

# 5-HT in the dorsal raphe nucleus is involved in the effects of 100-Hz electro-acupuncture on the pain-depression dyad in rats

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**Abstract.** The pain-depression dyad is becoming widespread in the clinic and is attracting increasing attention. A previous study by our group found that 100-Hz electro-acupuncture (EA), but not 2-, 50- and 2/100-Hz EA, was effective against the reserpine-induced pain-depression dyad. This finding is in contrast to the fact that low-frequency EA is commonly used to treat supraspinal-originating diseases. The present study aimed to investigate the mechanism underlying the effects of 100-Hz EA on the pain-depression dyad. Repeated reserpine injection was found to induce allodynia and depressive behaviors in rats. It decreased 5-hydroxytryptamine (5-HT) levels and immunoreactive expressions in the dorsal raphe nucleus (DRN). 100-Hz EA alleviated the pain-depression dyad and upregulated 5-HT in the DRN of reserpine-injected rats. Intracerebroventricular injection of para-chlorophenylalanine, an inhibitor of 5-HT resynthesis, suppressed the upregulation of 5-HT in the DRN by 100-Hz EA and partially counteracted the analgesic and anti-depressive effects of 100-Hz EA. The present study was the first to demonstrate that 5-HT in the DRN is involved in mediating the analgesic and anti-depressive effects of 100-Hz EA on the pain-depression dyad. This

finding provided a scientific basis for high-frequency EA as a potential treatment for the pain-depression dyad.

## Introduction

The pain-depression dyad is characterized by widespread pain, tenderness to palpation and various concomitant symptoms, including affective disorders such as depression (1). This syndrome is becoming increasingly widespread in the clinic and is attracting increasing amounts of attention (2-5). It is usually treated with antidepressants and antiepileptics. However, due to heavy side effects of antidepressants, it is difficult for patients to adhere to the medication for a long duration (6). Researchers have begun to focus on non-pharmaceutical therapies such as music therapy and psychotherapy, in an attempt to identify an ideal and systemic therapy for the pain-depression dyad.

Acupuncture, particularly electro-acupuncture (EA), has been approved worldwide to be effective for pain or emotional problems (7-9). Low-frequency EA can relieve pain mainly at the supraspinal level and the spinal cord, while high-frequency EA mainly acts via the spinal cord (10). Low-frequency EA also effectively attenuates depression (11). Considering that the pain-depression dyad is associated to lesions at the supraspinal level (1,12), it was hypothesized that low-frequency EA is also more effective than high-frequency EA in treating the pain-depression dyad. However, by comparing the effects of 2-, 50-, 100- and 2/100-Hz EA on the pain-depression dyad, a previous study by our group found that only 100-Hz EA effectively alleviated allodynia and depression (13). Consequently, the present study was performed to investigate the underlying mechanism of this effect of 100-Hz EA.

Accumulating evidence has shown that the serotonergic system in the dorsal raphe nucleus (DRN) participates in the descending modulation of pain (14,15). It also has an important role in the induction and manipulation of emotional disorders (16,17). Studies have shown that the serotonergic system in DRN has a role in the effects of EA on pain or emotional disorders (18,19). Whether the serotonergic system in DRN is also involved in the effects of 100-Hz EA on the pain-depression dyad has remained elusive, which was therefore the focus of the present study.

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**Abbreviations:** 5-HT, 5-hydroxytryptamine (serotonin); DRN, dorsal raphe nucleus; EA, electro-acupuncture; PWT, paw withdrawal threshold; pCPA, para-chlorophenylalanine; OF, open field; EZM, elevated zero maze; ST 36, Zusanli, 5 mm lateral to the anterior tubercle of tibia; SP 6, Sanyinjiao, 10 mm proximal to the prominence of medial malleolus; HPLC-ECD, high-performance liquid chromatography and electrochemistry detection; TBST, Tris-buffered saline and Tween-20

**Key words:** pain-depression dyad, electro-acupuncture, 5-hydroxytryptamine, dorsal raphe nucleus, rat

## Materials and methods

**Animals.** A total of 50 male Sprague-Dawley rats (weight, 250±20 g; age, 8 weeks old) were purchased from the Shanghai Laboratory Animal Center (Shanghai, China) and housed in 40x50x25 cm cages at room temperature (25±1°C) with *ad libitum* access to food and water. The animals were housed in groups of 5-6 rats with a 12-h light/dark cycle. All animal experiments were performed in accordance with the regulations of the State Science and Technology Commission for the Care and Use of Laboratory Animals (no. 2, 1988). The present study was approved by the Ethics Committee of Zhejiang Chinese Medical University (Hangzhou, China).

