POSSIBLE ANTIDEPRESSANT EFFECTS AND MECHANISMS OF MEMANTINE IN BEHAVIORS AND SYNAPTIC PLASTICITY OF A DEPRESSION RAT MODEL

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Abstract—Glutamatergic processes are strongly implicated in the pathophysiology and treatment of depression, including the antidepressant effects of N-methyl-D-aspartate (NMDA) receptor antagonists. This study was designed to see whether memantine, a noncompetitive NMDA antagonist, has antidepressant effects in behaviors and synaptic plasticity. Rats were randomly divided into control, stressed, and stressed+memantine groups. The animal model was established by memantine. Rats in stressed group performed worse in reversal learning related stages, while rats in stressed+memantine group performed worse in reversal learning related stages. LTP test showed lower amplitude of field excitatory postsynaptic potential in prefrontal cortex in stressed group. Immunohistochemistry showed lower expression of NR2B receptor in prefrontal cortex in stressed group, and higher expression in hippocampus in stressed+memantine group. In conclusion, memantine in dose of 20 mg/kg improves the sucrose consumption, reversal learning and prefrontal cortical synaptic plasticity, but impairs spatial memory, which is probably due to different extent of up-regulating NR2B receptor expression in prefrontal cortex and hippocampus in stressed rats. © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: depression, memantine, sucrose consumption, water maze, long term potentiation, NR2B.

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Abbreviations: CUS, chronic unpredictable stress; fEPSP, field excitatory postsynaptic potential; IOD, integrated optical density; IT, initial training; LD, laterodorsal thalamic nucleus; LDDM, laterodorsal thalamic nucleus, dorsomedial part; LTP, long-term potentiation; mPFC, medial prefrontal cortex; MWM, Morris water maze; NE, northeast; NMDA, N-methyl-D-aspartate; PFC, prefrontal cortex; PrL, prelimbic area; RET, reversal exploring test; RT, reversal training; SET, space exploring test; SW, southwest.

Depression is a multifaceted neurobiological disorder characterized by behavioral, neurochemical and other physiological abnormalities (Nestler et al., 2002), which affects more than 15% of the population at some point in life (Kessler et al., 1994). Stress is known to be one of the causal factors for development of major depression (Kendler et al., 1999; Caspi et al., 2003). Based on this observation, the chronic unpredictable stress (CUS) animal model has been developed to mimic the development and progress of clinical depression (Willner et al., 1992). The CUS involves subjecting normal rodents to a series of repeated physical stresses over a period of weeks or longer. At the end of the stress, the animals show signs of anhedonia such as reduced sucrose preference, which can be reversed by chronic, but not acute, administration of antidepressant medications (Willner, 1997). Accordingly, we use CUS as an animal model of depression to examine behavioral, electrophysiological and pathological changes, which might shed light on the mechanism and treatments of depression.

There is substantial evidence that the glutamatergic system may be involved in the pathophysiology and treatment of depression. This includes the delayed, indirect effects of many antidepressants on the glutamatergic system (Javitt, 2004). N-methyl-D-aspartate (NMDA) is one of glutamate receptors, and its antagonists have demonstrated antidepressant-like properties, with potential common downstream mechanisms of action (Skolnick et al., 2009). It is found that ketamine, a high-affinity NMDA receptor antagonist, has a rapid-albeit transitory-antidepressant effect in patients with major depression (Berman et al., 2000) as well as depression model rats (Garcia et al., 2009; Li et al., 2010). Unfortunately, ketamine’s psychotomimetic effects preclude its use as a chronic antidepressant (Berman et al., 2000). Memantine, an uncompetitive antagonist of NMDA receptors, has been shown to be well tolerated and beneficial in global, cognitive, functional, and behavioral measures in patients with Alzheimer’s disease (Reisberg et al., 2003; Tariot et al., 2004). In contrast to ketamine, memantine is devoid of psychotomimetic effects at therapeutic doses (5–20 mg/d) (Parsons et al., 1999). Based on these, our study is designed to test whether memantine has therapeutic efficacy in animal model of depression, more specifically, in behavioral, electrophysiological and pathological changes.

