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Antidepressant effect of helicid in chronic unpredictable mild stress model in rats

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ABSTRACT

Helicid (4-formylphenyl-β-D-allopyranoside) is a bioactive constituent of *Helicid nilgirica* Bedd that has been used in Chinese traditional herbal medicine to treat headache, insomnia, and depression. However, the underlying mechanisms of these effects are unclear. We have now investigated the effect of helicid on depression-related behaviors in rats exposed to chronic unpredictable mild stress (CUMS) and have also explored possible underlying mechanisms that involve neurotrophin expression. After 6 weeks isolation, body weight and sucrose preference were significantly reduced in rats with CUMS-induced depression compared with controls. The CUMS rats also showed significant inhibition of locomotory parameters in open field tests (involving behavioral assays). Helicid significantly regulated levels of corticosterone (CORT), inflammatory cytokines and 5-hydroxytryptamine (5-HT). Helicid also reversed CUMS-induced decreases of 5-HT1A receptor expression and promoted brain derived neurotrophic factor (BDNF) expression in the hippocampus The significant reversal of depressive-like behaviors by helicid is similar to that achieved by fluoxetine. The antidepressive effects are likely attributable to the promotion of hippocampal neurotrophin expression through activation of the serotonergic system. Helicid thus has potential for treating depressive disorders.

1. Introduction

Depression, which involves emotional, cognitive and physical symptoms, and is characterized by sadness, loss of motivation, feelings of worthlessness, guilt and suicidal thoughts, places both emotional and financial costs on society [1].

Current treatments for depression are chiefly tricyclic antidepressants, monoamine oxidase inhibitors, selective norepinephrine reuptake inhibitors and selective serotonin reuptake inhibitors. However, not all the patients respond well to current interventions. The effects of the drugs may also take weeks to manifest and may be accompanied by unwanted side effects [2,3]. The neurobiological underpinnings, molecular pathophysiology and therapeutic mechanisms involved in the action of antidepressant drugs are not fully understood. It is, however, clear that new strategies to treat depression are urgently needed.

Mechanisms of depression and antidepressant action are multifactorial. Chronic stress can cause dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, which is followed by excessive production of glucocorticoids, damage to the hippocampus and depressive-like behaviors [4,5]. Both fluoxetine and imipramine have been shown to improve these behaviors [6]. The relationship between inflammatory processes and depression has been gradually recognized [7]. Evidence suggests that major depressive disorder may be associated with dysregulation of the immune system, and CUMS has been shown to increase levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α, or to significantly decrease levels of 5-HT, noradrenaline and dopamine, in the hippocampus [8,9]. 5-HT receptors, especially 5-HT1A and 5-HT2A, are known to participate in the action of antidepressants [10,11]. It is a widely accepted hypothesis that the antidepressant-induced elevation of monoamine transmitter levels results in enhanced expression of neurotrophins. This leads to increased hippocampal neurogenesis [12,13], and alterations in neuronal plasticity are believed to often be necessary for a therapeutic response [14]. The

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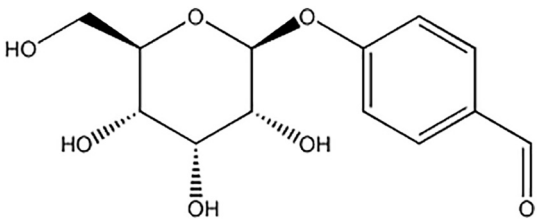

Fig. 1. Structure of helicid ($C_{13}H_{16}O_7$). SID:126631785 [CID:7573800]. MW = 284.2 g/mol, melting point = 199 °C–200 °C.

transcription factor cyclic adenosine monophosphate (cAMP)-response element binding protein (CREB) is critical for cell survival and neuroplasticity, as well as for adaptation of neurons and neuronal networks in both short- and long-term responses to environmental stimuli. These responses are induced via activation of different signaling mechanisms, including cAMP-dependent protein kinase A (PKA), extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) and Ca^{2+}/calmodulin-dependent protein kinase pathways [15–17]. The downstream protein factor, BDNF, was found to be decreased in the CUMS model and gradually restored after antidepressant treatment [18,19].

Recently, studies have been undertaken to identify effective antidepressants from medicinal and herbal plants and it has been suggested that traditional Chinese medicine may be somewhat helpful for depression. The underlying mechanisms may involve modification of monoamine neurotransmitters in the HPA axis via hippocampal neurons and neurotrophic factors [20,21]. There are thus many potential targets for the treatment of depression.

Helicid (4-formylphenyl-β-D-allopyranoside) (Fig. 1), which is a major constituent of Helicia nilgirica Bedd [22], is widely recognized for its sedative-hypnotic and analgesic properties and it has also been used to treat psychoneurosis [23,24]. Both helicid and its derivative, W0620, have been shown to have antidepressant effects in the mouse tail suspension test and the forced swimming test (FST) [25–27]. Helicid reversed depression-like behaviors, including deficient locomotion, reduced sucrose preference, reduced emotional behavior scores and loss of body weight [28], and significantly increased BDNF expression and the number of bromodeoxyuridine (BrdU)-positive cells in the hippocampal dentate gyrus of chronically stressed mice [29]. The antidepressant-effects of helicid in the CUMS model have, however, not yet been investigated. This is the first study to investigate the effect of helicid on depressive-related behaviors in rats exposed to CUMS and to explore the possible underlying molecular involving neurotrophin expression.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (180–220 g) were supplied by the Experimental Animal Center of Zhejiang Province, China. The animals were offered food and water ad libitum and maintained under standard laboratory conditions (12-h light/12-h dark cycle with lights on at 08:00 h, temperature 22 ± 2 °C, relative humidity 50 ± 10%). The animals were acclimated for 1 week, then randomly divided into different groups. All animal protocols were carried out in strict accordance with the guidelines established by the National Institutes of Health and were approved by the Wannan Medical College Animal Care Committee. The experimental timelines are depicted in Fig. 2.

