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Electroacupuncture attenuates the decrease of hippocampal progenitor cell proliferation in the adult rats exposed to chronic unpredictable stress

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Abstract

The present study was designed to investigate whether electroacupuncture (EA) was beneficial to extenuate the behavioral deficit in a rat model of depression induced by chronic unpredictable stress (CUS) and to observe the effect of EA on progenitor cell proliferation in the dentate gyrus (DG) of hippocampus. EA was performed on acupoints "Bai-Hui" (Du 20) and unilateral "An-Mian" (EX 17) once daily for 3 consecutive weeks, 2 weeks post CUS procedure. Open field test and forced swimming test were employed to evaluate the behavioral activity during a stress period or EA treatment. The results revealed that exposure to CUS resulted in a decrease of behavioral activity, whilst a daily session of EA treatment significantly reversed the behavioral deficit of these depression model rats. Moreover, as shown by 5-bromo-2-deoxyuridine (BrdU) labeling immunohistochemistry, hippocampal progenitor cell proliferation was decreased in the DG of depression model rats. Intriguingly, EA treatment effectively blocked this decrease. The study demonstrated a potential antidepressant-like effect of EA treatment on CUS induced depression model rats, which might be mediated by up-regulating the hippocampal progenitor cell proliferation.

Keywords: Electroacupuncture; Depression; Neurogenesis; Stress; Dentate gyrus

Introduction

Depression is a severe illness with a lifetime prevalence of between 10 and 20% according to large epidemiological studies (Paykel, 2003). Suicide is a major risk in depression, with about 15% of depressed patients committing suicide (Mueller and Leon, 1996). Given facts that 30% of depressed patients do not respond to pharmacological treatment and that side effects are common, an evaluation of alternative methods of treatment is warranted (Pohl and Nordin, 2002). Acupuncture has long been used as a treatment for somatic and mental disorders in Traditional Chinese Medicine (TCM). This approach generates few side effects, and is considered a new, alternative method of medicine in Western countries (Kaptchuk 2002; Lacey et al. 2003; NIH Consensus Conference 1998). During the past 50 years, electric stimulation has been applied to acupuncture needles, and appears to be more effective for the treatment of depression (Luo et al., 1998). Many clinical data indicate that complementary, alterative therapies are effective in treating depression and a growing number of people with depression are choosing to be treated in this manner, notably with acupuncture (Han, 1986; Luo, 2000; Ulett et al., 1998). However, very little published data exists regarding basic experimental studies on the effect of acupuncture treatment on depression.

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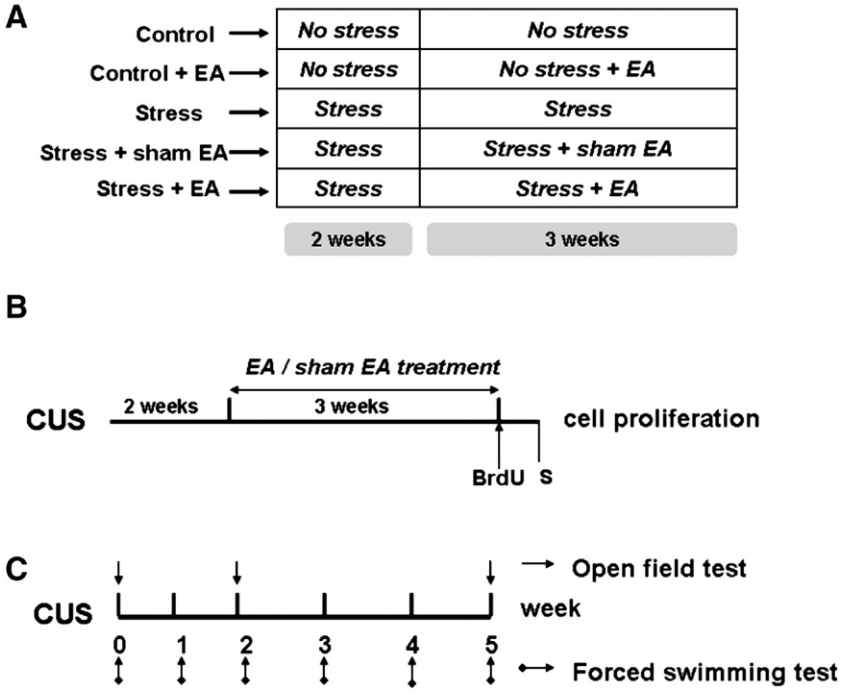

