

Effects of the κ -opioid receptor on the inhibition of 100 Hz electroacupuncture on cocaine-induced conditioned place preference

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Abstract. The administration of 100 Hz electroacupuncture has been demonstrated to suppress cocaine-induced conditioned place preference (CPP) in rats, and there is evidence that the κ -opioid receptor may have a role in cocaine addiction. The present study sought to explore the mechanisms underlying the inhibitory effects of 100 Hz electroacupuncture on cocaine-induced CPP in rats. A rat model of cocaine-induced CPP was used in the present study to investigate the following: i) Naloxone treatment (5 and 10 mg/kg) following 100 Hz electroacupuncture-mediated inhibition on cocaine-induced CPP, revealing that a high dose (10 mg/kg) of naloxone blocked the inhibitory effects of 100 Hz electroacupuncture on cocaine-induced CPP; ii) nor-binaltorphimine (nor-BNI) on 100 Hz electroacupuncture-mediated inhibition on cocaine-induced CPP, which indicated that administration of 10 μ g/5 μ l and 0.3 μ g/1 μ l nor-BNI intracerebroventricularly and via the nucleus accumbens, respectively, reversed the inhibitory effects of 100 Hz electroacupuncture on cocaine-induced CPP, and that injection of nor-BNI in different brain areas of rats blocks the inhibitory effects of electroacupuncture on cocaine-induced CPP; and iv) 100 Hz electroacupuncture on the mRNA expression levels of the κ -opioid receptor in the rat nucleus accumbens and amygdala, which established that mRNA expression levels of κ -opioid receptor in the nucleus accumbens were increased with 100 Hz electroacupuncture plus cocaine-induced CPP. Overall, the results of the present study indicated that 100 Hz electroacupuncture was able to suppress cocaine-induced CPP via the κ -opioid receptor in the nucleus accumbens.

Introduction

The dopamine pathway in the nucleus accumbens and ventral tegmental area serves a crucial role in the brain's reward system, where it elicits a rewarding effect (1-3). Cocaine is a central nervous system (CNS) stimulant and acts directly on the dopamine transporter in the nucleus accumbens to inhibit dopamine reuptake, thereby increasing the dopamine concentration in the synaptic cleft, stimulating the reward system, and ultimately leading to addiction (4). Administration of cocaine in dopamine transporter gene knockout mice does not increase dopamine in the nucleus accumbens, or induce a rewarding effect (5). Therefore, dopamine in the nucleus accumbens is of importance in the reward mechanism of cocaine (6). The activation of κ -opioid receptors in the CNS has been reported to inhibit various aspects of cocaine dependence, including cocaine-induced conditioned place preference (CPP) and self-administration, by reducing dopamine release in the nucleus accumbens (7,8). The amygdala is also important in the reward effect of cocaine; destroying the amygdala has been demonstrated to decrease cocaine induced CPP (9). The amygdala sends glutamatergic projections to the nucleus accumbens, and activation of the κ -opioid receptor inhibits glutamatergic transmission (10). The κ -opioid receptor is extensively distributed throughout the ventral tegmental area, nucleus accumbens and amygdala, all of which are markedly associated with reward.

A previous study has confirmed that high-frequency (100 Hz), but not low-frequency (2 Hz), electroacupuncture suppresses cocaine-induced CPP in rats (11). The κ -opioid receptor agonist, U50488 is able to block this effect (12,13), indicating that the κ -opioid receptor may be of importance in cocaine addiction. Thus, the present study hypothesized that 100 Hz electroacupuncture may suppress cocaine-induced CPP via the κ -opioid receptor in the CNS.

The present study aimed to further elucidate cocaine addiction, and a rat model of cocaine-induced CPP was employed to investigate the effects of the non-specific opioid receptor antagonist, naloxone, and the selective κ -opioid receptor antagonist, nor-binaltorphimine (nor-BNI) on the inhibitory effect of 100 Hz electroacupuncture in cocaine-induced CPP. The effects on the mRNA expression levels of the κ -opioid receptor in various regions of the brain were also assessed. Finally, the molecular mechanisms underlying the inhibitory

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effects of 100 Hz electroacupuncture on cocaine-induced CPP were investigated.

Materials and methods

Animals. A total of 36 male Sprague-Dawley rats (180–200 g) were provided by the Institute of Zoology, Chinese Academy of Sciences (Beijing, China) and were randomly assigned to 3 groups ($n=12$ per group). They were housed at $22\pm1^\circ\text{C}$ at a humidity of 50% in a 12 h light/dark cycle. They were acclimatized to their experimental surroundings for 5 days. The study was approved by the Ethics Committee of Peking University, Health Science Center (Beijing, China).

