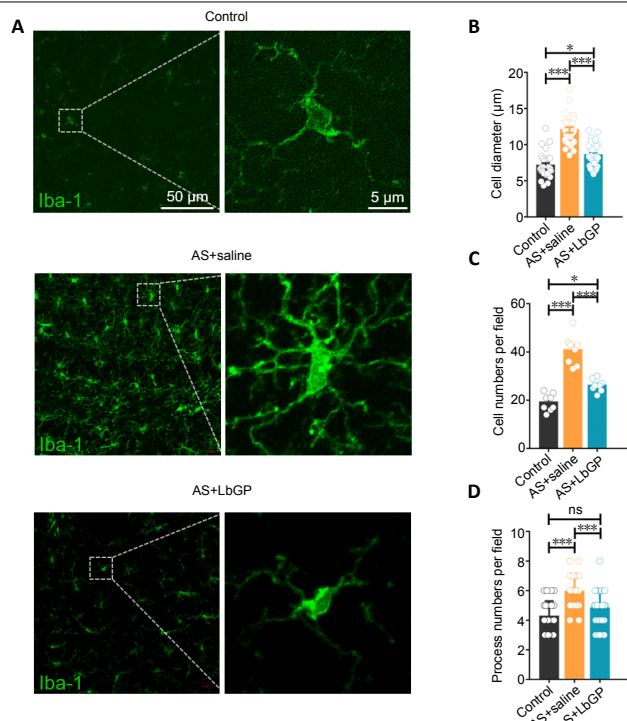
(A) Schematic diagram of the experimental design. LbGP was administered daily by gavage during 28 days of exposure to AS, followed by electrophysiological recordings. (B) The action potentials evoked by current pulses in brain slices of mice from the AS + saline and AS + LbGP groups ( $n = 10$  cells from three mice in the AS + saline group;  $n = 6$  cells from three mice in the AS + LbGP group,  $P = 0.0139$ ). (C) Left: Percentage of the three types of LHB neurons in mice from the AS + saline and AS + LbGP groups ( $n = 41$  cells from four mice in the AS + saline group;  $n = 39$  cells from four mice in the AS + LbGP group,  $P = 0.0435$ ). Right: Spontaneous firing frequency of burst neuron ( $n = 8$  cells from three mice for the AS + saline group;  $n = 7$  cells from three mice for AS + LbGP group,  $P = 0.0026$ ). Data are expressed the mean  $\pm$  SEM and analyzed by Student's  $t$ -test (B and C-right) or chi-square test (C-left). \* $P < 0.05$ , \*\* $P < 0.01$ . AS: Aversive stimuli; LbGP: *Lycium barbarum* polysaccharide-glycoprotein; LHB: lateral habenula.

term exposure to AS. The LHB consists of many different cell types (Weiss and Veh, 2011). Nevertheless, in this study, the neurons for recording in LHB were randomly chosen and did not characterize a specific subset of neurons whose activity might be modulated by LbGP. Identifying whether any neuronal type responds selectively should be determined in future studies.

Accumulating evidence indicates that glial cells, including astrocytes and microglia, play a pivotal role in the regulation of neuronal activity in the brain (Allen and Lyons, 2018; Fan et al., 2019; Cragolini et al., 2020). In addition, microglia, whose activation is involved in inflammatory responses, are considered to be resident macrophage-like immune cells in the brain (Bate et al., 2005). Our results thus confirm previous studies showing that long-term exposure to AS increased microglial activation in the LHB, which could be reduced by LbGP preventative treatment. Combined with the data described in this study, we postulate that LbGP preventative treatment might reduce aberrant neuronal activity in the LHB by reducing inflammatory reactions in the LHB. Indeed, recent studies have found that microglia can regulate neuronal activity by releasing proinflammatory cytokines, including TNF- $\alpha$  and IL-1 $\beta$  (Schäfers and Sorkin, 2008; Vezzani and Viviani, 2015). Future studies should be conducted to determine the causal relationship between the effects of LbGP on microglial inflammatory activation and the effects of LbGP on the neuronal activity of LHB neurons.

In conclusion, we found that LbGP preventative treatment could ameliorate the depressive- and anxiety-like behaviors caused by exposure to long-term AS. LbGP also reduced the aberrant neuronal activity and microglial inflammatory

## Research Article



**Figure 5 | LbGP preventative treatment suppresses microglial activation in the LHB of AS-induced depressant mice.**

(A) Representative figure of microglia (Iba-1<sup>+</sup>, green) in the LHB. (B) Quantitative data of the cell diameter per microglia in LHB:  $n = 29$  cells from three mice of each group,  $P < 0.0001$ ; Control vs. AS + saline:  $P < 0.0001$ , control vs. AS + LbGP:  $P = 0.0137$ , AS + saline vs. AS + LbGP:  $P < 0.0001$ . (C) Number of microglial cells in LHB:  $n = 12$  slices from three mice of each group,  $P < 0.0001$ . Control vs. AS + saline:  $P < 0.0001$ , control vs. AS + LbGP:  $P = 0.0006$ , AS + saline vs. AS + LbGP:  $P < 0.0001$ . (D) Number of processes per microglial cell in LHB:  $n = 30$  cells from three mice of each group,  $P < 0.0001$ . Control vs. AS + saline:  $P < 0.0001$ , control vs. AS + LbGP:  $P = 0.0978$ , AS + saline vs. AS + LbGP:  $P < 0.0021$ . Data are expressed the mean  $\pm$  SEM. \* $P < 0.05$ , \*\*\* $P < 0.001$  (one-way analysis of variance with Šidák's multiple comparisons test). AS: Aversive stimuli; Iba-1: ionized calcium binding adaptor molecule 1; *Lycium barbarum* polysaccharide-glycoprotein; LHB: lateral habenula; ns: no significant difference.

activation in the LHB of the AS mice. These findings may shed light on the potential use of *Lycium barbarum*, a commonly used Chinese herbal medicine, as an alternative preventative intervention for depression and anxiety.

**Author contributions:** Study design: CRR and KFS; behavioral experiments implementation: YWF, YFP, LH, YX, ZFH; physiological recordings: XDH; histology and microscopy: YWF, YFP, YY; data analysis: YWF, YFP, SL, XDH; manuscript writing: CRR, SL. All authors approved the final version of the manuscript.

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**Additional file 1:** Open peer review report 1.

**Additional file 2:** Original data.

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