**Experimental design.** All animals were implanted with a guide cannula and allowed to recover for seven days. The animals were then randomly assigned to a normal group or a reserpine treatment group. The reserpine treatment group included the following subgroups: Model group, EA group and pCPA + EA group. Mechanical allodynia was assessed via paw withdrawal threshold (PWT) to a mechanical stimulus. The reserpine solution was subcutaneously injected once daily for three consecutive days [day(d)1-d3]. The PWT was assessed 1 day after the last reserpine injection (d4). Intracerebroventricular (i.c.v.) injection of a para-chlorophenylalanine (pCPA) or sterile saline was administered immediately after the PWT tests. Then EA treatment was applied on the same day. Another PWT test was performed 30 min after EA treatment. On d5, the micro-injection and EA treatment were repeated. PWT, open field (OF) and elevated zero maze (EZM) tests were performed in sequence 30 min after EA treatment. All rats were immediately sacrificed after the behavioral tests to obtain DRN samples (Fig. 1).

**Guide cannula implantation.** The surgery was performed as described previously (20), with slight modifications. On the day of surgery, the animals were anesthetized with 0.5 ml/kg of 7% chloral hydrate (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). Each rat was then placed in a stereotaxic apparatus (68002; RWD Life Science Co., Ltd, Shenzhen, China). A small incision was made to expose the skull and a burr hole was drilled. And i.c.v. guide cannula (62001; RWD Life Science Co., Ltd) was implanted according to the following coordinates, which were based on a standard rat brain stereotaxic atlas (21): 0.9 mm posterior to the bregma, 1.5 mm lateral to the midline and 3.8 mm ventral from the surface of the skull. The guide cannula was affixed to the skull using two stainless steel screws and dental cement. All of the animals were allowed to recover for seven days after surgery.

**Induction of the pain-depression dyad.** Reserpine powder (100 mg; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was dissolved in 400 µl glacial acetic acid and then diluted with distilled water to 20 ml. The animals in the reserpine treatment group were injected subcutaneously with a reserpine solution (0.2 ml/kg daily) for three consecutive days as previously described (13). The rats in the normal group were injected subcutaneously with a vehicle solution (2% solution of glacial acetic acid in distilled water).

**Micro-injection of pCPA.** In the pCPA + EA group, pCPA (20 mg/ml; Sigma-Aldrich; Merck KGaA), an inhibitor of serotonin (5-hydroxytryptamine; 5-HT) resynthesis, was injected directly into the lateral ventricle. An injection cannula was connected to a 250-µl Hamilton syringe with polyethylene tubing [outer diameter, 0.85 mm; inner diameter (ID), 0.42 mm; RWD Life Science Co., Ltd] and back-filled with the pCPA solution. The injection cannula was inserted into the guide cannula and 10 µl of the pCPA solution was injected (i.c.v.) using a microsyringe infusion pump (UMP3; World Precision Instruments Inc., Sarasota, FL, USA) at a rate of 1 µl/min. The injection cannula was kept in the guide cannula for 10 min after injection. The rats in other groups were injected with 10 µl sterile saline.

**EA treatment.** The bilateral Zusanli (ST 36, 5 mm lateral to the anterior tubercle of the tibia) and Sanyinjiao (SP 6, 10 mm proximal to the prominence of medial malleolus) acupoints were selected as in the previous study by our group (13). Stainless steel acupuncture needles (0.25 mm in diameter, 13 mm in length) were inserted into the acupoints at a depth of 5 mm. The two ipsilateral needles were connected to the output terminals of a Han's Acupoint Nerve Stimulator (LH-202H; Huawei Co. Ltd., Beijing, China). The EA parameters were adopted as follows: Square wave current output (pulse width, 0.2 msec); stimulation intensities of 1.0, 1.5 and 2.0 mA, each for 15 min in sequence; stimulation frequency of 100 Hz. Animals were awake and calmed by placing their heads in black hoods with no physical restraint during EA treatment. EA was performed on d4 and d5.

**Assessment of mechanical allodynia.** Mechanical allodynia was measured using an electronic von Frey instrument (EVF-3; Bioseb *In Vivo* Research Instruments, Chaville, France) (22,23). Rats were placed on an elevated metal mesh floor and allowed to adapt for 15 min. The stimulus was applied to the left hind paw for 5 sec. The plastic rod was pushed against the left hind paw with linear ascending force until a robust and immediate withdrawal occurred. The PWT was calculated as the mean of three tests with intervals of 30 sec.

**Behavioral tests of depression and associated emotional disorders.** Emotional behavior was quantified using the OF test and EZM test, which are generally used to evaluate depression and anxiety (24,25). All exterior lights were blocked and the ambient noise in the testing room was maintained below 40 db; abrupt loud noises that may have altered locomotion or produce prolonged immobility were avoided during testing. The room temperature was maintained at ~25°C.

The OF test was performed as follows (13): Four square OF arenas (100 cm in diameter, 100 cm in width and 100 cm in height) constructed with black plexiglass were placed together to form the apparatus. The entire apparatus was wiped with 75% ethanol prior to each trial. Animals were placed in the testing room 1 h before the test. Each animal was placed in the center of the arena for 20 sec at the beginning of the trial to adapt to the environment and the behavior was then videotaped for 5 min and quantified by the SMART 3.0 system (Panlab Harvard Apparatus, Barcelona, Spain).