2.2. Drugs and reagents

Helicid (purity > 99.9%) was supplied by Kunming Baker Norton Pharmaceutical Co., Ltd., Kunming, Yunnan Province, China. The solubility was > 300 mg/mL and the bioactivity of a solution in sterile water remained unchanged after > 2 years at room temperature. Fluoxetine hydrochloride (FLU) was purchased from Sigma-Aldrich (St Louis, MO, USA). TNF-α, IL-1β and IL-6 enzyme linked immunosorbent assay (ELISA) kits were obtained from RayBiotech, Inc., (Norcross, GA, USA). The CORT ELISA kit was supplied by R&D Systems (Minneapolis, MN, USA). Anti-ERK, anti-phosphorylated-ERK, anti-CREB, anti-phosphorylated-CREB and anti-BDNF were obtained from Cell Signaling Technology (Beverly, MA, USA). Anti-5-HT1A and anti-5-HT2A were supplied by ABCAM (Cambridge, MA, USA). Horse radish peroxidase-conjugated secondary antibodies were purchased from ABclonal (Boston, MA, USA).

2.3. Groups and treatment

After the acclimation phase, the rats (n = 60) were randomly divided into CUMS (n = 50) and control groups (n = 10). CUMS animals were housed singly and subjected to 6 weeks of unpredictable chronic mild stressors. Control animals were housed 4 per cage and were not stressed. At week 7, the CUMS group was randomized into five subgroups (n = 10): CUMS + saline, CUMS + helicid (8, 16, 32 mg/kg/d) and CUMS + FLU (5 mg/kg/d). The animals were then dosed intragastrically with saline (controls), a saline solution of helicid, or a saline solution of fluoxetine once daily for 6 weeks, using a lavage apparatus. CUMS was applied continually, and normal controls received no treatment. Doses of helicid and fluoxetine were adjusted for the animal model as described in the literature, and doses were pre-tested prior to experiments [29,30].

2.4. CUMS procedure

The CUMS procedure used in the present study was performed as described previously [31], with modifications described in Table 1. Briefly, rats for CUMS were housed singly and exposed daily to one of the following stressors: 24 h periods of water or food deprivation, lights on overnight, wet bedding overnight, tilted cage overnight, unpredictable shocks (15 mA, one shock/20 s, 10 s duration for a total of 20 min), cold water (15 °C) swimming for 5 min, tail suspension for 1 min, tail pinch for 1 min, and reversal of diurnal cycle. Stress treatment was carried out for 12 weeks, in a pseudorandom order, between 8:30 am and 10:30 am. The control animals were housed in a separate room and had no contact with the stressed animals.

2.5. Sucrose preference test

The sucrose preference test (SPT) was used to assess anhedonia, which occurs in several affective disorders, including depression. Testing was performed at baseline as well as 2, 4, 6, 8, 10 and 12 weeks after initiation of CUMS. Briefly, the rats were housed and trained to drink a 1% sucrose solution by providing them with sucrose solution instead of water for 48 h. The rats then underwent a series of SPTs, which were preceded by 22 h of food and water deprivation. Each animal was presented simultaneously with two bottles, one containing 1% sucrose solution and the other containing water. The sucrose preference of the animals preferring sucrose was quantified as follows: % sucrose preference = sucrose solution consumption/(sucrose solution consumption + water consumption) × 100.

2.6. Open field test

The open field test (OFT) is frequently used to qualitatively and quantitatively measure general locomotor activity and willingness to explore in rodents [32]. Rats in the open field were monitored using an automated activity monitoring system (SuperMaze, Laboratory Animal Behavioral Analysis System, Shanghai, China). Each rat was placed at a randomized starting point in the center of a square black apparatus