Fig. 1. Animal groups (A), schematic representation of the experimental procedure (B) and behavioral test (C). Rats were randomly divided into five groups: Control, Control+electroacupuncture (EA), Stress, Stress+sham EA, Stress+EA (n=6 each group) and were subjected to a variety of chronic stressors (CUS) during 5 weeks, whereas animals of the normal group (Control) remained undisturbed. Animals received EA or sham EA treatment 2 weeks after the experiment started. For analysis of cell proliferation, groups of rats (n=4) received a single injection of BrdU (100 mg/kg i.p.) on the final day of 5-week period, and euthanized (S) 24 h after BrdU administration. Open field test was measured before stress, EA administration, and at the end of the experiment. Forced swimming test was measured respectively at the end of every week.

The dentate gyrus (DG) of the adult hippocampus contains undifferentiated, rapidly proliferating progenitor cells. Approximately 70–80% of the newly formed cells differentiate into granule neurons, which ultimately fully integrate into the hippocampal network (Cameron et al., 1993; Kaplan and Hinds, 1977; Van Praag et al., 2002). Hippocampal neurogenesis can be influenced by several environmental factors and stimuli, among which stress plays a key role in the regulation (Kempermann, 2002). Adult neurogenesis is decreased by many different types of stressors, including predator odor (Galea et al., 2001), social stress (Czeh et al., 2001; Gould et al., 1997), acute and chronic restraint stress (Pham et al., 2003; Vollmayr et al., 2003; Rosenbrock et al., 2005), footshock stress (Malberg and Duman, 2003; Vollmayr et al., 2003), and chronic mild stress (Alonso et al., 2004). In contrast to the effects of stress, antidepressant treatment increases adult neurogenesis. Administration of one of the several different classes of antidepressants, including selective serotonin or norepinephrine reuptake inhibitors, increases neurogenesis in adult hippocampus (Madsen et al., 2000; Malberg et al., 2000; Manev et al., 2001; Nakagawa et al., 2002; Santarelli et al., 2003). It is clear from the studies cited here that there is an interaction of stress, antidepressant action and neurogenesis (Warner-Schmidt and Duman, 2006). Interestingly, studies indicated that acupuncture increased cell proliferation in DG after transient global ischemia in gerbils (Kim et al., 2001a,b) and in DG of streptozotocin-induced diabetic rats (Kim et al., 2002). These observations raised the question of whether electroacupuncture (EA) can influence the cell proliferation in the DG of rats subjected to stress-induced depression. To address this question, we investigated the effect of EA on the behavior and progenitor cell proliferation in DG by using the rat model of chronic unpredictable stress (CUS) induced depression, which has been extremely useful in elaborating and detecting the effects of antidepressant drugs (Katz et al., 1981; Willner et al., 1992).

Methods and materials

Animals

Experiments were performed on adult 4-month-old (300–350 g) male Sprague-Dawley rats (Experimental Animal Center, Shanghai Medical College of Fudan University, China). Prior to experimental manipulation, rats were allowed to acclimatize for 1 week maintained in an environment with a 12:12 h light-dark cycle and free access to food and water. All rats were used strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Design of study

To observe the behavioral effects of EA on depression model rats, 30 rats (6 in each group) were randomly divided into five groups: the control group, the control plus EA group, the stress group, the stress plus sham EA group and the stress plus EA group. For analysis of the hippocampal progenitor cell

proliferation, a further 20 rats (4 in each group) were also divided into the five groups. The experimental design is shown in Fig. 1. EA or sham EA was administered once a day for 3 weeks starting 2 weeks after the beginning of the experiment. An open field test was done before stress, EA administration, and at the end of the experiment. A forced swimming test was done at the end of every week.

CUS procedure

The depression model was induced by CUS (Willner et al., 1992), which was designed to maximize the unpredictable nature of the stressors and consisted of the following stressors in random order: 40-h water deprivation, 30-min cage rotation, 40-h food deprivation, 5-min forced swim, reversal of the light/dark cycle, 5-min hot environment (40 °C). The CUS procedure was carried out in stressed animals once per day for 5 weeks. The mixture of psychological and physical stressors experienced during the CUS paradigm not only reduces the chances of adaptation but also better mimics the variability of stressors encountered in daily life (Joels et al., 2004). Non-stressed animals were left undisturbed in their home cages except for necessary procedures such as regular cage cleaning.