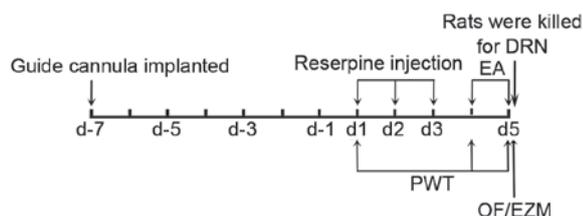


Figure 1. Experimental procedure. All animals were implanted with a cannula (d-6) and allowed to recover for seven days. Mechanical allodynia was assessed via PWT (d1). Reserpine solution was subcutaneously injected once daily for three consecutive days (d1-d3). PWT was assessed prior to and after EA treatment (d4). On d5, EA and PWT assessment were repeated, followed by the OF and EZM tests in sequence. All rats were sacrificed in order to obtain dorsal raphe nucleus samples. PWT, paw withdrawal threshold; EA, electroacupuncture; d, day; EZM, elevated zero maze; OF, open field.

The EZM test was performed as follows (13): A maze with a black metallic annular platform (100 cm in diameter, 25 cm in width and 55 cm in height) was equally divided into four quadrants. Two opposite quadrants (closed arms) were enclosed by black metallic walls (30 cm in height) on the inner and outer edges of the platform, while the remaining two opposite quadrants (open arms) remained uncovered (24). The animals were placed in the testing room 1 h prior to the test. The entire apparatus was wiped with 75% ethanol prior to each trial. The animal was placed in the center of a closed arm for 20 sec to adapt to the environment and its behavior was then videotaped for 5 min and quantified by the SMART 3.0 system.

**Detection of 5-HT levels by high-performance liquid chromatography and electrochemistry detection (HPLC-ECD).** HPLC-ECD was performed as described previously (26), with slight modifications. A stock solution of 5-HT (H9523; Sigma-Aldrich; Merck KGaA) at a concentration of 1  $\mu\text{g}/\text{ml}$  was prepared as a standard. 5-HT powder was added to 0.1 mol/l hydrochloric acid, which was then dissolved in 0.1 mol/l perchloric acid including 1 mol/l EDTA (Sigma-Aldrich; Merck KGaA). The working standard solutions were prepared by serially diluting the stock solutions to concentrations of 160, 80, 40, 20, 10, 5 and 2.5 ng/ml. All solutions were filtered through a 0.22- $\mu\text{m}$  Millipore filter prior to injection into the HPLC-ECD system (Agilent 1200 HPLC system; Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with an ESA Coulochem III detector (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Standard solutions (10  $\mu\text{l}$ ) were injected using an autosampler to generate a standard curve by HPLC-ECD analysis.

Samples were prepared in accordance with the procedure of Pani *et al* (27). The animals were anesthetized and transcardially perfused with ice-cold saline to remove circulating blood. The brains were quickly removed from the calvaria and placed in a cooled rat brain matrix (68711; RWD Life Science Co., Ltd). The DRN was dissected (21), weighed and stored at  $-80^{\circ}\text{C}$ . The samples were ultrasonically homogenized in 0.1 mol/l perchloric acid containing 1 mol/l EDTA (10  $\mu\text{l}$  solution for each milligram of tissue). The homogenate was centrifuged at 25,300  $\times$  g at  $4^{\circ}\text{C}$  for 15 min. The supernatant was filtered through a 0.22- $\mu\text{m}$  millipore filter and stored at  $-80^{\circ}\text{C}$ .

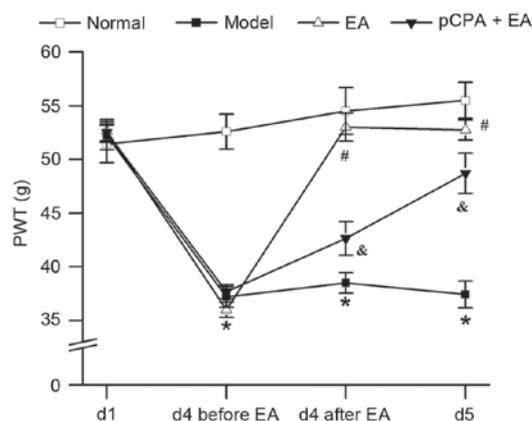


Figure 2. Effects of EA on the PWT in rats with pain-depression dyad. The PWT was measured with an electronic von Frey apparatus at d1, d4 before EA, d4 after EA and d5. Values are expressed as the mean  $\pm$  standard error of the mean (n=10). \*P<0.05, vs. normal group; #P<0.05, vs. model group; &P<0.05, vs. EA group. PWT, paw withdrawal threshold; d, day; EA, electroacupuncture; pCPA, para-chlorophenylalanine.

