Research report

Characteristics of electroacupuncture-induced analgesia in mice: variation with strain, frequency, intensity and opioid involvement

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Abstract

The present study was conducted to evaluate the characteristics of electroacupuncture (EA)-induced analgesia in mice. Three inbred strains of mice (DBA/2, C57BL/6J, BALB/c) and three outbred strains (ICR, LACA, NIH) were used in the experiment. Two pairs of metallic needles were inserted into acupoints ST 36 and SP 6 connected to an electric pulse generator. EA parameters were set as constant current output with alteration of a positive and negative square wave, 0.6 ms in pulse width for 2 Hz and 0.3 ms for 100 Hz. Tail-flick latencies evoked by radiant heat were measured before, during and after EA stimulation. We found that (1) DBA/2 mice showed a significantly more potent analgesic effect than the other five strains in response to both 100 and 2 Hz EA. In this case, the intensities were 1.0–2.0–2.0 mA, 10 min for each intensity totally 30 min. (2) EA analgesia increased as the intensity of stimulation increased from 0.5 to 2.0 mA, but it remained at this plateau when the intensity further increased from 2.0 to 3.0 mA. (3) 10.0 mg·kg^{-1} naloxone was needed to block the analgesic effect induced by 2 Hz EA of 2.0 mA, but to block that by 100 Hz, 25.0 mg·kg^{-1} was necessary. (4) A positive correlation was observed between analgesia induced by morphine at the dose of 5.0 mg·kg^{-1} and by 100 Hz EA in two tested strains DBA/2 and C57BL/6J. In conclusion, EA induces reliable, strain-dependent analgesia in mice. The naloxone-reversibility of EA, a measure of whether it is opioid or non-opioid mediated, is dependent upon intensity and frequency. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

Acupuncture has been used in the Orient to produce pain relief for more than two thousand years; though evidence of this historical use is mostly anecdotal. About 30 years ago Western scientists began testing these Oriental acupuncture theories as well as using acupuncture in Western medicine. In the past, most acupuncture experiments used rats, although early in 1970s, mice began to be used for the study of electroacupuncture (EA) analgesia, but less than 100 papers searched in MEDLINE in the category 'acupuncture and mouse' exist. In these papers, one outbred mouse strain (Kunming) was used mostly and there was no evidence of acupuncture testing in inbred mice. Most of these papers are not related to pain or analgesia and say nothing of systematic research on EA. Now people have more and more interest in pain research, because many inbred strains and knockout mice are available [1].

In the present study, six different strains of mice (three inbred and three outbred) were used to observe the EA (a modern version of acupuncture) analgesia. The influence of mouse strain, frequency and intensity of the electrical stimulus were evaluated. Naloxone blockade and correlation analysis with morphine was used to investigate the involvement of endogenous opioid peptides in EA analgesia.

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2. Materials and methods

2.1. Animals and chemicals

Female and male mice of the following inbred (DBA/2, C57BL/6J, BALB/c) and outbred (ICR, LACA, NIH) strains, weighing 20–25 g were supplied by the Animal Department of Health Science Center, Peking University. They were housed five per cage with food pellets and water available ad libitum. All experiments were carried out in accordance with the National Institute of Health Guidance for the Care and Use of Laboratory Animals (NIH Publication No. 80-23), revised in 1978.

Morphine hydrochloride (morphine) was purchased from Shenyang Pharmaceutical Company (China), naloxone hydrochloride (NLX) from Sigma (USA). All drugs were dissolved in normal saline (NS). Morphine (5.0 mg·kg^{-1}) was injected intraperitoneally (i.p.), and naloxone (0.1, 1.0, 5.0, 10.0, 25.0 mg·kg^{-1}) or NS were injected subcutaneously (s.c.) 15 min prior to EA. Injection volume was 0.1 ml kg^{-1} body weight.

