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Brief report

Glia atrophy in the hippocampus of chronic unpredictable stress-induced depression model rats is reversed by electroacupuncture treatment

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ABSTRACT

Background: Growing evidence indicates that glia atrophy contributes to the pathophysiology and possibly the pathogenesis of major depressive disorder. Electroacupuncture (EA), one of Chinese traditional therapy, has potent antidepressant-like effect in many clinical studies. The mechanism by which EA improves behavioral deficits is still unclear.
Method: Chronic unpredictable stress (CUS)-induced depression model rats were used to study the effect of EA treatment. EA was performed on acupoints 'Bai-Hui' (Du 20) and unilateral 'An-Mian' (EX 17) once daily for three consecutive weeks two weeks post CUS procedure. The antidepressant-like effect of EA treatment was analyzed by physical state (PS) and open field test (OFT). Astrocytic marker glial fibrillary acidic protein (GFAP) level in the hippocampus was detected by immunohistochemsitry, Western blot analysis and reverse transcription-polymerase chain reaction (RT-PCR).
Results: Exposure to CUS resulted in a decrease of behavioral activity, whereas a daily session of EA treatment significantly reversed the behavioral deficit of these depression model rats. Moreover, the levels of GFAP mRNA and protein were decreased in the hippocampus of depression model rats. Intriguingly, EA treatment blocked effectively the decreased GFAP level.
Limitation: The relative small number of the depression model rats may cause some bias of behavioral tests.
Conclusion: EA has potential antidepressant-like effect on CUS-induced depression model rats, which might be mediated by affecting the glial atrophy in the hippocampus.

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1. Introduction

Depression is a devastating illness that may be caused by genetic and environmental factors such as stress (Nierenberg and Alpert, 2000; Berton and Nestler, 2006). Although significant progress has been made in treating depression, about 20–30% of depressed patients are resistant to current drug therapies and there is a 2–3 week lag of onset to therapeutic efficacy (Duman et al., 2000; Wong and Licinio, 2001). It is crucial to look for the alternative treatments with balanced impact between the antidepressant therapeutic effects and side effects (Han, 1986). Acupuncture is a traditional Chinese therapy that has become increasingly popular in Western countries. Sometimes an electric current passed through the needles and this is known as electroacupuncture (EA) (Ulett et al., 1998). A number of studies have found that acupuncture is an effective remedy for depression and it may be as effective as antidepressant drugs (Mukaino et al., 2005).

However, there are few basic experimental studies regarding the mechanism of acupuncture treatment in depression. A recent study showed the notable changes of astroglial structural plasticity in response to stress and antidepressant treatment, which supports the notion that glial changes may

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contribute to the pathophysiology of affective disorders as well as to the cellular actions of antidepressants (Czeh et al., 2006). A previous study also indicated that EA could enhance VEGF expression through the activation of astrocytes during treatment with transient middle cerebral artery occlusion in rats (Wang et al., 2003). These results raised the question of whether EA can influence astrocytes in the hippocampus during treatment with depression model rats. To address this question, we investigated the effect of EA on the behavioral activity and GFAP level in the hippocampus of the chronic unpredictable stress (CUS)-induced depression rats model, which had been extremely useful in elaborating and detecting the effects of antidepressant drugs (Katz and Sibel, 1982; Willner, 1990).

2. Methods

Experiments were performed on adult 3 month old (300–320 g) male Sprague–Dawley rats (Experimental Animal Center, Shanghai Medical College of Fudan University, China). All rats were used strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize the number of animals used and their suffering. The CUS procedure was carried out in animals used to induce depression model and to treat with EA or sham EA once per day for 3 consecutive weeks (Willner, 1995; Liu et al., 2007). Physical state was measured about once a week using a scale from 1 to 3: a healthy state was noted as 3 and damaged state with piloerection and/or dirty fur was noted as 1. Intermediate state was noted as 2 (Alonso et al., 2004). The open field test was performed as previously described (Redmond et al., 1997), which included the number of crossings (defined as at least three paws in a quadrant) and the number of rearings (defined as the animal standing upright on its hind legs) during the 3 min session, and was measured before stress, EA or sham EA treatment and at the end of the experiment (Fig. 1A).

