

Effect of different charges of modified electroconvulsive seizure on the cognitive behavior in stressed rats: Effects of GluR1 phosphorylation and CaMKII α activity

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Abstract. Electroconvulsive therapy (ECT) is an efficient therapy for major depression and modern ECT requires anesthesia to enhance safety. However, the commonly used anesthetic, propofol, may weaken the treatment efficacy. A recent study confirmed that ketamine rapidly reduced the symptoms of depression in affected patients. A previous study found that electroconvulsive seizure (ECS), the animal model for ECT, under anesthesia of low-dose ketamine combined with propofol could enhance the antidepressant efficacy and improve the cognitive performance. The present study aimed to investigate the responses to different charges (0, 60, 120, 180 or 240 mC) of ECS under compound anesthetics, ketamine combined with propofol, in stressed rats and the underlying mechanisms to aid in optimization of treatment regimens. The results indicated that ECS exhibited an improved antidepressant effects at 120 mC compared with 60 mC, however, no significant differences in antidepressant effects were identified among the 120, 180 and 240 mC groups. Furthermore, rats subjected to ECS at 120 mC exhibited the best cognitive performance. The phosphorylation levels of calcium/calmodulin-dependent protein kinase II α (CaMKII α) at Thr286, glutamate receptor 1 (GluR1) at Ser831 and cAMP-response element-binding protein (CREB) at the Ser133 were higher in the 120-mC group compared with all other groups. These results indicated that the ECS at medium intensity (120 mC) with administration of compound anesthetics may exert an improved therapeutic effect on depression compared with other intensities (0, 60, 180 and 240 mC). The

results also suggested that the improvement in cognitive function in stressed rats may be attributed to the phosphorylation of CaMKII α (Thr286), GluR1 (Ser831) and CREB (Ser133).

Introduction

Depression is a prevalent and recurrent psychological illness that results in a significant burden for individuals and the society (1). Previous studies indicated that electroconvulsive therapy (ECT) is an efficient and rapid treatment for major depression, especially for patients with drug-resistant and refractory depression (2,3). Modern ECT requires administration of anesthetic agents and muscle relaxants to avoid the side effects of treatment, including headache, muscle ache and bone fracture (4). At present, propofol is the most frequently used general anesthetic during ECT (5). Previous studies indicated that propofol could alleviate the cognitive impairment induced by electroconvulsive seizure (ECS) (6,7). However, certain studies suggested that propofol may interfere with the efficacy of ECT due to its anticonvulsant properties (8,9). Therefore, an improved anesthetic regimen for ECT is required.

In the last 40 years ketamine has been administered as an anesthetic to several million people (10,11). Ketamine is considered an anesthetic and a rapid-acting antidepressant (12,13). A clinical study revealed that a single ketamine infusion could rapidly reduce the levels of anhedonia in treatment-resistant major depression (14). Notably, the anesthetic dose of ketamine was positively associated with significant adverse psychological effects, including delusion and hallucination (15,16). These disadvantages may limit the clinical use of ketamine, especially for patients with psychiatric disorders. Based on these concerns, a previous study revealed that compared with propofol anesthesia, a low, subanesthetic dose of ketamine combined with propofol could enhance the antidepressant efficacy and further improve the cognitive performance following ECS, an animal analog of human ECT, in stressed stressed rats (17). As the stimulus intensity is germane to both therapeutic and adverse effects of ECT (18), the appropriate stimulus intensity of this modified ECS (MECS) with the introduction of compound anesthetics, requires further study. Furthermore, the biological processes

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underlying the protective effect of MECS on cognitive function remain to be elucidated.

Previous studies revealed that the glutamatergic transmitter system, including N-methyl-D-aspartic acid (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors, was crucial in the pathological physiology of depression and cognitive function (19,20). It is commonly accepted that ketamine exerts its biological effects by inhibiting the NMDA receptor (14). However, another study suggested that ketamine may exert rapid antidepressant-like effects by enhancing the AMPA receptor relative to NMDA receptor throughput in critical neuronal circuits (21). Furthermore, previous studies indicated that the antidepressant effects induced by ketamine or MECS were associated with glutamate receptor 1 (GluR1) (17,21). The AMPA receptor is made up of heterotetrameric complexes comprising at least two of four subunits (GluR1-4) (22). Differences in subunit composition confer specific physiological properties to AMPA receptor function and the phosphorylation of subunits is necessary for maintaining the function of this receptor (23). GluR1 was reported to be involved in the cognitive function (24,25). GluR1 subunit is recruited into the hippocampal synapses to alter the cognitive behavior with the phosphorylation at Ser831 [GluR1 (Ser831)] by calcium/calmodulin-dependent protein kinase II (CaMKII), and at Ser845 [pGluR1 (Ser845)] by protein kinase A (PKA) (24). In addition to the expression of CaMKII and PKA, the activities of these two protein kinases could also affect the phosphorylation of GluR1 (25). Therefore, the current study hypothesized that the improved cognitive performance induced by MECS may be associated with the phosphorylation levels of GluR1 and the expression and activities of these two protein kinases in the hippocampus. CaMKII α is the dominant isoform of CaMKII in the brain (26). The hallmark feature of CaMKII regulation is the generation of autonomous kinase activity by Thr286 autophosphorylation (26). In contrast to CaMKII, the activity of PKA can be determined by the relative expression of the regulatory subunit to the catalytic subunit (25). The effects of different charges of MECS on PKA- and CaMKII α -dependent GluR1 phosphorylation have not been previously reported.

In the present study, five different stimulus intensities of MECS were used to evaluate their efficacies on depression-like and cognitive behaviors in stressed rats, and to investigate the potential roles of the expression of GluR1, phospho (p)GluR1 (Ser831), pGluR1 (Ser845), PKA regulatory subunit (PKR2), PKA catalytic subunit (PKA β), CaMKII α , pCaMKII α (Thr286), cAMP-response element-binding protein (CREB) and pCREB (Ser133) in these processes.

