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Antidepressant-like effects and possible mechanisms of amantadine on cognitive and synaptic deficits in a rat model of chronic stress

Mei Yu*, Yuan Zhang*, Xiaoyu Chen, and Tao Zhang

College of Life Sciences and Key Laboratory of Bioactive Materials Ministry of Education, Nankai University, Tianjin, PR China

Abstract

The aim of this study was to examine whether amantadine (AMA), as a low-affinity noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, is able to improve cognitive deficits caused by chronic stress in rats. Male Wistar rats were divided into four groups: control, control + AMA, stress and stress + AMA groups. The chronic stress model combined chronic unpredictable stress (CUS) with isolated feeding. Animals were exposed to CUS continued for 21 days. AMA (25 mg/kg) was administrated p.o. for 20 days from the 4th day of CUS to the 23rd. Weight and sucrose consumption were measured during model establishing period. Spatial memory was evaluated using the Morris water maze (MWM) test. Following MWM testing, both long-term potentiation (LTP) and depotentiation were recorded in the hippocampal CA1 region. NR2B and postsynaptic density protein 95 (PSD-95) proteins were measured by Western-blot analysis. AMA increased weight and sucrose consumption of stressed rats. Spatial memory and reversal learning in stressed rats were impaired relative to controls, whereas AMA significantly attenuated cognitive impairment. AMA also mitigated the chronic stress-induced impairment of hippocampal synaptic plasticity, in which both the LTP and depotentiation were significantly inhibited in stressed rats. Moreover, AMA enhanced the expression of hippocampal NR2B and PSD-95 in stressed rats. The data suggest that AMA may be an effective therapeutic agent for depression-like symptoms and associated cognitive disturbances.

Keywords

Amantadine, chronic stress, cognition, synaptic plasticity, NR2B, PSD-95

Introduction

Stressful events predispose individuals to a variety of mental disorders, including depression, which is one of the most serious and recurring psychiatric disorders with tremendous personal and socioeconomic consequences. Exposure to aversive experiences induces profound behavioral changes in humans and rodents, including depressive-like symptoms, anxiety and cognitive deficits, which are paralleled by physiological abnormalities (Mizoguchi et al., 2000; Quan et al., 2011a). Based on these observations, the chronic unpredictable stress (CUS) animal model has been developed to mimic the development and progress of clinical depression (Willner, 1997). Moreover, there is considerable evidence that cognition deficits are an essential component of major depression, and antidepressant treatment may mitigate cognitive impairments in parallel to mood improvement of depressive patients (Airaksinen et al., 2004). Rodents that are exposed to chronic unpredictable stressors may elicit depression-like symptoms (Willner, 2005). When the intensity and duration of stressors on brain structures exceed a certain individual-specific threshold, they can impair hippocampal-dependent spatial memory (Kim & Diamond, 2002; McEwen & Magarinos, 2001). There is strong evidence that stress-induced effects on cognition are at least in part mediated by the stress-evoked excessive release of glutamate (Sapolsky, 2000; Szakacs et al., 2012). Meanwhile, synaptic plasticity, including long-term potentiation (LTP) and long-term depression, is impaired by CUS (Alfarez et al., 2003; Marsden, 2013). Existing studies suggest that synaptic weakening, manifest as depotentiation (defined activity-dependent reversal of previously established LTP) in the hippocampus, is required for cognitive flexibility (An et al., 2013a) and may be impacted by stress as well. Stress-associated cognitive deficits are observed with alterations of protein expression. The NR2B receptor plays an important role in the regulation of LTP (Fox et al., 2006). As a major postsynaptic scaffolding protein and principal mediators of the N-methyl-D-aspartate (NMDA) receptor, postsynaptic density protein 95 (PSD-95) is also essential for synaptic plasticity in the hippocampus (Ehrlich et al., 2007). It is well known that a reduction of these proteins expression in the synapse can impair plasticity and cognition.

