



Effects of Baihui electroacupuncture in a rat model of depression

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Background: This study aimed to investigate the effect of electroacupuncture (EA) on behavior in a rat model of chronic unpredictable mild stress (CUMS) and to explore the underlying molecular mechanisms.

Methods: A total of 45 adult male Sprague-Dawley rats were randomly divided into three groups: the control, CUMS, and CUMS plus EA groups. Rats in the CUMS and EA groups were subjected to a 3-week CUMS condition, while rats in the EA group received EA at the Baihui (GV 20) acupoint (2 Hz, 0.6 mA) for 10 min once daily before being subjected to the CUMS condition. The sucrose preference test (SPT) was used as a measure to infer activation of the pleasure response to depression-like behaviour. After the behavioral test, 5-bromodeoxyuridine (BrdU) was intraperitoneally injected (100 mg/kg) and brain samples were collected 24 h later for the detection of hippocampal BrdU. Cell proliferation was determined according to the proportion of BrdU-positive cells. Brain-derived neurotrophic factor (BDNF) expression was detected.

Results: The severity of anhedonia, BDNF⁺ cells, and BrdU⁺ neurons in DG significantly decreased in CUMS rats, and was accompanied by a reduced BDNF and BrdU⁺ expression ($P < 0.05$). After EA, the low levels of BDNF⁺ cells and BrdU⁺ expression and the depression-like behavior increased markedly ($P < 0.05$).

Conclusions: EA contributes to neuroprotection against CUMS by enhancing BDNF expression and improving hippocampal neurogenesis.

Keywords: Major depressive disorder (MDD); electroacupuncture (EA); chronic unpredictable mild stress (CUMS); brain-derived neurotrophic factor (BDNF); neurogenesis; rat

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Introduction

Major depressive disorder (MDD) is associated with poor social function, low quality of life, increased economic burden, and a high risk of suicide (1). Acupuncture has been used in China for thousands of years as a widely recognized alternative therapy in clinical practice, and has been used to treat various neurological diseases including depressive disorders (2-5). Due to recent advances in technology, a new

form of acupuncture, electroacupuncture (EA), is now being applied in clinical practice. It has proved that EA could be used in improving cognitive and emotional symptoms in patients with organic brain diseases like Alzheimer's disease (6), anxiety disorders (7) and several physical diseases comorbid mood disorders including irritable bowel syndrome (8), hypertension (9) and stroke (10). Although exercise, electroconvulsive seizure therapy and transcranial magnetic stimulation are used to treat

depression, psychopharmacological interventions are still the main stay of treatment options for most patients (11) by updating of evidence (12) and guideline (13,14). Evidence-based medicine involving in the efficacy and tolerance on treatments of depression remains largely empirical. Several first-line pharmacological treatments are available, they are not effective for a significant proportion of patients (15) and concomitant adverse reactions limit the use in clinical practices (16). Acupuncture can activate a patient's own pain control system, stimulate blood flow, repair the physical damage of emotional distress, reduce pain, lift mood, and boost the immune system. If the combination of low-dose antidepressants plus EA could produce the same efficacy as solo antidepressants treatment with conventional dose, while reducing drug-related adverse reactions, this will be a relatively ideal treatment option. Evidences show that EA not only significantly improve depression symptoms and has fewer side effects than antidepressants (2) but when combined with a low dose of antidepressants mimic efficacy appear as compared to the same drug treatment with conventional dosage meanwhile showing lower incidences of adverse reactions and relapse (17). For these reasons, it has become a widely applied alternative therapy and is generating significant interest from Western countries (18). However, the antidepressant mechanism of EA is poorly understood, and evidence of its effectiveness remains weak (19). Further clinical and experimental studies are warranted to confirm the effectiveness of EA. Studies of animal models have indicated that EA exerts its neuroprotective effect against cerebral ischemia (20), hemorrhage (21), hypoxia, chronic stress (2), inflammation (22), and cognitive impairment (23) by upregulating the brain-derived neurotrophic factor (BDNF) expression in the hippocampus. Given that BDNF downregulation-induced hippocampal synaptic plasticity impairment plays an important role in the pathogenesis of MDD, the present study was undertaken to investigate the anti-depression-like effects of EA in rats exposed to chronic unpredictable mild stress (CUMS) and to explore the underlying mechanisms. Although different kinds of animal models of depression are used in laboratory experiments including surgical bilateral olfactory bulbectomy, maternal separation early life stress, learned helplessness, repeated restraint stress, social isolation, chronic social defeat stress, witness defeat, genetic and pharmacological intervene and CUMS (24), CUMS modal centers on the idea that low level chronic and unpredictable stressors, similar to what vulnerable individuals might experience in everyday life,