Materials and methods

Animals. A total of 104 healthy adult male Sprague-Dawley rats (weight, 200-250 g; age, 2-3 months) were obtained from the Laboratory Animal Center of Chongqing Medical University (Chongqing, China). Animals were housed under standard laboratory conditions (temperature, 22-24°C; air humidity, 55-60%; 12-h light/dark cycle) with food and water available *ad libitum*. Rats were allowed to acclimatize for 1 week before further experiments. All procedures were approved by the Ethics Committee of Chongqing Medical University in

accordance with the animal care guidelines of the National Institutes of Health and Use of Laboratory Animals (27).

Chronic unpredictable mild stress procedure. The rat model of depression was established by chronic unpredictable mild stress (CUMS). This experimental protocol was adopted from previous studies with a minor modification (28-30). Briefly, the rats were randomly administered the following stressors for 28 days: Damp sawdust for 24 h of continuous lighting, swimming in cold water (4°C) for 5 min, water deprivation for 24 h, food deprivation for 24 h, shaking for 20 min (once per sec), tail pinching for 1 min, cage tilting (45°) for 24 h, or social crowding for 24 h. All stressor stimuli were randomly scheduled and repeated within 4 weeks to prevent habituation and increase unpredictability.

Drugs and treatment. Propofol and ketamine were purchased from AstraZeneca (cat no. H20080473; Cambridge, UK) and Jiangsu Hengrui Medicine Co., Ltd. (cat no. H32022820; Lianyungang, China), respectively. Based on previous results, a constant dosage of anesthetics, ketamine (10 mg/kg) and propofol (80 mg/kg), was administered intraperitoneally (i.p.) 10 min prior to ECS treatment in each group (17). On the day of the experiment, the rats were anesthetized to prepare them for MECS treatments. Anesthesia time was defined as the time from the loss of righting reflex until the regaining of it. The animals were ventilated with a small animal ventilator (TKR-200C; Jiangxi Teli Anaesthesia & Respiration Equipment Co., Ltd., Nanchang, China). The mean arterial pressure, heart rate and body temperature were continuously monitored using a multichannel electrophysiological recording system (model, RM6240; Chengdu Instrument Factory, Inc., Chengdu, China). The levels of PaO₂, PaCO₂ and the acid-base status were adjusted to ensure hemodynamic stability. The rats were excluded from the experiment if they were hemodynamically unstable (mean arterial pressure <10.7 kPa) or if their blood gas levels were not within the normal range [PaO₂ >13.6 kPa; pH 7.35-7.45; PaCO₂ 4.67-6.00 kPa; base excess of -2-2 mEq] (31). In the present study, no rats were excluded due to hemodynamic instability or gas levels.

The experimental design is summarized in Fig. 1. Following the completion of the CUMS procedure, one group of stressed rats was used for measuring seizure threshold in ECS with the compound anesthetics (ST group, n=8). The other stressed rats were allocated to six groups (n=96): i) Group D received normal saline solution (8 ml/kg, i.p.) once a day; ii) groups M0, M60, M120, M180 and M240 received ECS treatment with stimulus intensities of 0, 60, 120, 180, and 240 mC, respectively, following treatment with the same anesthetic protocol [ketamine (10 mg/kg) and propofol (80 mg/kg), i.p.]. ECS was delivered via ear clip electrodes using a Niviqure ECT system (Niviqure Meditech, Pvt., Ltd., Bangalore, India) (32). The following stimulus parameters were used: Bidirectional square wave pulses (amplitude, 0.8 A; width, 1.5 ms; 125 Hz) with a duration of 0.4, 0.8, 1.2 or 1.6 sec to generate charges of 60, 120, 180 and 240 mC, respectively. These treatments were administered once daily for 7 days. Oxygen was given to the rats that underwent ECS and their saturation of blood oxygen (SpO₂) was monitored. Only rats with a value of SpO₂ \geq 95% were included.