Amantadine (AMA) is a low-affinity noncompetitive NMDA receptor antagonist that is widely used in treatment...
of Parkinson’s disease. Previous studies indicate that AMA has an antidepressant activity (Huber et al., 1999; Moryl et al., 1993). Moreover, AMA can enhance the therapeutic effect of antidepressants when included as an adjunct therapy in treatment-resistant depression (Rogoz et al., 2007; Stryjer et al., 2003). One of our previous investigations reports that memantine and its derivatives exhibit an antidepressant effect and reverse the behavioral and synaptic deficits in the CUS rats, but with side-effects (Quan et al., 2011b). Hence, we focused on exploring the potential therapeutic effects of AMA in impeding the cognition deficits and aberrant synaptic plasticity following chronic stress in the rodent. A hypothesis was raised that AMA was able to effectively impede the development of depression-like behavior and the spatial cognitive deficits, and to some extent restore impaired synaptic plasticity in stressed rats. Accordingly, a rat model of chronic stress was established. Both body weight and sucrose consumption were measured. Afterwards, spatial learning and memory were tested with Morris water maze (MWM). The LTP and depotentiation from hippocampal Schaffer collaterals to CA1 region were recorded to examine the bidirectional synaptic plasticity. Finally, the protein expression of NR2B and PSD-95 in the hippocampus was assayed by Western to explore the possible mechanism.

Materials and methods

Animal and drug treatments

Adult male Wistar rats weighing 220–230 g were purchased from the Laboratory Animal Center, Academy of Military Medical Science of People’s Liberation Army. Before all experiments, animals were allowed 3 days of habituation and kept in groups of four to five under standard laboratory conditions (24 ± 2 °C room temperature, 12 h light/dark cycle with lights on at 7:00 a.m., and freedom to food and drink) in the Medical School of Nankai University. All procedures adhere to the National Institute of Health Guide for the Care and Use of Laboratory Animals, and are approved by the Committee for Animal Care at Nankai University. Every effort has been made to minimize animal suffering and the number of animals.

After the habituation, they were arbitrarily divided into four groups: control group (Con), control with AMA group (Con + AMA), stress group (Str) and stress with AMA group (Str + AMA). The animal model of stress was established by combining CUS and isolated feeding. The animals of both Str and Str + AMA groups were reared in one cage separately with each individual, and the rats of other two groups were kept in groups of four to five. AMA was bought from Jiangsu Peng Harrier Pharmaceutical Corporation, China. The rats treated with AMA in both the Con + AMA and Str + AMA groups were administered daily by the gavage for 20 days at a dose of 25 mg/kg dissolved in water from the 4th day of CUS to the 23rd. At the same time, both Con and CUS animals received vehicles by gavage at the same dose.

Chronic unpredictable stress procedure

The stress procedure was performed as described (Willner, 1997) with substantial modification and conducted for 3 weeks. The animal model of stress consists of seven different kinds of stressors: food deprivation (24 h), water deprivation (24 h), noise (40 kHz, 90 dB, 9 min), reversed light/dark cycle (dark: 7:00 am–19:00 pm; light: 19:00 pm~next day 7:00 am), ice water swimming (4 °C, 5 min) in a 40-cm-diameter and 50-cm-depth glass tank, tilted cage with their own homecage (45°, 7 h) and tail pinch (1 min) with hemostatic forceps. Each rat received one stressor per day individually, which was sufficient alone to induce long-lasting effects. In order to achieve the unpredictability, the stressors were applied in a different sequence each week to avoid any habituation. One thing to note here was that both food deprivation and water deprivation stressors were not arranged in the day of sucrose intake procedure to avoid continuous hunger. On the other hand, rats in the Con and Con + AMA groups did not receive any stressors and were housed in normal conditions.

Body weight measurement and sucrose preference test

The body weight was measured every 3 days at 9:00 a.m. from the 1st to the 21st days. The sucrose preference test has been slightly modified from the previous description (Casarotto & Andreatini, 2007). Prior to the first sucrose preference test in all experiments, all rats in the four groups were trained to habituate sucrose water by placing a bottle of normal water and a bottle of sucrose solution (1%, w/v) with them for 2 days. Additionally, the position of the bottles was changed several times during the period. Afterwards, they were deprived of food and water for 23 h from 10:00 a.m. until 9:00 a.m. on the next day. Each rat was provided with 1% sucrose solution and normal water individually for 1 h, and the weights of sucrose solution and water consumed were recorded accordingly. During the 3 weeks of the CUS process, the sucrose consumption test was carried out at the end of each week in the same way, but without the 2-day habituation. Based on, there were four sucrose consumption tests. Finally, the total fluid intake and the percentage of sucrose consumption were calculated as follows: total fluid intake = sucrose solution + normal water; sucrose consumption percentage = sucrose solution intake/total fluid intake × 100%.