For behavioral changes, we focus on cognitive dysfunction, which is increasingly found to be associated with depression (Drevets et al., 1997; Austin et al., 2001; Dolan, 2002), yet hasn’t been studied much in animal models of
depression. Reversal learning, which examines the cognitive flexibility in learning contingencies, is of particular interest in animal models (Bondi et al., 2008; Murray et al., 2008). In clinical field, deficits in such processes, which can lead to perseverative cognitive and emotional biases, are thought to be important in the development and maintenance of depression (Beck et al., 1987). Prefrontal cortex (PFC) has been shown to play an important role in reversal learning (Watson and Stanton, 2009; Quan et al., 2010). Spatial memory is also frequently used in animal models to evaluate cognitive function, and hippocampus is well known for its role in spatial memory (Morris et al., 1982; Clark et al., 2007). Thus we mainly examine PFC and hippocampus related behaviors.

For electrophysiological changes, we focus on synaptic plasticity, which is widely believed to comprise the cellular basis for learning and memory. Long-term potentiation (LTP) of synaptic transmission is one of the prime candidates for mediating learning and memory as well as many other forms of experience-dependent plasticity (Malenka and Bear, 2004). Growing attention has been focused on LTP of PFC in recent years, because of the hypothesis that cellular alterations in the PFC contribute to the cognitive dysfunction (Drevets et al., 1997; Bondi et al., 2008) and clinical symptoms of depression (Manji et al., 2001; Duman, 2004). The laterodorsal thalamic nucleus (LD) provides massive projections to the frontal cortex, which constitute one of the pathways that are involved in spatial cognition (Shibata and Kato, 1993; Van Groen et al., 2002). Our previous studies have further shown that the synaptic plasticity of LD to PFC pathway might be involved in reversal learning (Quan et al., 2010; Zheng et al., 2011). Therefore, we want to see if this thalamocortical pathway is impaired in depression rats, and whether it will be modified by memantine.

For pathological changes, we focus on NR2B receptor expression. NMDA receptor is a tetrameric membrane-inserted protein complex comprising two NR1 and NR2 (Laube et al., 1998) or NR3 subunits (Nishi et al., 2001). NR2B is one of the major NR2 subunits in neocortex and hippocampus (Monyer et al., 1994), and there is currently great evidence supporting the contribution made by NR2B to synaptic plasticity and cognitive functions (von Engelhardt et al., 2008). Furthermore, some recent evidence suggests that some NMDA antagonists exhibit antidepressant-like actions especially targeting the NR2B subtype (Skolnick et al., 2009). These findings lead us to a hypothesis that NR2B receptor expression might be affected in our animal model of depression, which might be reversed by memantine.

**EXPERIMENTAL PROCEDURES**

**Animals and drug treatments**

Eighteen male Wistar rats (180–220 g body weight) were purchased from the Laboratory Animal Center of Academy of Military Medical Science of People’s Liberation Army, and were reared in standard rodent cages in animal house of Medical School, Nankai University, under the condition of a constant temperature of 24 °C (±2 °C) and a 12 h light/dark cycle (lights on at 7 AM). Food and water were freely available during all phases of the experiment, with the exception of model establishing and sucrose consumption phases. After 2 days’ habituation to the environment, the rats were randomly divided into three groups: control group (Con, n = 6) rats were housed with three individuals in one cage; stressed group (Str, n = 6) and stressed + memantine treated group (Str+MEM, n = 6) rats were housed separately with each individual in one cage. All efforts were made to minimize the number of animals used and their suffering. The experiments were carried out according to the guidelines of the Beijing Laboratory Animal Center, and approved by the Ethical Commission at Nankai University.

