Neurochem Res (2008) 33:2166–2170
DOI 10.1007/s11064-008-9791-9

ORIGINAL PAPER

Electroacupuncture Reduces Voluntary Alcohol Intake in Alcohol-preferring Rats via an Opiate-sensitive Mechanism

David H. Overstreet · Cai-Lian Cui · Yao-Ying Ma · Chang-Yong Guo · Ji-Sheng Han · Scott E. Lukas · David Y.-W. Lee

Accepted: 19 June 2008 / Published online: 17 July 2008

Abstract Electroacupuncture (EA) has been shown to modify the effects of various drugs of abuse, including alcohol. Inbred P rats were trained to drink alcohol voluntarily and then subjected to two periods of alcohol deprivation lasting 3 days. During the second deprivation, the rats received either EA or sham EA. The rats were pretreated with naltrexone (5 mg/kg) or saline 30 min before each of the EA or sham EA sessions. Approximately 6 h after the last naltrexone or saline treatment, the alcohol tubes were returned and alcohol and water intakes were recorded later at 2, 4, 6, and 24 h. Only EA led to a decrease in alcohol intake, which was most prominent at 6 and 24 h, and this inhibitory effect of EA was blocked by naltrexone, suggesting that activation of the endogenous opiate system may be responsible for EA's effects on alcohol intake in the alcohol-dependent iP rats.

Keywords Electroacupuncture · iP rat · Alcohol · Naltrexone

Special issue article in honor of Ji-Sheng Han.

D. H. Overstreet
Bowles Center for Alcohol Studies, UNC School of Medicine, Chapel Hill, NC 27599-7178, USA

C.-L. Cui · Y.-Y. Ma · C.-Y. Guo · J.-S. Han · D. Y.-W.Lee (✉)
Bio-Organic and Natural Products Laboratory, Mailman Research Center 317, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont, MA 02478, USA
e-mail: dlee@mclean.harvard.edu

C.-L. Cui · J.-S. Han
Neuroscience Research Institute and Department of Neurobiology, Peking University Health Science Center, 38 Xueyuan Road, Beijing 100083, People's Republic of China

S. E. Lukas
Behavioral Psychopharmacology Research Laboratory, McLean Hospital/Harvard Medical School, Belmont, MA 02478, USA

Introduction

Alcohol and drug abuse pose serious medical, social, and economic problems in the United States and around the world. A great deal of effort has been directed toward developing effective therapies; however, because of the complexity of drug dependence and the lack of effective remediation, especially for relapse, which is often precipitated by withdrawal and/or intense craving even after prolonged abstinence, poses a serious therapeutic challenge. In a consortium effort focusing on a systematic evaluation of alternative therapies, we have been investigating both traditional Chinese medicine and acupuncture for their complementary role in the treatment of alcohol and drug abuse.

Acupuncture (derived from "acus", meaning a sharp point, and "punctura", meaning puncturing), consists of stimulating certain points on the body by means of needles. This therapeutic art, together with other techniques such as moxibustion and massage aimed at stimulation of those specific points (the acupoints), has been used in China for over 3,000 years. Ancient Chinese health care providers employed acupuncture (acupoint stimulation with needles), massage (acupoint stimulation with the hand), food therapy (adjusting food and drink for health), gymnastic therapy (exercise for fitness), and herbs (for medication) as major weapons to fight illnesses. While acupuncture and related acupoint therapies are most commonly recognized for analgesic effect, their medical applications are by no means limited to pain treatment.

The discovery of morphine like substances (endorphins) in the mammalian brain in 1975 [1] had a great impact on

acupuncture research. It was soon made clear that acupuncture-induced analgesia (by manual needling) can be blocked by the narcotic antagonist naloxone, suggesting the involvement of endogenous opioid substances [2]. In animal experiments, manual acupuncture or acupuncture combined with electrical stimulation (electroacupuncture, EA) was shown to accelerate the production and release of endorphins that can interact with different kinds of opioid receptors to ease pain [3]. It was further clarified that endorphins are, in fact, a group of neuropeptides possessing different characteristics. Among these neuropeptides, beta-endorphin and enkephalin are primarily agonists at mu and delta opioid receptors, whereas dynorphin is an agonist at kappa receptors [4]. Interestingly, electrical stimulation of different frequencies can induce the release of different kinds of endorphins. For example, low frequency (2–4 Hz) EA accelerates the release of enkephalins to interact with mu and delta receptors, whereas high frequency (100 Hz) EA accelerates the release of dynorphin to interact with kappa receptors [5]. These findings strengthen the scientific basis of this ancient healing art and point the way to its use in areas beyond pain control such as alcohol and drug abuse.