Open-field test

The open-field test was performed as previously described (Redmond et al., 1997), and was carried out before stress (0 week), 2 weeks after stress (2 weeks) and 5 weeks after stress (5 weeks). The open-field apparatus consisted of a four-sided $100 \times 100 \times 40$ cm^3 wooden box, which was covered inside with folium to increase the reflectivity of the walls. The floor of the box was divided into 16 squares. A 60 W light bulb was positioned 90 cm above the base of the apparatus, and was the only source of illumination in the room. Each animal was placed in the center of the apparatus and allowed to explore freely for 3 min. During the test time 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) were measured. After each animal, the test apparatus was cleaned with a 10% ethanol solution and water to remove any olfactory cues.

Forced swimming test

This procedure was performed as described previously (Hansen et al., 1997). Briefly, rats were forced to swim in a glass box ($19 \times 29 \times 39$ cm) containing 25 cm of fresh water maintained at 25 °C. On the first day of the experiment, rats were placed in water for 15 min. After the swimming session, each rat was removed from the water, partially dried with a paper towel, placed in a drying environment for 30 min (pre-test) and returned to its home cage. The rats were placed again in the box individually 24 h later for 5 min (test). The total duration of immobility during the first 5 min of the swimming session was recorded. The rat was judged to be immobile when it made only the movements necessary to keep its head above water level.

BrdU injection

To examine the proliferation of hippocampal progenitor cells, rats were injected with 5-bromo-2-deoxyuridine (BrdU; 100 mg/kg, i.p.) on the final day of the 5-week period and were sacrificed 24 h after the injection. The short survival time following BrdU injection allowed us to determine the effect of different manipulations on the cell proliferation rate of progenitor cells.

EA delivery

For EA treatment, rats were placed in wood holders. The rat was bound moderately to the holder so that the movement of the rat's body was restrained while the head could move freely. Rats were allowed to acclimate for 30 min before EA treatment. According to previous studies, "Bai-Hui" and "An-Mian" acupoints were selected during EA treatment. 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 strenocleidomastoideus and muscle splenius capitis). The two needles 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. In order to exclude the possibility of depression induced by stress such as animal fixation, sham EA group animal was given the same manipulation as the EA group except without electrical current during sham EA treatment.

Perfusion and tissue storage

Rats were given an overdose of urethane (1.5 g/kg, i.p.) and perfused through the ascending aorta with 200 ml of normal saline followed by 300 ml 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The brains were removed from the skulls, and postfixed for 4 h in the identical fixative solution at 4 °C. Following this, brains were immersed in 30% sucrose in PB for 24–48 h at 4 °C for cryoprotectant. Serial sections of the brains were cut (35 μm sections) through the entire DG on a freezing microtome (Leica CM1900, Germany) and stored in cryoprotectant (25% ethylene glycol, 25% glycerol, 0.05 M PB, pH 7.4) at −20 °C until use.

BrdU immunohistochemistry

Free-floating tissue sections were processed for BrdU immunohistochemistry. DNA denaturation was conducted by incubation for 2 h in 50% formamide/2 × SSC at 65 °C, followed by several PBS rinses. Sections were incubated for 30 min in 2 N HCl at 37 °C followed by 10 min in boric acid. After washing in PBS, sections were incubated for 10 min in 0.3% H_2O_2 to eliminate endogenous peroxidases. After blocking with 3% normal horse serum (NHS) with 0.01% Triton X-100 for 1 h, sections were incubated with sheep anti BrdU (Biodesign, USA;

1:200) overnight at 4 °C. Sections were then incubated for 1 h with a secondary antibody (biotinylated donkey anti-sheep, Jackson Immunoresearch; 1:200), followed by signal amplification with an avidin–biotin complex (Vector Laboratories). Samples and were visualized by catalysis of 3, 3-diaminobenzidine (DAB) by horseradish peroxidase in the presence of 0.03% H_2O_2. The sections were then mounted, dehydrated and covered. To test the specificity of the primary antibody, controls were performed including the substitution of NHS for the primary antibody and omission of the primary antibody, neither of which showed any trace of immunohistochemical reaction.