could induce a wide range of behavioral deficits including decreased sucrose preference, motivation for reward stimuli and sexual behaviors along with increased aggression, anxiety-like behaviors and altered sleep patterns (25). The depression-like phenotype can be assessed with strong validity in deferent maze tests for cognition, open-field test for motivation and sucrose preference for anhedonia.

We present the following article in accordance with the ARRIVE reporting checklist (available at <http://dx.doi.org/10.21037/atm-20-7459>).

Methods

Animals and grouping

Forty-five adult male Sprague-Dawley rats weighing 200–240 g were purchased from the Animal Center of Zhejiang University and randomly divided into three groups: control (n=15), CUMS (n=15), and EA plus CUMS (n=15) using random number table method. After a baseline sucrose preference test (SPT), all rats except those in the control group were subjected to a 3-week CUMS condition and housed individually. During this time, the EA group received daily EA at the Baihui acupoint. SPT was administered again at 7-, 14-, and 21-day intervals after the initiation of the CUMS condition to assess the severity of depression-like behavior. After the final SPT, 5-bromodeoxyuridine was intraperitoneally administered (100 mg/kg) to all animals. Twenty-four hours later the rats were killed with an overdose intravenous injection of 10% chloral hydrate at 3 mL/kg, and the bilateral hippocampi were collected for immunohistochemistry and western blotting. Experiments were performed under two projects the Traditional Chinese Drug Administration Bureau of Zhejiang Province (license No. 2017ZA083) and the Major Science and Technology Project in Medical and Health of Zhejiang Province (co-constructed Project by the Province and the Ministry, license No. WKJ-ZJ-1922) and granted by Animal Ethics Committee of Zhejiang University of Chinese Medicine (license No. 2016-327), in compliance with the guidelines of Zhejiang University for the care and use of animals.

CUMS procedures

Twenty-four hours after the baseline SPT, rats in the CUMS and EA plus CUMS groups were subjected to CUMS to induce behavior reflective of the core depression symptom:

anhedonia. The CUMS protocol was designed to maximize the unpredictable stress according to previous reports (26,27) with a minor modification. One of the following stressors was administered every day (in a random order) for 3 weeks: food deprivation for 20 h; water deprivation for 17 h; swimming at 4 °C for 5 min; experiencing at high temperature (40 °C) for 5 min; remaining in the cage at a 45° tilt for 17 h; remaining on a shaking platform (horizontal shaking at high speed) for 10 min; being restrained for 2 h; remaining in a soiled cage (200 mL water mixed with 100 g sawdust bedding) for 5 h; remaining in an environment with persistent bright light 17 h; tail pinching for 2 min; and exposure to unpleasant noise for 5 min. Immediately after each stress session, rats were returned to their usual cage and maintained in standard conditions until the next stress session.

EA treatment

Acupuncture was performed as previously reported (22) at the Baihui (GV-20) acupoint in the EA group once daily during the 3-week CUMS exposure. GV-20 is located above the apex auriculate at which the fractional amplitude of low-frequency fluctuation could fully spread to mood relevant hippocampus and prefrontal cortex circuits (28). The sterilized disposable stainless steel needles (0.3 mm in diameter) were inserted to 2–3 mm, and EA was conducted for 10 min once daily with a dense-dispersed wave at a frequency of 2 Hz and a current of 1 mA (low frequency 5-channel TENS Unit and Electrical Needle Stimulator, Huatuo Medical Technology Co., Ltd., Suzhou, China).