As evidenced, EA has been reported to counteract the effects of a variety of drugs of abuse, including morphine [6] and alcohol [7–11]. Because EA can suppress alcohol withdrawal symptoms [7] and inhibits alcohol-stimulated release of dopamine in nucleus accumbens [8], it was predicted that the application of EA would reduce alcohol intake in alcohol-preferring rats. Furthermore, because there is evidence that opiate mechanisms may be involved in the actions of EA [12–14], it was predicted that pretreatment with the opiate antagonist naltrexone prior to EA application would counteract the effects of EA. The findings presented here provide strong support for both of these predictions.

Experimental Procedure

Animals

The rats were inbred alcohol-preferring P rats (iP). They were selected from the breeding colonies at UNC-Chapel Hill at about 50 days of age and trained to drink alcohol (10%, v/v) voluntarily using standard methods [15–18]. The rats were drinking alcohol or water for at least 1 month before experimental procedures were employed. They were housed individually in standard cages and stable temperature ($22 \pm 1°C$) and humidity ($40 \pm 4\%$). They were subjected to a reverse light-dark cycle (lights off from 10:00 to 22:00) so that recordings of intake could be made at intermediate time points. These procedures were approved by the UNC-Chapel Hill Institutional Animal Care and Use Committee.

Two-Bottle Choice Study

The rats were first exposed to water only for 1 day and then to alcohol (10%) only for 3 days to ensure that they were exposed to alcohol. Thereafter, they had continuous access to alcohol and water tubes, except as described below. Generally, water and alcohol volumes were recorded on a daily basis between 08:00 and 10:00. When necessary, recordings were also taken at 12:00, 14:00, and 16:00 to obtain a time course of the drinking.

EA Treatment

Rats were restrained in rodent holders (see Fig. 1) with their hind legs and tails protruding. Two stainless-steel needles of 0.3 mm diameter were inserted into each hind leg, one in a place around the acupoint ST36 (5 mm lateral to the anterior tubercle of the tibia), and the other around SP6 (2 mm to the posterior border of the tibia and 3 mm above the medial malleolus). Constant current square-wave electrical stimulation produced by a programmed pulse generator (HANS LH-800, produced by Peking University of Astronautics and Aeronautics Aviation) was given via the two needles for a total of 30 min. The frequency alternated between 2 and 100 Hz with a 0.6 and 0.2 ms pulse width, respectively. The intensity of the stimulation was increased stepwise from 0.5 to 1.0 mA and 1.5 mA, with each step lasting for 10 min. The EA stimulation was given three times (24, 48, and 72 h) after the last alcohol intake session. Control rats were placed in the restrainer only for 30 min in the first experiment or had needles inserted but no current passed in the third experiment (sham).

The EA treatment was carried out by experienced acupuncturists, Cai-lian Cui, Yao-Ying Ma and Chang-Yong Guo from Peking University and working on the CERC Project at Bowles Center for Alcohol Studies, UNC School of Medicine, Chapel Hill.

Experiment 1

This experiment contained two groups of nine rats, which received either EA treatment or restraint only. Rats were trained to drink alcohol voluntarily and allowed to achieve stable intake of alcohol over a 1-month period. Rats were then deprived of alcohol for 3 days and drinking was monitored during the 24-h period following the replacement of the alcohol tubes. Two weeks later the deprivation of alcohol was repeated. However, during this 3-day session the rats were subjected to either EA ($n = 9$) or restraint only ($n = 9$) for 30 min a day. The alcohol tubes were replaced 1 h after the last EA treatment and alcohol intake was monitored for 24 h.