Quantitation of BrdU labeling

A modified unbiased stereology protocol was used that has been reported to successfully quantify BrdU labeling (Malberg et al., 2000; West et al., 1991). Every eighth section through the rostral-caudal extent of the hippocampus was examined (Bregma −2.8 to −4.8) (Banasr et al., 2006). All BrdU-labeled cells in the granule cell layer together with the subgranular zone, defined as a two-cell-body-wide zone along the border of the granule cell layer, were counted by an experimenter blinded to the study code. To distinguish single cells within clusters, all counts were performed at 40× with a 10× zoom and 100× with 10× zoom magnification under a light microscope (Leica, Germany), omitting cells in the outermost focal plane. The total number of BrdU-labeled cells was estimated by multiplying the number of cells counted in every eighth section by eight. To confirm that the BrdU was labeling newborn cells and not cells undergoing DNA repair, mitotic figures were observed in each hippocampal slice.

Statistical analysis

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.

Results

CUS decreases behavioral activity: reversal by EA treatment

In the open field test, stressed rats demonstrated a typical decrease in the number of crossings and rearings, whereas EA treatment increased these behavioral activities (Fig. 2). Before CUS, the number of crossings of all rats were not significantly different ($F_{4,25}=0.891$, $P>0.05$). Animals in stressed groups showed a significant decrease in the number of crossings in the 2nd week, which continued to the 5th week when the experiment ended ($F_{4,25}=44.875$, $P<0.05$). In the stress plus EA group, the number of crossings decreased significantly after 2 weeks exposure to the stressors ($F_{4,25}=24.091$, $P<0.05$), and the number of crossings showed a significant increase compared to the stress plus sham EA group after EA treatment for 3 weeks, ($F_{4,25}=31.588$, $P<0.05$) (Fig. 2A). Similar changes were seen in the vertical activity (number of rearings) for all groups of rats (Fig. 2B).

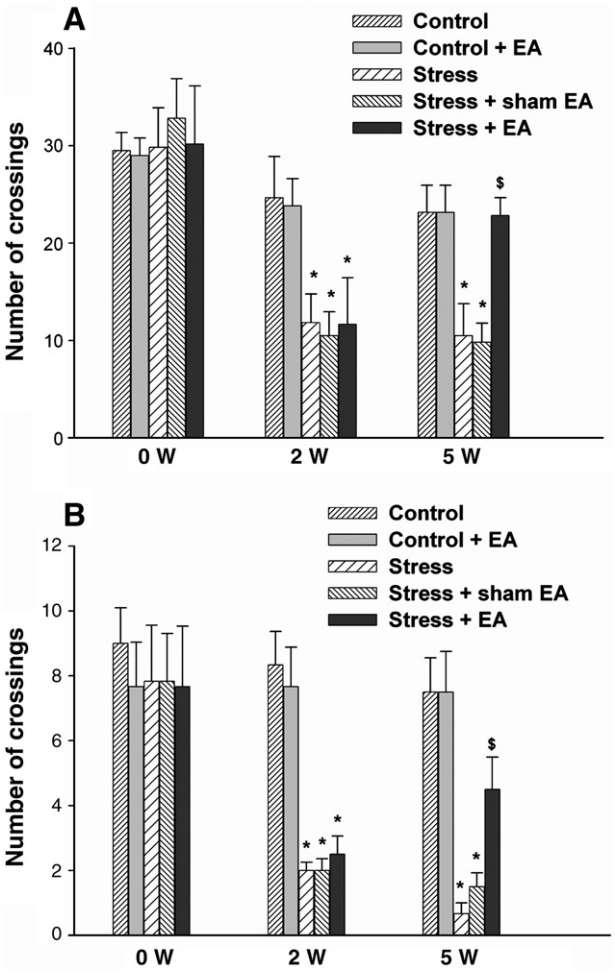

Fig. 2. Open field behavior in rats after CUS procedure and EA treatment. Rats were treated with either EA or sham EA 2 weeks after the stress procedure started. Animals performed the open field test before CUS, 2 weeks and 5 weeks after CUS. (A) Number of crossings during the 3 min session. (B) Number of rearings during the 3 min session. Results are given as mean±SEM ($n=6$ per group). *$P<0.05$, the stress group compared to the control group; $^{\$}P<0.05$, the stress plus EA group compared to the stress plus sham EA group.

