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Electroacupuncture combined with clomipramine enhances antidepressant effect in rodents

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Abstract

The present study was designed to evaluate the antidepressant effect of electroacupuncture (EA) and the potential additive or synergistic effects of EA and clomipramine (CLO, a tricyclic antidepressant) in the mouse forced swimming test (FST) and chronic mild stress (CMS) induced depression-model rats. The FST is an antidepressant screening procedure performed initially to observe the immediate effects of EA and/or CLO on the immobility time. CLO (2.5, 5, 10, 20 and 60 mg/kg intraperitoneally) were administered at 23, 6 and 1h respectively prior to each test. EA was given at the 'Bai-Hui' (Du 20) and unilateral 'An-Mian' (EX 17) acupoints 1 h before each test. Immobility time was significantly reduced by EA and CLO at 2.5, 5, 10, 20 or 60 mg/kg, respectively. EA combined with 2.5 mg/kg CLO exhibited additive effects on the immobility time. In addition, rats were exposed chronically (1st–11th week) to a variety of mild unpredictable stressors. Depressed mood and anhedonia were recognized as a decrease in sucrose intake in the CMS rats. CLO at 2.5, 5 mg/kg and EA at the same acupoints and parameters were administrated on the CMS rats once every other day for 6 weeks (5th–11th week). The intake of 1% sucrose solution was reduced by CMS, which was restored to normal level after 6 weeks treatment with 5 mg/kg CLO or EA combined with 2.5 mg/kg CLO. However, neither the sucrose intake nor the sucrose preference in the depressive rats was significantly changed by the treatment with EA or 2.5 mg/kg CLO alone. These results demonstrated that EA combined with CLO at low doses has an additive or synergistic antidepressant action, and this combination may provide an effective strategy for depression management.

Keywords: Depression; Electroacupuncture; Clomipramine; Forced swimming test; Chronic mild stress

The prevalence of depression is consistently high worldwide, and is associated with considerable morbidity and mortality. In the United States, the estimated lifetime prevalence is 21.3% in women and 12.7% in men [29]. The goal of pharmacotherapy is the reduction and ultimate removal of all signs and symptoms of depression. Although options for pharmacological treatment for depression have grown exponentially over the past several decades, the current antidepressants which belong to four different classes—tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) and miscellaneous antidepressants continues to have limitations of both efficacy and tolerability, such as (1) efficacy rate remains at 60–70%, (2) lack of immediate onset of efficacy and (3) side effects of drugs [6,11,23]. The delayed onset of therapeutic effects and severely side-effects of antidepressants often result in problems with compliance of patients taking part in clinical trails [19].

The use of complementary and alternative medicine (CAM) is widespread. Those with psychiatric disorders are more likely to use CAM than those with other diseases [15]. There are both benefits and limitations to CAM. For example, EA could alleviate the symptoms of depression with very few side effects, whilst there is incomplete efficacy in many patients as with other antidepressants [12,18]. In addition, certain psychiatrists advise that clinicians may choose augmentation and combination strategies in treatment-resistant depressions [4,7], for example, a combination of two classic antidepressants or a combination of two

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therapies. Therefore, we try to combine EA with antidepressants for the treatment of experimental depression.

The forced swimming test and CMS model have been used extensively for examining antidepressant drug action and probable new mechanisms for several decades [8,10,24,25]. The stress regime is a realistic experimental model of stressors in every day life, and may be analogous to "life difficulties" implicated in the etiology of depression [17,27]. Furthermore, the use of sucrose consumption is valid in relation to the two core symptoms of major depression, i.e. anhedonia and depressed mood [2]. These parallels suggest that the CMS model could be used as screening tests in the context of antidepressant discovery and development programs, and as simulations within which to investigate aspects of depression. The CMS induced depression model rats, an ethologically relevant animal model of depression, have been used widespread to test the effectiveness of different antidepressant action or to find potential mechanisms of depression [10,13,24], although there are some limitations involved with this procedure [1,26]. Therefore, in the present study, these tests were used to evaluate the antidepressant effects of EA and/or CLO in depression.

Male KM mice (20–30 g) and Male Sprague-Dawley (SD) rats (200–250 g) from the Experimental Animal Center of Fudan University were kept under controlled environmental conditions (25 °C, 12 h: 12 h light–dark cycles, water and food *ad libitum*). Prior to experimental manipulation, the animals were given a period of 1 week to adjust to the new surroundings. All of follow protocols were approved by the Committee on Research Animal Care of Fudan University, and followed by the principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory Animals.