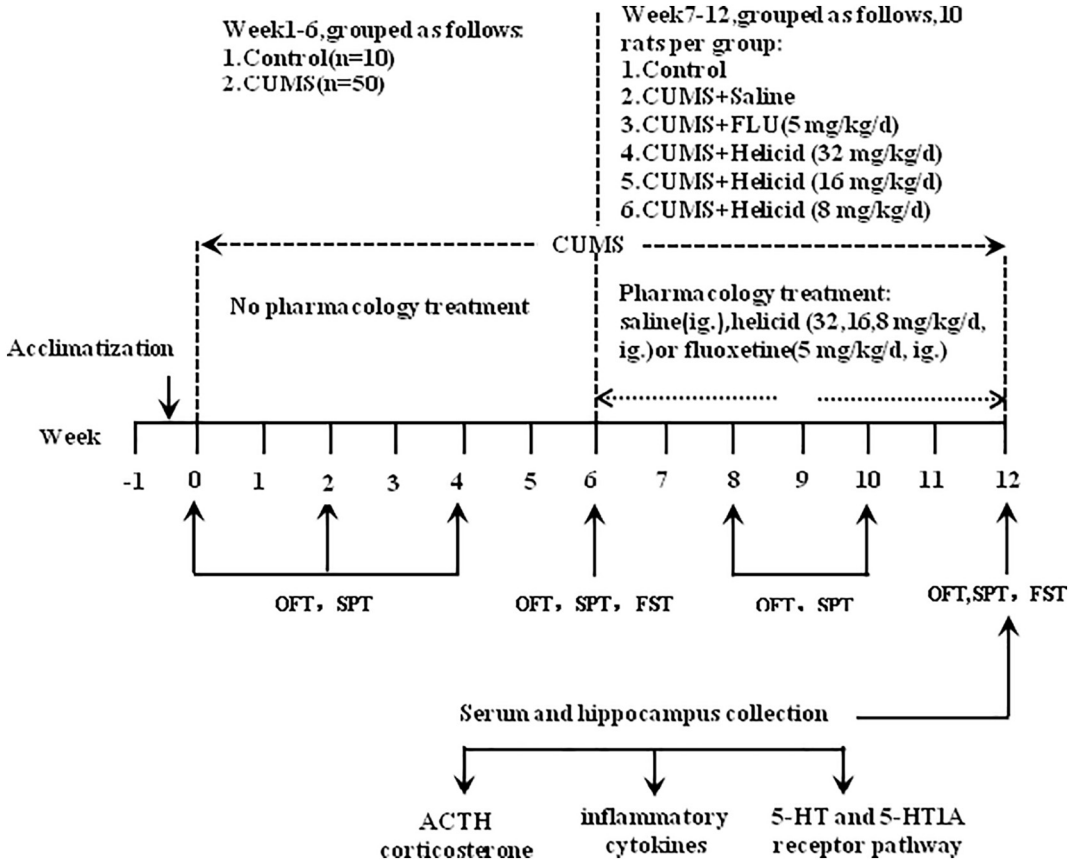

Fig. 2. Experimental timeline. Rats were acclimatized for 1 week prior to the experiment. CUMS lasted for 12 weeks (except for control rats). OFT and SPT were carried out every 2 weeks as described in the text, FST was performed at week 6 and week 12. From week 7 to 12, rats were dosed with saline, helicid, or fluoxetine, except for the control rats. After 6 weeks treatment, rats were anesthetized and killed by decapitation, and serum and hippocampus tissues were collected.

(100 cm × 100 cm), with walls 50 cm high. Animal behaviors were recorded automatically for 5 min and analyzed using the software provided. Total distance traveled and rearing were used as indicators of spontaneous activity, and zone crossing was used as an indicator of willingness to explore. All activities were recorded by a video camera mounted above the open field and scored in real-time by an advanced motion recognition software package that detects and analyzes movement. Between tests, the apparatus was cleaned with ethanol and water to remove olfactory cues.

2.7. Forced swimming test

The FST is widely used for evaluating depressive behavior and the efficacy of antidepressant drugs in rodents. The test is based on the observation that animals in an enclosed environment become immobile after an initial period of vigorous activity, and that the duration of immobility is reduced by effective antidepressant drugs [33]. Rats were subjected to a pretest session for 15 min followed by a 5 min test session 24 h later. The rats were individually placed in a cylinder (60 cm in height, 20 cm in diameter) filled with water at 25 °C to a depth of 30 cm. The duration of immobility during the 5 min test session was measured. Immobility was defined as the absence of all movement, except minor movements required for the rat to keep its head above the surface of the water.

Table 1
Chronic unpredictable mild stress paradigm.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week1	F	H	B	A	G	D	C
Week2	B	E	F	C	G	H	A
Week3	C	F	E	B	D	A	G
Week4	A	F	H	B	G	E	D
Week5	C	A	H	F	E	D	B
Week6	B	G	D	A	C	B	F
Week7	A	E	B	C	H	F	G
Week8	A	C	E	B	F	D	G
Week9	B	C	A	D	E	F	G
Week10	C	E	D	F	A	B	E
Week11	H	C	A	B	F	G	D
Week12	E	A	C	B	F	D	G

A, 24 h periods of water or food deprivation; B, wet bedding overnight; C, tilted cage overnight; D, unpredictable shocks (15 mA, one shock/20 s, 10 s duration for a total of 20 min); E, cold water (4 °C) swimming for 5 min; F, tail suspension for 1 min; G, tail pinch for 1 min; H, reversal of diurnal cycle.

2.8. Collection of serum and hippocampus

Blood samples were collected from all animals after 6 weeks treatment with helicid or fluoxetine. When the behavioral tests were finished, the rats were anesthetized by intraperitoneal administration of 10% chloral hydrate (300 mg/kg) and killed by decapitation. Blood samples were immediately collected and serum was obtained by centrifugation at 3000*g* for 10 min. The bilateral hippocampi were quickly removed and placed in labeled tubes. The serum and hippocampi were quickly frozen in liquid nitrogen for 15 min and then stored in a freezer at −80 °C for further analyses.

2.9. ELISA determination

Serum CORT levels were measured using a CORT ELISA kit, according to the manufacturer's instructions. The sensitivity of the measurement was 47 ng/mL. The intra- and inter-assay coefficients of variation were 6.0% and 6.2%, respectively.

The frozen hippocampi were thawed to room temperature, quickly rinsed with physiological saline, homogenized in lysis buffer and then incubated on ice for 30 min. The homogenates were centrifuged at 14000*g* for 20 min at 4 °C and the supernatants were collected for determination of TNF-α, IL-6, IL-1β and 5-HT. Measurements of cytokine levels were conducted in duplicate using ELISA in the same run, to avoid inter-assay variability. All cytokines were assessed using a sandwich ELISA, following the manufacturer's protocol. The plates were measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

2.10. Western blotting

The hippocampi were prepared in a similar manner to that used for the ELISA to measure inflammatory cytokines. Total protein was measured, and the same amount was loaded and separated with 10% SDS-PAGE gels followed by transfer to a polyvinylidene fluoride membrane. After blocking in 5% BSA solution at room temperature for 3 h, membranes were incubated with the appropriate primary antibodies at 4 °C overnight: anti-ERK (1:1000), anti-phosphorylated-ERK (1:2000), anti-CREB (1:1000), anti-phosphorylated-CREB (1:1000) and anti-BDNF (1:5000). The membranes were washed three times with TBS-Tween buffer, incubated with goat anti-mouse (1:5000) or anti-rabbit secondary antibody (1:2000) for 2 h at room temperature, and then washed three times in TBST buffer for 10 min each time. Blots were washed again three times with TBST buffer, and immunoreactive bands were visualized using an ECL detection system (Bio-Rad, Hercules, CA, USA).