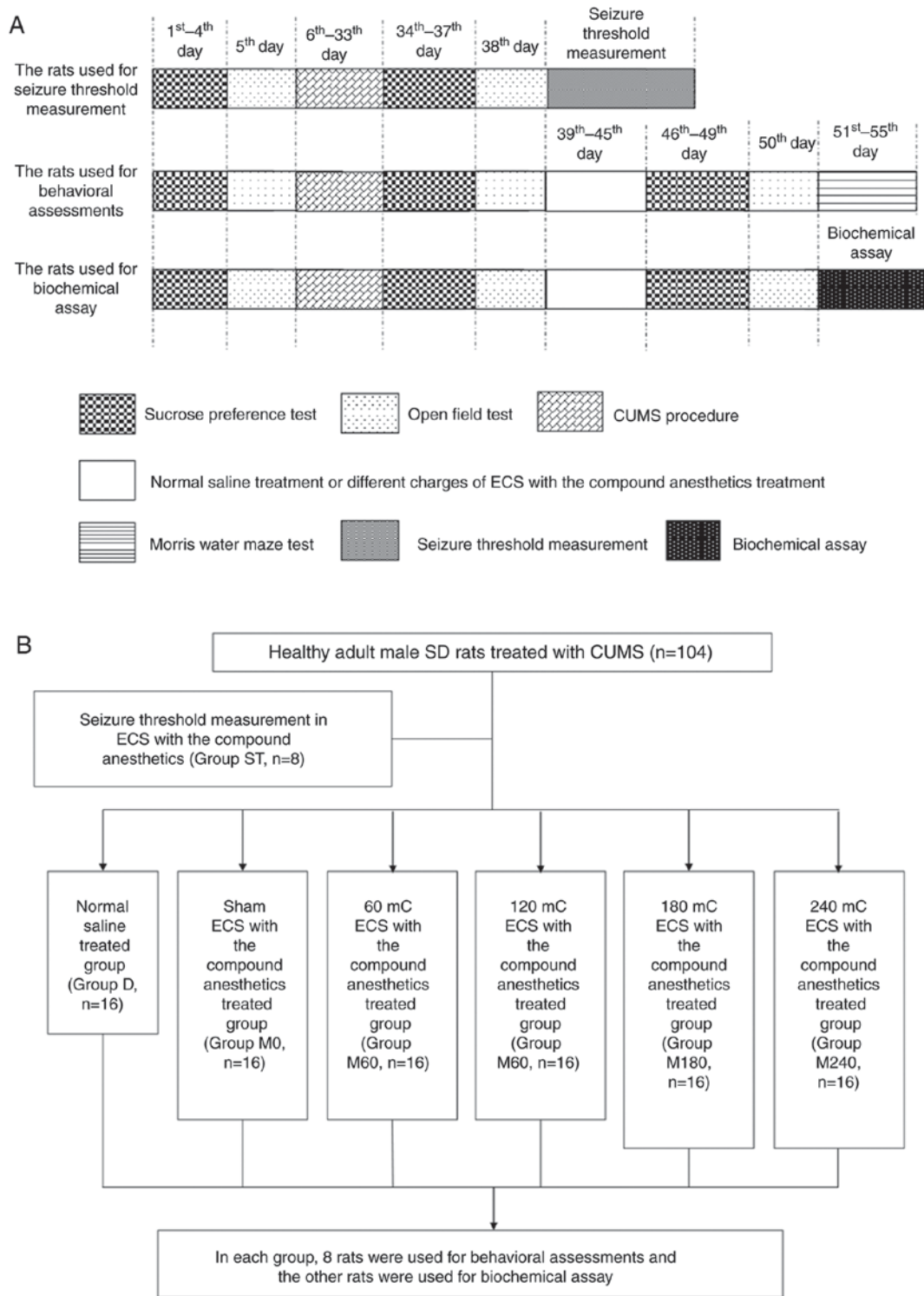


Figure 1. Schematic overview of all group assignments and experimental protocols. (A) Treatment timeline for the use of stressed rats for ECS seizure threshold measurement, behavioral tests and biochemical assays. (B) Group allocation and experimental protocols. SD, Sprague-Dawley; CUMS, chronic unpredictable mild stress; ECS, electroconvulsive seizure; compound anesthetics, ketamine (10 mg/kg) and propofol (80 mg/kg) administered via intraperitoneal injection.

A previous study demonstrated that the seizure threshold of rats with propofol anesthesia was ~40 mC (33). Furthermore, a clinical study reported that ketamine may slightly increase the seizure duration induced by ECT (13). Therefore, the current study tested the initial ECS stimuli at 30 mC in each rat of the ST group. If this stimulus did not induce a general tonic seizure, the charge was increased by 1 mC for another ECS on the following day. ECS stimulation was increased by 1 mC at a

time until the rat exhibited an obvious tonic seizure. The ECS charge at which a general tonic seizure occurred was recorded as the seizure threshold of the rat.

Sucrose preference test. Anhedonia, an indication of depressive disorder in rats, was defined as a reduction in sucrose preference. Sucrose preference was measured as described in a previous study (33). Following deprivation of water and food

for 23 h, the rats were given free access to two preweighed bottles for 1 h, one filled with 1% sucrose solution and the other filled with water. The position of the two bottles was rotated every 30 min to prevent place preference. The two bottles were subsequently weighed to measure the percentage of the consumed sucrose solution. The sucrose preference percentage (SPP) was calculated according to the following formula: $SPP (\%) = \frac{\text{sucrose solution consumption (g)} \times 100}{[\text{water consumption (g)} + \text{sucrose solution consumption (g)}]}$ (7).

Open field test (OFT). For the OFT, a spontaneous locomotor activity was measured as described in a previous study (34). Experimental animals were placed at the center of an open box (100x100x50 cm³). The floor was divided into 25 equal squares with the walls painted black. The horizontal locomotor activities (segments crossed with all four paws) and vertical exploratory activities (standing on hind paws) were scored. The number of horizontal activities (crossing) and vertical activities (rearing) performed by each rat was observed for 3 min.

Morris water maze (MWM). To assess the spatial performance function, the rats were tested in a MWM, which was a circular pool (diameter, 150 cm; height, 50 cm) with a platform 1-2 cm below the surface of the warm water (22±1°C) (35). The maze was divided into four equal quadrants: SW, NW, SE and NE. Each rat was placed into the water randomly from a quadrant and allowed a maximum of 60 sec to find the submerged platform located at the center of the NE quadrant. If the rats failed to complete the task within 1 min, they were gently guided to the platform where they remained for 15 sec. Each rat was given four trials/day for 5 consecutive days. The swimming speed, swimming distance and escape latency (EL) to find the platform were recorded using a video tracking system device with a data analysis program (Anhui Zhenghua Bio-Tech Co., Ltd., Anhui, China). On day 6, the platform was removed and the probe trial was performed for 60 sec. The swim time spent in the platform quadrant was recorded as the space exploration time.

Western blot analysis. Following the spatial memory retention test, eight rats from each group were humanely sacrificed and the bilateral hippocampus was rapidly removed on ice prior to storage at -80°C. Tissues were homogenized using the RIPA lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) and the supernatants were collected after centrifugation at 12,000 x g for 15 min at 4°C. After a bovine serum albumin microassay (Pierce BCA Protein Assay Kit; cat. no. 23225; Pierce; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and spectrophotometry to assess the protein levels, total protein was separated by 4-12% SDS-PAGE and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% nonfat milk for 1 h at 37°C and subsequently incubated with appropriate primary antibodies overnight at 4°C: GluR1 (cat. no. ab31232), pGluR1 (Ser831; cat. no. ab109464), pGluR1 (Ser845; cat. no. ab76321), PKR2 (cat. no. ab38949), PKAβ (cat. no. ab75993), CREB (cat. no. ab31387), pCREB (Ser133; cat. no. ab32096; all Abcam, Cambridge, UK), CaMKIIα (cat. no. sc-9035), pCaMKIIα (Thr286; cat. no. sc-12886-R; both Santa Cruz Biotechnology, Inc., Dallas,

TX, USA) and GAPDH (cat. no. AF1186; Beyotime Institute of Biotechnology; all 1:1,000). After washing, the membranes were incubated with biotinylated secondary antibodies (cat. no. A0208; 1:2,000; Beyotime Institute of Biotechnology) for 1 h at 37°C. Blots were developed using a chemiluminescent substrate (BeyoECL Plus kit; cat. no. P0018; Beyotime Institute of Biotechnology) and analyzed semi-quantitatively using the Bio-Rad Quantity One software (version 4.4.0; Bio-Rad Laboratories, Inc., Hercules, CA, USA). To determine the relative expression of these proteins, they were normalized to GAPDH.