The rats received daily i.p. injections of saline solution (NaCl 0.9%) and memantine hydrochloride (20.0 mg/kg, Zhuhai Federal Pharmaceutical Corporation, Shenzhen, China) for 21 days from the 2nd to 22nd day counting from the beginning day of CUS in Str and Str+MEM group respectively. The dose of memantine, 20.0 mg/kg in 2.0 ml/kg volume dissolved in saline solution, was chosen on the basis of previous studies (Parsons et al., 1999; Lukoyanov and Paula-Barbosa, 2001; Piétà Dias et al., 2007). We didn’t include Con+MEM group in our study, mainly based on previous animal study that didn’t show significant difference between Con and Con+MEM groups (Rosí et al., 2009), and memantine’s excellent clinical safety profile for the treatment of Alzheimer’s disease (Reisberg et al., 2003).

**CUS procedure**

The CUS procedure was conducted for 21 days, according to the modification method of Willner (1997). Briefly, the weekly stress regime consisted of seven different kinds of stressors: food deprivation (24 h), water deprivation (24 h), electric foot-shock (36 V, 10 s) for 10 times with 10 s interval, reversed light/dark cycle (24 h), ice water swimming (4 °C, 5 min), cage tilt (45°, 7 h) and tail pinch (1 min). The stressors were applied in seemingly random order and at varying times, with each stressor conducted once a week. Food deprivation and water deprivation stressors were not arranged during the sucrose consumption procedure (Table 1).

<table>
<thead>
<tr>
<th>Days in 1st wk</th>
<th>Days in 2nd wk</th>
<th>Days in 3rd wk</th>
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</thead>
<tbody>
<tr>
<td>1. Ice water swimming</td>
<td>8. Ice water swimming</td>
<td>15. Water deprivation</td>
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</table>

Experimental schedule for the chronic unpredictable stress (CUS) procedure last for 3 wk on stressed group and stress + memantine treated group rats. Seven different kinds of stressors were conducted, with each stressor conducted once a week in a semirandom order.
The rats of Con group were left undisturbed throughout the model establishing period.

Body weight measuring and sucrose consumption experiment

Body weights were measured everyday at 9 AM and recorded on the 1st, 4th, 8th, 11th, 15th, 18th and 22nd day.

Four days before CUS, all the three groups of rats were habituated to drink 1% sucrose solution by replacing normal water for 2 days (48 h). On the last 2 days of each week during the CUS period, sucrose consumption procedure was performed. In all, there were three times of sucrose consumption test. In this procedure, all rats were deprived of food and water for 23 h, starting at 10 AM. Twenty-three hours later, at 9 AM, the rats were provided with the 1% sucrose solution for 1 h. The amount of sucrose solution each rat drank was monitored by weighing the bottle at the beginning and the end of the test. Sucrose intake = beginning bottle weight – ending bottle weight. Sucrose consumption = sucrose intake/body weight.

Morris water maze experiment

The day after CUS and the last time of drug treatments, rats were trained and tested in Morris water maze (MWM) to monitor the spatial cognition. The water maze tank was 150 cm in diameter and 60 cm in height, filled with water (23 ± 1 °C) to the depth of 45 cm. The water was made opaque by black nontoxic ink. The tank was divided into four quadrants by two imaginary perpendicular lines crossing in the center of the tank. The four quadrants were clockwise named as northeast (NE), southeast, southwest (SW) and northwest respectively. A movable black circular platform (10 cm in diameter) was located in the center of one quadrant. The top of the platform submerged 2–3 cm below the water surface for the rat to climb on so as to escape from the water. The swimming path was recorded using a camera mounted 2.0 m above the center of the pool and analyzed using a computerized video tracking system (Ethovision 2.0, Noldus, Wageningen, Netherlands). The room was furnished with several extra-maze cues immobile throughout the entire experiment process.