Reagents. Cocaine hydrochloride (Qinghai pharmaceutical factory Co., Ltd., Xining, China), nor-binaltorphimine (nor-BNI; Sigma-Aldrich, St. Louis, MO, USA), and the reverse transcription-polymerase chain reaction (RT-PCR) kit (GK8030-20; Sangon Biotech, Co., Ltd., Shanghai, China). The following reagents were used for the reaction: 200 U/ μl M-MLV reverse transcriptase (20 μl), 5X RT reaction buffer (100 μl), 25 U/ μl RNase inhibitor (20 μl), 25 mM dNTP (20 μl), 60 μM oligo(dT)₁₈ (20 μl), 250 μM random primer (20 μl), ddH₂O (RNase and DNase free; 1 ml) (all from Generay Biotech Co., Ltd., Shanghai, China). β -actin primer, κ receptor primer and dynorphin primer were all purchased from Sangon Biotech, Co., Ltd. Cocaine hydrochloride and nor-BNI were prepared with physiological saline.

CPP. The CPP shuttle box (Med Associates, Inc., St. Albans, VT, USA) was made of resin glass, separated into two conditioned training boxes of equal size as follows: Box A and box C (33x22x26 cm), and a middle box, box B (12x22x26 cm), separated by two movable (up and down) partitions. If the partitions were lowered, the animals would be restricted within the box. When the partitions were elevated, the animals are able to run freely in the shuttle box. One of the two conditioned training boxes consisted of a white wall and a squared floor made of stainless steel. The second consisted of a black wall and a striped floor made of stainless steel. The middle box consisted of a gray wall and a gray resin glass floor. A row of infrared light emission (infrared LED monitoring system) and a receiver system were located on the anterior and posterior walls in each box, 1.5 cm above the floor. This system transmitted the locomotor activity of the animals to a computer via a light-electric signal transducer. The crossing frequencies and time spent in each box were recorded and the MED-PC software (Med Associates Inc., St. Albans, VT, USA) was used for the analysis.

Cocaine CPP model. Model preparation occurred in 3 stages, including pretest, training and test, over a period of 10 days. Pretest stage: The natural preference of rats was tested prior to training. Partitions were removed, and the rats were placed in the middle box with the partition in the up position, enabling them to run freely in the shuttle box for 15 min. Their time spent in box A or C was measured. The preference value was calculated by the following formula: $A \text{ or } C / (A + C)$, and rats scoring between 0.4 and 0.6 were included in the proceeding analyses. Training: CPP training commenced 24 h after the

pretest. The rats in the cocaine group were intraperitoneally injected with 5 or 10 mg/kg cocaine or 0.5 ml physiological saline every other day. The rats administered cocaine were placed in the non-preference box (white box), whilst those administered physiological saline were placed in the preference box (black box). The rats in the saline control group were injected with physiological saline daily, and alternatively placed in the non-preference (white) or preference (black) box every other day. The CPP stage occurred daily over eight days, each for 30 min (timing began immediately after the rats were placed in the training box). Test: The CPP test was performed 24 h after the final training. The rats were placed in the middle box and were able to run freely in the shuttle box for 15 min. The time spent in box A or C was recorded. The preference value was calculated according to the aforementioned equation. The CPP model was successfully established if the preference value in the cocaine group was significantly increased compared with that of the control group.

Electroacupuncture. Rats were placed in a specially made plastic cylinder with their tails and hind limbs outside the cylinder. Stainless steel needles were inserted at the bilateral hind limbs at positions identical to the human Zusanli (ST36; Lateral tibial anterior 5 mm;) and Sanyinjiao (SP6; Posterior tibial 1 mm, 3 mm above the medial malleolus), and the other end was connected to an Acupoint Nerve Stimulator (model HANS LH800, Beihang University, Beijing, China). Electroacupuncture was conducted for 30 min at 100 Hz square wave, and a 0.1 msec wave width. Stimulus intensity was increased by 1 mA every 10 min up to 3 mA. A simple restraint group in which rats were placed in the cylinder with no acupuncture was used as a control.

Administration of nor-BNI in the lateral ventricle and nucleus. Rats were anesthetized with 50 mg/kg sodium pentobarbital and then fixed with ear bars onto the stereotaxic apparatus in the abdominal position. In accordance with Paxinos and Watson Stereotaxic Coordinates (lateral ventricle: P:-1.0 mm, L:1.6 mm, H:4.0 mm, nucleus accumbens: P:1.2 mm, L: \pm 0.8 mm, H:5.5 mm, ventral tegmental area: P:-5.2 mm, L: \pm 0.8 mm, H:6.8 mm, amygdala central nuclei: P:-2.3 mm, L: \pm 4.8 mm, H:7.0 mm, basolateral amygdala nuclei: P:-2.3 mm, L: \pm 3.7 mm, H:7.0 mm), the lateral ventricle or nucleus was selected, and a 24GA plastic sleeve was inserted. Administration of nor-BNI was performed with an inner trocar (1 mm longer than the plastic sleeve) through the plastic sleeve.