2.11. Statistical analyses

All values are presented as mean ± SEM. The Statistical Package for the Social Sciences (IBM, Chicago, IL, USA) and GraphPad Prism5.0 (GraphPad Software, Inc., La Jolla, CA, USA) were used for statistical analyses and graph generation. Data were analyzed using unpaired *t*-test or one-way ANOVA followed by Tukey's *post-hoc* tests. Results were considered to be significant if $p < 0.05$.

3. Results

3.1. Depression-like behaviors after 6 weeks of CUMS

CUMS reduced sucrose preferences to 64.10% of the control value after 6 weeks ($p < 0.001$) (Fig. 3A). CUMS significantly prolonged the duration of immobility in the FST, compared with the control group ($p < 0.05$) (Fig. 3B). Rats exposed to CUMS weighed less than controls from the second week ($p < 0.05$, $p < 0.001$) (Fig. 3C). In the OFT, animals exposed to CUMS covered less distance and exhibited a decreased total distance, number of zone crossings and rearing events, compared with the control group ($p < 0.01$, $p < 0.001$) (Fig. 3D1–D3). All of these results indicate that the depressed rat model had been established.

3.2. Effects of helicid on the behaviors of rats exposed to CUMS

SPT, FST and OFT were performed during the experimental period to examine whether helicid treatment reversed CUMS-induced depression-like behaviors in rats. In the SPT, sucrose preference was significantly reduced in rats subjected to CUMS compared with control animals. The sucrose preference of rats exposed to CUMS that were treated with helicid (32 mg/kg/d) or fluoxetine (5 mg/kg/d) for 6 weeks was restored to a level similar to that of control animals ($p < 0.05$). Although treatment with lower doses of helicid (16 and 8 mg/kg/d) increased sucrose preference, these differences were not statistically significant (Fig. 4A). In the FST, a decrease in immobility times was observed with administration of helicid (32 mg/kg/d) or fluoxetine (5 mg/kg/d) compared with untreated CUMS rats ($p < 0.05$, $p < 0.01$) (Fig. 4B). Rats exposed to CUMS that were treated with helicid (32 and 16 mg/kg/d) or fluoxetine (5 mg/kg/d) had significantly increased body weights compared with untreated CUMS animals ($p < 0.05$, Fig. 4C). In the OFT, CUMS significantly reduced the distance traveled and decreased the number of zone crossings and rearing events compared with control animals. These effects were significantly reversed by treatment of CUMS rats with helicid (32 mg/kg/d) or fluoxetine (5 mg/kg/d) ($p < 0.05$), but no such effects were seen with lower doses of helicid (16 and 8 mg/kg/d) (Fig. 4D1–D3). Based on these results, we concluded that helicid improves CUMS-induced depression-like behavior in rats.

3.3. Effects of helicid on CORT levels

The effects of helicid on CORT levels in rat serum are shown in Fig. 5. CUMS significantly increased levels of CORT compared with the control group ($p < 0.001$). Treatment with helicid (32 mg/kg/d) or fluoxetine (5 mg/kg/d) significantly reduced the level of CORT compared with the CUMS group ($p < 0.05$, $p < 0.01$).

3.4. Effects of helicid on levels of inflammatory cytokines

The effects of helicid on levels of inflammatory cytokines in rat hippocampi are shown in Fig. 6 (A, B, C). CUMS significantly increased levels of TNF-α, IL-6 and IL-1β compared with the control group ($p < 0.001$, $p < 0.001$, $p < 0.01$, respectively). Treatment with helicid (32, 16, 8 mg/kg/d) reduced levels of TNF-α, IL-6, and IL-1β, but only the highest dose (32 mg/kg/d) reached statistical significance ($p < 0.05$, $p < 0.01$, $p < 0.05$, respectively). Treatment with fluoxetine (5 mg/kg/d) also significantly reduced levels of TNF-α, IL-6, and IL-1β ($p < 0.01$, $p < 0.001$, $p < 0.01$, respectively). A reduction in the level of IL-6 was also seen in the helicid (16 mg/kg/d) group ($p < 0.05$).

3.5. Effects of helicid on levels of 5-HT

The effects of helicid on levels of 5-HT in rat hippocampi are shown in Fig. 6(D). CUMS significantly reduced the level of 5-HT compared with the control group ($p < 0.01$). Treatment with helicid (32 mg/kg/d) or fluoxetine (5 mg/kg/d) significantly increased the level of 5-HT compared with the CUMS group ($p < 0.01$ $p < 0.001$).

3.6. Western blot analysis

There was a significant difference in 5-HT1A receptor expression between the CUMS group and the control group ($p < 0.05$). Although CUMS + helicid treatment (8, 16, 32 mg/kg/d) reversed these changes, only CUMS + helicid (32 mg/kg/d) significantly increased