The FST, also called behavioral despair or the Porsolt test was first proposed as a simpler variation of the learned helplessness test, and is probably the most widely used screening test of antidepressant potential of novel compounds [2,17]. The FST schedules were performed according to the method described by Porsolt et al. [20,21]. The mice were placed individually in glass cylinders (height: 25 cm, diameter: 10 cm) containing 6 cm of water maintained at 25 °C, and 15 min later they were moved to a 30 °C drying environment for 30 min (pre-test). Twenty four hours later, the mice were placed again in the cylinder individually for 5 min (test). The mouse was considered immobility when floating and making only the necessary movements to keep its head above the water surface level. The duration of immobility was timed during the 5 min by an unbiased operator. A decrease in the duration of immobility was indicative of an antidepressant-like effect.

In FST, the male mice were randomly assigned to the following groups: (1) normal saline (NS) ($n = 10$); (2) CLO 2.5, 5, 10, 20, 60 mg/kg, $n = 10$ for each dose; (3) EA ($n = 10$); (4) EA plus 2.5 mg/kg CLO ($n = 10$). The intraperitoneal injections of CLO of different doses were performed 23 h, 6 h and 1 h before the test. The EA administration was started 1 h before the test and maintained for 40 min. Throughout EA administration, the mice were relaxed in a cage (191 mm × 292 mm × 127 mm). A pair of stainless steel needles of 0.3 mm diameter was inserted at a depth of 5 mm into acupoint of "Bai-Hui" (Du 20), (on the midline of the head, approximately on the midpoint of the line connecting the apices of the two auricles) and the unilateral acupuncture point "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 G 6805-2, Shanghai Medical Electronic Apparatus Company, China). Alternating trains of dense–spare frequencies (60 Hz for 5 s and 4 Hz for 2.5 s alternately) were selected. The intensity of stimulation ($\leq$1 mA) was adjusted to provoke slight twitches of the mouse's ear [5].

After the FST, the CMS-induced depression-model for rats was performed to further evaluate the effect of EA and combined treatment with EA and CLO. The standard CMS protocol, as described by Willner et al. [28], consists of the sequential application of a variety of mild stresses: (1) food deprivation, (2) water deprivation, (3) continuous lighting, (4) cage tilt (30°), (5) paired housing (Thursday 5:00 pm–Friday 10:00 am), (6) soiled cage (100 ml water spilled onto bedding), (7) exposure to reduced temperature (18 °C), (8) intermittent white noise (85 dB), (9) stroboscopic lighting (300 flashes/min), (10) exposure to an empty water bottle following a period of water deprivation, (11) restricted access to food (scattering of a few 45 mg precision pellets in the animal's home cage), (12) presence of a foreign object in the home cage (e.g., piece of wood or plastic). Details of the schedule are detailed in Table 1.

In this part of the experiment, male SD rats ($n = 8$ per group) were randomly divided into following groups: (1) NS; (2) CMS; (3) CMS plus 2.5 mg/kg CLO; (4) CMS plus 5 mg/kg CLO; (5) CMS plus EA; (6) CMS plus EA and 2.5 mg/kg CLO.

Seventy-two hours prior to the start of CMS, rats were given a continuous 48 h exposure to two bottles: one containing 1% solution of sucrose, the other tap water. The bottles were counterbalanced across the left or right sides of the feeding compartment. This procedure was adopted for two-bottle tests throughout the experiment. Prior to testing for fluid consumption, animals were deprived of food and water for 23 h. Testing was carried out in the animals' home cage 6 h into its light cycle, from 2:00 pm to 3:00 pm every Tuesday. The volume of fluid consumption was measured repeatedly three times as the pre-administration control. Sucrose preference rate was calculated according to the formula:

$$\%\text{Preference} = \left[\left(\frac{\text{sucrose intake}}{\text{total intake}}\right) \times 100\%\right]$$

EA was applied at the same acupuncture points and parameters as the above experiments on performed mice; In CMS rats, CLO and EA treatment was commenced at the 5th week from the start of CMS. CLO at 2.5 or 5 mg/kg was administered at 4:00 p.m. every day for 6 weeks. EA was applied at 4:00 p.m. e.o.d. for 6 weeks. The test of CMS was continued and sucrose consumption was measured from 2:00 p.m. to 3:00 p.m. every Tuesday throughout 11 weeks.

Data were presented as mean ± S.E.M. Data referring to the immobility time were analyzed by a two-way ANOVA (CLO × EA) with repeated measures, followed by Student–Newman–Keuls (S–N–K) test. Data of sucrose intake

Table 1
Schedule of chronic mild stress procedures

	Food deprivation	water deprivation	continuous lighting	cage tilt	grouped housing	soiled cage	cold room	intermittent white noise	stroboscobic lighting	empty water bottles	restricted access to food	foreign object in cage
a.m. MON p.m.	15:00	15:00		10:00		17:00	15:00-15:30		10:00-12:00			
a.m. TUES p.m.	15:00		17:00			10:00		15:00-18:00				
a.m. WED p.m.	17:00	11:00 17:00	10:00						10:00 17:00	10:00-11:00		17:00
a.m. THURS p.m.		10:00		10:00 17:00	17:00		10:00-10:30					10:00
a.m. FRI p.m.	12:00				10:00			12:00 17:00			10:00-12:00	
a.m. SAT p.m.	10:00	10:00	17:00									
a.m. SUN p.m.	12:00	12:00	12:00	17:00								

and preference were analyzed by one- or two-way ANOVA (stress × week) (CLO × EA), followed by S–N–K test.