2.2. EA stimulation [2]

Two stainless steel needles 0.3 mm in diameter and 3 mm in length were inserted in each hind leg, one at the acupoint Zusanli (ST 36), 2 mm lateral to the anterior tubercle of the tibia which is marked by a clear notch, and the other at the acupoint Sanyinjiao (SP 6), 2 mm proximal to the upper border of medial malleolus, at the posterior border of the tibia [3,4]. They were connected to the output of a Han's Acupoint Nerve Stimulator (HANS, LH 800, manufactured at the Health Science Center, Peking University) and transmitted consistent square wave current to simultaneously to the two acupoints. The frequency was either 2 or 100 Hz, and the corresponding pulse width was 0.6 or 0.2 ms. For the EA analgesia in six strains of mice (Section 3.1), the intensity of stimulation ranged from 1.0–2.0–2.0 mA, increased in a stepwise manner (10 min for each intensity, 30 min in total). For experiment of analgesic effects induced by EA at different intensities (Section 3.2), the intensities were 0.2, 0.5, 1.0, 2.0, 3.0 mA in different groups, and the stimulation time was 20 min for each group. In all experiments, insertion of the needle alone, without current stimulation was used as a control.

2.3. Nociceptive testing

Nociceptive sensitivity was assessed by recording the tail flick latency (TFL) induced by radiant heat. Room temperature was carefully maintained at 21±1 °C to minimize the influence of ambient temperature on TFLs [5]. Mice were gently immobilized in a plastic restrainer with hindlimbs and tail extending. A focused beam of light (2 mm in diameter) from a 12.5 W projection bulb was applied to the tail 2–3 cm from the tip. TFL was measured to the nearest 0.1 s with a digital timer which was electrically connected in series with the radiant heat. Basal latency was kept within the range of 3.5–5.5 s by adjusting the electrical voltage of radiant heat at the start of the experiment. The average of three consecutive measures taken at 5 min intervals was taken as the mean basal TFL. TFL was measured every 10 min during the 30 min period of EA, with the electrical stimulation temporarily switched off during the TFL measurement. After the EA session, TFL was measured three more times to show the after-effect of EA analgesia. To avoid tissue damage, a 10 s cut-off value was employed. The percentage change of TFL increase induced by EA was calculated as follows: [(EA latency−basal latency)/basal latency]×100% [3].

2.4. Naloxone blockade experiment

To test the effect of naloxone blockade on EA at low (2 Hz) or high (100 Hz) frequency, 74 female C57BL/6J mice were assigned to four groups with low frequency EA: (1) EA+NS; (2) EA+NLX 0.1 mg·kg^{-1}; (3) EA+NLX 1.0 mg·kg^{-1}; (4) EA+NLX 10.0 mg kg^{-1}. Eighty four female C57BL/6J mice were divided into four groups for high frequency EA: (1) EA+NS; (2) EA+NLX 1.0 mg·kg^{-1}; (3) EA+NLX 5.0 mg·kg^{-1}; (4) EA+NLX 25.0 mg·kg^{-1}. After basal TFL assessment, NLX or NS was injected s.c. 15 min prior to EA application.

In the experiment of naloxone blockade on EA with low (2.0 mA) or high (3.0 mA) intensity for each frequency, 40 female C57BL/6J mice were randomly divided into four groups: EA 2.0 mA+NS, EA 2.0 mA+NLX, EA 3.0 mA+NS, and EA 3.0 mA+NLX. NLX at doses of 1.0 mg·kg^{-1}, 25.0 mg·kg^{-1} or NS was injected the same as mentioned above.

2.5. Morphine analgesic experiment and the correlation analysis

First, EA at 100 Hz was tested in four stains of mice (LACA, BALB/c, DBA/2 and C57BL/6J) using the same protocol described above (Section 2.1). One week later, morphine (5.0 mg·kg^{-1}, i.p.) analgesia was tested in these mice. Each group had 10–12 mice, half of them were tested with EA first and the other half received morphine first. Area under the curve (AUC) relative to maximum possible AUC was used to represent the analgesic effect. All data were analyzed with Pearson correlation coefficients.

2.6. Statistical analysis [6]

The data were expressed as mean±S.E. and analyzed by one-way analysis of variance (ANOVA) followed by the

Newman–Keuls post-hoc test. The criterion value of alpha was chosen to be $P<0.05$.

3. Results

3.1. Analgesic effects induced by EA at 2 and 100 Hz in six strains of mice

As shown in Fig. 1A and B, electroacupuncture-induced analgesia (EAA) is significantly different among the six strains of mice for 2 and 100 Hz. DBA/2 mice showed significantly more analgesic effect than the other five strains for 100 Hz [$F(17,18)=6.83$, $P<0.01$]. For 2 Hz, DBA/2 mice were also the highest responders, but only significantly stronger than ICR mice which displayed the least amount of analgesia [$F(17,18)=2.45$, $P<0.05$].