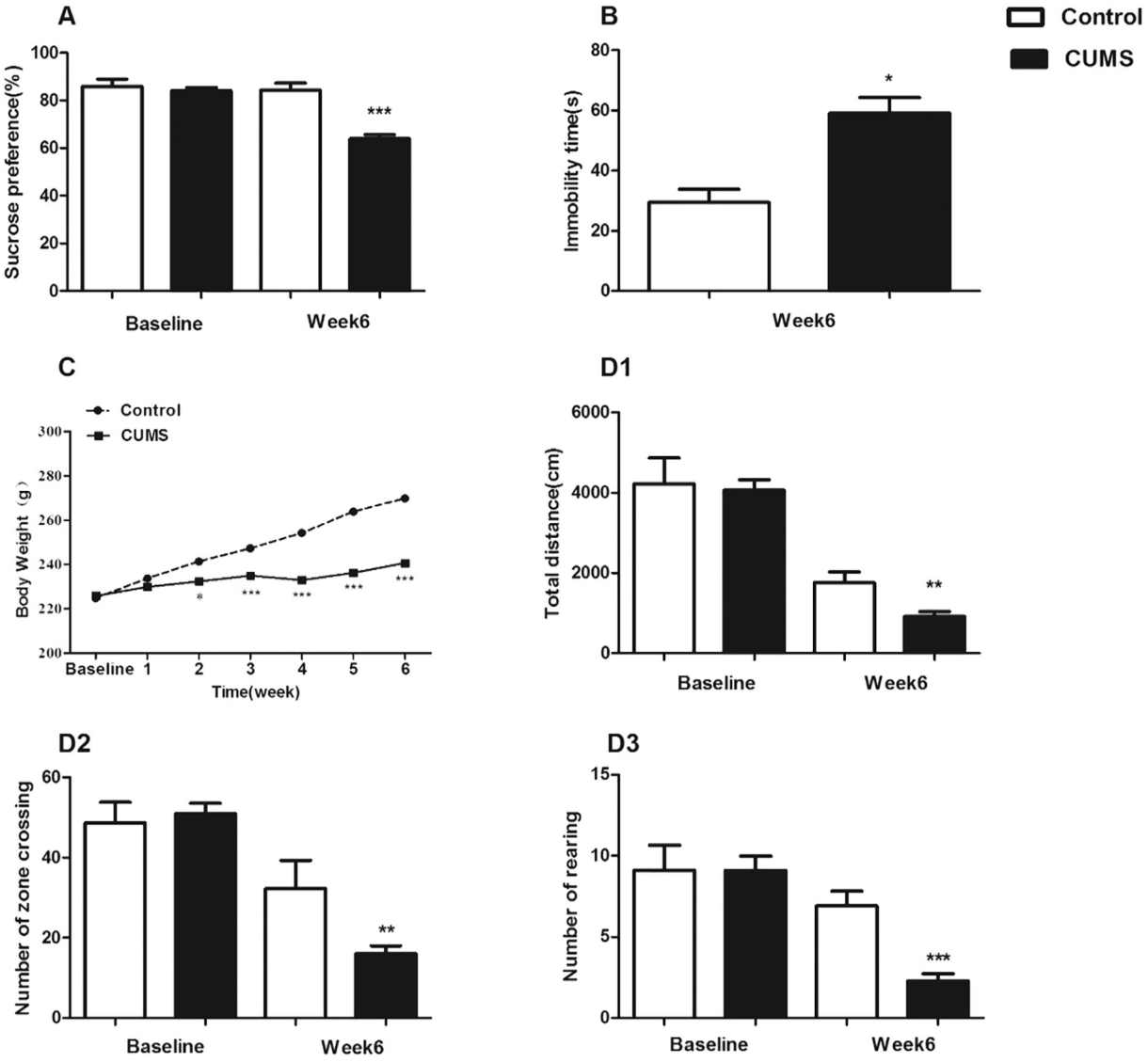

Fig. 3. Depression-like behaviors after 6 weeks of CUMS. Sucrose preferences (A), Forced swimming (B), Body weight (C), OFT: Total distance covered (D1), Zone crossing (D2), Rearing (D3). Data are shown as means ± SEM; n = 10 control, n = 50 CUMS. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control group. Data were analyzed using unpaired *t*-test.

hippocampal 5-HT1A receptor expression ($p < 0.05$). A similar result was observed with fluoxetine treatment. There were no significant differences in 5-HT2A receptor expression between the CUMS, control, CUMS + helicid (32, 16, 8 mg/kg/d) or fluoxetine (5 mg/kg/d) groups ($p > 0.05$). Expression of p-ERK was significantly decreased in the CUMS group ($p < 0.001$) and administration of either helicid (32, 16, 8 mg/kg/d) or fluoxetine (5 mg/kg) increased p-ERK expression. Treatment with helicid (32 mg/kg/d) or fluoxetine (5 mg/kg) significantly increased p-ERK expression compared with the CUMS group ($p < 0.05$, $p < 0.001$). Treatment with helicid (32 mg/kg/d) or fluoxetine (5 mg/kg) was also found to significantly increase p-CREB expression in rats exposed to CUMS (each $p < 0.01$). Importantly, the decrease in BDNF levels in the hippocampus induced by CUMS was reversed by treatment with helicid (32, 16, 8 mg/kg/d) or fluoxetine (5 mg/kg/d), but only helicid (32 mg/kg/d) resulted in significant differences ($p < 0.05$) (Fig. 7).

4. Discussion

Rats reared in isolation and subjected to CUMS were used as a model of depression to compare the effects of an established antidepressant (fluoxetine) with those of helicid, which has not previously been tested in this model [33]. The model is widely used for studying antidepressant candidates because of its reliability [31,34]. Kim's group [35] reported that isolation is relevant to several neuropsychiatric disorders, such as schizophrenia, anxiety and depression. After 6 weeks of chronic mild stress, the animals had a reduced preference for sucrose and did not gain weight rapidly, suggesting depression-like effects. An OFT confirmed that stressed rats covered less total distance and displayed reduced zone crossing and rearing, suggesting a change in emotion or security. Previous studies have shown that chronic treatment with the selective serotonin reuptake inhibitor fluoxetine reversed depression-related behaviors in rats exposed to CUMS and our own data are consistent with these earlier studies [36,37].Notably, chronic treatment with helicid significantly reversed CUMS-induced depression-like behaviors, confirming the antidepressant-like effects of helicid.