Immunohistochemistry. The remaining rats in each group (n=8) were anesthetized and transcardially perfused using 4% paraformaldehyde (PFA) in normal saline. Their brains were removed and stored for at least 24 h in 4% PFA at 4°C before dehydration and embedding in paraffin. The hippocampi were cut into 10-μm thick sections in the coronal plane and mounted on glass slides. The immunoreactivity of pGluR1 (Ser831) and pCaMKIIα (Thr 286) was determined as described in a previous study (36). Briefly, sections of the rat brain were incubated overnight at 4°C with anti-pGluR1 (Ser831) and anti-pCaMKIIα (Thr 286; both 1:100) antibodies, and subsequently incubated for another 1 h at 37°C with biotin-labeled secondary antibodies (1:50; Beyotime Institute of Biotechnology, cat. no. A0208). The primary antibody was visualized using brown 3,3'-Diaminobenzidine substrate (Wuhan Boster Biological Technology, Ltd., Wuhan, China). The CA1 region in the hippocampus was observed using a light microscope (Olympus BX60; Olympus Corporation, Tokyo, Japan) at a magnification of x400 and analyzed using the Image-Pro Plus software (version 6.0; Media Cybernetics, MD, USA).

Statistical analysis. Statistical analysis was performed using SPSS (version 17.0; SPSS Inc., IL, USA). All data are presented as the mean ± standard deviation. Statistical significance was determined using one-way analysis of variance. The Student-Newman-Keuls test was used to compare differences among the groups. The repeatedly measured data were analyzed using repeated-measures analysis of variance. P<0.05 was considered to indicate a statistically significant difference.

Results

Seizure threshold. The seizure threshold of CUMS rats treated with compound anesthesia was 41.25±5.50 mC.

Sucrose preference test. The SPP in each CUMS group exhibited a significant decrease compared with the respective group before CUMS (all P<0.05), however no significant differences were found among the groups (F=0.735, P=0.601; Fig. 2A). Following the administration of MECS at different charges, the SPP in groups M60, M120, M180 and M240 significantly increased compared with the respective groups before MECS treatments (all P<0.05). Compared with group M60, the SPP increased in group M120 (P<0.05). Furthermore, no significant differences were identified in SPP between groups D and M0 (P=0.823).

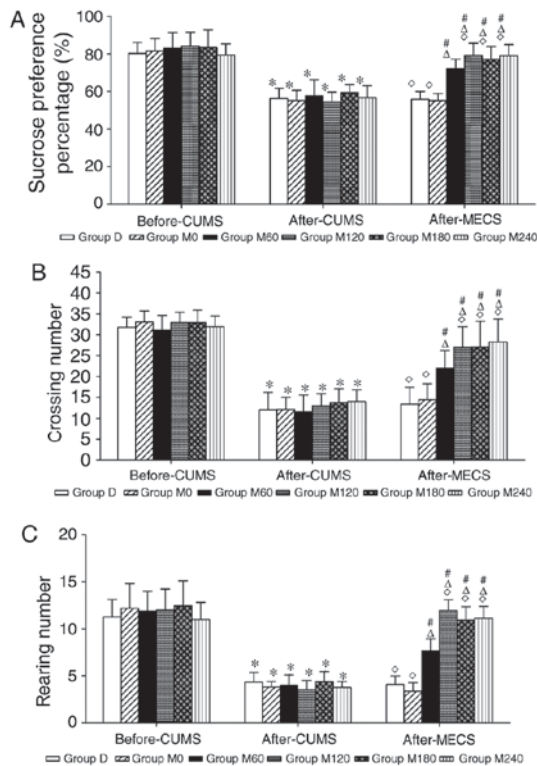


Figure 2. Effects of different charges of MECS on the behavioral performances of animals based on SPP and OFT. (A) SPP. (B) Crossing numbers. (C) Rearing numbers. Data are expressed as the mean \pm standard deviation (n=8 each group). *P<0.05 vs. the respective group before CUMS; #P<0.05 vs. the respective group after CUMS; ^P<0.05 vs. group D after MECS; and °P<0.05 vs. M60 group after MECS. MECS, modified electroconvulsive seizure; SPP, sucrose preference percentage; OFT, open field test; CUMS, chronic unpredictable mild stress.

Open field test. Following the CUMS treatment, rats exhibited decreased locomotor activity (P<0.05) and rearing numbers (P<0.05) in the OFT compared with the respective groups before the CUMS administration (Fig. 2B and C). No significant differences in OFT scores were found among the CUMS groups (number of crossings, F=0.698 and P=0.628; rearing, F=1.038 and P=0.408). Following the MECS treatment at different charges, the number of crossings and rearing in groups M60, M120, M180 and M240 significantly increased compared with the respective groups before the MECS treatment (all P<0.05). The number of crossings and rearing increased in the M120 group compared with the M60 group (number of crossings, P<0.05; rearing, P<0.05). Furthermore, no significant differences were observed in the number of crossings and rearing between groups D and M0 (number of crossings, P=0.672; rearing, P=0.235).

Morris water maze. During the MWM test, the swimming speed of CUMS rats exhibited no significant differences among the groups (data not shown). The mean latencies to find the submerged platform decreased progressively during the five consecutive training days for all animals. Following the MECS treatment, the time to find the platform was shorter in the M120 group compared with the other groups (P<0.05). Furthermore, group M0 exhibited no significant decrease in EL compared with group D each day (P>0.05; Fig. 3A). Similar