The following working conditions were maintained in the HPLC-ECD system for the detection of 5-HT: Gradient elution; mobile phase: 75 mmol/l  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 1.7 mmol/l sodium octane sulfonate; 100  $\mu\text{l}/\text{l}$  triethyl amine; 25 mmol/l EDTA; 10% acetonitrile; pH 3.0; C18 reversed-phase column (inner diameter, 3.2 mm; length, 150 mm; MD-150 ODS; Thermo fisher Scientific, Inc.); flow rate, 0.6 ml/min; temperature,  $33^{\circ}\text{C}$ ; injection volume, 10  $\mu\text{l}$ ; detector Model, 5014B (analytical cell) and 5020 (guard cell); cell potentials  $E_1$ ,  $E_2$  and  $E_{\text{GC}}$ : -150, +220 and +270 mV, respectively; full scale/range, 100 nA; signal output voltage, 1.0 V. The 5-HT levels are expressed as ng/g of wet tissue.

**Immunofluorescence of 5-HT expression in the DRN.** After the behavioral tests, the animals were anesthetized with chloral hydrate (3.5 mg/kg, intraperitoneal injection) and transcardially perfused with 150 ml pre-cooled saline followed by 400 ml of a 4% paraformaldehyde solution. The brains were removed and post-fixed in paraformaldehyde for 24 h prior to being placed in a 15% sucrose solution overnight. The brains were transferred to a 30% sucrose solution and incubated for 72 h prior to embedding in optimal cutting temperature matrix. Cryostat sections were cut at 30  $\mu\text{m}$  around the DRN region (bregma -7.50, 8.00 and 8.50 mm) on a sliding microtome and blocked in 5% donkey serum (ab7475; Abcam, Cambridge, UK) in Tris-buffered saline containing Tween-20 (TBST)/Triton for 60 min. The sections were then incubated at  $4^{\circ}\text{C}$  overnight in TBST containing an anti-5-HT primary antibody (1:100 dilution; cat. no. ab10385; Abcam). Immunoreactivity to the antigen was visualized using an Alexa Fluor 488-conjugated secondary antibody (1:1,000 dilution; cat. no. 103715; Jackson ImmunoResearch Laboratories, Inc., Bar Harbor, ME, USA). Images were obtained using a fluorescence microscope (Olympus IX71; Olympus, Tokyo, Japan) equipped with Image-Pro Insight 8.0 software (Media Cybernetics, Rockville, MD, USA).

**Statistical analysis.** Values are expressed as the mean  $\pm$  standard error of the mean. SPSS 16.0 software (SPSS, Inc.,

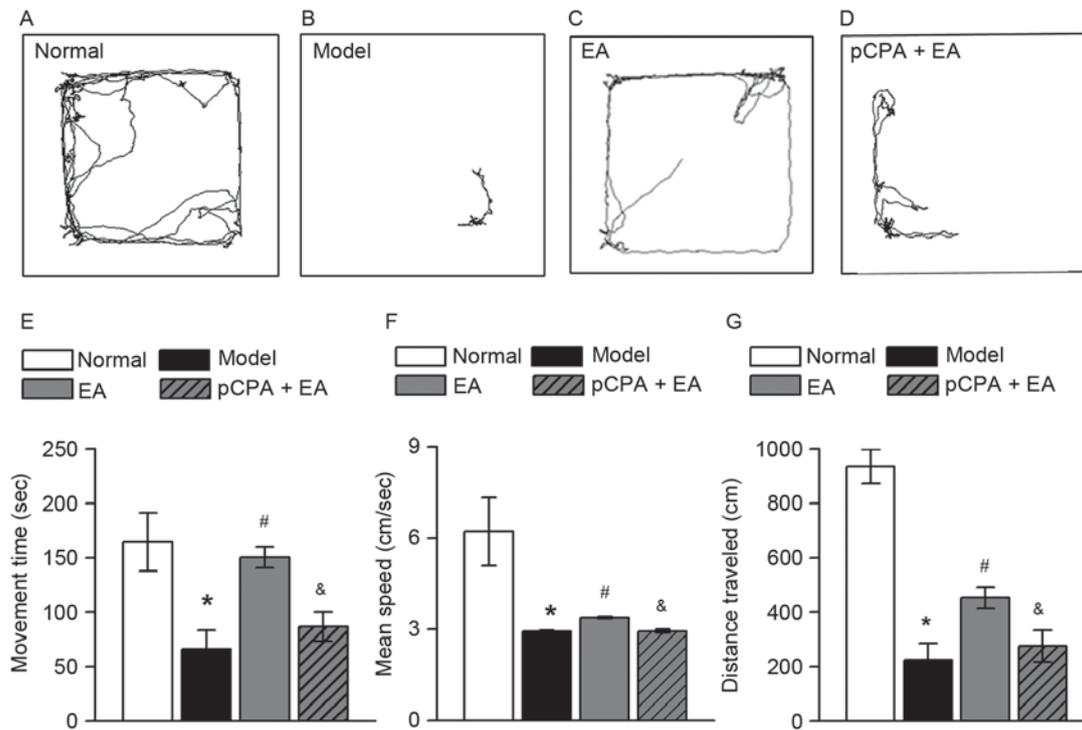


Figure 3. Effects of EA on depressive-like behavior in rats quantified using the OF test on day 5. Trajectories in OF test from (A) the normal group, (B) model group, (C) EA group and (D) pCPA + EA group. Effects of EA on (E) movement time, (F) mean speed and (G) distance traveled by rats with pain-depression dyad. Values are expressed as the mean  $\pm$  standard error of the mean (n=10). \*P<0.05, vs. normal group; #P<0.05, vs. model group; &P<0.05, vs. EA group. EA, electroacupuncture; pCPA, para-chlorophenylalanine; OF, open field.