Training and testing in the MWM comprised of four consecutive stages: initial training (IT); space exploring test (SET); reversal training (RT); reversal exploring test (RET). This protocol was adopted and modified on the basis of our previous study (Quan et al., 2010; Zheng et al., 2011). Following urethane anesthesia, the rat was placed in a stereotaxic frame (Narishige, Japan). After a 1–2 cm long incision was made along the midline, two small holes were drilled in the skull at the electrode inputting regions on the left side. Following the atlas (Paxinos and Watson, 2005), a concentric bipolar stimulating electrode was slowly implanted into the laterodorsal thalamic nucleus, dorsomedial part (LDDM), [AP −2.3 to −2.8; L 1.4 to 2.0; H 4.2 to 4.7]. The recording electrode was implanted into prelimbic area (PrL) in medial prefrontal cortex (mPFC), [AP 3.0 to 3.3; L 0.7 to 1.0; H 2.8 to 3.4]. The test stimuli were delivered to the LDDM region every 30 s at an intensity that evoked a response of 70% of its maximum (range 0.2–0.5 mA). After a 30 min baseline, high-frequency stimulation to induce LTP consisted of two series of 10 trains (250 Hz, 200 ms) at 0.1 Hz, delivered at test intensity. The field potentials were recorded every 20 min after LTP induction (n = 6 for each group) for 2 h. The evoked responses were stored as the averages of four. Field excitatory postsynaptic potential (fEPSP) slope was measured as the average slope from 20% to 80% of the first positive deflection of the potential. The fEPSP amplitudes were expressed as a percentage change of the mean baseline slopes.

Immunohistochemistry

After electrophysiological experiment, each rat was deeply anæsthetized and perfused through the ascending aorta with 200 ml of PBS followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Then the brains were removed and immersed in the same buffer for immunohistochemical analysis. The brains were sectioned coronally, and each 2-mm-thick section was then embedded in paraffin blocks, maintaining proper orientation so that the anteriorside most side could be sectioned. A single 5 μm section was then cut from each block. Brain sections were analyzed by immunohistochemical staining using PV-9000 detection kit (ZSJO-Bio, China) according to the manufacturer’s instructions.

### Table 2. The skeleton of Morris water maze experiment design

<table>
<thead>
<tr>
<th>Stages</th>
<th>Sessions and time points</th>
<th>Parameters</th>
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<tbody>
<tr>
<td>IT (initial training)</td>
<td>S1 - 1st day 10 AM</td>
<td>S2 - 1st day 6 PM  &lt;br&gt; Escape latency and swimming speed</td>
</tr>
<tr>
<td>SET (space exploring test)</td>
<td>5th day 6 PM (a single probe trial)</td>
<td>Platform crossings and SW quadrant dwell time</td>
</tr>
<tr>
<td>RT (reversal training)</td>
<td>S1 - 7th day 10 AM</td>
<td>S2 - 7th day 6 PM  &lt;br&gt; Escape latency and swimming speed</td>
</tr>
<tr>
<td>RET (reversal exploring test)</td>
<td>8th day 10 AM (a single probe trial)</td>
<td>Platform crossings, NE and SW quadrant dwell time</td>
</tr>
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Each session (S) of IT and RT stages of Morris water maze (MWM) experiment consisted of four probe trials, and each trial lasted for 1 min.
with some modifications based on previous studies (Dyall et al., 2007). Briefly, the deparaffinized sections were boiled in citrate buffer in microwave oven for antigen retrieval. After that, peroxidase activity was inactivated by incubation with 3% H2O2 for 10 min at room temperature. Then, the sections were incubated with rabbit anti-NR2B antibody (diluted 1:500, ZSJQ-Bio, China) in a moist chamber at 4 °C overnight. Negative controls were conducted by exchange of primary antibody for PBS. The sections were then incubated with poly-horseradish peroxidase conjugated secondary antibody, anti-rabbit IgG (1:400). The immunoperoxidase reaction was visualized using diaminobenzidine tetrahydrochloride for 5 min. Between steps, the sections were washed twice in PBS. Finally, the sections were counterstained with Hematoxylin and mounted. Sections were viewed on a Leica microscope (Wetzlar, Germany) and digitized using a charge-coupled device camera (Olympus DP71, Germany). Quantification was performed using Image-Pro Plus software. Following stereotaxic coordinates vs. bregma (Paxinos and Watson, 2005): PFC (3.0 to 3.3), hippocampus (−2.8 to −3.1), we chose the PrL in mPFC (layers 5/6) and CA1 region of hippocampus for analysis. One section of each region was selected from each rat in each group, and five fields per section were taken for analysis. We examined the sum of integrated optical density (IOD) of NR2B-positive neurons in each field of a selected section.