In FST, CLO produced a dose-dependent decrease in the immobility time ($p < 0.01$). The immobility time of EA group was also significantly decreased compared with the NS group ($p < 0.01$). The immobility time of EA plus CLO 2.5 mg/kg group was decreased further in comparison with that of EA or CLO (2.5 mg/kg) groups alone ($p < 0.01$) (Fig. 1). Two-way ANOVA (CLO × EA) showed a non-significant interaction of CLO 2.5 mg/kg and EA groups ($p > 0.05$).

Further investigation was performed in CMS rats. In the initial baseline test, the mean basal intakes of sucrose and water were 8.2 ± 2.1 ml and 1.9 ± 1.1 ml, respectively, and the sucrose preference rate was 81 ± 14.6%. Two-way ANOVA (stress × week) revealed a significant effect for sucrose intake ($p < 0.05$). Further analysis indicated a significant decrease of the sucrose intake of rats due to stress. After 4 weeks of stress exposure, sucrose intake (3.16 ± 1.42 ml) and sucrose preference rate (45 ± 16.3%) were decreased significantly in the CMS group ($p < 0.05$, versus NS group) (Fig. 2). Water consumption did not change obviously. When treatments with EA or CLO (2.5 mg/kg, 5 mg/kg or 2.5 mg/kg plus EA) were performed, the sucrose intake began to increase at the end of the first week of treatment. However, this was not maintained in the EA or CLO 2.5 mg/kg groups alone ($p > 0.05$ versus CMS group) (Fig 2). After 6 weeks of treatment, sucrose intake and sucrose preference rate were significantly restored in CLO 5 mg/kg group and CLO 2.5 mg/kg plus EA group ($p < 0.05$ versus CMS group) (Figs. 2 and 3). The sucrose intake of EA plus CLO 2.5 mg/kg

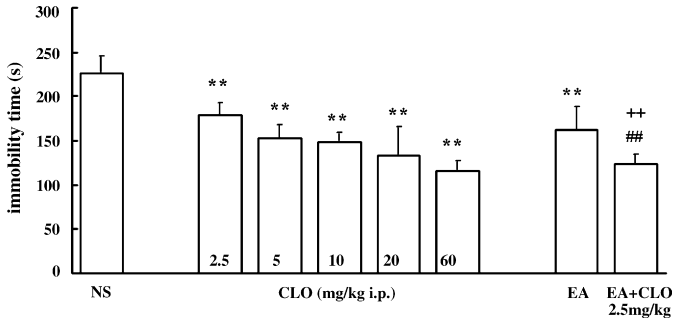

Fig. 1. Immediate effects of clomipramine (CLO) and EA on immobility time in the mouse forced swimming test ($n = 10$ for each group). Data are expressed as mean ± S.E.M. $^{**}p < 0.01$ vs. NS group; $^{\#\#}p < 0.01$ vs. EA alone group; $^{++}p < 0.01$ vs. CLO 2.5 mg/kg alone group.

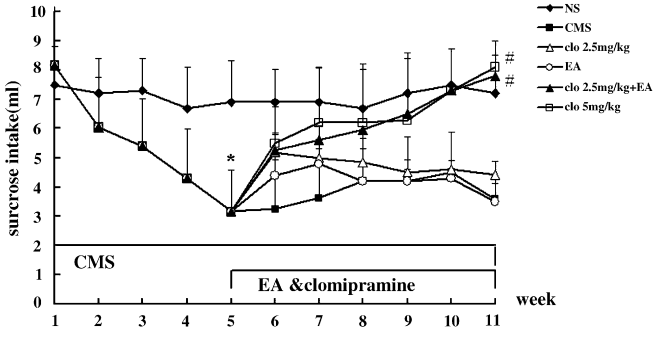

Fig. 2. Cumulative effects of chronic clomipramine (CLO) and repetitive EA on sucrose (1.0%) consumption of rats under chronic mild unpredictable stresses (CMS, $n = 8$ for each group). Data are expressed as mean ± S.E.M. $^{*}p < 0.05$ vs. NS group; $^{\#}p < 0.05$ between CLO 5 mg/kg group or CLO 2.5 mg/kg plus EA group and CMS group.