In the forced swimming test, the immobility time was measured during the first 5 min of swimming. At the beginning of experimental procedure, there was no significant difference among the groups exposed to forced swimming ($F_{4,25}=0.337$, $P>0.05$). After CUS for 2 weeks, stressed animals showed a significant increase in immobility time ($F_{4,25}=23.839$, $P<0.01$). From this time, EA and sham EA were administered to the stressed animals for 3 weeks. At the end of the 5th week, the stress group and sham EA-treated animals showed a significant increase in immobility time ($F_{4,25}=64.032$, $P<0.01$). However EA-treated animals revealed a significant decrease in immobility time compared with the sham EA-treated animals ($F_{4,25}=35.554$, $P<0.05$), which suggested that EA blocked the stress-induced change of behavioral activity. In addition, the control plus EA group displayed a significant decrease in the immobility time of forced swimming test during the experiment ($F_{4,25}=64.032$, $P<0.05$) (Fig. 3).

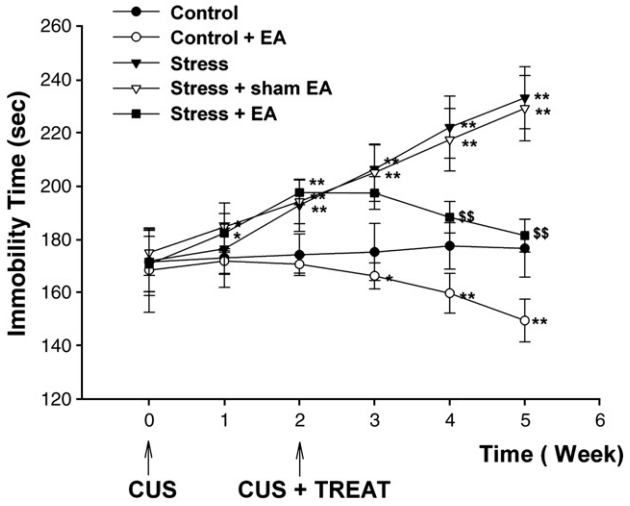

Fig. 3. Immobility time in the forced swimming test during the experimental procedure. Rats were treated with either EA or sham EA 2 weeks after CUS was initiated for a further 3 weeks. Immobility time was measured during the first 5 min of forced swimming at the end of every week. Results are expressed as mean±SEM (n=6 per group). *P<0.05, **P<0.01, the stress group compared to the control group; $^{\$}P$<0.05, $^{\$\$}P$<0.01, the stress plus EA group compared to the stress plus sham EA group.

CUS significantly decreases hippocampal progenitor cell proliferation: EA treatment blocks the stress-induced effect

BrdU immunohistochemistry revealed dividing cells in the DG. The majority of the BrdU-positive cells were located in the subgranular zone (SGZ) and generally occurred isolated or in small clusters of three to five cells (Fig. 4A, D). Chronic stress for 5 weeks resulted in a significant 49.9% ($F_{4,15}$=17.425, P<0.01) decrease in the number of BrdU-positive cells relative to the control group (Fig. 4B, E). EA-treated rats showed a 48.6% (P<0.01) increase in the number of BrdU-positive cells in the DG compared with the stress plus sham EA group (Fig. 4C, E). No differences were observed between the control group and the control plus EA group in the cell proliferation (4250.37±125.92 vs 4472.25±378.32, P>0.05), although differences in the forced swimming behavior were observed. The progenitor cell proliferation of the hippocampus was evaluated at the end of the experimental period of 5 weeks, during which animals were treated with EA or sham EA for 3 weeks (Fig. 4E). Comparison of BrdU-labeled cells in sham EA-treated animals with EA treatment group showed that EA noticeably increased the number of BrdU-positive cells in the DG, suggesting that the EA treatment blocked the decrease of hippocampal progenitor cell proliferation induced by CUS.