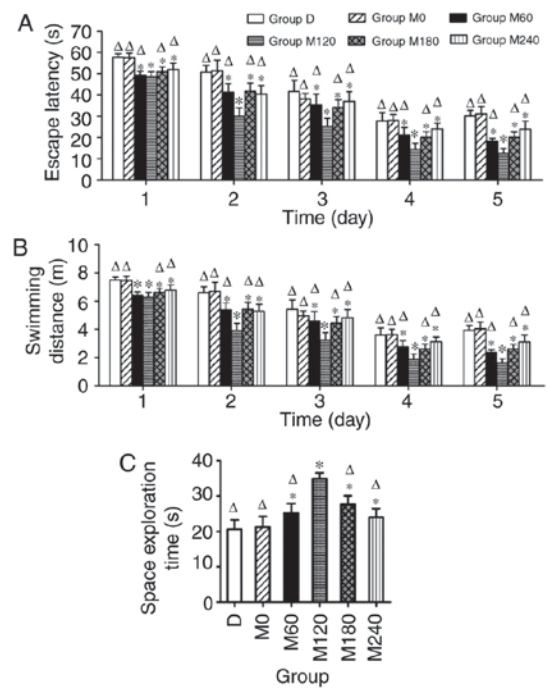


Figure 3. Effects of different charges of modified electroconvulsive seizure on the behavioral performance of animals based on the Morris water maze experiment. (A) Escape latency. (B) Swimming distance. (C) Space exploration time. Data are expressed as the mean \pm standard deviation (n=8 each group). °P<0.05 vs. group D and ΔP<0.05 vs. group M120.

results were found for the swimming distance (Fig. 3B). When the platform was removed for spatial memory testing, group M120 exhibited a significantly longer dwelling time in the former platform quadrant. No differences were identified between groups D and M0 (Fig. 3C).

Expression levels of PKA subunits and GluR1, and phosphorylation of GluR1 (Ser 845) in the hippocampus. In the present study, PKAR2, PKAβ (Fig. 4A), GluR1 and pGluR1 (Ser845; Fig. 4B) were assessed via western blotting. No significant differences were identified in the expression of PKAR2 (F=0.448 and P=0.810) and PKAβ (F=0.803 and P=0.562) subunits of PKA (Fig. 4C). Furthermore, analysis of variance did not reveal any statistically significant effects of MECS on the expression of GluR1 (F=1.059 and P=0.415) and pGluR1 (Ser 845; F=0.365 and P=0.866) in the hippocampus (Fig. 4D).

CaMKIIα activity and GluR1 (Ser831) phosphorylation in the hippocampus. In the present study, CaMKIIα, pCaMKIIα (Thr286; Fig. 5A) and pGluR1 (Ser831; Fig. 5B) were assessed by western blotting. As presented in Fig. 5B, anesthesia combined with ECS treatments did not induce significant effects on the hippocampal expression of CaMKIIα (F=0.462 and P=0.799), however the treatments altered the pCaMKIIα (Thr 286) levels (F=34.597 and P<0.01). Additionally, compared with the other five groups, pCaMKIIα (Thr 286) expression significantly increased in the M120 group (P<0.01), while the expression of this protein was lower in groups D and M0 compared with all other groups (all P<0.01). The expression of pCaMKIIα (Thr286) and pGluR1 (Ser831) were also examined via immunohistochemistry (Fig. 5C). The expression of pGluR1 (Ser831)

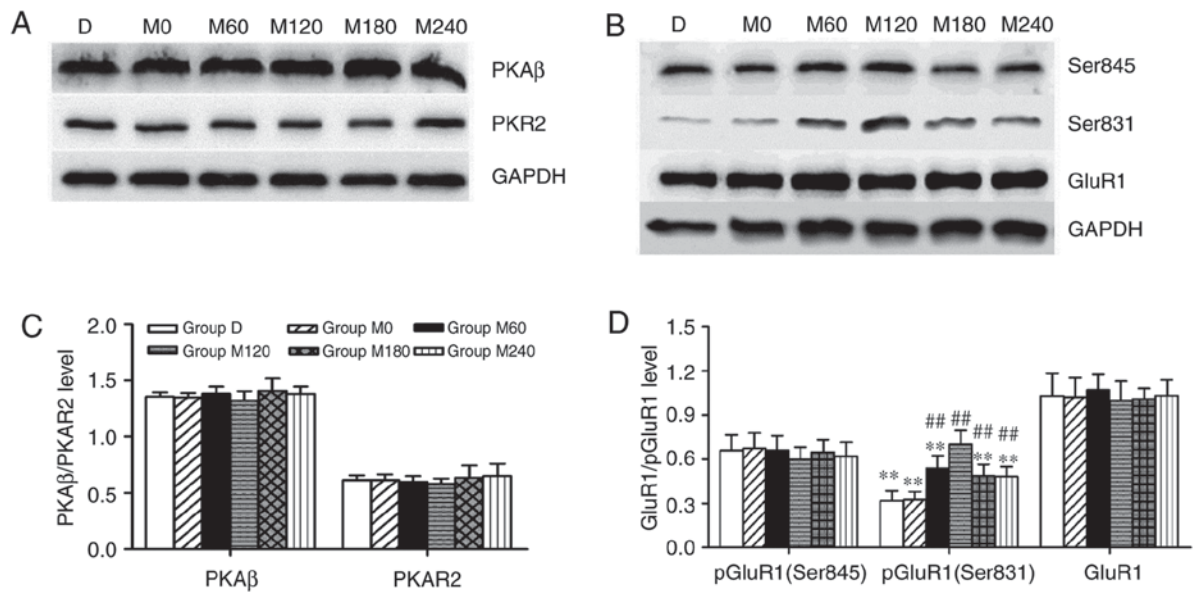


Figure 4. Effects of different charges of modified electroconvulsive seizure on the expression levels of PKA, GluR1 and the phosphorylated forms of GluR1 in the hippocampus. (A) Western blot analyses were conducted to examine the expression levels of pGluR1 (Ser845), pGluR1 (Ser831) and GluR1 in hippocampal tissue. (B) Western blot analyses were conducted to examine the expression levels of PKAβ and PKR2 in hippocampal tissue. (C) Quantification of PKAβ and PKR2 expression relative to GAPDH. (D) Quantification of pGluR1 (Ser845), pGluR1 (Ser831) and GluR1 expression relative to GAPDH. Data are presented as the mean ± standard deviation (biochemical assay, n=8 in each group). ##P<0.01 vs. group D and **P<0.01 vs. group M120. GluR1, glutamate receptor 1; p, phosphorylated; PKA, protein kinase A; PKR2, PKA regulatory subunit; PKAβ, PKA catalytic subunit.

