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# Harmine produces antidepressant-like effects via restoration of astrocytic functions

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ABSTRACT

Depression is a world-wide disease with no effective therapeutic methods. Increasing evidence indicates that astrocytic pathology contributes to the formation of depression. In this study, we investigated the effects of harmine, a natural β-carboline alkaloid and potent hallucinogen, known to modulate astrocytic glutamate transporters, on chronic unpredictable stress (CUS)-induced depressive-like behaviors and astrocytic dysfunctions. Results showed that harmine treatment (10, 20 mg/kg) protected the mice against the CUS-induced increases in the immobile time in the tail suspension test (TST) and forced swimming test (FST), and also reversed the reduction in sucrose intake in the sucrose preference experiment. Harmine treatment (20 mg/kg) prevented the reductions in brain-derived neurotrophic factor (BDNF) protein levels and hippocampal neurogenesis induced by CUS. In addition, harmine treatment (20 mg/kg) increased the protein expression levels of glutamate transporter 1 (GLT-1) and prevented the CUS-induced decreases in glial fibrillary acidic protein (GFAP) protein expressions in the prefrontal cortex and hippocampus, suggesting that restoration of astrocytic functions may be a potential mechanism underlying the antidepressant-like effects of harmine. This opinion was proved by the results that administration of mice with L-Alpha-Aminoadipic Acid (L-AAA), a gliotoxin specific for astrocytes, attenuated the antidepressant-like effects of harmine, and prevented the improvement effects of harmine on BDNF protein levels and hippocampal neurogenesis. These results provide further evidence to confirm that astrocytic dysfunction contributes critically to the development of depression and that harmine exerts antidepressant-like effects likely through restoration of astrocytic functions.

## 1. Introduction

Major depression is a common disease affecting numerous persons in the world-wide. At present, nearly all of the clinical antidepressants are developed out of the monoaminergic deficit hypothesis of depression, and these agents have been used as classical antidepressants for a very long time (Lanni et al., 2009; Kern et al., 2012). However, more and more studies and clinical reports show that these antidepressants exhibit numerous limitations (Fava, 2010; Fabbri et al., 2013; Sanchez et al., 2015). For example, the first application of traditional antidepressants is effective only in about one-third of patients, and approximately two-third of patients fail to achieve clinical improvements after trying several times (Schwartz et al., 2016). Thus, it is necessary to develop novel mechanism-based antidepressants in order to improve the present status of drug therapy of depression.

Increasing evidence indicates that astrocytic dysfunction is actively involved in the pathogenesis of depression. Decreased numbers of hippocampal astrocytes have been observed in rodents treated with chronic stresses or maternal deprivation (Ye et al., 2011; Leventopoulos et al., 2007). Post-mortem studies of tissues from depressed patients

*Abbreviations:* ALS, amyotrophic lateral sclerosis; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CUS, chronic unpredictable stress; DCX, doublecortin; DMSO, Dimethyl Sulphoxide; EAATs, excitatory amino-acid transporters; FITC, fluorescein isothiocyanate; FST, forced swimming test; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; GLAST, glutamate/aspartate transporter; GLT-1, glutamate transporter-1; GS, glutamine synthetase; L-AAA, L-Alpha-Aminoadipic Acid; TST, tail suspension test

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describe reduced numbers of astrocytes in the brain (Oh et al., 2012; Ménard et al., 2016), and these reductions are supported by the finding that the glial fibrillary acidic protein (GFAP)-expressing cells are reduced by chronic stresses in both prefrontal cortex and hippocampus (Banasr and Duman, 2008; Ardalan et al., 2017). In other studies, researchers show that expressions of glial-specific excitatory amino-acid transporters (EAATs: $EAAT_1$/glutamate/aspartate transporter (GLAST), $EAAT_2$/glutamate transporter-1 (GLT-1)) and glutamine synthetase (GS) can be altered in brain tissues from depressed patients (Choudary et al., 2005; Bernard et al., 2011). Functionally, the astrocytic abnormality has been shown to induce a decrease in glutamate uptake and cycling, an accumulation of glutamates, as well as an impairment in brain-derived neurotrophic factor (BDNF) signals and hippocampal neurogenesis (Lutgen et al., 2016; Martin et al., 2012). Thus, inhibition of astrocytic dysfunction may be a potential strategy for depression therapy.

Harmine is a hallucinogenic alkaloid found in the seed of *Peganum harmala* and *Banisteriopsis caapi*, both of which are traditionally used for ritual and medicinal preparations in the Middle East, Central Asia, and South America (Sourkes, 1999). In past years, a wide range of pharmacological effects of harmine, such as antioxidation (Moura et al., 2007; Kim et al., 2001), antigenotoxicity (Moura et al., 2007), and antidiabetes (Waki et al., 2007), have been revealed. Preclinical findings show that harmine has potential antidepressant-like activities in acute and chronic depression models (Fortunato et al., 2009; Fortunato et al., 2010; Aricioglu and Altunbas, 2003; Farzin and Mansouri, 2006). More strong correlations between harmine and depression have been evidenced by recent studies in depressed patients, in which a single dose of ayahuasca, a harmine-containing hallucinogenic plant, has been reported to produce rapid and sustained antidepressant-like activities (Osório Fde et al., 2015; Sanches et al., 2016). Mechanistically, the antidepressant-like effects of harmine may be mediated by restoration of BDNF signals (Fortunato et al., 2009; Fortunato et al., 2010). An inverse-agonistic mechanism located in the benzodiazepine receptors may also mediate the antidepressant-like effects of harmine, as flumazenil, an inhibitor of the benzodiazepine receptor, has been shown to abrogate the antidepressant-like effects of harmine in the forced swimming test (FST) (Aricioglu and Altunbas, 2003). Recently, several different studies show that harmine increases GLT-1 gene and protein expression as well as glutamate uptake activity in animal models of amyotrophic lateral sclerosis (ALS) (Li et al., 2011) and cerebral ischemia (Sun et al., 2014), suggesting that harmine may exert neuroprotective effects via enhancement of GLT-1 functions.