Concentrations of cortisol have been reported to be elevated in patients with depression, and these are reduced by antidepressants, such as fluoxetine [38]. We found higher concentrations of plasma CORT in rats subjected to CUMS, and these were significantly reduced by helicid or fluoxetine. Increasing numbers of studies have

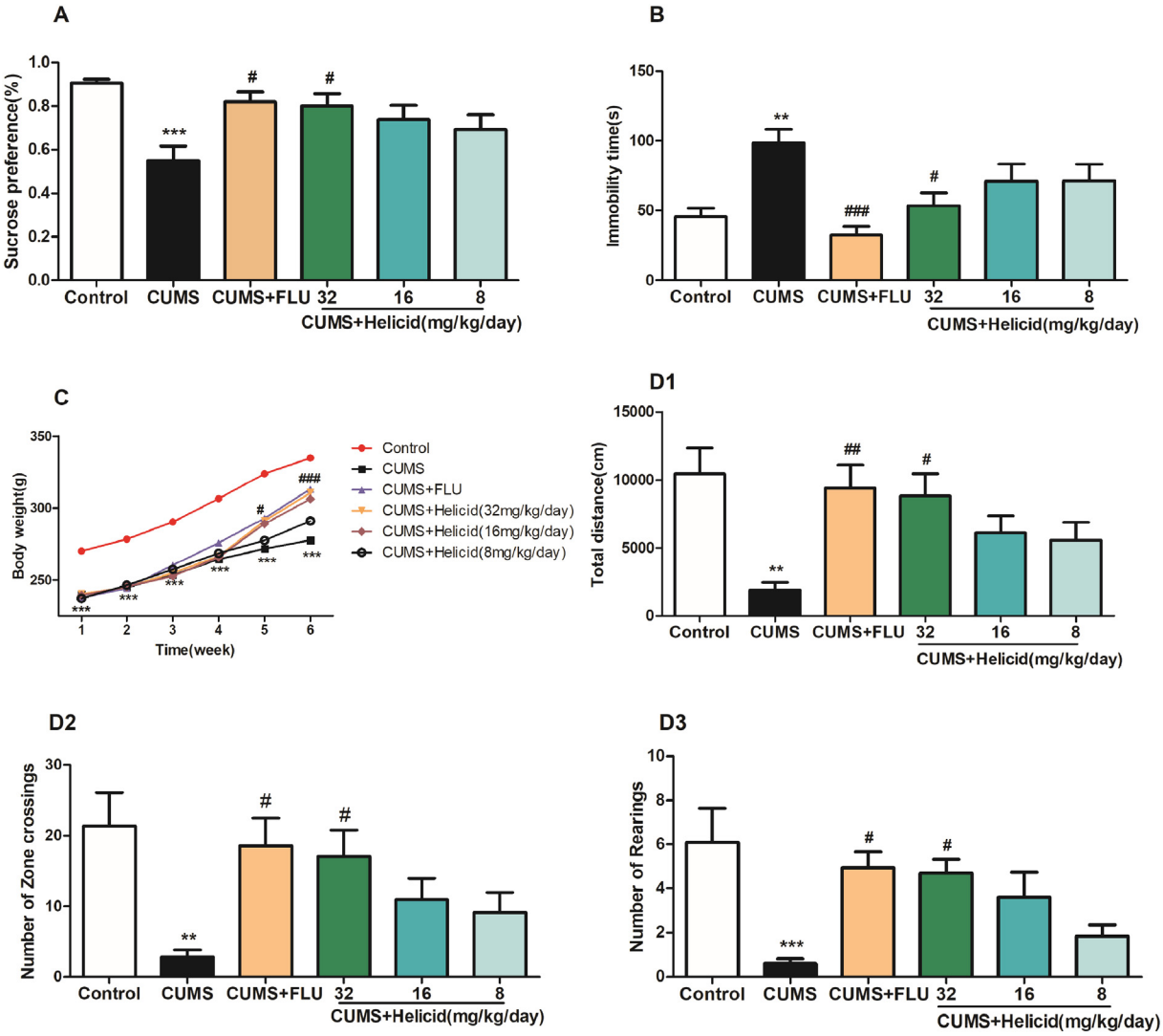

Fig. 4. Effects of helicid on depression-like behaviors of CUMS rats. Sucrose preferences (A), Forced swimming (B), Body weight (C), Open field test: Total distance covered (D1), Zone crossing (D2), Rearing (D3). Control: normal control group, without stressors or any treatment; chronic unpredictable mild stress (CUMS): CUMS group, exposed to stressors and without any treatment; CUMS + FLU (5 mg/kg/d): CUMS + fluoxetine group, exposed to stressors and treated with fluoxetine (5 mg/kg/d) for 6 weeks; CUMS + helicid (8, 16, 32 mg/kg/d): CUMS + helicid group, exposed to stressors and treated with helicid (8, 16, 32 mg/kg/d) for 6 weeks. Data are shown as means ± SEM, n = 10 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$versus CUMS group. Data were analyzed using one-way ANOVA followed by Tukey's *post-hoc* tests.

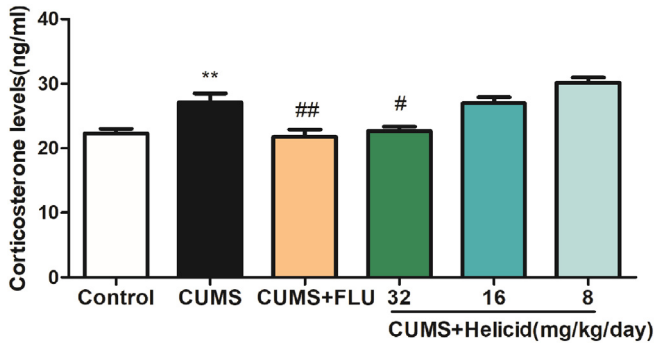

Fig. 5. Effects of 6-week treatment with helicid (8, 16, 32 mg/kg/d) or fluoxetine (5 mg/kg/d) on CORT levels in serum. Values are shown as means ± SEM, n = 10 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with CUMS group. Data were analyzed using one-way ANOVA followed by Tukey's *post-hoc* tests.

demonstrated higher levels of cytokines in patients with depression [39], and we showed here that helicid or fluoxetine can reduce levels of IL-1β, IL-6 and TNF-α in the hippocampi of rats exposed to CUMS.