3.2. Analgesic effects induced by EA at different intensities

Fig. 2A shows the results of 2 Hz EA using different intensities. Significant analgesia was produced only when the intensity reached 0.5 mA. Analgesia increased linearly up to 2.0 mA and then leveled off [$F(9)=7.62$, $P<0.01$].

Fig. 2. Analgesia induced by electroacupuncture at 2 and 100 Hz with different intensities in female C57BL/6J mice. Panel A: 2 Hz EA. Panel B: 100 Hz. Intensities were 0.2~3.0 mA, stimulus of each intensity lasted for 20 min, the percentage increase in tail flick latency (TFL) for EA 20 min was calculated. Bars represent mean and vertical lines represent S.E. *$P<0.05$, **$P<0.01$, compared with the group without EA stimulation.

Similar results were obtained with 100 Hz EA [$F(9)=5.92$, $P<0.01$] as shown in Fig. 2B.

3.3. Naloxone blockade effects on analgesia induced by EA at different frequencies

Low dose NLX (0.1 mg kg) produced a significant (approximately 50%) decrease in the analgesic effect produced by 2 Hz EA. The low (0.1 mg·kg^{-1}), medium (1.0 mg/kg) or high (10.0 mg·kg^{-1}) dose of NLX decreased the analgesic effect 50, 82 and 85%, respectively [$F(17,18)=12.90$, $P<0.01$] (Fig. 3A). For 100 Hz, the analgesic effect was reversed neither by the medium (1.0 mg·kg^{-1}) nor by the higher (5.0 mg·kg^{-1}) NLX, but there was a significant (80%) reduction in analgesia by 25.0 mg·kg^{-1} NLX [$F(19,20)=8.11$, $P<0.01$] (Fig. 3B). In other words, much higher concentration of NLX was needed to block the same analgesic effect induced by 100 Hz EA compared to block that induced by 2 Hz EA.
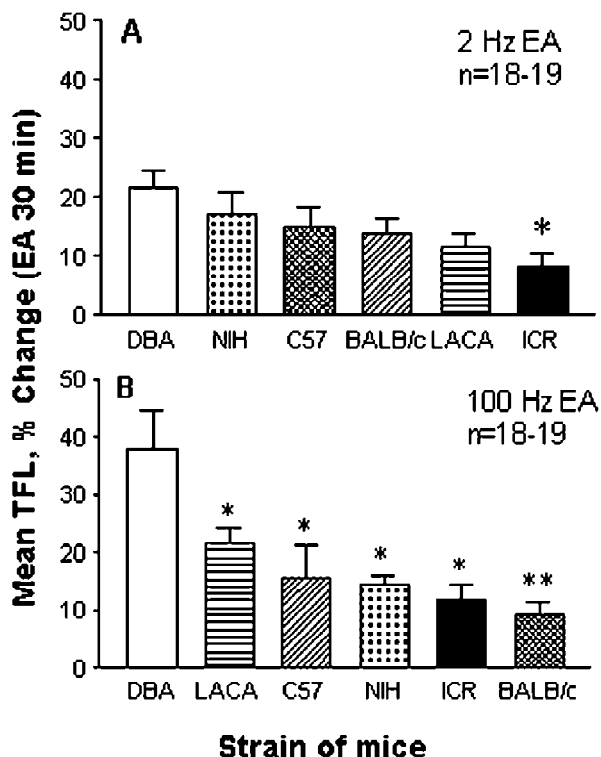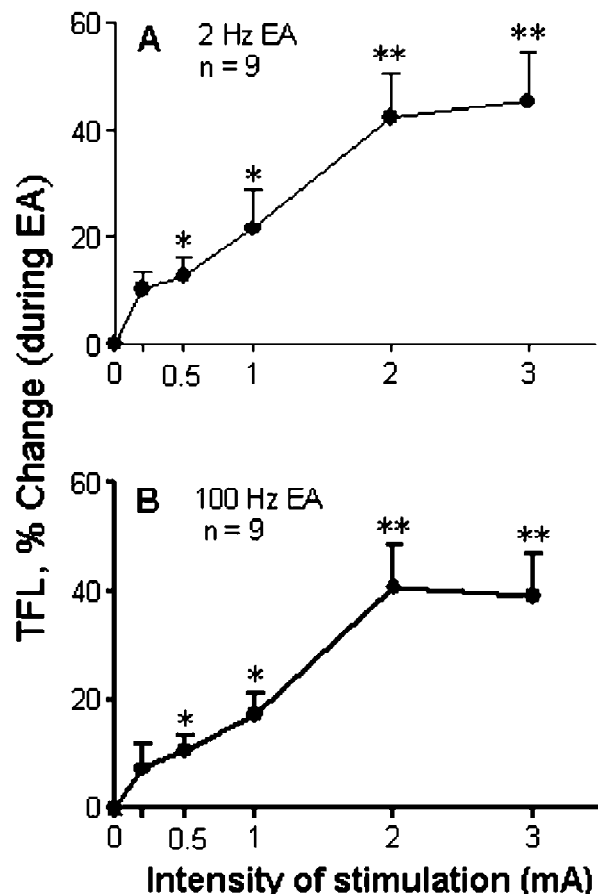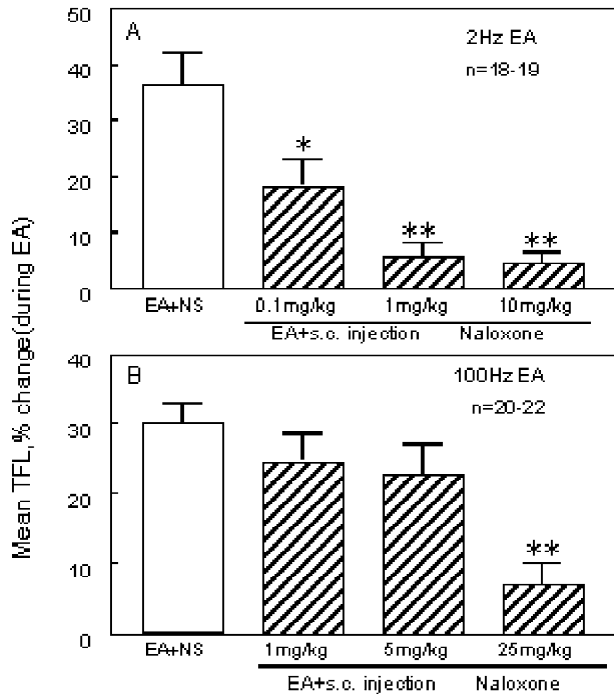