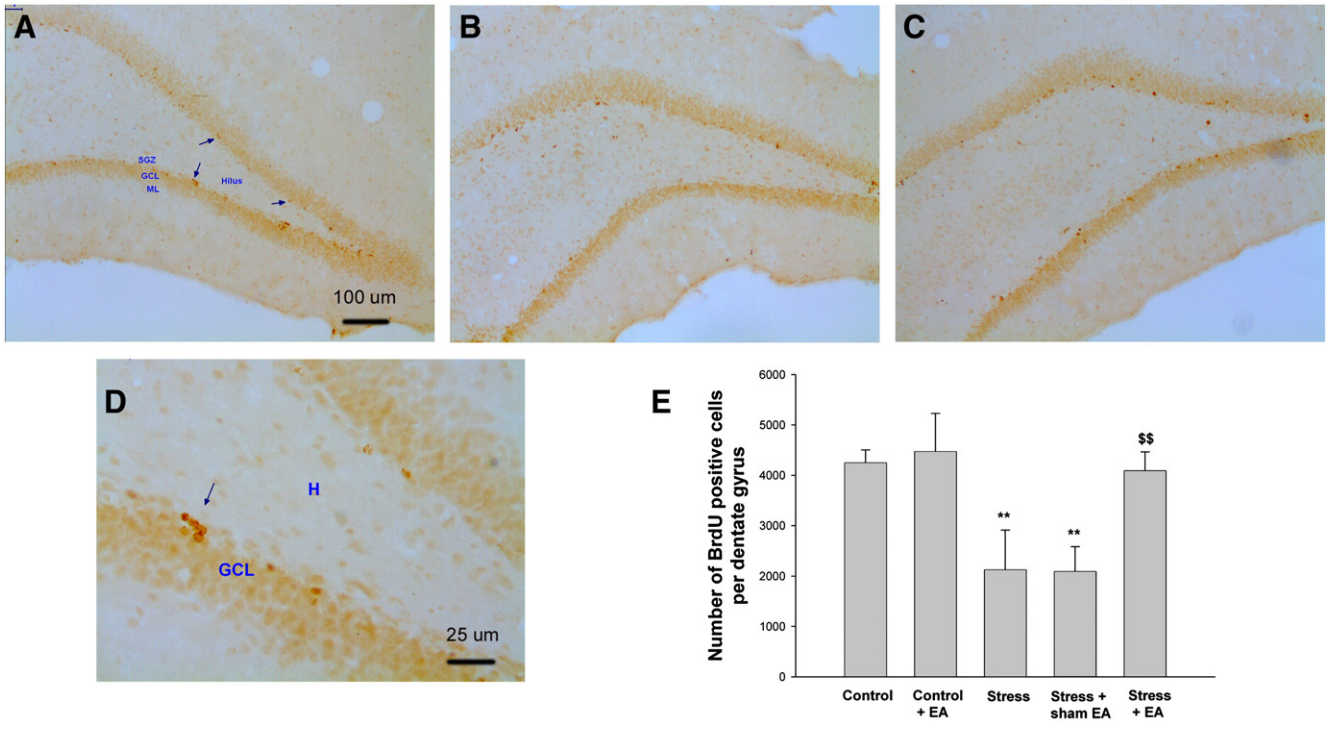

Fig. 4. Exposure to chronic unpredictable stress decreases the number of BrdU-positive cells in the adult hippocampus, whereas EA treatment reversed the stress-induced effect. Rats received a single injection of BrdU on the last day of a 5-week stress period and were sacrificed for BrdU immunohistochemical staining. Panels A, B and C are representative photographs showing the distribution of BrdU-labeled cells in the hippocampus of the rats from the groups Control (A), Stress (B) and Stress+EA (C). The BrdU-labeled cells, as indicated by arrows, appear in pairs or clusters in the SGZ of the DG, the region between the granule cell layer (GCL) and hilus (H). Under a 40× with a 100× zoom magnification, individual cells in the clusters are clearly visualized (D). The BrdU-labeled cells in the SGZ were counted under the high magnification. Panel E shows the number of BrdU-labeled cells in the SGZ of rats. The results are expressed as the estimated mean total number (±SEM) of BrdU-labeled cells per DG region (n=4 per group). **P<0.01, the stress group compared to the control group; $^{\$\$}P$<0.01, the stress plus EA group compared to the stress plus sham EA group.