Chicago, IL, USA) was used for all data analysis. Statistical analysis was performed by one-way analysis of variance followed by a post-hoc test of the least significant differences for multiple comparisons. The data were analyzed using a two-tailed student's t-test for the behavior results, 5-HT levels and 5-HT cell expression levels. P<0.05 was considered to indicate a statistically significant difference.

## Results

*EA increases the PWT in rats with pain-depression dyad via 5-HT.* As is shown in Fig. 2, repeated injection of reserpine resulted in a significant decrease in the PWT of rats (P<0.05). 100-Hz EA significantly increased the PWT in reserpine-injected rats (P<0.05, vs. the model group). Injection of pCPA (i.c.v.) significantly restrained the effect of 100-Hz EA on PWT (P<0.05, vs. the EA group).

*EA reduces depressive-like behavior in rats with pain-depression dyad via 5-HT.* The OF test was performed to observe depressive-like behavior in rats. Representative trajectories from the OF test for the normal group, model group, EA group and pCPA + EA group are presented in Fig. 3A-D, respectively. Repeated injection of reserpine significantly reduced movement time, mean speed and distance traveled in rats (Fig. 3E-G, respectively; P<0.05). Movement time, mean speed and distance traveled were significantly increased by 100-Hz EA (P<0.05, vs. the model group). Injection of pCPA (i.c.v.) significantly restrained the effect of 100-Hz EA on the three parameters mentioned above (P<0.05, vs. the EA group).

*EA reduces anxiety-like behavior in rats with pain-depression dyad via 5-HT.* In certain rodent models of depression, anxiety-like responses are observed (28,29). To determine whether anxiety-like behaviors accompany the pain-depression dyad, rats were subjected to the EZM test. Three-dimensional activities in the EZM test of animals from the normal group, model group, EA group, and pCPA + EA group are presented in Fig. 4A-D, respectively. Repeated injection of reserpine resulted in significant decreases in entries in the open arms, stretching time and distance traveled in rats (Fig. 4E-G, respectively; P<0.05). 100-Hz EA significantly increased the number of entries in the open arms, stretching time and distance traveled in rats with pain-depression dyad (P<0.05, vs. the model group). Although a declining trend of entries into the open arms existed in the pCPA + EA group, no significant difference was found between the EA group and the pCPA + EA group (Fig. 4E). Injection of pCPA (i.c.v.) significantly restrained the effects of 100-Hz EA on stretching time and distance traveled (Fig. 4F and G, respectively; P<0.05, vs. the EA group).

*EA increases 5-HT in the DRN of rats with pain-depression dyad.* HPLC-ECD was adopted to determine the effect of 100-Hz EA on 5-HT levels in the DRN in rats with pain-depression dyad. Repeated injection of reserpine significantly decreased the 5-HT levels of DRN in rats (Fig. 5; P<0.05). 100-Hz EA significantly increased the 5-HT levels of DRN in reserpine-injected rats (P<0.05, vs. the model group). The upregulation of 5-HT levels in DRN by 100-Hz EA was completely abrogated by injection of pCPA (i.c.v.) in the pCPA + EA group (P<0.05, when compared to the EA group).