Sucrose preference testing

The sucrose preference test (SPT) has been used as a measure of the severity of anhedonia, and details of the procedure have been described previously (29). In brief, animals were housed individually with two bottles containing either 1% sucrose solution or tap water with standard lab chow available continuously. At baseline, an initial SPT was performed before the CUMS condition, and then further SPTs were performed three times under similar conditions (two-bottle test, 2-h periods) on every weekend after the commencement of the CUMS exposure.

Hippocampal immunohistochemistry

Immunohistochemistry was performed for the evaluation of BDNF or BrdU-positive cells. A SABC-kit (Wuhan

Boster Biological Technology, China) was applied to the immunohistochemistry for BDNF evaluation. After deparaffinization and hydration, sagittal sections (5 µm) were blocked in 5% albumin from bovine serum for 20 min. After incubation with rabbit-anti-rat BDNF immunoglobulin G (IgG) (1:200) (4 °C) overnight, sections were treated with biotinylated goat-anti-rabbit IgG and streptavidin-biotin peroxidase complex (SABC) for 2 h (37 °C), followed by visualization with 3,3'-diaminobenzidine. Counterstaining was done with hematoxylin. This was followed by dehydration in alcohol, transparency in xylene, and mounting with neutral resin. The hippocampal neurogenesis was evaluated on the coronal sections (5 µm). In brief, the sections were treated with 0.3% hydrogen peroxide in phosphate buffer saline (PBS) for 30 min and then with 10% normal goat serum in 0.05 M PBS for 30 min. After incubation with anti-BrdU monoclonal antibody (1:200) overnight at room temperature, sections were exposed to SABC for 2 h (37 °C). The sections were mounted in neutral resin after dehydration and then observed under an Olympus microscope. The BDNF⁺ cells and BrdU⁺ cells in the same region of the dentate gyrus were counted and analyzed with the FIVE software (Thermo Fisher Scientific).

Western blotting

Western blotting was performed as previously reported (30). Brain tissues were lysed in lysis buffer (0.05% Igepal, 50 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl, 1% deoxycholic acid, 0.1% SDS, 1% Triton X-100). The lysates were then centrifuged at 10,000 rpm for 30 min at 4 °C, and the supernatants were harvested for the detection of protein concentration. Total proteins were separated on 12.5% SDS-polyacrylamide gels and then transferred onto a nitrocellulose membrane. Membranes were then blocked in PBS containing 0.1% Tween and 5% nonfat milk for 30 min. Thereafter, membranes were treated with either anti-Cx43 monoclonal antibody (1:1,000, Sigma) or anti-actin antibody (1:500, Sigma) overnight. After washing, membranes were incubated with SABC for 1 h, followed by visualization with enhanced chemiluminescence. The expression of BDNF and BrdU was quantified by measuring the optical density (OD) at 260 nm.

Statistical analysis

All statistical analyses were carried out with SPSS software

version 15.0 (IBM SPSS Software), and all data are expressed as mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) was used for between-group comparisons. A P value <0.05 was considered statistically significant.

Results

Behavioral changes on SPT

At baseline, there was no significant difference among

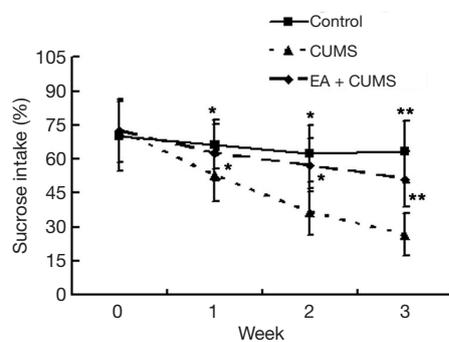


Figure 1 Sucrose intake in the three groups at different time points. One-way ANOVA, *, $P<0.05$; **, $P<0.01$ vs. CUMS group. ANOVA, analysis of variance; CUMS, chronic unpredictable mild stress; EA, electroacupuncture.