Within these contexts, we hypothesized that harmine exerts antidepressant-like effects likely through restoration of astrocytic functions. As anticipated, we showed that harmine not only prevented mice depressive-like behaviors, but also reversed the reductions in BDNF protein levels and hippocampal neurogenesis induced by CUS. Harmine also prevented CUS-induced decreases in GFAP protein expressions and up-regulated GLT-1 protein expressions in mice hippocampus and prefrontal cortex. Specific inhibition of astrocytic functions abrogated the protective effects of harmine in depressed mice. Since astrocytes contribute critically to the integrity of neuronal functions (Ben Haim and Rowitch, 2017; Yang et al., 2015), our studies indicate that the astrocyte may be a potential target for the antidepressant-like effects of harmine.

## 2. Materials and methods

### 2.1. Animals

8–10 weeks old male C57BL/6J mice were housed five per cage under standard conditions (12-h light/dark cycle; lights on from 07:00 to 19:00; 23 ± 1 °C ambient temperature; 55 ± 10% relative humidity) for 1 week with free access to food and water. Each experimental group consisted of 10 mice. Behavioral experiments were carried out during the light phase. Animal experiments were conducted in accordance with internationally accepted guidelines for the use of animals in toxicology as adopted by the Society of Toxicology in 1999 and approved by the University Animal Ethics Committee of Nantong University (Permit Number: 2110836).

### 2.2. Materials

Harmine and fluoxetine were purchased from MedChem Express (Princeton, NJ, USA). L-Alpha-Aminoadipic Acid (L-AAA) was the product of Sigma (Saint Louis, MO, USA). The doses of fluoxetine, harmine and L-AAA were chosen as previous studies (Takada and Hattori, 1986; Khurgel et al., 1996; Jiang et al., 2015a, 2015b). Harmine and fluoxetine were prepared in 10% dimethyl sulphoxide (DMSO) and were administered intraperitoneally (i.p.) in a volume of 10 mL/kg. L-AAA was dissolved in a phosphate buffer and was administered intracerebroventricularly (i.c.v.). Antibodies against doublecortin (DCX), GFAP, GS, GLAST, and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibody against GLT-1 was the product of Abcam (Cambridge, MA, USA). Hoechst 33,258 was purchased from Santa Cruz Biotechnology (Santa Cruz, California, USA).

### 2.3. Tail suspension test (TST) and FST

The TST and FST were performed according to the methods of Steru (Steru et al., 1985) and Porsolt (Porsolt et al., 1977), respectively. In the FST, the experimental mice were individually placed in a clear glass cylinder (height 25 cm, diameter 10 cm) filled to 10 cm with water at 25 ± 1 °C for 6 min. In the TST, the experimental mice were suspended 50 cm above the floor for 6 min by adhesive tape placed approximately 1 cm from the tip of the tail 2 h after the last injection. The immobile time was recorded during the last 4 min by an investigator blind to the study. For FST, the immobile time was defined as the time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above the water. For TST, the mice were considered immobile only when they hung passively and were completely motionless, and any mice that try to climb their tails were removed from statistical analysis. The concrete schematic diagram for the experimental timeline in the TST and FST in CUS models was presented in Fig. 1A.

### 2.4. Chronic unpredictable stress (CUS)

The CUS, consisting of daily exposure to two of the following stressors in a random order over a 5-week period: cage shaking (1 h), lights on during the entire night (12 h), placement in cold room (4 °C, 1 h), mild restraint in small cages (2 h), 45° cage tilt (14 h), lights-off during the daylight phase (3 h), wet cage (14 h), flashing light (6 h), noise in the room (3 h), and water deprivation during the dark period (12 h), was used to induce depressive-like behaviors in C57BL/6J mice.

### 2.5. Sucrose preference experiment

The sucrose preference experiment was performed at day 36. The experimental mice were given the choice to drink from two bottles in individual cages, one with 1% sucrose solution and the other with water (Yang et al., 2017). All were acclimatized for 2 days to two-bottle choice conditions, and the position of two bottles was changed every 6 h to prevent possible effects of side preference in drinking behavior. The experimental mice were then deprived of food and water for 24 h, and on day 39, the mice were exposed to pre-weighed bottles for 1 h with their position interchanged. Sucrose preference was calculated as a percentage of the consumed sucrose solution relative to the total amount of liquid intake. The schematic diagram for sucrose preference experiment was presented in Fig. 1A.