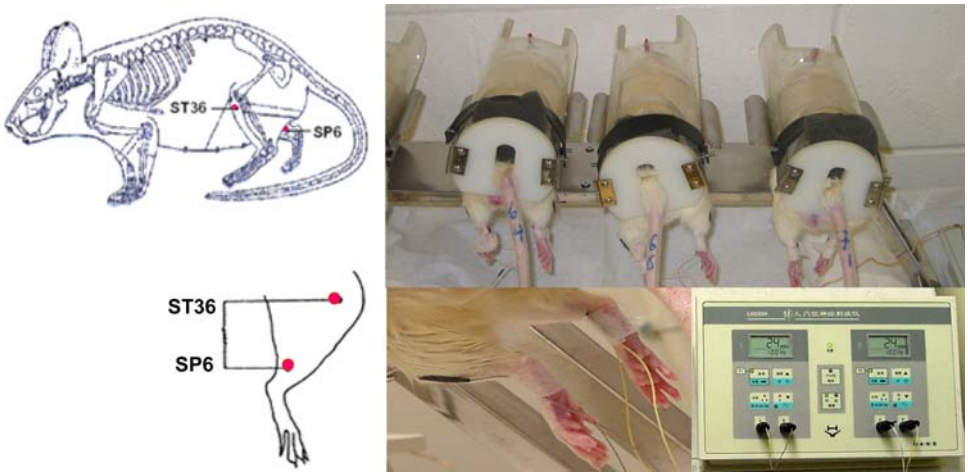

Fig. 1 Anatomical chart and diagram of the EA points ST36 and SP6 that are located on the hind legs where the needles were inserted (top and bottom left two panels). Photograph of a rat prepared for EA while restrained (top right panel). Close up of the needle insertion point and HANS LH-800 device (bottom right panels)

Experiment 2

This experiment was designed to determine whether the effects of EA on alcohol intake in iP rats are dependent on the duration of voluntary alcohol drinking. Naïve P rats were selected from the breeding colonies and subjected to three consecutive days of EA or restraint. They were then trained to drink alcohol in the standard manner.

Experiment 3

This experiment contained four groups of rats in a factorial design. One factor was sham treatment or the application of EA. The second factor was vehicle or naltrexone (5 mg/kg). As in Experiment 1, these treatments were employed during the second deprivation period. Naltrexone (5 mg/kg) or vehicle was given via i.p. injection 30 min prior each EA or sham treatment. However, the alcohol tube was not returned until 6 h after the injections to minimize the effects that naltrexone might have in this study [17].

Data Analysis

Alcohol intake (g/kg) was calculated from the volume of 10% alcohol consumed using 0.79 for the density of alcohol. Data were presented as means ± SEM and analyzed by appropriate ANOVAs. If the main finding was significant, follow-up tests were carried out with Tukey's protected *t*-tests.

Results

In Experiment 1, EA had a large inhibitory effect on alcohol intake as illustrated in Fig. 2. Although the groups exhibited similar time-dependent increases in alcohol intake during the baseline period, the alcohol intake of rats exposed to EA was much lower (F [3, 32] = 6.89, $P < 0.01$). In contrast, the alcohol intake of the rats subjected to restraint only was similar to that during baseline (Fig. 2). The inhibitory effect of EA on alcohol intake continued throughout 24 h, as illustrated in Fig. 3 (F [3, 32] = 2.84, $P = 0.05$). Not only did EA counteract the appearance of deprivation-induced drinking, it reduced overall drinking.

However, in Experiment 2 as illustrated in Fig. 4, EA treatment had no effect at all on the alcohol intake of iP rats that were just learning to drink alcohol. Thus, a history of alcohol drinking is required for EA treatment to be effective in reducing alcohol intake. Figure 4 should be near here.

In Experiment 3, the voluntary intake of alcohol in the rats that were treated with EA and vehicle was consistently lower than the other treatment groups during the first 6 h

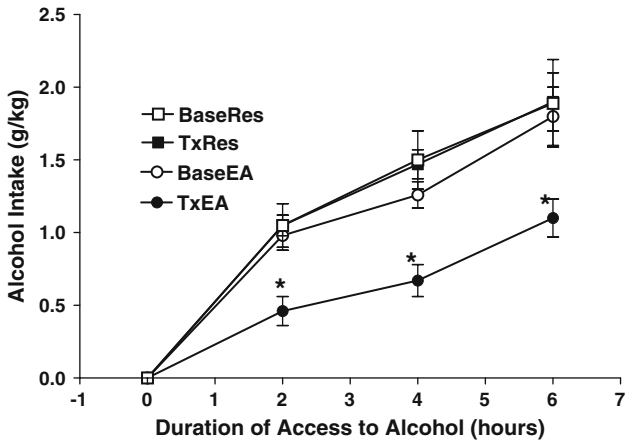

Fig. 2 Time course of the first 6 h of access to alcohol in iP rats after deprivation during baseline conditions and after EA treatment or restraint only. *Significantly different, $P < 0.01$, from other groups. BaseRes, baseline condition for restraint group; BaseEA, baseline condition for EA-treated group; T × Res, restraint treatment; T × EA, EA treatment