Morris water maze test

After the last day of gavage, the MWM test was performed to monitor the spatial cognition. The MWM consists of a metal pool (150 cm in diameter and 60 cm in height) filled with tap water that dissolved black nontoxic ink (22 ± 2 °C, 45 cm deep). As described in our previous papers (An et al., 2013b; Quan et al., 2011b), the maze is divided into four quadrants with two imaginary perpendicular lines crossing in the center of the tank. The end of each line demarcates four cardinal points: North (N), South (S), East (E) and West (W). A 10-cm-diameter platform that could be moved is positioned in the middle of one quadrant, and its top is submerged 2 cm below the water surface. The rat had to learn to use distal cues to navigate a direct path to this hidden platform and escape from the water. In the maze, the rat’s movement is monitored by a CCD camera connected to a personal computer, through which data are collected and analyzed (Ethovision 2.0,
Noldus, Wagenigen, Netherlands). The four start locations of S, E, NE and SW were chosen, as these were the distal start locations that were closer to being equal in length with regard to distance from the platform (Vorhees & Williams, 2006).

The MWM test is composed of four consecutive stages: initial training (IT), space-exploring test (SET), reversal training (RT) and reversal exploring test (RET). Firstly, the IT stage is conducted for 5 days with two sessions every day. Each session consists of four trials from the four start locations described earlier, which is carried out at 10 a.m. and 6 p.m., respectively. In each trial, the rats were given 60 s to find the hidden platform that was placed in the northwest (NW) quadrant before the start of the test. The rat was taken to the cage if it found the platform and stayed on it for ~3 s. However, rats would be guided to the platform and stayed for at least 10 s when they failed to find the platform. The intervals among trials lasted for 8–10 min. At this stage, the escape latency and swimming speed were recorded. Secondly, the SET stage was performed using one trial without the platform after the last session of the IT stage ~24 h later. The rats were released individually into the water from one of the starting points and allowed to swim freely for 60 s. Two parameters were recorded: platform crossings and NW quadrant dwell time (the percentage of time spent in the target quadrant). Thirdly, the RT stage was conducted for 3 days from the seventh day in the same way and with the same parameters in the IT stage. The difference was that the platform was moved into the opposite quadrant in the center of the SE quadrant. Finally, in the RET stage, the method used and the parameters recorded were the same as those in the SET stage.

In vivo LTP and depotentiation recordings

Hippocampal synaptic plasticity between Schaffer collaterals and CA1 pyramidal neurons, including LTP and depotentiation, was measured using in vivo electrophysiological techniques after the MWM test. The protocol was adopted and modified on the basis of our previous study (An et al., 2013a). Rats were administered with 30% urethane anesthesia (4 ml/kg, i.p., Sigma-Aldrich, St. Louis, MO). After the anesthesia, the animals were placed in stereotaxic frames (Narishige, Japan) for surgery. An incision of ~2 cm long was made and a small hole in the skull was drilled on the left side of the brain for both the recording and stimulating electrodes. According to Paxinos and Watson coordinates (Paxinos & Watson, 2005), the recording electrode was implanted into the hippocampus CA1 region (3.5 mm posterior to the bregma, 2.5 mm lateral to midline, 2.0–2.2 mm ventral below the dura), and the bipolar stimulating electrode was implanted into the hippocampus Schaffer collaterals region (4.2 mm posterior to the bregma, 3.5 mm lateral to midline, 2.3–2.6 mm ventral below the dura). The optimal stimulating intensity (range 0.3–0.5 mA) that could evoke a response of 50% of its maximum amplitude was delivered at single-pulse stimulation (stimulus pulse with 0.2 ms, at 0.03 Hz) to record the evoked response every 20 min baseline. After the baseline, a theta burst stimulation (TBS), consisting of 30 trains of 12 pulses (200 Hz) at 5 Hz, was applied to induce LTP. Following TBS, the single-pulse stimulation was resumed to record the evoked response every 20 min baseline. A theta burst stimulation (TBS), consisting of 30 trains of 12 pulses (200 Hz) at 5 Hz, was applied to induce LTP. Following TBS, the single-pulse stimulation was resumed to record the evoked response every 20 min baseline. A theta burst stimulation (TBS), consisting of 30 trains of 12 pulses (200 Hz) at 5 Hz, was applied to induce LTP. Following TBS, the single-pulse stimulation was resumed to record the evoked response every 20 min baseline. A theta burst stimulation (TBS), consisting of 30 trains of 12 pulses (200 Hz) at 5 Hz, was applied to induce LTP. Following TBS, the single-pulse stimulation was resumed to record the evoked response every 20 min baseline. A theta burst stimulation (TBS), consisting of 30 trains of 12 pulses (200 Hz) at 5 Hz, was applied to induce LTP. Following TBS, the single-pulse stimulation was resumed to record the evoked response every 20 min baseline. A theta burst stimulation (TBS), consisting of 30 trains of 12 pulses (200 Hz) at 5 Hz, was applied to induce LTP. Following TBS, the single-pulse stimulation was resumed to record the evoked response every 20 min baseline.
body weight, sucrose consumption percentage and MWM escape latencies. Other data, obtained from the SET/RET stage, electrophysiological experiments and Western, were analyzed by two-way factorial ANOVA for multiple comparisons with stress or drug. To detect significant differences between groups, ANOVAs were supported by post hoc tests. All analyses were performed using SPSS 20 software (IBM Corp., Armonk, NY). A p value of <0.05 was taken to indicate statistical significance.