For EA treatment, according to our previous studies, 'Bai-Hui' and 'An-Mian' acupoints were selected (Liu et al., 2007; Yu et al., 2007). A pair of stainless steel needles of 0.3 mm diameter was inserted with a depth of 3 mm and 2 mm respectively into the acupuncture points 'Bai-Hui' (Du-20, located above the apex auriculate, on the midline of the head) and "An-Mian" (EX 17, between muscle sternocleidomastoideus and muscle splenius capitis). The two needles

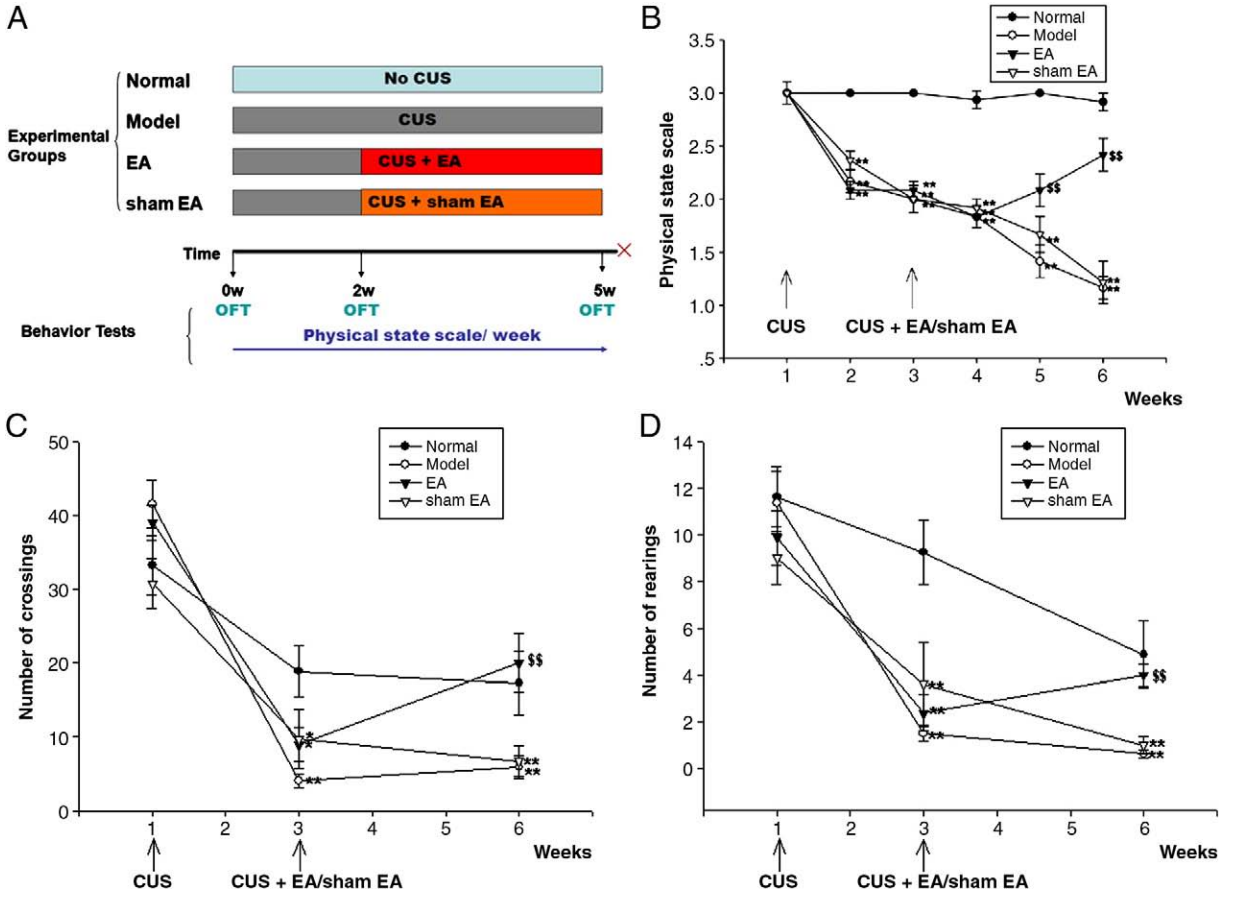

Fig. 1. Rats were randomly divided into four groups: Normal, Model, sham EA, EA ($n = 6$ per group). Stress groups were subjected to CUS during 5 weeks, whereas the normal group remained undisturbed. Treatment groups received EA or sham EA treatment 2 weeks after the experiment started. For analysis of GFAP expression, groups of rats were sacrificed at the end of a 5-week period (A). Physical state scores (B). Number of crossings or rearings in the open field behavior (C&D). Results are given as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ vs. Normal group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. sham EA group.