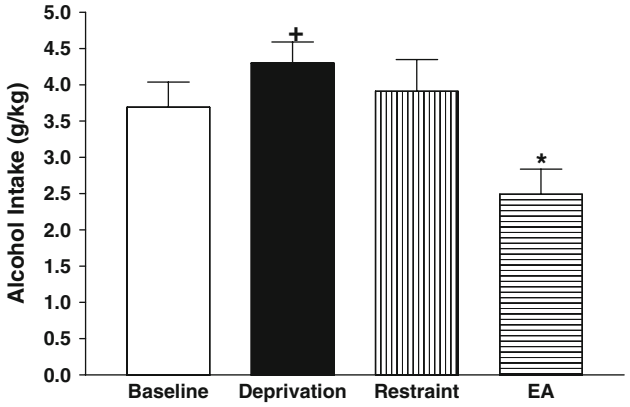

Fig. 3 Twenty-four hour Alcohol intake in iP rats at baseline or after being treated with either active EA or to restraint only after being deprived of alcohol for 3 days. *Significantly different, $P < 0.01$, other groups. $^{+}$Significantly different from baseline group

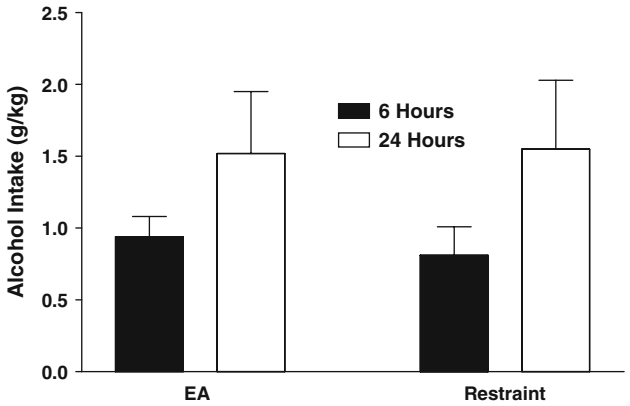

Fig. 4 Initial 6 and 24 h intake in iP rats subjected to EA or restraint prior to exposure to alcohol

after the return of alcohol, replicating the previous finding of reduction in alcohol intake by EA (Fig. 5). A two-way ANOVA, with time and treatment as the two factors, was conducted on these data. As expected, there was a highly significant time effect (F [3, 81] = 11.65, $P < 0.0001$), but the treatment effect was also significant (F [2, 81] = 3.21, $P < 0.05$). However, the interaction effect was also significant (F [6, 81] = 4.99, $P < 0.001$), which is consistent with the differences between the treatments becoming larger with time (Fig. 5). Indeed, the EA + vehicle group was significantly different from the sham + vehicle group at 4 and 6 h only.

There were also group differences in alcohol intake at 24 h (F [3, 27] = 8.12, $P < 0.001$) (Fig. 6). The group exposed to EA and injected with vehicle exhibited the lowest intake. Note also that the rats, which received sham treatment and injected with naltrexone did not differ from the control group, suggesting that the 6 h delay was adequate to prevent the inhibitory effects of naltrexone on alcohol intake.

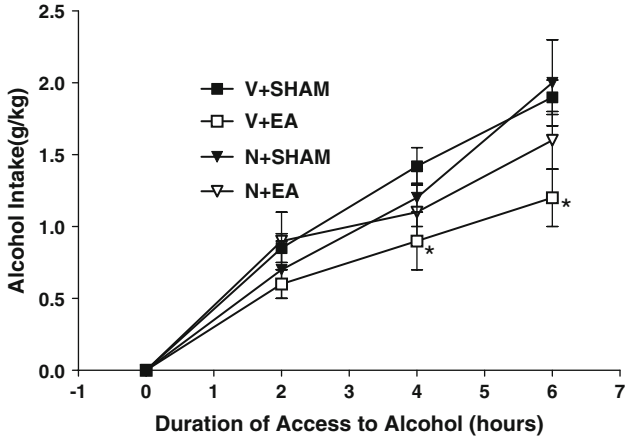

Fig. 5 Time course of alcohol intake over the first 6 h in iP rats subjected to EA or sham treatment (SHAM) and/or injected with naltrexone (N, 5 mg/kg) or vehicle (V). *Significantly different, $P < 0.01$, from V + SHAM group