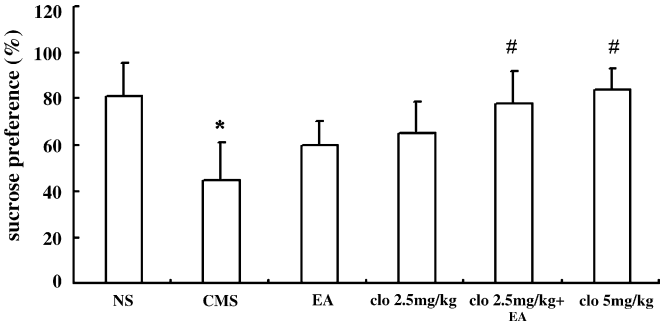

Fig. 3. After 11 weeks, cumulative effects of chronic clomipramine (CLO) and repetitive EA on preference for sucrose (1.0%) over water to chronic mild unpredictable stresses (CMS, $n = 8$ for each group). $^{*}p < 0.05$ vs. NS group; $^{\#}p < 0.05$ vs. CMS group.

group was decreased further in comparison with that of EA or CLO (2.5 mg/kg) groups alone ($p < 0.05$) (Fig. 2). Two-way AVONA (CLO × EA) on the sucrose intake showed a significant interaction of CLO 2.5 mg/kg plus EA ($p < 0.05$). But Two-way AVONA (CLO × EA) on the sucrose preference showed a non-significant interaction of CLO 2.5 mg/kg plus EA ($p > 0.05$).

It is considered that depression as a chronic psychological disorder was induced by varied stressors, i.e. life's difficulties. In this study, animals were exposed to stress regime which mimic the stressors in every day life. The symptom of anhedonia and depressed mood is the two core symptoms of major depression, which were manifested as sucrose intake in CMS model in this study. After 4 weeks of stress exposure, sucrose intake of CMS model is significant lower than that of NS group.

Acupuncture is a traditional Chinese treatment that has become increasingly popular in Western nations. More and more depression patients in Western commence seeking help from acupuncture or EA. But its scientific evidence and laws of action are not very clear at this point. Investigations about the effects of acupuncture or EA on patients indicated that acupuncture leads to improvement analogous to certain antidepressants [12,16]. Nevertheless, a larger trial of acupuncture in the acute- and maintenance-phase treatment of depression is warranted before further recommendations can be made [9,18]. Data in the present study showed that EA has an immediate effect on immobility time in FST and the intermediate chronic antidepressant efficacy on sucrose preference in CMS model. But further investigations need to do before make further conclusion.

The effect of combination of EA and the CLO in low dose was also observed in this study. Two-way AVONA exhibited that the effects of EA and clo2.5 mg/kg were additive on immobility time and sucrose preference, and synergistic on sucrose intake, and suggested combination of EA and the CLO in low dose could be a new therapeutic option for the treatment of depression. It is also reported in previous literature that mianserin (a tetracyclic antidepressant) combined with acupuncture improved the course of depression in patients more than pharmacological treatment with mianserin alone [22]. Their data also suggested combination of two strategies could be an improved strategy for the treatment of depression.

The body weights of rats were also observed weekly during the experiment. The data of body weight-gain of the last week did not show significant different between CMS model (25.3 ± 8.27 g) and NS control groups (22.93 ± 3.75 g). All of CLO alone groups got less body weight-gain (CLO 2.5 mg/kg: 7.22 ± 3.63 g, CLO 5 mg/kg: 6.38 ± 1.99 g) ($p < 0.01$ versus NS and CMS groups). The EA alone (21.42 ± 3.04 g) and combined with the CLO groups (30.42 ± 3.69 g) got similar body weight-gain with the NS and CMS groups ($p > 0.05$ versus NS and CMS groups). These data was very interesting but not similar with the other papers [26] and suggested the more investigations need to do to determine the body weight is a good target to evaluate the depression model and the effect of the antidepressants.

Understanding the fundamental biology of major depression has been proved to be a challenging scientific problem of enormous clinical relevance. Psychobiological research on depression has traditionally concentrated on the monoamine transmitter [3]. It has long been proposed that depression in humans may result from deficient activity in the serotonergic and noradrenergic system. The mechanistic action of TCAs (including CLO) in clinical depression is to augment monoamine function, which hence alleviates symptoms. TCAs could also inhibit the uptake of serotonin (5-HT) and norepinephrine (NE). However, the enhanced activity of monoamine also produces unwanted side effects on the autonomic nervous system and central nervous system. Interestingly, EA has been previously reported to show selective and differential modulatory effects on monoamine transmitters by increasing the release of 5-HT and decreasing the release of NE [14]. It is perhaps this mechanism which results in the additive or synergistic effects of EA and CLO.

In summary, the combination of a low dose of CLO and EA treatment produces greater antidepressant effect than either treatment alone. These results suggest that the combination of EA and CLO may provide an improved strategy for treating depression.

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