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were connected with the output terminals of an EA apparatus (Model G6805-1A, Shanghai Medical Electronic Apparatus Company, China). Alternating strings of dense-sparse frequencies (60 Hz for 5 s and 4 Hz for 2.5 s alternately) were selected. The intensity was adjusted to induce slight twitch of the ear (≤1 mA), with the intensity lasting for 30 min.

The expression of GFAP in the hippocampus was observed by immunohistochemistry. The sections were subsequently incubated overnight with rabbit anti-GFAP polyclonal antibody (1:1000, DAKO, USA) at 4 °C for 24 h. The secondary antibody was biotinylated goat anti-rabbit immunoglobulin G (IgG) (1:200, Vector Laboratories, Burlingame, CA). Levels of GFAP protein and mRNA were measured by Western blot analyses and reverse transcription-polymerase chain reaction (RT-PCR) essentially as described in our previous study (Liu et al., 2009). For Western blot analyses, the primary antibody was diluted 1:5000 (TTBS, incubated at 4 °C) and the secondary antibody diluted 1:2000 (TTBS, incubated 45 min R.T.). For RT-PCR analyses, the sequences of primers for GFAP were as follows: forward: 5′-TGAGGCAGAAGCTCCAAGATGAAA-3′ 1178–1201 reverse: 5′-CTGGTTTCTCGGATCTGG-3′ 1599–1622 (U59486); β-actin forward: 5′-CACCATGTACCCTGGCATTG-3′ reverse: 5′-TAACGCAACTAAGTCATAGT-3′. The PCR results were expressed as ratios of the intensity of the GFAP bands to that of β-actin band.

Data are presented as mean ± S.E.M. and analyzed by SPSS 11.0. Repeated measures analysis of variance (ANOVA) followed by S–N–K test was used for post-hoc analysis for differences between groups. $p<0.05$ was considered statistically significant.

3. Results

For the behavioral tests, at the beginning of experimental procedure, there was no significant difference among the groups according to the physical state score ($F_{3,20}=1.667$, $p>0.05$). After CUS for 2 weeks, stressed rats showed a significant degradation of the physical state due to stress ($F_{3,\ 20}=27.439$, $p<0.01$). Since this time, EA and sham EA were administered to the stressed rats for 3 weeks. At the end of the 5th week, the model rats and sham EA-treated rats showed significantly decreased scores in physical state ($F_{3,\ 20}=79.872$, $p<0.01$). In contrast, the degradation of the physical state was significantly improved by EA after 2 weeks of treatment. This effect lasted for the remainder of the stress period (Fig. 1B). Similar changes were seen in the open field test for all groups of rats, which demonstrated a typical decrease in the number of crossings and rearings, whereas EA improved these behavioral activities (Fig. 1C, D).

Immunohistochemistry revealed that CUS for 5 weeks led to a significant decrease in the expression of the astroglial marker, GFAP, in the DG (Fig. 2A, B, C) and CA3 region (Fig. 2E, F, G) of hippocampus, whereas EA treatment for 3 weeks reversed this effect. GFAP-immunoreactive astroglial cells showing fine branches were sparsely distributed in the normal rats (Fig. 2D).

Quantification of protein by Western blot showed that a single protein band of the expected size (~50 kDa) for GFAP was detected with the GFAP-specific primary antibody (Fig. 2H). The statistical results demonstrated that GFAP protein levels in the hippocampus of model rats and sham EA-treated rats showed significant decrease. However, EA treatment for 3 weeks reversed the GFAP protein level in the hippocampus compared to the sham EA treatment ($p<0.05$) (Fig. 2I). Also as shown in Fig. 2H, an expected 726-bp PCR product was found in RT-PCR analysis. Semi-quantification showed that similar changes were observed among these groups as the statistical results of GFAP mRNA ($p<0.01$) (Fig. 2J).