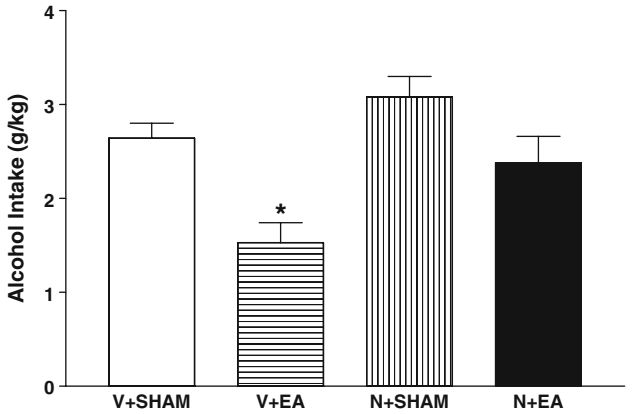

Fig. 6 Twenty-four hour Alcohol intake in iP rats subjected to EA or sham treatment (SHAM) and pretreated with either naltrexone (N, 5 mg/kg, i.p.) or vehicle (V).*Significantly different, $P < 0.01$, from V + SHAM group

Discussion

The application of EA during a 3-day abstinence/deprivation period clearly reduced alcohol intake in iP rats. Not only did it block the small alcohol deprivation effect, it reduced alcohol below the 24 h baseline level. Although the effects were somewhat smaller in the third experiment, EA also reduced alcohol intake. The lower amounts of alcohol intake in this experiment are most likely related to the design of the study that required a 6 h delay after the injections. Thus, the rats only had about 8 of the 12 h of darkness to drink and iP rats drink less than 20% of their fluid during the light phase. Nevertheless, both studies revealed that EA significantly reduced alcohol intake.

In contrast to Experiments 1 and 3, where the iP rats had had access to alcohol for at least 1 month, the iP rats in Experiment 2 had minimal experience with alcohol, and EA

did not significantly affect alcohol in these rats. The reason for this difference may be that the rats with over 1 month of experience may be physically dependent on alcohol, as previous studies have documented that anxiety-like behavior emerges after acute withdrawal [15]. Indeed, at the end of the study, alcohol was withdrawn and anxiety-like behavior was confirmed using the social interaction test (data not shown). This finding is consistent with reports of others that the effects of several drugs on alcohol intake can be observed more clearly in alcohol-dependent rats [19, 20]. The fact that EA is more effective in alcohol-dependent rats provides support for the practice of using acupuncture in alcohol-dependent patients.

The rats that were pretreated with naltrexone 30 min prior to the application of EA did not exhibit a reduction in alcohol intake, suggesting that the release of endogenous opioids during the application of EA was responsible for the reduced alcohol intake. Previous work [13] demonstrated that the EA frequencies used in the present study likely released a range of endogenous opioids and so multiple opioid receptors may be involved with this process. Additional studies with more selective opioid receptor antagonists may provide more precise information about which opiate systems are most responsible for EA's effects on alcohol consumption.

Although we have argued that our data support the involvement of opiate mechanisms in the reduction of alcohol intake by EA, we concede that other effects of EA may contribute to the observed reductions in alcohol drinking. Alternative interpretations include the notion that EA increases the release of serotonin [11] or reduces the release of dopamine in the nucleus accumbens [8]; either of these might well contribute to the effects of EA on alcohol intake. Only further studies can determine the relevance of these effects.

Even though the observations in the present study are promising and support the conduct of clinical studies, the following caveat must be considered. There have been many positive studies of EA on cocaine- or morphine-related effects [21]. However, two recent reviews have suggested that there is no strong clinical data to support the use of acupuncture in either opiate or cocaine abuse [22, 23]. It is important that any clinical studies in alcoholics using acupuncture should be properly controlled and carefully done.

Acknowledgements This study was funded jointly by NCCAM and NIAAA (P01-AT002038) through a Center of Excellence Program on "Alternative Therapies for Alcohol and Drug Abuse." Additional Supports provided by NIDA Grant KO5 DA00343 (SEL), and by the National Basic Research Programme (2003-CB515407) of China.