Statistical analysis

Of the weight, sucrose consumption and MWM experiment (during IT and RT stages), a two-way repeated measures ANOVA was used with “group” as the between-subject factor and “day”/“session” as repeated measure. The two-way ANOVA was performed on the data from individual day/session. Of the LTP test, fEPSP amplitudes were analyzed by one-way ANOVA, and calculated at the time points of 20 min, 40 min, 60 min, 80 min, 100 min and 120 min after baseline for each rat. Each time point represents mean±SEM of averaged to four consecutive evoked responses. To detect significant differences among three groups, ANOVAs were supported by the Bonferroni post hoc tests. Of the immunohistochemistry analysis, sum IOD of five fields in a selected section was averaged, and group differences were analyzed by one-way ANOVA. All the data were expressed as mean±SEM. All the analyses were performed using SPSS 16.0 software. The significant level was set at 0.05.

RESULTS

Weight and sucrose consumption results

The effects of chronic stress and memantine treatment on body weight were shown in Fig. 1A. Two-way repeated measures ANOVA confirmed the statistical difference of day ($F_{(6,60)}=274.300, P<0.001$), day $\times$ group interaction ($F_{(12,90)}=89.096, P<0.001$), and group ($F_{(2,15)}=114.593, P<0.001$). The one-way ANOVA for individual day analyzing showed that there were significant differences between groups in all days except the 1st day. Subsequent Bonferroni post hoc test showed that, during the 3 weeks of CUS, rats gained weight in all three groups, but rats in Con group gained significantly more weight than those in Str and Str+MEM groups. There was no significant difference between Str and Str+MEM group.

The effects of chronic stress and memantine treatment on sucrose intake were shown in Fig. 1B. Two-way repeated measures ANOVA confirmed the statistical difference of day ($F_{(2,30)}=8.451, P<0.01$), day $\times$ group interaction ($F_{(4,30)}=5.755, P<0.01$), and group ($F_{(2,15)}=40.88, P<0.001$). The one-way ANOVA for individual day analyzing showed that there were significant differences between groups in all three times ($P<0.001$). Bonferroni post hoc test showed that, during the 3 weeks of CUS, rats’ sucrose intake was significantly higher in Con group than those in Str group and Str+MEM group. Of the last time, there was significant difference between Str group and Str+MEM group ($P<0.05$). Similar results were got on sucrose consumption (Fig. 1C).

MWM experiment results

During IT stage, mean escape latency was calculated for each of the nine training sessions (Fig. 2A). Two-way repeated measures ANOVA confirmed the statistical difference of session ($F_{(6,120)}=58.653, P<0.001$), session $\times$ group interaction ($F_{(8,120)}=6.201, P<0.001$), and group ($F_{(2,15)}=5.352, P<0.05$), as all three groups did improve over the nine sessions of training. The one-way ANOVA for individual session showed significant difference among groups on session 1 ($F_{(2,17)}=63.935, P<0.001$), session 2 ($F_{(2,17)}=12.120, P<0.01$) and session 3 ($F_{(2,17)}=7.628, P<0.01$). We omitted the result of session 1, as it was used for rats to adapt to the environment. There were no significant differences between three groups in the last six sessions. Bonferroni post hoc test showed that on session 2 and 3, escape latency was longer in Str+MEM group than that in Str group (session 2, $P<0.01$; session 3, $P<0.05$) and Con group (session 2, $P<0.01$; session 3, $P<0.05$). Two-way repeated measures ANOVA conducted on the
swimming speed confirmed the significant difference of session \((F(8,120) = 64.046, P<0.001)\), session \times group interaction \((F(16,120) = 6.956, P<0.001)\), and group \((F(2,15) = 8.108, P<0.01)\) (Fig. 2B). Bonferroni conducted on individual session showed that on session 2, 3 and 4, swimming speed was faster in Str+MEM group than that in Str group \((P<0.05)\) and Con group \((P<0.01)\) (session 2, \(P<0.01\); session 3, \(P<0.001\); session 4, \(P<0.05\)) and Con group (session 2, \(P<0.001\); session 3, \(P<0.001\); session 4, \(P<0.05\)). During SET stage, platform crossings were calculated for each rat (Fig. 2C). One-way ANOVA confirmed that the difference between groups was significant \((F(2,15) = 6.500, P<0.01)\). Bonferroni showed that platform crossings were fewer in Str+MEM group than that in Str group \((P<0.05)\) and Con group \((P<0.05)\). ANOVA showed no statistical difference of SW quadrant dwell time between groups \((F(2,17) = 0.069, P=0.934, \text{Fig. 2D})\).