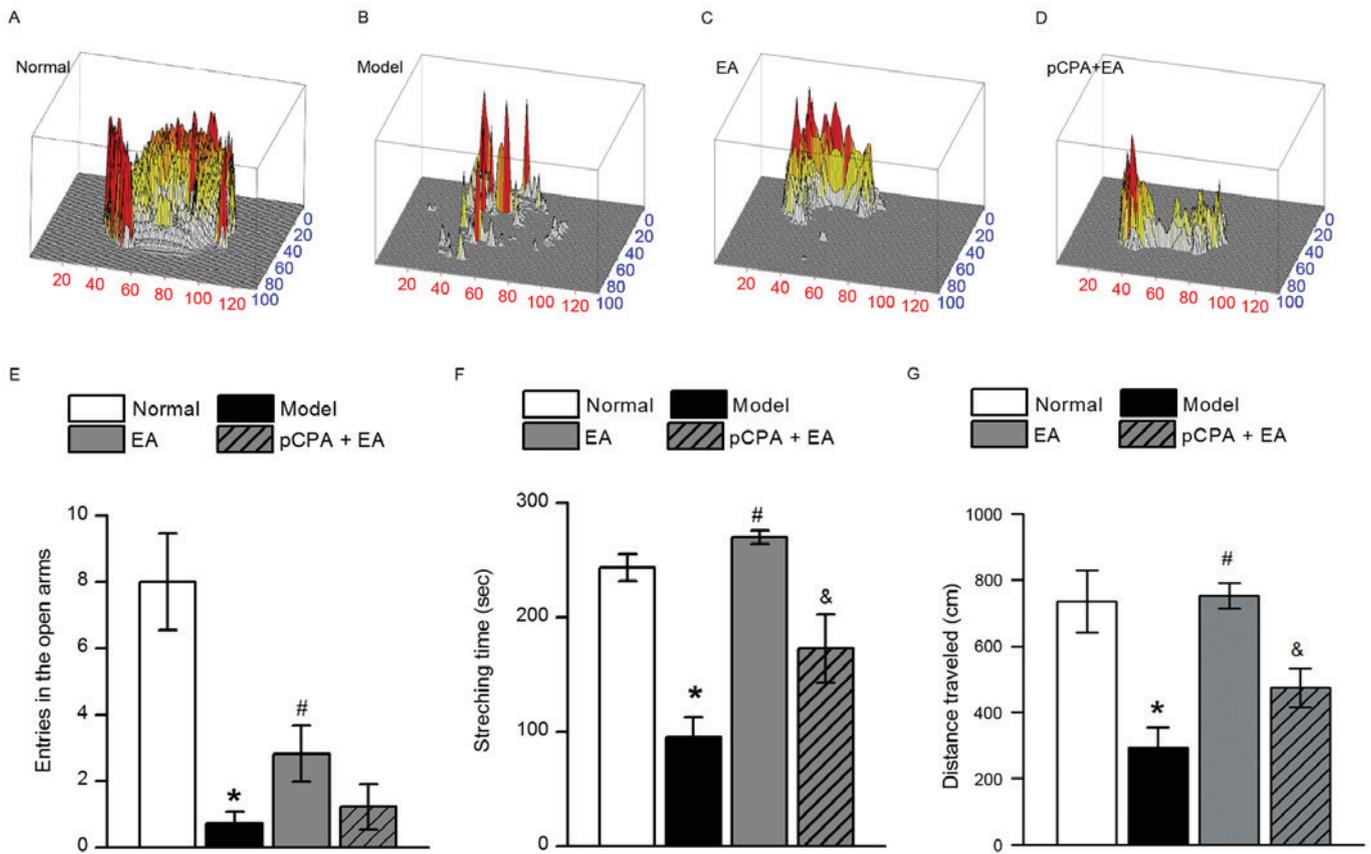


Figure 4. Effects of EA on the anxiety-like behavior in rats quantified by the EZM test on day 5. Three-dimensional activities in EZM test in (A) the normal group, (B) model group, (C) EA group and (D) pCPA + EA group. Effects of EA on (E) entries in the open arms, (F) stretching time and (G) distance traveled of rats with pain-depression dyad. Values are expressed as the mean  $\pm$  standard error of the mean (n=10). \*P<0.05, vs. normal group; #P<0.05, vs. model group; &P<0.05, vs. EA group. EA, electroacupuncture; pCPA, para-chlorophenylalanine; EZM, elevated zero maze.

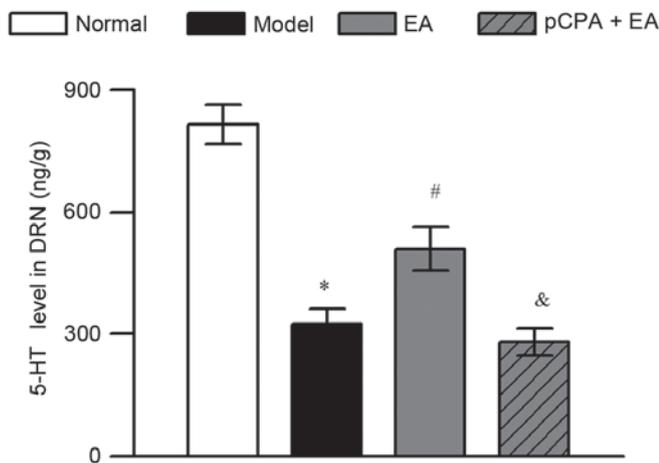


Figure 5. Effect of EA on 5-HT levels in the dorsal raphe nucleus of rats as determined by high-performance liquid chromatography circular electrochemical detection on day 5. Values are expressed as the mean  $\pm$  standard error of the mean (n=7). \*P<0.05, vs. normal group; #P<0.05, vs. model group; &P<0.05, vs. EA group. EA, electroacupuncture; 5-HT, 5-hydroxytryptamine; pCPA, para-chlorophenylalanine.

EA enhances the number of 5-HT-immunoreactive cells in the DRN of rats with pain-depression dyad. Distribution of 5-HT-immunoreactive cells in the DRN of rats was investigated at bregma -7.5, -8.0 and -8.5 mm (Fig. 6A-C,

respectively). The abundance of 5-HT-immunoreactive cells in the DRN was higher at bregma -8.00 mm than at bregma -7.50 and -8.50 mm. A significant decline of 5-HT-immunoreactive cells in the DRN compared with the normal group was found at bregma -8.00 mm (Fig. 6D, P<0.05), which was greater than that observed at the other points. Bregma -8.0 mm was therefore determined to be the optimal location for examining the effect of 100-Hz EA on 5-HT-immunoreactive cells in the DRN of rats with pain-depression dyad.