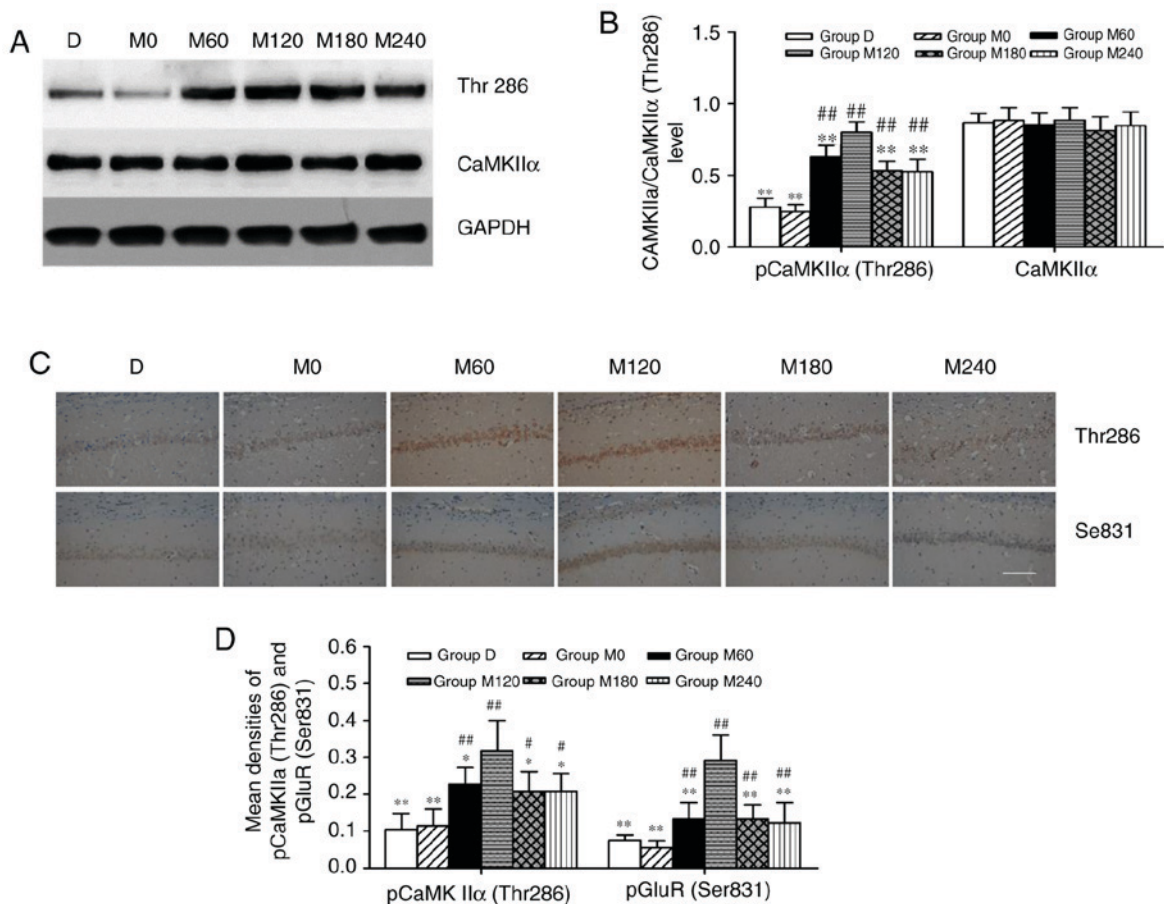


Figure 5. Effects of different charges of modified electroconvulsive seizure on the levels of CaMKIIα and pCaMKIIα (Thr286) in the hippocampus. (A) Western blot analyses were conducted to examine the expression levels of CaMKIIα and pCaMKIIα (Thr286) expression in hippocampal tissue. (B) Relative expression of CaMKIIα and pCaMKIIα (Thr286). (C) Representative images of pGluR (Ser831) and pCaMKIIα (Thr286) expression in the CA1 region of the hippocampus (magnification, x400). (D) Quantitative analysis of pGluR (Ser831) and pCaMKIIα (Thr286) expression levels in the hippocampus. Data are presented as the mean ± standard deviation (biochemical assay, n=8 in each group). *P<0.05 and ##P<0.01 vs. group D; *P<0.05 and **P<0.01 vs. group M120. CaMKIIα, calcium/calmodulin-dependent protein kinase IIα; p, phosphorylated; GluR1, glutamate receptor 1.

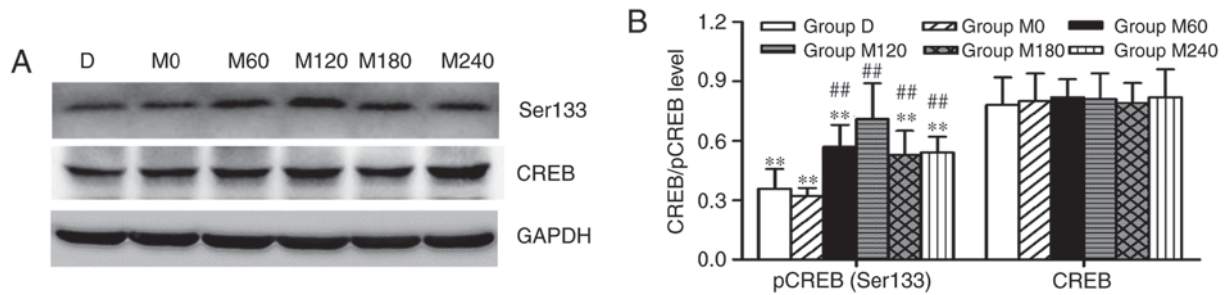


Figure 6. Effects of different charges of modified electroconvulsive seizure on the expression levels of CREB and pCREB (Ser133) in the hippocampus. (A) Western blot analyses were conducted to examine the expression levels of CREB and pCREB (Ser133) in hippocampal tissue. (B) Relative expression of CREB and pCREB (Ser133). Data are presented as the mean \pm standard deviation (biochemical assay, $n=8$ in each group). ## $P<0.01$ vs. group D; ** $P<0.01$ vs. group M120. CREB, cAMP-response element-binding protein; p, phosphorylated.

was the highest in group M120 compared with all other groups (all $P<0.01$; Fig. 4D). The results of immunohistochemistry were similar to those of western blotting, and indicated that the expression levels of pCaMKII α (Thr 286) and pGluR1 (Ser831) in the CA1 region of the hippocampus increased in group M120 compared with other groups ($P<0.05$ and $P<0.01$; Fig. 5D).