Previous studies have shown that CUMS often induces alterations in expression of the 5-HT1A receptor, which is thought to play a central role in modulating depressive/anxiety-related behaviors [40]. It has also been demonstrated that the 5-HT1A receptor is required for the effects of serotonergic antidepressants on hippocampal neurogenesis [41]. In our previous study, we found that helicid promoted neurogenesis and upregulation of BDNF in the dentate gyrus [29]. We found that 5-HT1A receptor expression in the hippocampus was downregulated in the CUMS group, but that this was significantly reversed by helicid (32 mg/kg/d), similar to fluoxetine. This indicates that activation of the 5-HT1A receptor might be involved in the adult hippocampal neurogenesis that is responsible for the antidepressant effects of helicid, similar to fluoxetine.

The 5-HT1A receptor directly modulates hippocampal ERK activities, which affects the neurogenesis of newborn cells in the hippocampus [42]. The ERK pathway is commonly associated with neurogenesis and cell survival [43]. ERKs may thus be important for the

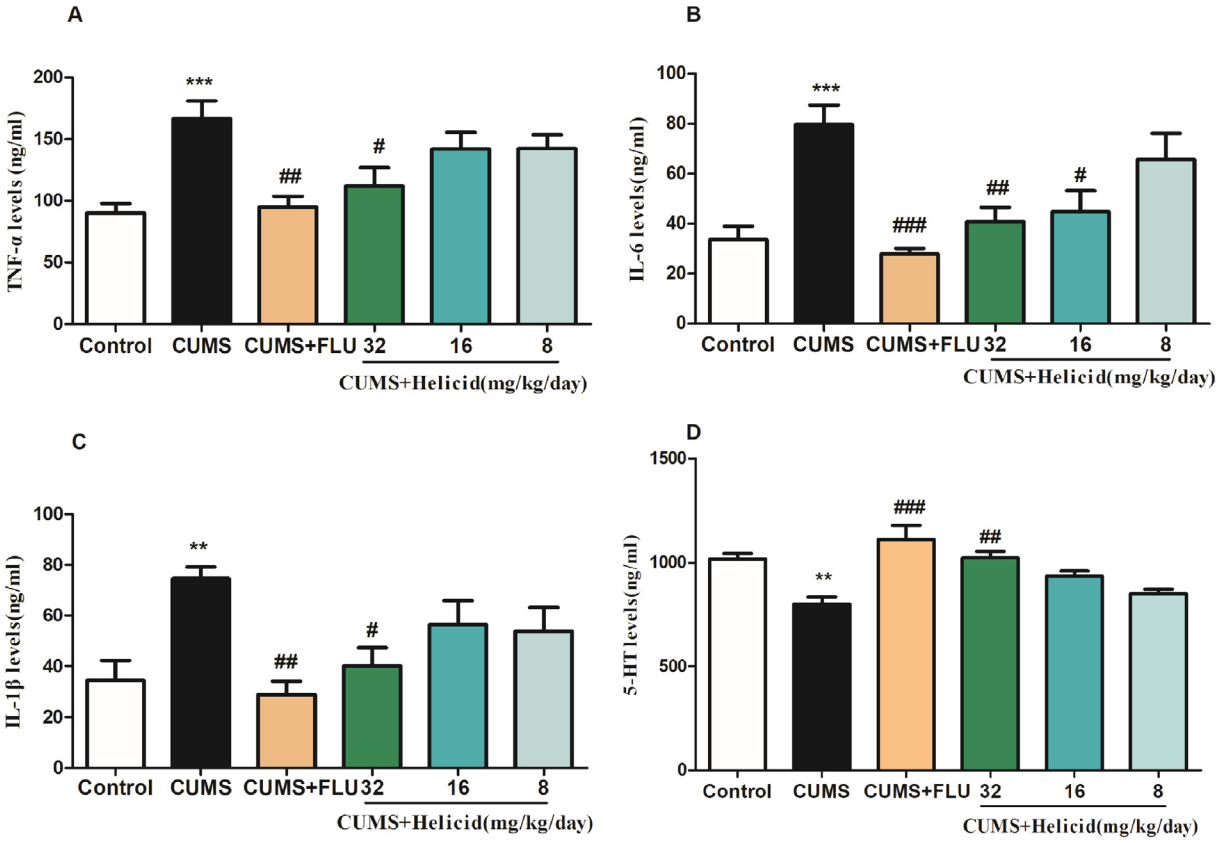

Fig. 6. Effects of 6-week treatment with helicid (8, 16, 32 mg/kg/d) or fluoxetine (5 mg/kg/d) on TNF-α, IL-6, IL-1β and 5-HT levels in hippocampus. Data are shown as means ± SEM, n = 10 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with chronic unpredictable mild stress (CUMS) group. Data were analyzed using one-way ANOVA followed by Tukey's *post-hoc* tests.