Fig. 1. Analgesia induced by electroacupuncture at 2 and 100 Hz in six strains of mice. The mean percentage increase in tail flick latency (TFL) for EA 30 min was taken as index of EA analgesia. Bars represent means and vertical line S.E. *$P<0.05$, **$P<0.01$, compared with DBA/2 mice, ANOVA followed by Newman–Keuls post-hoc test.

Fig. 3. The naloxone blockade effect of analgesia induced by EA at 2 or 100 Hz in mice. Bars represent mean and vertical lines represent S.E. *$P<0.05$, **$P<0.01$, compared with corresponding NS group. ANOVA followed by Newman–Keuls post-hoc test.

3.4. Naloxone blockade effects on analgesia induced by EA at different intensities

As shown in Fig. 4A, 1.0 mg·kg^{-1} NLX reversed 68% of the analgesic effect induced by 2.0 mA EA ($P<0.05$, $n=10$, $F=2.46$), but none of that by 3.0 mA EA. Similarly with 100 Hz EA (Fig. 4B) 25 mg/kg NLX reversed 67% of the analgesic effect produced 100 Hz EA at 2.0 mA [$F(9)=7.62$, $P<0.01$], but none of that by 100 Hz EA at 3.0 mA.

3.5. Correlation analysis between 100 Hz electroacupuncture-induced analgesia and morphine-induced analgesia (MIA)

A positive within-strain correlation, as shown in Fig. 5, was obtained between 100 Hz EAA and MIA using C57BL/6 and DBA/2 mice (for female C57BL/6, $\gamma=0.89$, $n=9$, $P<0.01$; for male C57BL/6, $\gamma=0.93$, $n=9$, $P<0.01$, for female DBA/2, $\gamma=0.92$, $n=10$, $P<0.01$; for male DBA/2, $\gamma=0.86$, $n=9–10$, $P<0.01$). Similar results were obtained in LACA and BALB/c mice (data not shown). No such positive correlation was observed between 2 Hz EAA and MIA.