Expression levels of CREB and pCREB (Ser 133) in the hippocampus. The western blot analysis was used to analyze and quantify the protein expression of CREB and pCREB (Ser133) in the hippocampus. No significant alterations in the expression levels of CREB were detected ($F=1.036$ and $P=0.427$). However, significant differences were identified in the expression levels of pCREB (Ser133) among the six groups ($F=13.156$ and $P<0.01$). Group M120 exhibited a significantly increased level of pCREB (Ser133) in the hippocampus ($P<0.01$) compared with the other five groups. Additionally, the expression levels of pCREB were lower in groups D and M0 compared with other groups (all $P<0.01$; Fig. 6A and B).

Discussion

The current study primarily demonstrated that different charges of MECS affected the behavioral performance and protein expression levels in stressed rats. Notably, the medium stimulus intensity (120 mC) of MECS was optimal for improving the antidepressant efficacy and cognitive performance in rats with depression. Furthermore, the underlying cognitive protective mechanisms of the stimulus intensity were associated with the upregulation of pCaMKII α (Thr286), pGluR1 (Ser831) and pCREB (Ser133).

The CUMS procedure, which has been successfully used in previous studies to establish animal models of depression (17,29,37), was first successfully established by Willner *et al* (38) in 1987. However, it should be noted that CUMS procedures may differ slightly in certain aspects between different laboratories, primarily due to convenience and logistics (29,39). While a certain extent of standardization of the procedure may be desirable, there are no standard factors that distinguish the laboratories in which the procedure operates reliably from those where it does not (39). Anhedonia is one of the primary symptoms of depression, which can be inferred from a reduction in the intake of sweet liquid food (1,40). As expected, the SPP of rats decreased significantly following the

CUMS procedure in the present study, which was consistent with results of a previous study (7). In addition, the CUMS model could also be used to simulate human bradykinesia, which is one of the core symptoms of depression (41,42). OFT is widely used to evaluate the locomotor and exploratory behavior in experimental animals, a reduction in which is a sign of depression (34,37). The stressed rats in the present study exhibited decreased crossing and rearing numbers, indicating apathy and reduced exploration. These results indicated that the depression-like behaviors were successfully modeled using the CUMS procedure. Furthermore, following administration of different charges of MECS, the SPP and OFT performances differed between groups treated with different charges. In addition, the SPP and OFT performances remained unaltered following an increase in charges from medium to high (≥ 20 mC). These results indicated that the ceiling effect of the antidepressant therapy using ECS with the compound anesthetics was reached at the 120 mC charge. One possible explanation for the lack of improvement in antidepressant efficacy following administration of higher stimulus intensities (180 and 240 mC) in the present study may be associated with the seizure duration, which was an important determinant of the treatment efficacy. A previous study reported that the ECT seizure duration did not exhibit a linear association with the stimulus intensity, and increased intensities may reduce seizure duration (43). However, further studies are required to clarify the underlying mechanism of this phenomenon, as the present study could not monitor the brain electrical activity of the stressed rats due to the lack of equipment. A previous study indicated that pretreatment with propofol could further improve the antidepressant effect induced by ketamine in the forced swimming test (44). However, no statistically significant improvement was found in the depression-like behaviors in rats administered the compound anesthetics only (group M0). The present study hypothesized that this result may be associated with the anesthetic regimen and animal models (exposed to acute or chronic stressors). Wang *et al* (44) used a subanesthetic adjuvant dose of propofol (5, 10, 15 and 20 mg/kg, i.p.) combined with ketamine (15 mg/kg, i.p.) to evaluate their safety and efficacy in rats with acute depression-like behavior. In the current study, a low dose of ketamine (10 mg/kg, i.p.) combined with propofol (80 mg/kg, i.p.), which was equivalent to the clinical dose in humans, was selected as the anesthetics during ECS in chronically stressed rats. Furthermore, Wang *et al* (44)

found that the subanesthetic adjuvant dose of propofol with ketamine could not increase the crossing and rearing of the rats compared with the control group. Therefore, combined with clinical-dose propofol, a lower-dose ketamine may not be able to exert a direct antidepressant effect in the chronic stress model of depression. To the best of the authors' knowledge, the anesthetic regimen used in the present study has not been previously reported. However, according to previous studies and relevant preliminary experiments, propofol (100 mg/kg, i.p.) or ketamine (50-100 mg/kg, i.p.) could be safely used as general anesthetics for rats (45-47). To minimize the possible adverse effects of ketamine including delusions and hallucinations, a lower dose (10 mg/kg, i.p.) was selected in the present study. Using the transformation formula for ketamine between rats and human patients, the dosage used in the present study was equivalent to the low-dose ketamine commonly used in clinical research for the induction of antidepressant effects in patients (17). A previous study has indicated that administration of 10 mg/kg, i.p. ketamine could prevent stress-evoked dysfunctions in rats (48). Due to the administration of compound anaesthetics, 80 mg/kg, i.p. propofol was used in the current study. This dose has been previously demonstrated to be safe and effective (47). No animal mortality was observed, and, therefore, the compound dosage used in the current study may be appropriate for MECS.

The therapeutic efficacy of ECT is associated with the degree to which the stimulus intensity exceeds the seizure threshold (i.e., the relative intensity) (49). The initial seizure threshold of the stressed rats treated with the compound anesthetic was ~41 mC. The relative intensities of 60, 120, 180 and 240 mC used in the present study were ~1.5-, 3-, 4.5- and 6-fold the seizure threshold, respectively. Although the highest charge was 240 mC, all rats survived under the high intensities in the present study, which was in line with the results reported by Luo *et al.* (33). A previous study found that the initial seizure threshold of solo ECS (without the administration of anesthetics) was 10 mC on average (18), and the threshold increased to 40 mC when ECS was administered with propofol (33), suggesting that this anaesthetic may exhibit antiepileptic effects. Based on the result that the initial seizure threshold of ECS co-administered with propofol and low-dose ketamine was 41 mC in the current study, it was hypothesized that low-dose ketamine may not interfere in the seizure effects of ECS.