Representative images of 5-HT-immunoreactive cells in the DRN for the normal group, model group, EA group and pCPA + EA group are shown in Fig. 7A-D, respectively. As is shown in Fig. 7E, repeated injection of reserpine significantly reduced the number of 5-HT-immunoreactive cells in the DRN of rats (P<0.05). Of note, 100-Hz EA significantly increased the number of 5-HT-immunoreactive cells in the DRN in reserpine-injected rats when compared to that in the model group (P<0.05). The upregulation of the number of 5-HT-immunoreactive cells in DRN by 100-Hz EA was totally abrogated by injection of pCPA (i.c.v.) in the pCPA + EA group (P<0.05, when compared to the EA group).

## Discussion

Pain-depression dyad is a complex illness with symptoms of pain overlapping with emotional disorders such as depression

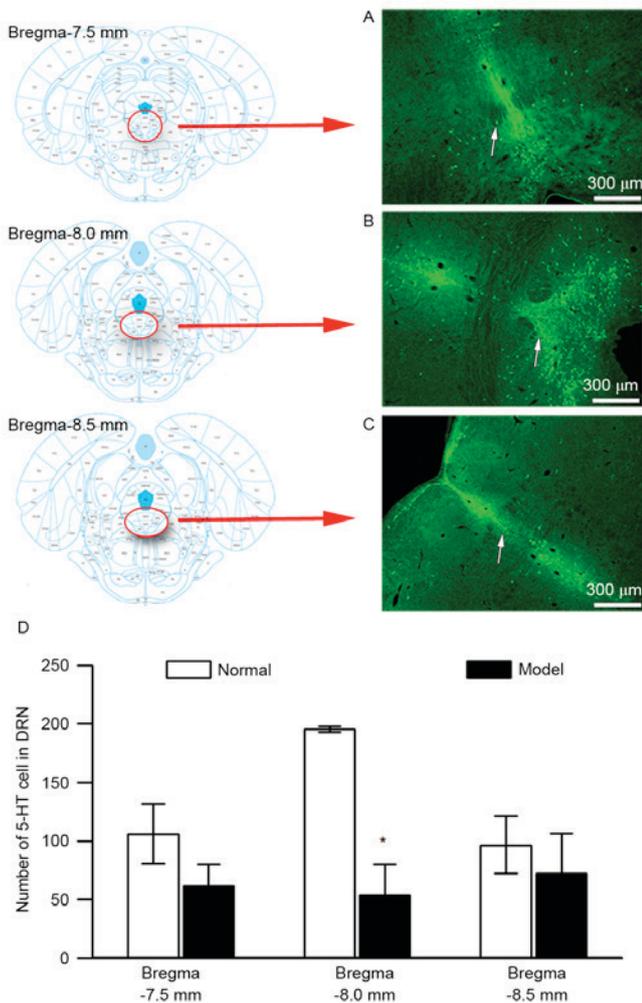


Figure 6. Distribution of 5-HT-immunoreactive cells in the DRN of rats detected by immunofluorescence on day 5. (A-C) Immunofluorescence of 5-HT expression in the DRN at bregma -7.5, -8.0 and -8.5 mm, respectively (scale bar, 300  $\mu$ m for all). White arrows indicate the 5-HT-immunoreactive cells. Insets (images from Paxinos and Watson, 1986) depict areas in which quantification was performed. (D) Quantification of 5-HT-immunoreactive cells in DRN. Values are expressed as the mean  $\pm$  standard error of the mean (n=3). \*P<0.05, vs. normal group. DRN, dorsal raphe nucleus; 5-HT, 5-hydroxytryptamine.

and anxiety (1). It has been reported that 52% patients with chronic pain suffer from depression, which is a costly health problem (30). While antidepressants and antiepileptics substantially reduce the symptoms, numerous patients cannot tolerate the side effects of their long-term administration. This dissatisfaction compelled researchers to explore complementary and alternative medicines (1,6,9). A previous study by our group initially demonstrated that EA with high frequency but not low frequency effectively relieves pain-depression dyad (13). The present study found that 5-HT in the DRN was involved in the analgesic and anti-depressant effects of 100-Hz EA.

Several modeling methods are used for studying the mechanisms of the pain-depression dyad, including nerve injury (31), stress load (32,33) and administration of reserpine (34), monosodium iodoacetate (35) and Freund's complete adjuvant (36). Reserpine-injected rats, an ideal model of pain-depression dyad, display hyperalgesia, allodynia and depressive behaviors accompanied with anxiety (28,34,37).