References

1. Hughes J, Smith TW, Kosterlitz HW et al (1975) Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258:577–580
2. Mayer DJ, Price DD, Rafii A (1977) Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. Brain Res 121:368–372
3. Han JS, Terenius L (1982) Neurochemical basis of acupuncture analgesia. Annu Rev Pharmacol Toxicol 22:193–220
4. Herz A (ed) (1993) Handbook of experimental pharmacology, vol 104/I. Springer-Verlag, Berlin
5. Han JS, Wang Q (1992) Mobilization of specific neuropeptides by peripheral stimulation of different frequencies. News Physiol Sci 7:176–180
6. Chen JH, Liang J, Wang GB et al (2005) Repeated 2 Hz peripheral electrical stimulations suppress morphine-induced CPP and improve spatial memory ability in rats. Exp Neurol 194:550–556
7. Kim JH, Chung JY, Kwon YK et al (2005) Acupuncture reduces alcohol withdrawal syndrome and c-Fos expression in rat brain. Am J Chin Med 33:887–896
8. Yoon SS, Kwon YK, Kim MR et al (2004) Acupuncture-mediated inhibition of ethanol-induced dopamine release in the rat nucleus accumbens through the GABAB receptor. Neurosci Lett 369:234–238
9. Zhao RJ, Yoon SS, Lee BH et al (2006) Acupuncture normalizes the release of accumbal dopamine during the withdrawal period and after the ethanol challenge in chronic ethanol-treated rats. Neurosci Lett 395:28–32
10. Yoshimoto K, Kato B, Sakai K et al (2001) Electroacupuncture stimulation suppresses the increase in alcohol-drinking behavior in restricted rats. Alcohol Clin Exp Res 25:63S–68S
11. Yoshimoto K, Fukuda F, Hori M et al (2006) Acupuncture stimulates the release of serotonin, but not dopamine, in the rat nucleus accumbens. Tohoku J Exp Med 208:321–326
12. Cabyoglu MT, Ergene N, Tan U (2006) The mechanism of acupuncture and clinical applications. Int J Neurosci 116:115–125
13. Han JS (2004) Acupuncture and endorphins. Neurosci Lett 361:258–261
14. Ulett GA, Han S, Han JS (1998) Electroacupuncture: mechanisms and clinical application. Biol Psychiatry 44:129–138
15. Kampov-Polevoy AB, Matthews DB, Gause L et al (2000) P rats develop physical dependence on alcohol via voluntary drinking: changes in seizure thresholds, anxiety, and patterns of alcohol drinking. Alcohol Clin Exp Res 24:278–284
16. Overstreet DH, McArthur RA, Rezvani AH et al (1997) Selective inhibition of alcohol intake in diverse alcohol-preferring rat strains by the 5-HT2A antagonists amperozide and FG 5974. Alcohol Clin Exp Res 21:1448–1454
17. Overstreet DH, Kampov-Polevoy AB, Rezvani AH et al (1999) Suppression of alcohol intake by chronic naloxone treatment in P rats: tolerance development and elevation of opiate receptor binding. Alcohol Clin Exp Res 23:1761–1771
18. Rezvani AH, Overstreet DH, Lee YW (1995) Attenuation of alcohol intake by ibogaine in three strains of alcohol-preferring rats. Pharmacol Biochem Behav 52:615–620
19. Funk CK, Zorrilla EP, Lee MJ et al (2007) Corticotropin-releasing factor 1 antagonists selectively reduce ethanol self-administration in ethanol-dependent rats. Biol Psychiatry 61:78–86
20. Walker BM, Koob GF (2007) The gamma-aminobutyric acid-B receptor agonist baclofen attenuates responding for ethanol in ethanol-dependent rats. Alcohol Clin Exp Res 31:11–18
21. Ren YH, Wang B, Luo F et al (2002) Peripheral electric stimulation attenuates the expression of cocaine-induced place preference in rats. Brain Res 957:129–135
22. Jordan JB (2006) Acupuncture treatment for opiate addiction: a systematic review. J Subst Abuse Treat 30:309–314
23. Kim YH, Schiff E, Waalen J et al (2005) Efficacy of acupuncture for treating cocaine addiction: a review paper. J Addict Dis 24:115–132