MWM test is frequently used as to study learning and memory function of animal models (1,36,50). However, the MWM test can lead to epigenetic alterations in the hippocampus and influence the cognitive behavior of animals. Zots *et al.* (51) found that the MWM test caused the formation of both spatial and nonspatial memory. Carter *et al.* (50) demonstrated that MWM training caused distinct epigenetic and gene expression alterations in rat hippocampal neurons (50). Therefore, repeated MWM tests may not accurately reflect the alteration of learning and memory function induced by ECS and anesthetics. Based on these concerns, the current study used 8 rats from each group for the behavioural tests, including the MWM test, and 8 rats from each group for the biochemical assays. The MWM test was performed only once to prevent changes to the epigenome. This protocol prevented the behavioral tests negatively affecting memory,

which can be associated with differential protein expression in the brain of the stressed rats (50). Preliminary studies indicated that the combination of ketamine and propofol exhibited a better cognitive performance compared with the use of propofol alone in the ECS procedure (17,52). The present study further identified an appropriate stimulus intensity of ECS to be applied in combination with anesthetics to improve the cognitive function of stressed rats. The results of the present study confirmed that the medium charge of 120 mC was the optimal stimulus intensity for the improvement in cognitive function. Receiving the compound anesthetics alone could not improve the cognitive performance and depression-like behaviors in stressed rats. The results of the current study indicated that low-dose ketamine combined with propofol was an appropriate anesthetic protocol for ECS, and 120 mC was the optimal charge under this novel protocol.

A previous study suggested that ketamine, an NMDA receptor antagonist, when administered at a low dose, may induce rapid antidepressant effects (14). Furthermore, low-dose ketamine could ameliorate the cognitive impairment induced by ECS (17). Previous studies suggested that the NMDA receptor is one of the key factors regulating the cognitive function. One study indicated that chronic exposure to arsenic could reduce the learning and memory function accompanied by downregulated levels of NMDA receptor subunit 2 (NR2) and memory-associated proteins proto-oncogene *c-Fos* and *c-Jun* (53). However, Maeng *et al.* (21) revealed that both nonselective NMDA antagonists and NR2B-selective antagonists exerted antidepressant effects by enhancing the activity of AMPA receptors relative to NMDA receptors. Furthermore, Chen *et al.* (17) found that low-dose ketamine combined with propofol could improve the cognitive impairment induced by ECS, which may be attributed to modulating the expression of pGluR1. GluR1 receptor subunit is expressed in the majority of brain regions, predominantly in the hippocampus (54). Previous studies suggested that the phosphorylation of GluR1 was necessary for synaptic expression, channel properties of AMPA receptors, synaptic plasticity, spatial learning and memory function (24,54,55). GluR1 Ser831/Ser845 double phosphomutant mice exhibited a significant cognitive deficit (56). Therefore, the GluR1 phosphorylation states modulated by CaMKII and PKA may serve roles in cognitive function. The current study indicated that the expression level of pCaMKII α (Thr286) in group M120 was the highest among the study groups, and was coincident with the cognitive performance in the MWM test. However, no significant alterations in total CaMKII α protein levels were observed. Therefore, these results suggested that the effects of different charges of MECS may be associated with alterations in CaMKII α activity. The results of the present study are consistent with the results of a previous study where an increase in CaMKII activity was associated with the induction of long-term potentiation (LTP) as a model of synaptic plasticity (57). Activation of CaMKII resulted in an increased expression of AMPA receptors, which caused enhanced function of AMPA and was necessary for the induction of LTP (57). Previous studies indicated that PKA acted in parallel with CaMKII in controlling synaptic incorporation of GluR1 (24,58). Therefore, the activation of PKA may also

affect activity-dependent AMPA receptor trafficking (24,58). The current study identified no marked alterations in the expression levels of the catalytic or regulatory subunits of PKA, suggesting that the alteration in cognitive function among the rats may not be associated with the PKA activity. However, it has been previously demonstrated that the activation of either PKA or CaMKII could modulate the GluR1 phosphorylation (24). One possible explanation for the above results was that repeated ECS, similar to single ECS, exerted its clinical effect primarily through the modulation of AMPA activity mediated by the GluR1 phosphorylation at Ser831 (59). The biochemical results of the present study indicated that GluR1 was significantly phosphorylated at Ser831 following MECS treatment, whereas the phosphorylation of GluR1 at Ser845 was unaltered. Therefore, MECS improved the cognitive performance in stressed rats, which may be due to the activation of the CaMKII-mediated phosphorylation of GluR1 in the hippocampus.

CREB is a key modulator of development, plasticity and neuroprotection in the central nervous system. Alterations in CREB-mediated transcription have been implicated in multiple cognitive and psychiatric disorders including depression and cognitive decline (60). It has been previously demonstrated that CREB activity decreased in the brains of depressed patients, was upregulated following administration of a number of antidepressants, and induced antidepressant effects in rodent hippocampi (61). Qiu *et al* (62) indicated that CREB could be phosphorylated in response to the activation of GluRs, leading to alterations in synaptic plasticity and neuronal structure. Therefore, the current study detected the expression of CREB and pCREB and found that the medium charge of 120 mC administered with the novel anesthetic protocol markedly upregulated the level of pCREB and did not alter the expression of CREB in the experimental animals. These results further confirmed that the combination of ketamine, propofol and ECS affected the cognitive function of depressed animals associated with synaptic plasticity. The present study suggested that optimal neuroprotective effects require administration of an appropriate stimulus intensity for the improvement of antidepressant activity.

In conclusion, the present study provided evidence that the medium stimulus of 120 mC was the optimal intensity for the treatment of stressed rats using this novel anesthetic protocol. The improved cognitive function was associated with CaMKII-mediated phosphorylation of GluR1 in the hippocampus.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

FZ was performed the experiments, data analysis and manuscript preparation. GH and XZ wrote and revised the manuscript, and produced the experimental design.

Ethics approval and consent to participate

The experiment protocols were approved by the Ethical Committee of Chongqing Medical University (reference no. 2017-004).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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