During RT stage, mean escape latency was calculated for each of the two training sessions (Fig. 3A). Two-way repeated measures ANOVA confirmed the significant difference of session \((F(1,15) = 55.632, P<0.001)\), session \times group interaction \((F(2,15) = 3.210, P<0.05)\), and group \((F(2,15) = 4.407, P<0.01)\), as all three groups did improve over the two sessions of training. The one-way ANOVA for individual session showed significant difference between three groups on session 1 \((F(2,17) = 5.367, P<0.05)\) and session 2 \((F(2,17) = 10.346, P<0.01)\). Bonferroni showed longer escape latency in Str group than that in Str+MEM and Con groups (session 1, \(P<0.05\); session 2, \(P<0.01\)). Two-way repeated measures ANOVA showed that there was no significant difference in session or group on swimming speed (Fig. 3B). During RET stage, the platform crossings were calculated for each rat (Fig. 3C). One-way ANOVA confirmed that the difference between groups was significant \((F(2,17) = 8.247, P<0.01)\). Bonferroni showed that platform crossing was less in Str group than that in Con group \((P<0.01)\). ANOVA showed statistical difference in NE \((F(2,17) = 4.407, P<0.05)\) and SW quadrant dwell time \((F(2,17) = 4.180, P<0.05)\) between groups (Fig. 3D). Bonferroni showed that rats in Str group spent shorter time in NE quadrant \((P<0.05)\) and longer time in SW quadrant \((P<0.05)\) than those in Con group.

**LTP experiment results**

In LTP test, stimulation of LDDM evoked a basal fEPSP in PrL of mPFC and high-frequency stimulation induced LTP of the stimulated synapses for at least 2 h. The time course of fEPSP amplitudes normalized to the 30 min baseline period was shown in Fig. 4A. The fEPSP amplitudes increased immediately after the high-frequency stimulation and stabilized to a level above the baseline period. For the 2 h of LTP, there was significant difference of group \((F(1,15) = 5.027, P<0.05)\), while not time \((F(5,75) = 2.239,
Since there was no statistical difference of time, we did further analysis of mean fEPSP amplitudes of the six time points in three groups (Fig. 4B). The difference was significant ($F(2,17) = 31.430, P < 0.001$). Bonferroni showed that mean fEPSP amplitudes were smaller in Str group than those in Con group ($P < 0.001$) and Str+MEM group ($P < 0.01$). The fEPSP amplitudes in Str+MEM group were smaller than those in Con group ($P < 0.05$).

