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Antidepressive-like effects of electroacupuncture in rats

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Abstract

Here, we investigate the effects of electroacupunture on the depressive-like symptoms in learned helplessness and forced swim tests in rats. Electroacupuncture stimulation (EA) was provided at ST-36 (*Zusanli*) and SP-6 (*Sanyinjiao*) acupoints. A positive control group was treated with imipramine. To verify the effects of EA over serotonergic system, other additional groups received daily, for three days, *p*-chlorophenylalanine and after two days, were submitted to behavioral tests. EA, like imipramine, enhanced the successful active avoidance in the learned helplessness and diminished the time spent in immobility position in the forced swim test, without affecting the number of squares crossed in the open field test. The administration of *p*-chlorophenylalanine abolished the antidepressive-like effect of EA. EA generates a clear antidepressant effect in two different animal models of depression, and this effect is related, at least in part, to the serotonergic system.

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1. Introduction

Depression represents a serious condition that often requires medical intervention. Clinically, it is treated with antidepressants, however, not all patients respond to antidepressant treatment, the therapeutic effects take several weeks to manifest and these effects are often accompanied by unwanted side effects [1]. Thus, new strategies for the treatment of depression are needed.

The serotonergic system plays an important role in depressive disorders (for review, see [2]). Given the importance of the serotonergic system in several physiological effects of electroacupuncture [3–5], this ancient Chinese method could represent an additional strategy for the treatment of depression. Indeed, a few clinical studies have already reported on an antidepressant effect of electroacupuncture [6–9]. In a recent review however, Mukaino and colleagues suggested that isolated controlled trials are insufficient to conclude whether electroacupuncture is an effective treatment for depression, but justifies further trials of electroacupuncture [10].

Here, we investigated the effects of electroacupuncture at ST-36 (*Zusanli*) and SP-6 (*Sanyinjiao*) acupoints in two animal models of depression in rats, and the importance of serotonergic system for these effects.

2. Materials and methods

2.1. Animals

Male Wistar EPM-1 rats (250–300 g) were kept on a standard light/dark cycle (12/12 h) with lights on at 07:00 AM, with free access to rat chow pellets and tap water. The animal care and experimental protocols were conducted under protocols approved by the Animal Care and Use Ethics Committee of the University, according to National Institute of Health Guide for the Care and Use of Laboratory Animals, 1996.

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2.2. Treatment and electroacupuncture procedures

To investigate the influence of the serotonergic system in the action of electroacupuncture, some groups received *p*-chlorophenylalanine (Sigma). Daily treatment for three consecutives days, at dose of 100 mg/kg., i.p., was initiated five days prior to the behavioral procedures. This scheme of *p*-chlorophenylalanine administration is an effective means for serotonin depletion [3,5].

The electroacupuncture procedure was provided in a single session (2 Hz, 1 V, 20 min electrostimulator Plexus AP 585 apparatus — Accurate Pulse/Biotherapy Lautz-Brazil) at the acupoints ST-36 (*Zusanli*) and SP-6 (*Sanyinjiao*), located according to anatomical references [11,12]. In the rat, ST-36 (*Zusanli*) is located approximately 1 mm lateral to the tibial tuberosity, whereas SP-6 (*Sanyinjiao*) is located in the posterior border of the tibia, 0.5 cm above the medial malleolus, both of which are easily located by manual inspection [12]. The needles of acupuncture used were 0.25 mm in diameter and 2 cm long, made of stainless steel stem and copper handle (Lautz-Brazil) and were bilaterally inserted at a depth of approximately 0.5 mm at the ST-36 (*Zusanli*) points, and just above the skin at the SP-6 (*Sanyinjiao*) points. Sham electroacupuncture [5] was similarly provided, however at a location (that was not an acupoint) in neighboring skin areas (0.5 cm laterally of the ST-36 (*Zusanli*) points and in the medial side of the hind limbs, 0.3 cm proximal, on a diagonal line to the SP-6 (*Sanyinjiao*).