the three groups in sucrose intake [$F_{(2,42)} = 0.47$, $P>0.05$]. However, sucrose intake reduced significantly in the CUMS group compared to the control group after 1 week of stress exposure, and this effect lasted until the end of the experiment ($P<0.05$). After 1 week of CUMS exposure, sucrose intake was significantly different among the three groups ($P<0.05$), and EA treatment significantly increased the sucrose intake compared to the CUMS group at the same time point (all $P<0.05$) (Figure 1).

Change of BDNF-positive cells in DG

BDNF-positive neurons had a large number of brown granules in the cytoplasm. These neurons were mainly found in the granular layer and nerve processes (Figure 2). The BDNF expression was at a high level in the control group but was significantly reduced in the CUMS group compared to the control group ($P<0.05$). After EA treatment, the BDNF expression in CUMS rats was significantly increased ($P<0.05$ vs. CUMS group).

Neurogenesis in DG

Newly generated neurons (BrdU positive) had dark brown granules in the nuclei. The presence of newly generated neurons suggests neurogenesis. The number

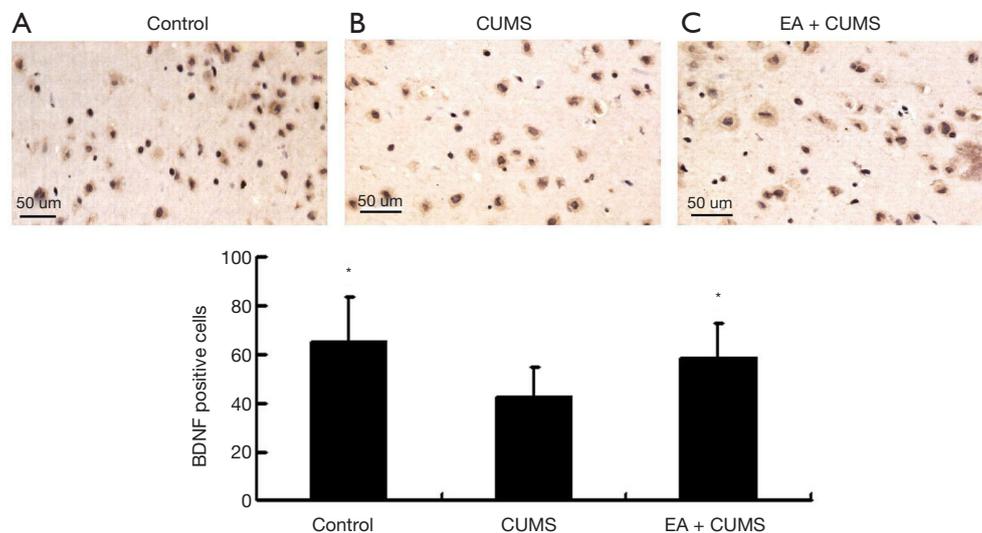


Figure 2 Immunohistochemistry for BDNF-positive cells (mean \pm SD) in DG of three groups. (A) Control group; (B) CUMS group; (C) EA plus CUMS group. BDNF⁺ cells had brown granules in the cytoplasm around the black nuclei. *, $P<0.05$ vs. CUMS group. $n=15$, Scale bar = 50 μm . CUMS, chronic unpredictable mild stress; EA, electroacupuncture; BDNF, brain-derived neurotrophic factor; SD, standard deviation; DG, dentate gyrus.