Fig. 6 presents the between-strain correlation of EAA and MIA in both sexes of four strains of mice. The analgesic effect of EA at 100 Hz was plotted against the
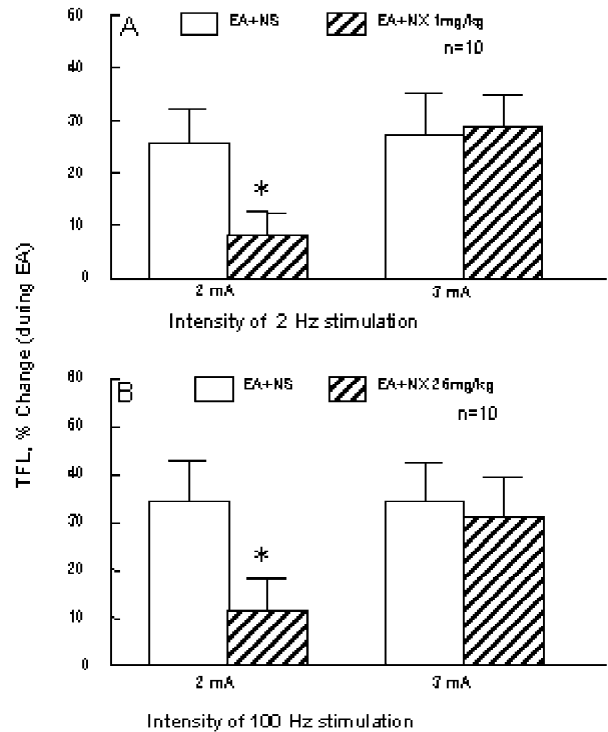

Fig. 4. The naloxone blockade effect of analgesia induced by EA at 2 Hz with low (2.0 mA) or high (3.0 mA) intensity. (A) EA 2.0 mA, naloxone 1 mg kg^{-1}. (B) EA 100 Hz, naloxone 25 mg kg^{-1}. Bars represent mean and vertical lines represent S.E. *$P<0.05$, compared with corresponding NS group, ANOVA followed by Newman–Keuls post-hoc test.

effect of morphine. Female DBA/2 mice displayed the strongest analgesia both for EAA and MIA, while male BALB/c mice the weakest EAA and MIA. This positive
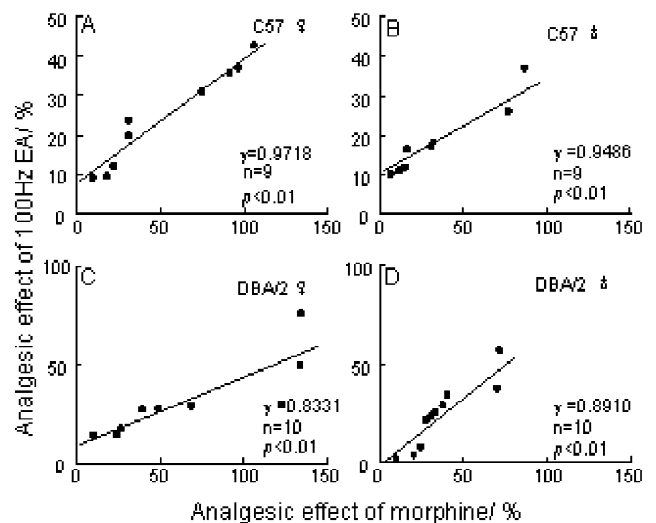