Results
Effects of AMA administration on body weight and sucrose test in stressed rats
As shown in Figure 1(A), the body weights in all groups were increased during the 3 weeks, however, a three-way repeated measures ANOVA showed that there were statistical differences for day \(F_{(6,21)} = 279.970, p < 0.001\), day \times stress interaction \(F_{(6,21)} = 13.695, p < 0.001\), day \times drug interaction \(F_{(6,21)} = 4.129, p < 0.001\) and day \times stress \times drug interaction \(F_{(6,21)} = 4.897, p < 0.001\). Additionally, there were significant differences of the stress effect \(F_{(1,26)} = 25.382, p < 0.001\), the drug effect \(F_{(1,26)} = 4.482, p < 0.05\) and the stress \times drug interaction effect \(F_{(1,26)} = 7.6, p < 0.05\) on the body weight. LSD post hoc analysis showed that the rat’s weight was statistically lower in the Str group compared to the Con group \((p < 0.01 on the 6th day; p < 0.001 from the 9th–21st day). Moreover, the weight was significantly enhanced by AMA in the Str + AMA group compared to the Str group \((p < 0.05 on the 6th day and the 9th day; p < 0.01 from the 12th–18th day; p < 0.01 on the 21st day).

Figure 1(B) shows the effects of CUS + isolated feeding and AMA treatment on the sucrose consumption percentage. A three-way repeated measures ANOVA showed that there were statistical differences for day \(F_{(3,36)} = 4.930, p < 0.05\), day \times stress interaction \(F_{(3,36)} = 5.805, p < 0.01\), day \times drug interaction \(F_{(3,36)} = 4.421, p < 0.05\) and day \times stress \times drug interaction \(F_{(3,36)} = 3.904, p < 0.05\). Moreover, there were significant differences of the stress effect \(F_{(1,38)} = 42.29, p < 0.001\), the drug effect \(F_{(1,38)} = 39.263, p < 0.001\) and the stress \times drug interaction effect \(F_{(1,38)} = 7.431, p < 0.01\) on the sucrose consumption percentage. LSD post hoc analysis showed that the sucrose consumption was significantly lower in the Str group than that in the Con group \((p < 0.05 on the 7th day; p < 0.01 on the 14th day; p < 0.001 on the 21st day). Furthermore, it was significantly increased by AMA in the Str + AMA group compared to the Str group \((p < 0.05 on the 7th day; p < 0.01 on the 12th day; p < 0.001 on the 21st day). There was no significant difference in the sucrose consumption percentage between the Con and the Con + AMA groups \((p > 0.05). Moreover, it was found that there was no significant difference of total fluid intake among these 4 groups (Figure 1C, \(p > 0.05\)).

Effects of AMA administration on the MWM in stressed rats
Figures 2 and 3 show the data obtained from the MWM test in all groups on each test day. During the IT stage, the escape latencies were visibly decreased with training (Figure 2A). A three-way repeated measures ANOVA showed that there were no statistical differences of day \times stress interaction \(F_{(4,37)} = 0.884, p > 0.05\), day \times drug interaction \(F_{(4,37)} = 1.551, p > 0.05\) and day \times stress \times drug interaction \(F_{(4,37)} = 0.814, p > 0.05\), except for day \(F_{(4,37)} = 237.926, p < 0.05\) as these four groups improved over the 5 days of training. However, it was found that there was a significant difference for the effect of stress \times drug interaction \(F_{(1,40)} = 12.687, p < 0.01\) on the escape latencies. Subsequent LSD post hoc analysis showed that there were significant differences of the escape latencies between the Str group and the Con group (Figure 2A; \(p < 0.05 on the 2nd day; p < 0.01 on the 3rd day; p < 0.001 on the 4th day; p < 0.01 on the 5th day)), indicating that rats in the Str group needed more time to find the platform compared to that in the Con group. However, the escape latencies were considerably reduced in the Str + AMA group compared to that in the Str group (Figure 2A; \(p < 0.05 on the 2nd day; p < 0.01 on the 3rd day; p < 0.001 on the 4th day; p < 0.01 on the 5th day)). Additionally, there was no significant difference of swimming speed among these 4 groups (Figure 2B, \(p > 0.05\)).