Discussion

In the present study, we investigated the effects of EA on the behavior of CUS induced depression model rats by using open field and forced swimming tests as well as examining progenitor cell proliferation in the DG of hippocampus. The results demonstrated that EA treatment not only had a potent antidepressant-like effect on CUS induced depression model rats but also attenuated the decrease of progenitor cell proliferation in the DG of adult rats exposed to CUS procedure.

That EA is a promising method for the treatment of mental disorders, such as major depression, has been supported by clinical trials (Han et al., 2004; Luo, 2000). The present research showed that EA treatment had an antidepressant-like effect on depression model rats. The results confirmed that chronically stressed rats exhibited a marked degradation of behavior — an effect which lasted until the end of the stress period. In line with the idea that stress-induced behavioral change may represent a valid measure of depression in rats are the present findings that treatment with EA significantly improved the behavioral deficit of stressed animals. A series of trials compared the treatment of depression using EA to treatment with tricyclic antidepressant amitriptyline (AM) (Luo et al., 1985). The results showed that EA was as effective as AM in the treatment of depression, and even more effective in the alleviation of symptoms of anxiety, without the side effects of drug treatment. The present study provided further evidence for the antidepressant efficacy of EA in a CUS induced depression model.

It was proposed that a stress-induced decrease in DG neurogenesis might be an important causal factor in the development of depressive episodes (Gould et al., 2000; Jacobs et al., 2000). The present study tested this hypothesis in the CUS induced depression model rats. Our results showed that CUS noticeably reduced the rate of newborn cell proliferation, as evidenced by the marked decrease (49.9%) in the number of BrdU-positive cells in the DG. Accumulated investigations have shown the deleterious effect of stressful events on hippocampal newborn cell proliferation in various animal species (Alonso et al., 2004; Czeh et al., 2001; Galea et al., 2001; Pham et al., 2003; Rosenbrock et al., 2005; Vollmayr et al., 2003). Furthermore, in the present study, EA treatment of stressed rats showed a 48.6% increase in the number of BrdU-positive cells in the DG compared with the sham EA treatment group, which suggested that the decreased neurogenesis induced by CUS in rats was counteracted by repeated treatment with EA. Interestingly, this effect paralleled the time course of behavioral modification. Previous investigations had demonstrated that antidepressant treatment could block the stress induced decrease in neurogenesis of the adult brain. The influence of maternal separation stress on neurogenesis in young rats (14–21 days) was reversed by chronic fluoxetine administration (Lee et al., 2001). Chronic administration of an atypical antidepressant, tianeptine, blocked the effects of subordination stress on neurogenesis in the hippocampus of adult tree shrews (Czeh et al., 2001). Recent study also showed that agmatine could reverse the chronic stress-induced decrease of open-field behavior and hippocampal cell proliferation (Li et al., 2006). Our results suggested that the effect of EA treatment on the depression model rats was a specific response to normalize the CUS-induced behavioral deficits and the change of hippocampal progenitor cell proliferation. The present study also provided the first evidence of attenuating the decrease of hippocampal progenitor cell proliferation in the DG after EA treatment in depression model rats, indicating that EA might exert its antidepressant-like effect through regulating the progenitor cell proliferation in the DG of hippocampus.

The present study demonstrated that the change of progenitor cell proliferation in the DG might work in the EA treatment on the depression model rats. However, the mechanisms by which EA attenuated the decrease of progenitor cell proliferation in the DG of adult rats exposed to CUS procedure were still unclear. Duman and Monteggia demonstrated that brain-derived neurotrophic factor (BDNF), which had been shown to play an key role in the proliferation of newborn cells (Li et al., 2007; Morcuende et al., 2003; Shirayama et al., 2002; Tauber et al., 2005), might also be involved in the etiology of depression (Duman and Monteggia, 2006). Interestingly, EA stimulation could influence BDNF expression in the hippocampus of rats exposed to immobilization stress (Yun et al., 2002). A recent study also demonstrated that EA could activate endogenous glial cell line-derived neurotrophic factor (GDNF) and its receptor system during the EA treatment of rats with neuropathic pain (Dong et al., 2005). Taken together, further studies are required to delineate whether BDNF or other growth factors show promise in the regulation of hippocampal progenitor cell proliferation during EA treatment on depression model.

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