Fig. 5. Within-strain correlation between analgesia induced by electroacupuncture at 100 Hz and morphine-induced analgesia in C57BL/6J and DBA/2 mice of both sexes. The Analgesic effect was expressed by percentage increase of tail flick latency. AUC: Area under the curve. Dose of morphine: 5.0 mg·kg^{-1} s.c.
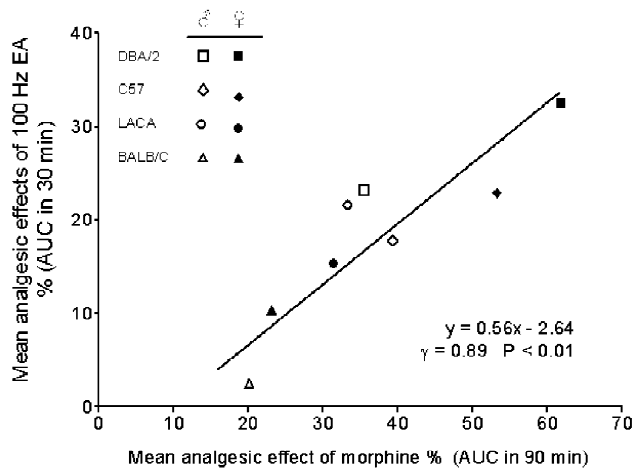
Fig. 6. The relationship between analgesia induced by electroacupuncture at 100 Hz and morphine induced analgesia in four strains of mice of both sexes. AUC: Area under the curve, with the analgesic effect expressed by percentage increase of tail flick latency during the whole period of assessment (30 min for EA, and 90 min for morphine). Dose of morphine: 5 mg kg^{-1} s.c.

correlation was very significant ($\gamma=0.89$, *XY* pairs$=8$, $P<0.01$).

4. Discussion

Recently we reported that EA at 2 or 100 Hz had a strain-dependent analgesic effect in 10 inbred strains of mice [7] and here we also show strain-dependent analgesia following 2 or 100 Hz EA in both inbred and outbred mice. The previous report and the present study all suggested the strain difference overall, but to a specific strain of mouse, the sensitivity to EA analgesia was not the same in two reports. For example, BALB/c was among the most sensitive strain to 100 EA while it is in the insensitive rank in the previous report finished in the US. Besides the different supplier of mice is one of the obvious explanations (genetic variation), environment can also be a very prominent factor.

EA analgesia increased as the intensities increased from 0.5 to 2.0 mA, EA with such low intensity (2.0 mA) could be blocked with naloxone at lower doses. When EA intensity was increased from 2.0 to 3.0 mA, the analgesia reached a plateau (Fig. 3), and EA with high intensity could be blocked with naloxone at higher doses (Fig. 4). In a similar study by Ernst and Lee [8], it was shown that a 27% pain threshold increase after 30 min of EA stimulation at low intensity could be partially blocked by 0.8 mg·kg^{-1} naloxone. We in conjunction with Ernst and Lee [8] believe that the nature of EA produced by 2.0 mA is opioid, and that above 2.0 mA is non-opioid. The high correlation between the analgesia induced by morphine and analgesia induced by 100 Hz EA at 2 mA supports that the idea that EA below 2 mA is opioid in nature.

In rats it is well known that analgesia induced by EA using different frequencies is mediated by different kinds of endogenous peptides, i.e., EA at low frequency mainly releases enkephalins and endorphins, and at high frequency mainly dynorphins. Goldstein et al. [9] found that in rats, the dose of naloxone required for a 50% blockade of 2 Hz EAA was 0.53 mg·kg^{-1}, whereas for 100 Hz EAA was 24 mg·kg^{-1}. It was also known that the dose of NLX required to block κ receptors was about 20-fold higher than to block μ receptors in vitro [10]. The present study reported that this frequency dependence may be also true for electroacupuncture analgesia in mice.

In rats, much evidences from cross-tolerance studies [11] and experiments with naloxone blockade [12] showed that morphine and μ-opioid receptor system was correlated with EA at low frequencies (e.g., 2 Hz), not at high frequencies (e.g., 100 Hz). But in the present study, MIA was positively correlated with high (100 Hz) EA analgesia at least in four strains of mice (Figs. 5 and 6). Why are they different? Firstly, morphine is by no means a pure μ-agonist [13]. Secondly, the efficacy of opioid analgesia is determined not only by the availability of opioid agonist and opioid receptors, but also by the potency of the anti-opioid system [14]. Mouse and rat are different in both opioid and anti-opioid aspects. For example the μ-receptor agonist DAMGO was approximately two times more potent in mice compared to rats, whereas δ-receptor agonist DPDPE was 15 times more potent than in the mouse [15].

To summarize this report, EA analgesia was observed in mice. This is a very good model for EA analgesia studies. The characteristics of EA analgesia in the mice are very similar, if not identical, to that in rats. When use this model, strain difference and the influence of parameters (i.e., intensity and frequency) should be considered.

Acknowledgements

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