In the SET stage, the reference memory was evaluated. A two-way factorial ANOVA revealed that there were significant differences for the stress effect \(F_{(1,40)} = 5.343, p < 0.05\), the drug effect \(F_{(1,40)} = 3.899, p < 0.05\) and the stress \times drug interaction effect \(F_{(1,40)} = 4.791, p < 0.05\) on the platform.
crossings. It was further found that there was a significant difference for the effect of stress/drug interaction $[F(1,40) = 7.262, p < 0.01]$ on the NW quadrant dwell time. The LSD post hoc test showed that both platform crossings ($p < 0.001$, Figure 2C) and NW quadrant dwell time ($p < 0.01$, Figure 2D) were significantly decreased in the Str group compared to the Con group. However, the above indicators were remarkably enhanced by administration of AMA (Figure 2C and D, $p < 0.01$).

With respect to the RT stage, the platform was moved into the contralateral quadrant to examine the learning flexibility. Average escape latencies are visibly decreased in all the groups (Figure 3A), indicating that animals learnt to find the new platform position following 3 days of training. A three-way repeated measures ANOVA showed that there were no statistical differences for day/stress interaction $[F(2,39) = 1.045, p > 0.05]$, day/drug interaction $[F(2,39) = 0.695, p > 0.05]$ and day/stress/drug interaction $[F(2,39) = 13.496, p > 0.05]$, except for day $[F(2,39) = 183.601, p < 0.001]$. However, it was found that there was a significant difference for the effect of stress/drug interaction $[F(1,40) = 5.979, p < 0.05]$ on the escape latencies. Subsequent LSD post hoc analysis showed that the escape latencies were longer in the Str group than that in the Con group (Figure 3A; $p < 0.05$ on the 1st day; $p < 0.01$ on the 2nd day; $p < 0.01$ on the 3rd day). Similarly, no differences in swimming speed were observed between these groups (Figure 3B, $p > 0.05$).

During the RET stage, a two-way factorial ANOVA showed that there were significant differences for the effect of stress $[F(1,40) = 5.319, p < 0.05]$ and the effect of stress/drug interaction $[F(1,40) = 4.181, p < 0.05]$ on the platform crossings. In addition, there was only a significant difference for the effect of stress $[F(1,40) = 3.253, p < 0.05]$ on SE quadrant dwell time. The LSD post hoc analysis showed that the both platform crossings ($p < 0.01$, Figure 3C) and SE quadrant dwell time ($p < 0.05$, Figure 3D) were significantly decreased in the Str group compared to those in the Con group. However, the platform crossings in the Str + AMA group were significantly increased ($p < 0.01$) compared to those in the Str group, while there was no significant difference for quadrant dwell time between these two groups. Additionally, there were no significant differences of behavior measurements between the Con group and the Con + AMA group in MWM test ($p > 0.05$).

**Effects of AMA administration on the LTP and depotentiation in stressed rats**

In the LTP test, stimulation of Schaffer collaterals evoked basal field excitatory postsynaptic potentials (fEPSPs) in the hippocampal CA1 region, while TBS stimulation induced LTP of the stimulated synapses for 1 h. The time course of
The fEPSPs slopes that has been normalized to the 20 min baseline period is shown in Figure 4(A left). It could be seen that the fEPSPs slopes were increased immediately after stimulation, and then more or less stabilized to a level above the baseline period. The last 15 min data were analyzed by two-way factorial AVONA. The results showed that there were significant differences for the effect of stress $[F(1,21) = 12.365, p < 0.01]$, drug $[F(1,21) = 6.293, p < 0.05]$ and stress × drug interaction $[F(1,21) = 10.023, p < 0.01]$ on the fEPSPs slopes. Bonferroni post hoc analysis showed that the mean fEPSPs slopes were significantly smaller in the Str group than that in both the Con group ($p < 0.001$) and the Str + AMA group ($p < 0.01$, Figure 4B). There was no significant difference of the mean fEPSPs slope between the Con group and Con + AMA group (Figure 4B).