2.3. Behavioral test

Two animal models of depression were utilized to evaluate the possible antidepressant effects of electroacupuncture: learned helplessness and forced swim.

2.3.1. Learned helplessness

The learned helplessness model was based on that described in previous literature [13]. We used the two-way shuttle box (56 × 21 × 25 cm; Ugo Basile, Italy). Rats were submitted over a period of 1 h to an inescapable footshock in the dark compartment of the apparatus (0.8 mA, 15 s duration), with an inter trial interval of 60 s. The intensity and duration of shock was determined based on a pilot study. An unconditioned stimulus (light) was also delivered in the same period the footshock was delivered. At the end of the 1 hour training session the animals were divided into one of the 5 different experimental groups (see below). Forty five minutes later animals were individually placed in the shuttle box, with free access to both compartments, given a 5-min adaptation period and just after that manually placed in the dark compartment of the apparatus. A light signal was given during the first 3 s of each trial. If there was no avoidance response within this period, the light signal remained on and a 0.8-mA shock (3 s duration) was delivered. A total of 30 trials with an inter trial interval of 30 s were done. The number of successful escapes was recorded. In this paradigm, the following experimental groups were formed: naïve (in the first 60 min, rats were maintained in the apparatus without presentation of shock or light; $N=10$), saline (as naïve rats, however light and shock were delivered; $N=14$), imob (as saline rats, however, just after the 60 min period, the animals were immobilized for 20 min; $N=10$), sham (as imob rats, however, during immobilization the animals received electroacupuncture at non acupoints; $N=10$), EA (as sham rats, except that the electroacupuncture procedure was performed at acupoints; $N=10$).

In a second experiment, to assess the influence of the effects of EA over serotonergic system, three additional groups were formed: pCPA (rats previously treated with *p*-chlorophenylalanine — see *p*-chlorophenylalanine treatment protocol; and maintained in the apparatus during the first 60 min without presentation of shock or light; $N=06$), pCPA + shock (as pCPA rats, however light and shock were delivered) and *p*-chlorophenylalanine + EA (as pCPA + shock rats, and just after the first 60 min period, rats were immobilized and received electroacupuncture at acupoints; $N=08$). As a positive control,

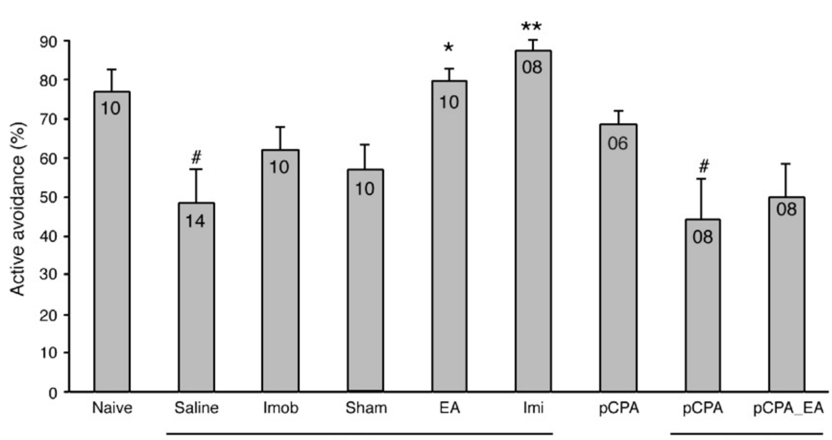

Fig. 1. Percentage of successful active avoidance in the learned helplessness test. Data expressed as mean ± SEM. Numbers within bars represent the number of animals in each group. Note the learned helplessness effect shown by naive *vs* saline rats, and by pCPA alone and with shock. Only animals that received electroacupuncture or imipramine avoided the shock more frequently than saline animals (depressive-like rats) (*$P<0.05$, **$P<0.01$ as compared to saline; #$P<0.05$ as compared to naïve).