4. Discussion

The main finding of our study is that EA treatment not only blocked the CUS-induced behavioral deficits but also reversed the glial atrophy in depression model rats.

Our results showed that EA reversed the CUS-induced decreased crossing and rearing behaviors in the open field test, as well as significantly improves the physical state of CUS model rats. These behavioral results will provide further cues of the potential value of EA for patients with depression.

Although the exact cellular mechanism by which EA exerts its antidepressant-like effect is not yet clear, the role of astrocytes in the etiology of depression may give the mechanism study some suggestions. Postmortem studies in major depression and bipolar disorder provide the first evidence for specific glial histopathology, including the prominent reductions in glial cell number and packing density in mood disorders (Ongur et al., 1998; Rajkowska et al., 1999; Cotter et al., 2001). The glial atrophy acts as an etiological factor in depression, which is also supported by observations of the number and volume reduction of hippocampal astrocytes after exposure to a variety of environmental stressors (Czeh et al., 2007). Furthermore, it was showed that the infusion of the astrocyte-specific gliotoxin, L-alpha-aminoadipic acid (L-AAA), directly into the prefrontal cortex of adult rats induced depressive-like behaviors in a recent study (Banasr and Duman, 2008). Some studies have revealed that astrocytes, which are the main producer of many neurotrophic factors, are the key regulators of postnatal neurogenesis, neuronal growth, maintenance, and plasticity, in addition to their housekeeping functions (Friedman et al., 1998; Althaus and Richter-Landsberg, 2000). The reduced availability of the neurotrophic factors can result in increased cellular vulnerability or even cell death. The glial atrophy can induce functional impairments of neuronal activity, increased neuronal vulnerability or even neuronal death and decrease of postnatal neurogenesis by reducing the production of neurotrophic factors. Stress can reduce the expression of neurotrophic factors produced by glia cells, astrocytic number and size in the hippocampus, which in turn can be prevented by long-term chronic antidepressant treatment (Duman et al., 1997; Russo-Neustadt and Chen, 2005; Czeh et al., 2006). Chronic treatment with lithium upregulates GFAP expression and modifies the morphology (orientation) of astrocytes alike (Rocha and Rodnight, 1994; Rocha et al., 1998). The antidepressant efficacy is likely based on the increased astrocytes contributing to the enhancement in neurotrophic support and the augmentation in synaptic plasticity.

There are numerous randomized clinical control trails suggesting that EA treatment is an effective therapeutic method for depressive disorders (Han, 1986; Pohl and Nordin, 2002; Han et al., 2004; Huang and Xia, 2004; Song et al., 2007).