To examine whether depotentiation, a form of LTP reversal, was involved in the process, an LFS induction protocol was employed for eliciting depotentiation (Figure 4A, right). Since there have been significant differences of fEPSPs slopes between stressed rats and normal ones before the LFS, the observed differences after the LFS cannot be used to properly evaluate the effect of CUS + isolated feeding or AMA treatment on synaptic plasticity. Accordingly, LTP-evoked responses in the last 15 min were normalized and used as the baseline of depotentiation (Figure 4A, left). A two-way factorial AVONA confirmed the statistically significant differences for the effect of stress $[F(1,21) = 8.084, p < 0.01]$ and the effect of drug $[F(1,21) = 8.225, p < 0.01]$. Bonferroni post hoc test indicated that there was significantly suppressive depotentiation in the Str group compared to that in the Con group ($p < 0.05$), while a considerably facilitated depotentiation existed in the Str + AMA group compared to that in the Str group ($p < 0.05$, Figure 4C). Furthermore, there was also no significant difference of the depotentiation between the Con group and Con + AMA group ($p > 0.05$).

Effects of AMA administration on NR2B and PSD-95 expression in stressed rats

In the Western-blotting assay test, two prominent bands at $\sim$180 and 95 kDa were detected by NR2B and PSD-95 antibodies, respectively (Figure 5A). A two-way factorial AVONA showed that there were significant differences for the effect of stress $[F(1,25) = 8.422, p < 0.01]$ and the effect of stress × drug interaction $[F(1,25) = 5.423, p < 0.05]$ on the NR2B expression. Subsequently, the LSD post hoc analysis showed that the expression of hippocampal NR2B was significantly decreased in the Str group compared to that in the Con group ($p < 0.01$, Figure 5B). It was further found that the expression of NR2B was significantly enhanced by amatadine ($p < 0.05$ between the Str + AMA group and the Str group, Figure 5B).

Moreover, A two-way factorial AVONA showed that there were significant differences for the effect of stress $[F(1,22) = 13.781, p < 0.01]$ and the effect of stress × drug interaction $[F(1,22) = 11.139, p < 0.01]$ on the expression of
Figure 4. The effects of chronic unpredictable stress and amantadine treatment on the long-term potentiation and depotentiation from Schaffer collaterals to the hippocampal CA1. (A) The changes of time coursing in fEPSPs slopes in four groups. The first 20 min of evoked responses were normalized and used as the baseline responses of LTP. The last 15 min of evoked responses during LTP were normalized and used as the baseline responses of depotentiation. (B) Magnitude of LTP was determined as responses between 45 and 60 min after the TBS. (C) Magnitude of depotentiation was determined as responses between 45 and 60 min after low-frequency stimulation. Data represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 comparison between the Con group and the Str group; #p < 0.05, ##p < 0.01, ###p < 0.001 comparison between the Str group and the Str + AMA group; the Con, Str and Str + AMA groups, n = 7; the Con + AMA group, n = 4.

Figure 5. The effects of chronic unpredictable stress and amantadine treatment on expression of the NR2B and PSD-95 in the hippocampus. (A) Results are immunoblots from single representative experiments. (1) the Control group, (2) the Str group, (3) the Str + AMA group, (4) the Con + AMA group. The expression values of the NR2B (B) and the PSD-95 (C) were normalized with β-actin value, then compared to control. Data are presented as mean ± SEM. **p < 0.01, ***p < 0.001, comparison between the Con group and the Str group; #p < 0.05, ##p < 0.01, comparison between the Str group and the Str + AMA group; the Con, Str and Str + AMA groups: PSD-95, n = 7/group; NR2B, n = 8/group; the Con + AMA group: PSD-95/NR2B, n = 5.
hippocampal PSD-95. LSD post hoc analysis showed that the expression of PSD-95 was significantly decreased in the Str group compared to that in the Con group ($p<0.001$, Figure 5C), while the expression of PSD-95 was significantly enhanced by AMA ($p<0.01$ between the Str + AMA group and the Str group, Figure 5C).

**Discussion**

The present study demonstrated that the NMDA receptor antagonist actionmatically impeded the development of depressive-like symptoms in a rat model of chronic stress after chronic AMA administration for 20 consecutive days. More importantly, the spatial memory deficits including spatial learning and re-acquisition in reversal learning were effectively attenuated by AMA treatment. The impairment of synaptic plasticity induced by CUS isolated feeding was considerably delayed by AMA administration. Finally, AMA treatment up-regulated the expression of NR2B and PSD-95 in the hippocampus of stressed rats.