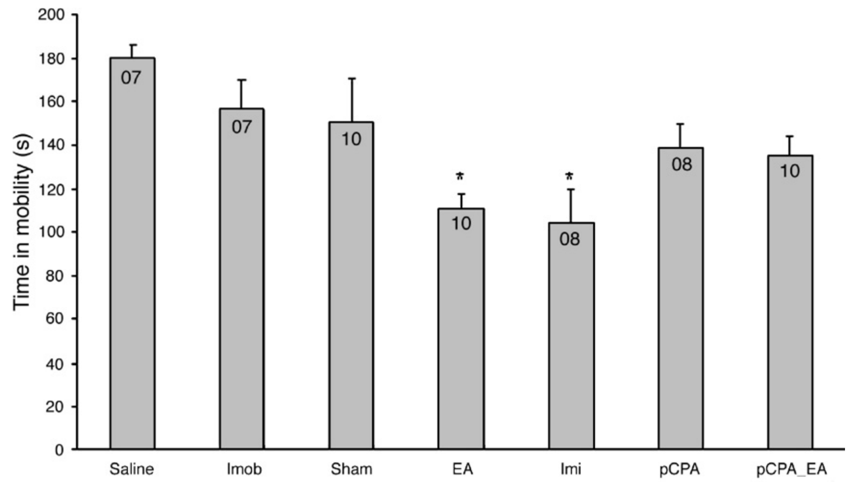

Fig. 2. Immobility in the forced swim test. Data expressed as mean ± SEM. Numbers within bars represent the number of animals in each group. Only electroacupuncture or imipramine decreased immobility, when compared to saline animals (* $P < 0.01$).

a group of animals received imipramine (Sigma) at a dose of 30 mg/kg i.p., 24 h, 5 h and 1 h, prior to the first 60 min period ($N = 08$).

2.3.2. Forced swimming test

In the forced swimming test, other animal model of depression, the rats were individually forced to swim 15 min inside a vertical Plexiglas cylinder containing 15 cm of water. They were again placed in the cylinders 24 h later and the total duration of immobility was measured during a 5 min test [14]. For this experiment, the groups were comprised of: saline (rats received saline 24 h, 5 h and 1 h prior to the 5 min test; $N = 07$), imob (just after the 15 min of forced swim, the rat was immobilized for 20 min; $N = 07$), sham (as imob rats, however, during immobilization the animals received electroacupuncture at a non acupoint; $N = 10$), EA (as sham rats, however the electroacupuncture was provided at acupoints; $N = 10$). As a positive control, a group of animals received imipramine (Sigma) at a dose of 30 mg/kg i.p., 24 h, 5 h and 1 h prior the 5 min test ($N = 08$). To assess the influence of the serotonergic system in the electroacupuncture effects in this model, two additional groups were formed: pCPA (rats previously treated with *p*-chlorophenylalanine — see *p*-chlorophenylalanine treatment protocol; and received saline 24 h, 5 h and 1 h prior to the 5 min test; $N = 08$) and *p*-chlorophenylalanine + EA (rats previously treated with *p*-chlorophenylalanine — see *p*-chlorophenylalanine treatment protocol and just after the15 min of forced swim, the rat was immobilized for 25 min and received electroacupuncture at acupoints; $N = 10$). Previous to the 5 min test in the despair swim, the animal was submitted to an open field test to measure locomotor activity of the animals [15].

2.4. Statistical analysis

The data obtained from behavioral tests were analyzed by a one-way ANOVA followed by a Tukey *post hoc* test. Significance was set at $P < 0.05$.