Immunohistochemistry results

Fig. 5 showed the expression of the NR2B subunit in layer 5/6 of PrL in mPFC and CA1 in hippocampus regions in the three groups. Decrease in sum IOD of 38% was seen in PFC in Str group as compared to that of the other two groups ($P < 0.001$), and increase in sum of IOD of 80% was seen in hippocampus in Str+MEM group as compared to that of the other two groups ($P < 0.001$).
DISCUSSION

In the present study, CUS successfully copied the state of depression by reducing sucrose intake, sucrose consumption and body weight gain. Previous study showed that decrease in intake of a sucrose solution reflected anhedonia or a decrease in the rewarding properties of the solution (Willner et al., 1992). Our study confirmed the validity of CUS as an animal model to induce depression-like symptoms, which is in agreement with previous studies (Willner, 1997; Nestler and Hyman, 2010). The last time of sucrose consumption test showed improvement in Str + MEM group, which indicated that memantine had antidepressant effects in rats of depression model. The dose chosen for the present study was based on memantine’s in vivo/vitro data of NMDA blockade (Parsons et al., 1999), memantine’s cognitive-enhancing (Lukoyanov and Paula-Barbosa, 2001) as well as neuroprotective effects (Pietà Dias et al., 2007) in animal models, which were judged to be sufficient. Only a few recent studies have examined memantine’s antidepressant effects. Some have shown its possible antidepressant effects basing on its similar mechanism with ketamine and dizocilpine (Skolnick et al., 2009;...
Résus et al., 2010), while some didn’t (Zarate et al., 2006). Possible explanation is memantine’s lower affinity for the NMDA receptor, faster open-channel blocking/unblocking kinetics and “partial trapping” type of channel closure as compared to ketamine (Zarate et al., 2006; Tsai, 2007). Thus, the discrepancies of antidepressant effects of memantine still need to be clarified. That memantine didn’t reverse the lack of weight gain in stressed rats, indicated that either 3 weeks was not long enough to see the improvement, or memantine could not improve weight gain loss. There is evidence demonstrating that stress-induced decreases in sucrose drinking and reversal of sucrose consumption by antidepressant drugs are independent of changes in body weight (Willner, 2005). Besides, a longer time of memantine treatment with weight monitoring will be needed in future studies.

In order to examine the character of cognitive function in depression, we did MWM test. On the IT and SET stages, no significant difference was seen between Str group and Con group, which indicated that spatial memory was not affected in stressed rats. That significant differences were seen in RT stage and RET stages between Str group and Con group suggested that reversal learning ability was affected. Animal studies have shown the important role of hippocampus in spatial memory (Morris et al., 1982; Clark et al., 2007) and PFC in reversal learning (Bussey et al., 1997; Watson and Stanton, 2009; Quan et al., 2010). The different results of CUS on spatial memory and reversal learning in our study might indicate that stress affects PFC related cognitive function to more extent than hippocampus related function. Only a few studies have investigated spatial cognition in animal models of depression, and most of them didn’t show any deficit in spatial memory (Bushnell et al., 1995; Hill et al., 2005). Our results also agreed with previous studies that reversal learning ability was impaired in animal models of depression (Hill et al., 2005; Bondi et al., 2008). Another report showed that stress was not found to adversely affect reversal learning (Liston et al., 2006), which is inconsistent with our results. Besides the reason of a different stress model, they pointed out that it is likely that reversal learning is mediated by multiple structures acting in concert, such as mPFC and orbitofrontal cortex.

IT and SET stages of MWM test also showed that escape latency was significantly prolonged in several sessions in Str+MEM group as compared to that in Str group. This result indicated that memantine didn’t improve spatial memory, but impaired it. The swimming speed results further suggested that the spatial memory was decreased not because of locomotor dysfunctions. Instead, memantine did elevate rats’ locomotor activity, which was consistent with other study (Morè et al., 2008). The hyperlocomotion effect was gone after session 4, probably due to drug washout. It should also be noted that the significant difference in body weight of Str+MEM group compared to controls may interfere with the locomotor activity and water maze behaviors. Results of RT and RET stages showed that memantine partly reversed rats’ reversal learning deficits. Previous studies didn’t reach an agreement about memantine’s effects on learning and memory. While some study showed that both low doses (Zoladz et al., 2006) and high doses (Lukoyanov and Paula-Barbosa, 2001) of memantine resulted in improvement of spatial cognition, some studies reported that high doses of memantine produced cognitive deficits (Creeley et al., 2006). Our results showed that memantine disrupted spatial memory, which indicated that the dose of 20 mg/kg was probably so high as to trigger cognitive deficits. Besides, since in our study memantine showed antidepressant effects in sucrose consumption, but different effects in spatial memory and reversal learning, we might support previous finding that cognitive changes distinct from clinical symptoms of depression (Koetsier et al., 2002).