It is well known that the CUS animal model can successfully simulate depression-like symptoms with decreased body weight and sucrose preference, which are the core symptoms of depression. Accordingly, our study further supports the validity of CUS as an animal model to study depression, consistent with the previously reported (Willner, 1997). After establishing a rat model of chronic stress, significant differences were observed in body weight and sucrose consumption between the Con group and the Str group. Moreover, the data showed that there was no statistical difference of total fluid intake among these groups. A previous study showed that reduced intake of a sucrose preference was a sign of anhedonia, or a decrease in the rewarding properties of the solution (Willner, 2005). Thus, our data indicate that the CUS rat model, combined with isolation rearing, simulated depression-like behavior.

AMA treatment caused significant antidepressant-like effect on depression related behaviors, similar to our previous findings related to memantine treatment (Quan et al., 2011b). An earlier study reported that AMA produced antidepressant-like activity, as shown by the decreased immobility time in the forced swim test (used to assess the behavioral despair, symptom of depression) (Moryl et al., 1993). Additionally, clinical data demonstrate that AMA exerts an antidepressant action in patients with Parkinson’s disease as well as in depressed patients (Zarate et al., 2002).

To evaluate whether AMA could impede the development of cognitive dysfunction in a rat model of chronic stress, we performed the MWM test to assess spatial memory. In both the IT and SET stages, our data indicated prolonged response latency and reductions in both quadrant dwell time and platform crossings in stressed rats relative to controls, indicating that spatial learning and reference memory were significantly impaired in the stressed rats. This finding is in line with that of previous studies (Henningsen et al., 2009; Mizoguchi et al., 2000). It has been reported that exposure to CUS over several weeks could produce a marked long-term deficit in learning and memory in rats (Conrad, 2010). Importantly, rats in the Str + AMA group required less time to find the platform, and platform crossings and quadrant dwell time were significantly enhanced compared to that in the Str group, suggesting that AMA could effectively impede the impairments of cognition induced by stress. The results were in accordance with the findings of previous studies, demonstrating that chronic administration of AMA could attenuate water maze performance deficits resulting from traumatic brain injury (Dixon et al., 1999; Wang et al., 2014). Furthermore, in both the RT and RET stages, the hidden platform was moved into the contralateral quadrant to examine learning flexibility, which can be attributed to erasure of previously acquired information and development of a new memory. Our data showed that the stressed rats failed to adjust the search strategy in response to the variation of platform position and resulted in impaired re-acquisition of a learned skill, indicating that the cognitive flexibility was significantly impaired. However, the escape latencies in the Str + AMA group were greatly reduced, suggesting that AMA could effectively improve cognitive flexibility. This is also in line with the findings of our previous study, in which memantine was applied in the treatment of stressed rats (Quan et al., 2011b). It is crucial to broaden our current knowledge of the potential effect of AMA on the potential treatment of cognitive dysfunction, not only as it shows a positive effect in traumatic brain injury (Dixon et al., 1999) and Parkinson’s patients (Cera et al., 2014) but also in stress-induced cognitive deficits.

Since LTP is recognized as the cellular basis of learning and memory (Bliss & Collingridge, 1993), LTP from Schaffer collaterals to hippocampal CA1 region was measured in the present study. A higher fEPSPs slope is commonly associated with more effective synaptic transmission and better learning and memory. Previous studies report that MWM training could increase the glutamate release from the presynaptic membrane, implying that behavioral training might enhance synaptic transmission (Richer-Levin et al., 1995, 1998). In the present study, behavioral training was performed before the LTP/depotentiation recordings for all the rats of four groups. The electrophysiological results showed that the strength of synaptic connections was significantly weaker in stressed rats, as shown by reduced fEPSPs slopes relative to controls. The data also illustrated that synaptic plasticity could be impaired in the hippocampus after prolonged exposure to aversive events (Alfarez et al., 2003). Interestingly, the negative effect of stress on LTP was remarkably attenuated by chronic AMA treatment, which may be related to the improvements in cognitive deficits in stressed rats. Hippocampal LTP is thought to be a cellular mechanism of learning and memory; however, the effect of depotentiation could be another crucial synaptic plasticity to erase memory by selectively reversing experience-dependent plasticity (Neves et al., 2008). Furthermore, depotentiation plays a role in the time- and state-dependent erasure of memory and enables the storage of new information by the hippocampus (Qi et al., 2013). In the present study, it was found that depotentiation was significantly suppressed in stressed rats, indicating that the resilience of synaptic structures was seriously damaged. This impairment of synaptic plasticity was greatly impeded by AMA intervention, consistent with improved performance in reversal learning of MWM. One of our previous studies showed that the balance...
between LTP and depotentiation played a key role in cognitive stability and flexibility (An et al., 2013a). Accordingly, the bidirectional synaptic plasticity, which shows integrated LTP and depotentiation, may be the possible cellular basis of cognitive function in learning and reversal learning of spatial information.