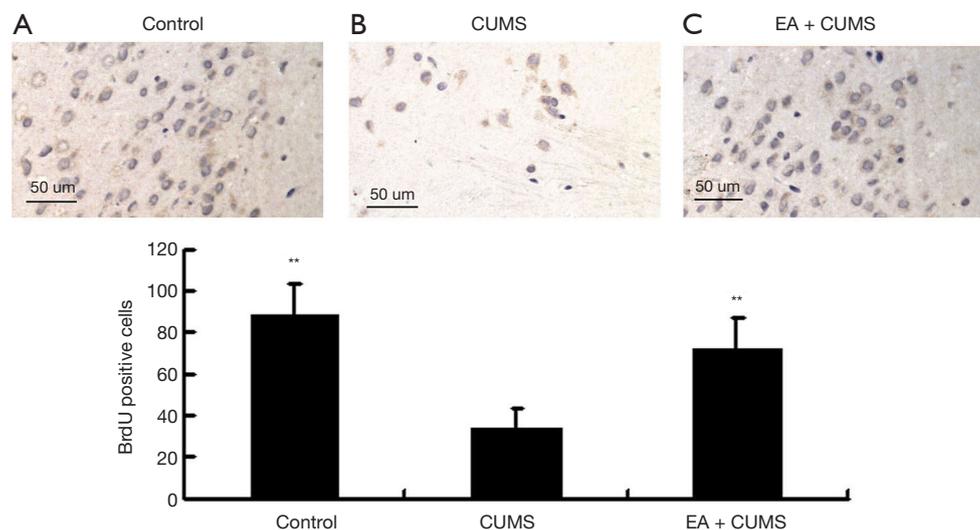


Figure 3 Immunohistochemistry for BrdU-positive cells (mean \pm SD) in the DG 24 h after BrdU administration in the three groups. (A) Control group; (B) CUMS group; (C) EA plus CUMS group. **, $P < 0.01$ vs. CUMS group. $n = 15$, scale bar = 50 μm . CUMS, chronic unpredictable mild stress; EA, electroacupuncture; BrdU, 5-bromodeoxyuridine; SD, standard deviation; DG, dentate gyrus.

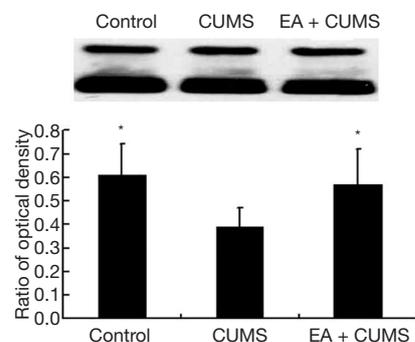


Figure 4 BDNF protein expression (western blotting) in the different groups. Mean \pm SD. *, $P < 0.05$ vs. CUMS group. BDNF, brain-derived neurotrophic factor; CUMS, chronic unpredictable mild stress, EA, electroacupuncture.

of BrdU-positive cells in the DG of the hippocampus was significantly lower in the CUMS group than in the control group, but EA treatment significantly increased the BrdU-positive cells ($P < 0.05$ vs. CUMS group) (Figure 3).

BDNF protein expression

The protein expression of BDNF in the hippocampus of the CUMS rats was markedly lower than in the control group ($P < 0.05$), but EA treatment significantly reversed the BDNF protein expression in CUMS-exposed rats (Figure 4).

Discussion

Investigations into the pathogenesis of MDD have made significant progress in recent times, with evidence suggesting that dysfunctional hippocampal neurogenesis and BDNF downregulation contribute to this disorder (31). It has reported exercise (32), antidepressants (33) and electroconvulsive seizure therapy (34) could reverse pathological synaptic remodeling and new born neuron formation modulated through BDNF elevation in depressed animals. In the CUMS model, animals may present with anhedonia, which is a core symptom of MDD (22), as indicated by their behavioral response to the SPT. In this study, the CUMS condition induced significant reduction in sucrose intake on the SPT, but EA treatment restored the sucrose intake in CUMS-exposed rats, suggesting that EA might also improve the symptoms of MDD. We further examined the mechanism underlying the neuroprotective effects of EA by evaluating hippocampal neurogenesis and BDNF expression. Results indicated that neurogenesis and the protein expression of BDNF was significantly reduced in rats exposed to the CUMS condition, which is consistent with previous findings that stress-induced depression is associated with decreased neurogenesis (35). A variety of studies have demonstrated the relationship between CUMS and reduced BDNF expression mediated by abnormal hippocampal plasticity (36). Research has confirmed