Since we’ve found deficit of reversal learning in our depression model rats, we want to further explore whether mPFC synaptic plasticity is changed. Our LTP results showed that CUS caused decrease in rats’ mPFC synaptic plasticity by decreasing fEPSP amplitudes, which supported our behavioral findings, and further indicated that synaptic projections from LD to mPFC might have participated in the regulation of reversal learning. This agreed with our previous studies (Quan et al., 2010; Zheng et al., 2011). Glutamate plays a principal role in modulating LTP, and is a likely key cellular mechanism for learning and memory (Malenka and Bear, 2004). Alterations in glutamate transmission, especially glutamate receptor activity, are found in depression (Li et al., 2010). Since CUS impaired prefrontal cortical LTP and reversal learning in the experiment, we could assume that there were alterations of glutamate receptor activity in the pathogenesis of depression. Together with the above studies, our study also indicated that the LTP of the LD-mPFC pathway was NMDA receptor dependent. This was confirmed by our study that memantine, an NMDA receptor antagonist, partly attenuated the deficit of synaptic plasticity caused by stress. Our results showed similarities of memantine’s effects with ketamine’s, in that they both have antidepressant effects. In contrast to ketamine, which has transient antidepressant effect (Berman et al., 2000; Garcia et al., 2009) and psychotomimetic side effect (Berman et al., 2000), memantine’s effects on behavior and synaptic plasticity were not transient. This is probably due to ketamine’s high-affinity while memantine is low-to-moderate-affinity (Parsons et al., 1999). Therefore, our results gave us a promising thought about memantine’s application in depression.

Reduced levels of NR2B subunit of NMDA receptor was demonstrated in PFC in major depression (Feyissa et al., 2009). Our immunohistochemistry results agreed with it, and indicated for the first time that memantine’s antidepressant effects might be related to its up-regulation of NR2B expression in PFC. Besides the high dose administration, our immunohistochemistry results further reconciled the evidence for memantine’s improvement of reversal learning and prefrontal cortical synaptic plasticity with impaired spatial memory, which indicated that memantine up-regulated NR2B expression to different extent in PFC and hippocampus. While NR2B expression returned to control
level in PFC, it exceeded the control level in hippocampus, which might lead to excessive LTP in hippocampus. A study showed that excessive LTP in hippocampus CA1 region had negative influences on hippocampus-dependend forms of learning and memory (Kim et al., 2009). The reason that chronic administration of memantine up-regulated NR2B expression is still unclear. Studies have shown that acute but not chronic administration of memantine increased brain-derived neurotrophic factor (BDNF) protein levels, which suggests adaptive mechanism (Réus et al., 2010). There is another hypothesis that glutamate-AMPA receptor activation is required for antidepressant effect, which also suggests that there is a subset of NMDA receptors, possibly on GABA-releasing interneurons, that when antagonized, block GABA release and lead to disinhibition of glutamate signaling (Farber et al., 1998; Li et al., 2010). In summary, memantine in dose of 20 mg/kg is effective in improving sucrose consumption, reversal learning and prefrontal cortical synaptic plasticity in chronic stressed rats, with the side effect of impairing spatial memory, which is probably due to different extent of up-regulating NR2B receptor expression in the PFC and hippocampus. Further studies will be needed focusing on: (1) characterization of the mechanisms of NMDA antagonists in antidepressant efficacy; (2) dose-finding studies of NMDA antagonists in an attempt to balance therapeutic efficacy with side effects. These will give us a better understanding of depression, as well as provide novel therapeutic targets for antidepressant drug development.

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