As a functional subunit of NMDA receptor complex, NR2B receptor is very important in LTP induction. It has been shown that memantine improves cognitive dysfunction by mediating the NR2B receptor (Quan et al., 2011b). In addition, a previous study reports that the GluN2B subunit-containing NMDA receptor is necessary for LFS to induce depotentiation (Qi et al., 2013). Accordingly, we examined expression of the NR2B subunit in the hippocampus. Expression of the NR2B subunit of NMDA receptor was significantly reduced in stressed rats relative to controls, suggesting that there was lower expression of NR2B protein in the depression-like animal model. It might be translated into reduced numbers of functional receptors (Dong et al., 2010; Lou et al., 2010), which is linked to impaired plasticity. Importantly, the expression of NR2B receptor was significantly enhanced by AMA, which could be part of an underlying mechanism that attenuates impairments of LTP and depotentiation as well as the cognitive deficits induced by chronic stress. Moreover, it is well known that PSD-95 plays a crucial role in the integration of NMDA receptor complexes, since it enables anchoring of NMDA receptors via the combination with NR2 subunits to the postsynaptic membrane. Consequently, PSD-95 may be essential for LTP induction and the regulation of synaptic function (Cousins & Stephenson, 2012). Our data showed that PSD-95 was markedly reduced in the hippocampus in the Str group compared to the Con group, which could explain why both LTP and depotentiation were damaged in stressed rats. AMA significantly up-regulated the expression of PSD-95, suggestive of enhanced communication/coupling of the NMDA receptors in the postsynaptic membrane. Consequently, the induction and maintenance of glutamate receptor-dependent plasticity could be preserved by AMA (Li et al., 2003). The AMA improvement of synaptic plasticity and resilience may be due to preventing the alteration of these two proteins expression in the hippocampus.

AMA, as a low-affinity NMDA receptor antagonist, has a neuroprotective effect with the ‘‘partial trapping’’ type of channel closure-like memantine, which may protect neurons from the glutamate excitotoxicity and consequently alleviate the cognitive deficits induced by chronic stress (Szakacs et al., 2012). Moreover, the fact that the NMDAR antagonists, like memantine, may enhance the cognition is by selectively inhibiting the ‘‘pathological activation’’ while preserving the ‘‘physiological activation’’ of NMDARs (Collingridge et al., 2013). Accordingly, we speculate that AMA has more or less similar characteristics of memantine. Additionally, AMA could enhance the release of dopamine (Baldessarini et al., 1972) and increase the expression of BDNF (Rogoz et al., 2005), which are important neurotransmitters to synaptic plasticity. In addition, NR2B expression may be up-regulated by chronically administrating AMA, which may function as an adaptive mechanism. Thus, AMA attenuation of impairments of synaptic plasticity and cognition following stress may act through several cellular mechanisms.

Additionally, a previous study showed that the low dose of AMA (15 mg/kg, 30 mg/kg, i.p.) was unable to impair memory, locomotion, exploration or motivation in mice, but the high dose (60 mg/kg) could produce neurotoxicity (Kaefer et al., 2010). Moreover, it was reported that the dose of AMA in human was in an average dose of 200 mg/day but not exceeding 400 mg/day (Hosenbocus & Chahal, 2013). The AMA dose used in the present study is converted from animal to human equivalently, based on the body surface area on the recommended daily dose of 200 mg in human (Nishikawa et al., 2009; Reagan-Shaw et al., 2008). Accordingly, we argue that AMA at a dose of 25 mg/kg generates no significant effect on either the LTP/depotentiation or behavior in the absence of prior stress exposure.

Conclusion

In summary, our results suggest that AMA at a dose of 25 mg/kg is effective in impeding the symptom of weight loss and anhedonia in the rat model of chronic stress, exhibiting potential antidepressant-like properties. Moreover, it significantly attenuates the impairments of synaptic plasticity and cognitive functions seen following chronic stress. A potential underlying mechanism is that AMA prevents the alterations of NR2B receptor and PSD-95 in the hippocampus of stressed rats. Nevertheless, further investigation is needed to explore the possible mechanism of NMDA antagonists in antidepressant efficacy. In conclusion, the data suggest that there are possible novel therapeutic targets for antidepressant drug development as well as a possibility for treating cognitive dysfunction in stress-related diseases.

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Declaration of interest

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References


Amantadine alleviates cognitive deficits