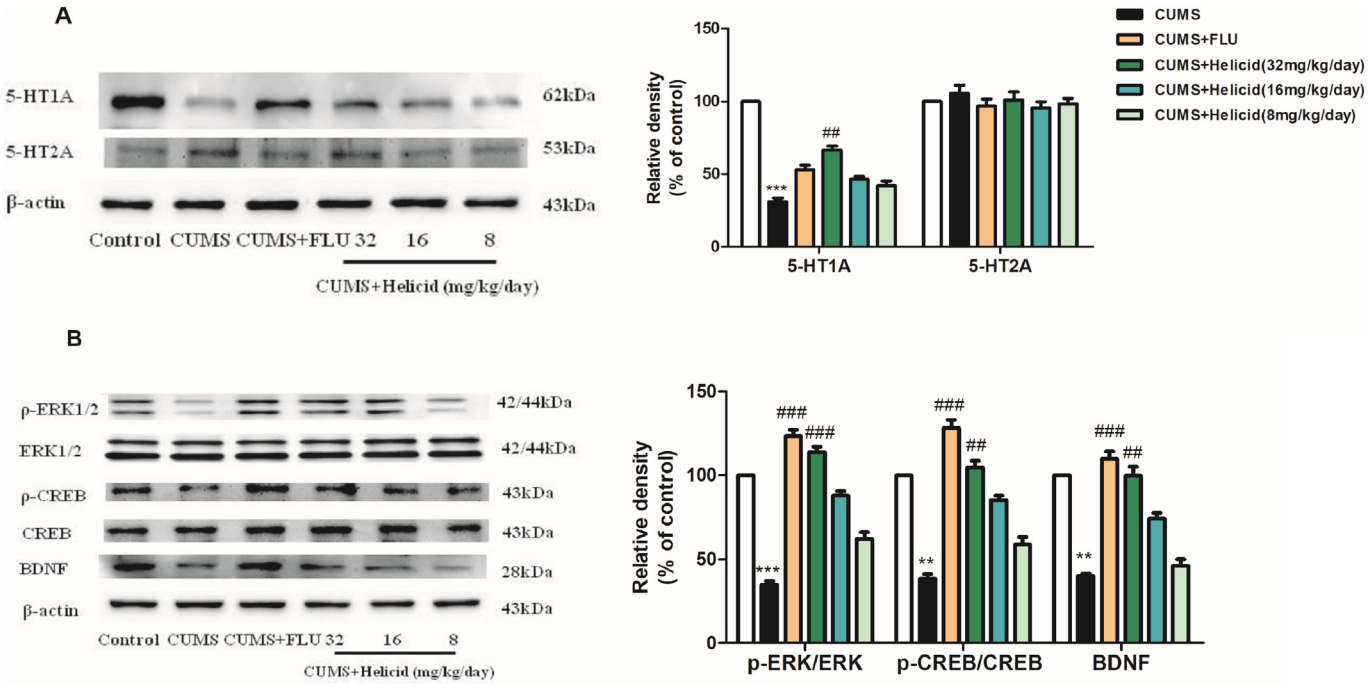

Fig. 7. Effects of 6-week treatment with helicid (8, 16, 32 mg/kg/d) or fluoxetine (5 mg/kg/d) on hippocampal protein expression. 5-HT1A and 5-HT2A (A), p-ERK1/2, p-CREB and BDNF (B). Values are shown as means ± SEM, n = 6 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with chronic unpredictable mild stress (CUMS) group. Data were analyzed using one-way ANOVA followed by Tukey's *post-hoc* tests.

activity of antidepressants and reduction of ERK phosphorylation caused by CUMS may define a pathophysiological mechanism of depression. We found that CUMS-mediated reduction of p-ERK1/2 was substantially recovered by helicid (32, 16, 8 mg/kg/d) or fluoxetine (5 mg/kg/d), although the effect was only statistically significant at the highest dose (32 mg/kg/d) of helicid and fluoxetine (5 mg/kg/d). CREB is a transcription activator that is involved in many signal transduction cascades activated by hormones, growth factors, synaptic activity, and other cellular stimuli implicated in neuronal plasticity. CREB protein expression in the brain has been shown to decrease in models of depression, and this effect was reversed by antidepressants [44]. We have shown that p-CREB was upregulated by helicid (32 mg/kg/d) and fluoxetine (5 mg/kg/d) in the CUMS-induced depression model.

It is also widely acknowledged that BDNF-mediated signaling pathways are implicated in alterations of neuroplasticity evoked by depression and antidepressants. Hippocampal BDNF expression was reduced in mice subjected to stress but increased with antidepressant treatment, and this was also observed in some patients with depression [45]. BDNF may thus be a major therapeutic target for antidepressant responses. We have confirmed that hippocampal BDNF is reduced in rats subjected to CUMS, and that chronic helicid treatment increases BDNF expression, suggesting that BDNF may participate in the antidepressant effects of helicid, which is consistent with our previous study [29], and similar to the effect observed with fluoxetine. Thus, helicid or fluoxetine may modulate ERK/CREB/BDNF signaling in the hippocampus.

In conclusion, helicid significantly reversed CUMS-induced depression-like behaviors in rats. Helicid treatment was found to be associated with changes in expression of IL-1β, IL-6 and TNF-α, and with cell proliferation in the hippocampus. These effects were correlated with increased levels of signaling in ERK/CREB/BDNF pathways, and these were similar to the effects produced by fluoxetine. In this study, the selective serotonin reuptake inhibitor (SSRI) fluoxetine was used as a positive control. Helicid produced similar effects to fluoxetine, although the effects of helicid were slightly less than those of fluoxetine. We speculate, therefore, that helicid might work in a similar way to fluoxetine, and will conduct further follow-up studies to verify this. In the present study, only effects on the hippocampus were used to explore the antidepressant mechanism of helicid. Effects on other brain regions, such as the prefrontal cortex and olfactory bulb [46], which are also affected by the CUMS procedure, and reversal of these changes by antidepressants, should also be considered. Future studies may confirm these suggested mechanisms at the gene level, and studies of other brain areas, other signaling pathways and blocker trials will be needed to fully elucidate the antidepressant mechanism of helicid.

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Conflicts of interest

The authors declare no conflicts of interest.

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