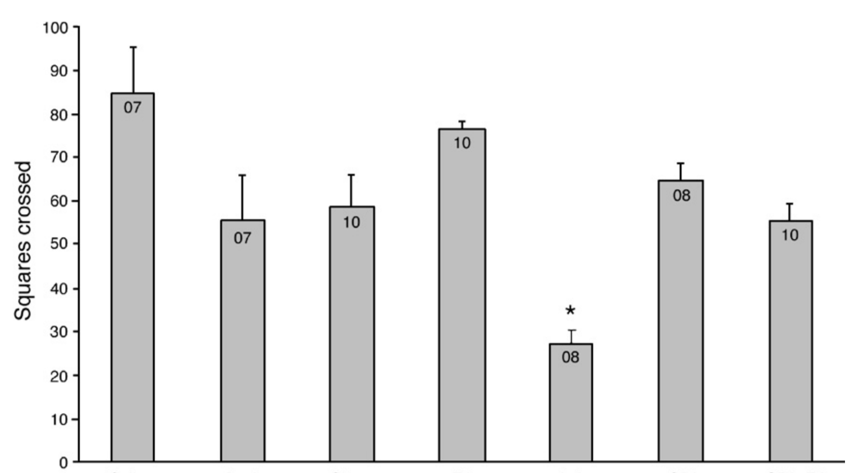

Fig. 3. Number of squares crossed during 5 min of session in the open field test. Data expressed as mean ± SEM. Numbers within bars represent the number of animals in each group. The animals that received imipramine showed a diminution of squares crossed when compared to the other experimental groups (* $P < 0.01$).

3. Results

3.1. Learned helplessness

The results obtained in the learned helplessness are shown in Fig. 1. ANOVA detected a significant difference between groups ($F_{(8,74)}$=4.69; $P<0.05$). As expected, animals that received saline and were submitted to shock presented an impairment in the percentage of successful active avoidance when compared to naïve animals, that did not receive a shock ($P<0.05$), a good indicative of depression-like symptoms. The imipramine treatment and electroacupunture procedure, but not immobilization and sham electroacupuncture significantly enhanced the percentage of successful active avoidance. Previous administration of *p*-chlorophenylalanine did not disrupt the learned helplessness effect: naïve *p*-chlorophenylalanine-treated rats avoided shock more effectively than treated rats exposed to shock. However, increased avoidance induced by electroacupuncture was abolished in *p*-chlorophenylalanine-treated rats.

3.2. Forced swimming

As expected, animals in the forced swimming test treated with imipramine or submitted to electroacupuncture spent less time in immobility when compared to the saline group ($F_{(6,54)}$= 3.68, $P<0.01$). As in learned helplessness, previous *p*-chlorophenylalanine administration abolished the effects of EA (See Fig. 2). These differences were not attributed to an augment of locomotor activity, taking into account that only rats treated with imipramine presented a diminution in the number of squares crossed over 5 min of session in the open field when compared to the other experimental groups ($F_{(6,54)}$=6.77 $P<0.01$), and these did not differ from each other (See Fig. 3).

4. Discussion

Here we demonstrate an effect of acupuncture in two rat models of depression. Not all patients respond to pharmacological antidepressant treatments. In addition, the therapeutic effects of prescribed drugs take several weeks to manifest and these effects are often accompanied by unwanted side effects. There is thus a need for additional therapeutic strategies for the treatment of depression. In a multi-centered clinical study, Luo and colleagues demonstrated EA treatment as an effective therapy for depressive disorders. That study however, was not controlled for the possible placebo effects of electroacupuncture [6]. Additional evidence describing an effect of acupuncture over depression resulted from uncontrolled clinical studies in small groups of patients [8,9]. Our work, although performed in rats, showed a clear antidepressant effect of electroacupuncture under controlled conditions, evidencing an efficacy similar to that of imipramine and indicating a possible neurochemical pathway required for these effects.

There is a well-documented involvement of the serotonergic system in depressive disorders [2]. The present work suggests the serotonergic system to play a role, at least in part, in the encountered antidepressant effects of electroacupuncture, given that the previous *p*-chlorophenylalanine treatment abolished these effects. Our results are further supported by a number of reports showing that electroacupuncture leads to an increment of Fos-positive cells in the raphe nuclei [16–18]. Interestingly, this pattern of Fos activation is similar to that encountered after chronic treatment with antidepressant drugs [19,20].

Our results provide a strong experimental basis for the further development of controlled clinical trials evaluating the effectiveness of acupuncture for treating depressive disorders. The use of several different controls and comparative groups indicate this effect of electroacupuncture to be specifically associated to the stimulation of acupoints, to require the serotonergic system and to have effectiveness similar to that of imipramine.

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