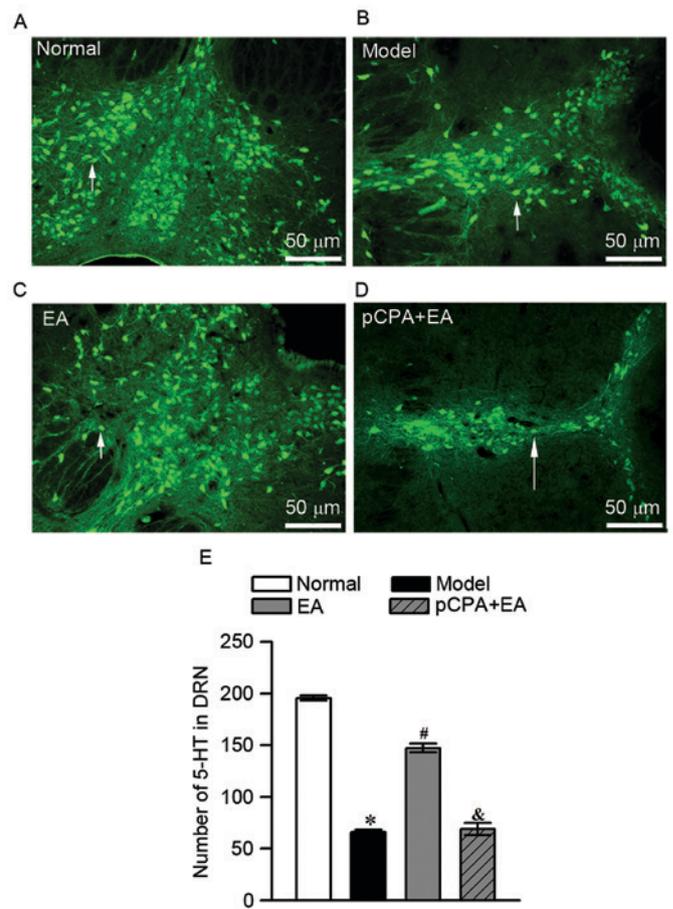


Figure 7. Effect of EA on 5-HT-immunoreactive cells in DRN assessed by immunofluorescence on day 5. (A-D) Immunofluorescence detection of 5-HT in the DRN of (A) the normal group, (B) model group, (C) EA group and (D) pCPA + EA group (scale bar, 50  $\mu$ m for all). White arrows indicate the 5-HT-immunoreactive cells. (E) Quantified amount of 5-HT-immunoreactive cells in DRN. Values are expressed as the mean  $\pm$  standard error of the mean (n=3). \*P<0.05, vs. normal group; #P<0.05, vs. model group; &P<0.05, vs. EA group. DRN, dorsal raphe nucleus; EA, electroacupuncture; 5-HT, 5-hydroxytryptamine; pCPA, para-chlorophenylalanine.

The response is also similar to that of pain-depression patients in the clinic. This model was thus selected for use in the present study. The results showed that reserpine injection resulted in mechanical allodynia in rats. The forced swimming test, OF test and EZM test have been widely used in animal psychology for decades (24,25,38). The forced swimming and OF tests are commonly adopted to evaluate depressive behaviors of animals (38). Considering that the forced swimming test can influence pain sensitivity in rodents (39,40), the present study used the OF test to evaluate depressive behaviors in rats with reserpine-induced pain-depression dyad. As the reserpine-injected rats exhibited not only depressive-like but also anxiety-like behavior in the EZM test, it was indicated that the pain-depression dyad model was successfully established.

5-HT has an important role in the central nervous system in the descending control of pain or emotion (18,41-43). DRN is abundant of serotonergic neurons (42). The present study found that reserpine, a monoamine depletor, caused a decline of 5-HT in the DRN, which contributes to reserpine-induced allodynia and depressive behaviors in rats (37,44). Acupuncture, particularly EA, is commonly used for pain or emotional

problems (7-9,19,45,46). The present and a previous study by our group showed that 100-Hz EA effectively improved mechanical allodynia and depressive behaviors in rats caused by reserpine injection (13). However, the mechanism underlying the effects of EA on the pain-depression dyad has been rarely assessed. Studies have shown that high-frequency EA at the spinal cord is highly effective and exerts its effects by segmental inhibition at the spinal cord (10,42,47,48) However, recent studies demonstrated that 100-Hz EA improved Parkinson's disease (a typical brain-derived disease) and may exert its effects through the cerebrum (49,50). In the present study, 100-Hz EA upregulated 5-HT DRN in rats with reserpine-induced pain-depression dyad. Furthermore, injection of pCPA (i.c.v.), an inhibitor of 5-HT resynthesis, suppressed the upregulation of 5-HT in the DRN by 100-Hz EA and partially abrogated the analgesic and anti-depressive effects of 100-Hz EA. These findings suggested that 100-Hz EA improves reserpine-induced pain-depression dyad partially via 5-HT DRN. It may be assumed that high-frequency EA on body parts other than the spinal cord is also efficient.

In conclusion, the present study was the first, to the best of our knowledge, to demonstrate that 5-HT in the DRN participates in mediating the effects of 100 Hz EA on the pain-depression dyad. The present study provided a scientific basis for the value of high-frequency EA in treating supraspinal-originating diseases.

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