combined Baihui and Dazhui acupuncture treatment may lead to a better therapeutic effect via co-regulation of neurotrophic factors in the prominent atrophic zones and promoting proliferation of neural progenitor cells in striatum in mice with ischemic stroke (37). Thus, the concomitant reduction in BDNF expression in rats exposed to CUMS in this study further confirms the role of BDNF in the pathogenesis of MDD, and therefore treatments targeting improvement in BDNF expression in the brain may prove to be promising developments for neuroprotection in MDD (38).

The neuroprotective effect of EA at GV 20 in MDD rats may possibly be related to the improvement of neural circuitry in the case of mood disorders (39). Functional and structural brain imaging has revealed that the cortex-hippocampal loop is associated with higher-order emotional and cognitive functioning (40), and stimulation at any acupoint including GV 20 in this loop may lead to the improvement of mood-related cerebral dysfunction. Furthermore, hippocampal neurogenesis is involved in the plasticity of hippocampal inner circuits. Neural progenitors in the DG originate from the subgranular layer. After proliferation, maturation, and migration to the CA3 area, the newly generated cells become mature and form a network with pyramidal neurons to integrate information into the cortex-hippocampal loop. In the growth and differentiation stage of neural stem cells, BrdU, an analogue of TTP that can be incorporated into DNA during the synthesis of DNA, has become a marker for newly generated cells, and the expression of BrdU therefore reflects the neurogenesis in local neural networks. A variety of factors are involved in the regulation of hippocampal neurogenesis including neurotransmitters, hormones, growth factors, and external environmental factors such as stress, social isolation, substance abuse, and physical exercise. A recent study reported that EA treatment after ischemic stroke may improve the post-stroke functional recovery by enhancing the proliferation and differentiation of neuronal stem cells via the BDNF signaling pathway (37).

CUMS reduces BDNF protein expression mainly via the dysfunctional hypothalamic-pituitary-adrenal (HPA) axis and the immune system (41). It is therefore possible that an increase in BDNF expression may exert a direct antioxidative effect (42) or indirectly improve the homeostasis of the neuroendocrine system (43) by inhibiting the hyperactivity of the HPA axis (44), decreasing the mRNA expression of adrenocorticotrophic hormone (ACTH), a corticotropin-releasing hormone, in the

hypothalamus and cortisol in the plasma, in order to control the immune response to tissue injury (45) and enhance the hippocampal serotonin system (46) which protects the brain against damage. This may partially explain why low-frequency EA is able to enhance mood and emotion, but further investigation is necessary to detail the exact mechanisms involved in EA and mood disorders.

Our study has several limitations. First, it must be cautious when the research conclusion is extended to human being due to certain racial differences and limitations of animal model in rats. Second, the exact sites of current diffusion involving in emotional control regions in brain and the activation level of cell currents are not precisely studied. Finally, we only selected Baihui acupoint for treatment and this fact is not consistent with the real situations that based on the theory of meridians several acupuncture sites are always simultaneously used to treat patients to achieve wider stimulus fields and bigger efficacy. A further research will be performed to overcome these shortcomings in the future.

Conclusions

In conclusion, our study shows that administration of EA at the Baihui acupoint may improve the symptoms of MDD through increasing BDNF expression in the brain to restore hippocampal neurogenesis.

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Footnote

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[org/10.21037/atm-20-7459](https://doi.org/10.21037/atm-20-7459)). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under two projects the Traditional Chinese Drug Administration Bureau of Zhejiang Province (license No. 2017ZA083) and the Major Science and Technology Project in Medical and Health of Zhejiang Province (co-constructed Project by the Province and the Ministry, license No. WKJ-ZJ-1922) and granted by Animal Ethics Committee of Zhejiang University of Chinese Medicine (license No. 2016-327), in compliance with the guidelines of Zhejiang University for the care and use of animals.

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