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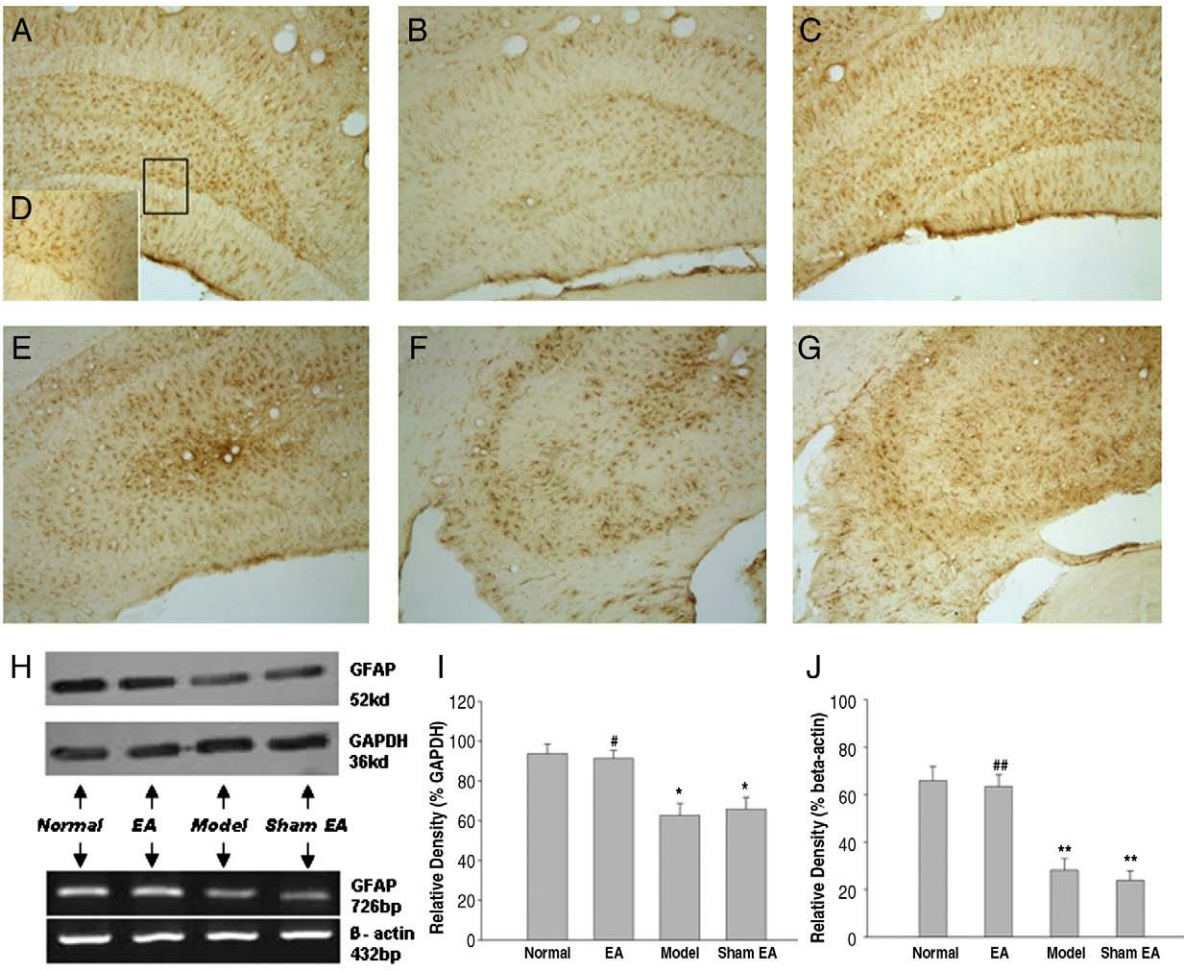

Fig. 2. GFAP immunostaining in the DG and CA3 of Normal group (A and E), sham EA group (B and F) and EA group (C and G). Under a 40× with a 100× zoom magnification, individual cells are clearly visualized (D). GFAP protein and mRNA detected by Western blot and RT-PCR respectively (H). The GFAP protein levels in different groups were expressed as a ratio to that of corresponding GAPDH (I). The GFAP mRNA level was expressed as a ratio to that of corresponding β-actin (J). Data were represented as mean ± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$ vs. Normal group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. sham EA group.

How EA reversed depression merited further basic and clinical researches. The present study showed that EA significantly improved the depressive-like behaviors in open field test and PS of CUS rats. The antidepressant-like effect of EA may correlate with the change of astrocytes in the hippocampus. It is likely that astrocytes contribute to the enhancement in neurotrophic support and associated augmentation in synaptic plasticity that may form the basis for antidepressant efficacy (Friedman et al., 1998; Althaus and Richter-Landsberg, 2000). As many researchers have shown the involvement of BDNF or GDNF in the EA treatment with some neurological diseases(Dong et al., 2005; Zhang et al., 2006; Dong et al., 2006; Yi et al., 2006; Wang et al., 2009), former research of our group also suggested that EA could reverse the reduced expression of the two neurotrophic factors in the hippocampus of depression model rats at the same time (data not shown), which further supports the role of astrocytes in the antidepressant-like effect of EA.

In summary, astroglia in the hippocampus might have a role in CUS-induced depression model rats. Further, the regulatory effect of EA on hippocampal astrocytes might be correlated with the antidepressant-like action of EA treatment.

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Conflict of interest

We declare that we have no duality or conflict of interest.

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