RT-qPCR. Rats were decapitated and the nucleus accumbens and amygdala were rapidly dissected apart and placed in liquid nitrogen and cryopreserved at -80°C . Total RNA was extracted with TRIzol according to the manufacturer's instructions (Thermo Fisher Scientific, Inc., Waltham, MA, USA). A Heraeus Biofuge Stratos high-speed refrigerated centrifuge at 10,000xg and 4°C (Thermo Fisher Scientific, Inc.) and ultraviolet spectrophotometer (Du 530, Beckman Coulter, Inc., Brea, CA, USA) were used. mRNA was reverse transcribed into cDNA. The following reagents were added to the reaction: RNA template, 1 μl random primer (10 μM), 1 μl oligo(dT)₁₈ (2.4 μM) and ddH₂O up to 13 μl . The reaction was heated at 65°C for 10 mins and quickly placed on ice for 5 mins. Then

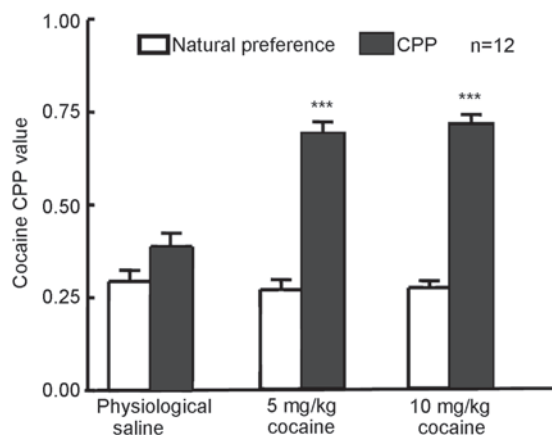


Figure 1. CPP values in rats exposed to different doses of cocaine. Rats were administered 5 or 10 mg/kg cocaine or physiological saline followed by CPP training. n=12 in each group. ***P<0.001, vs. natural preference values. CPP, conditioned place preference.

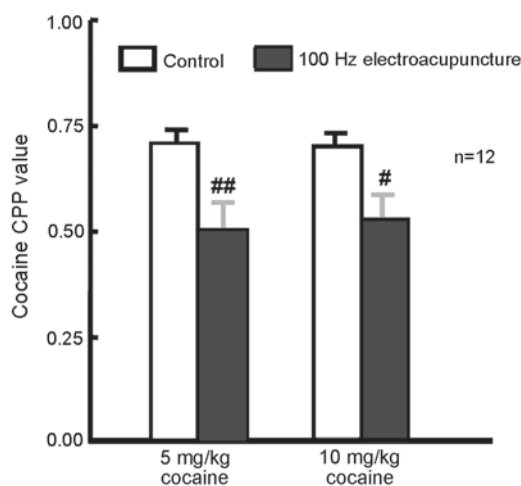


Figure 2. Inhibitory effects of 100 Hz electroacupuncture on cocaine-induced CPP. 100 Hz electroacupuncture was administered 24 h prior to the CPP testing. n=12 in each group. #P<0.05 and ##P<0.01 vs. the control group. CPP, conditioned place preference.

the following components were added: 5 μ l 5X RT reaction buffer, 1 μ l 25 mM dNTP, 1 μ l 25 U/ μ l RNase inhibitor, 1 μ l 200 U/ μ l IM-MLV RTase and ddH₂O up to 25 μ l. The reaction was incubated at 37°C for 1 h and then at 85°C for 5 mins to terminate the reaction. Once the reaction was complete it was stored at -20°C. Next, amplification of the target gene cDNA was performed with the PCR machine (Progene, Techn, NJ, USA), and PCR was performed. In brief, a denaturation step was carried out at 94°C for 3 mins, then the reaction was kept at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min. The cycle was repeated 20 times and the reaction was then kept at 72°C for an extension time of 5 min. The experiment was repeated 3 times. Product analysis was conducted as follows: PCR products (5 μ l) were mixed with X6 gel loading buffer (1 μ l); the samples were loaded into the wells of a 1% agarose gel for electrophoresis using a PowerPAC Basic Power Supply (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 80 V for 60 min. The samples were stained with 0.5 mg/ml ethidium bromide (Beijing Solarbio Technology Co., Ltd., Beijing,

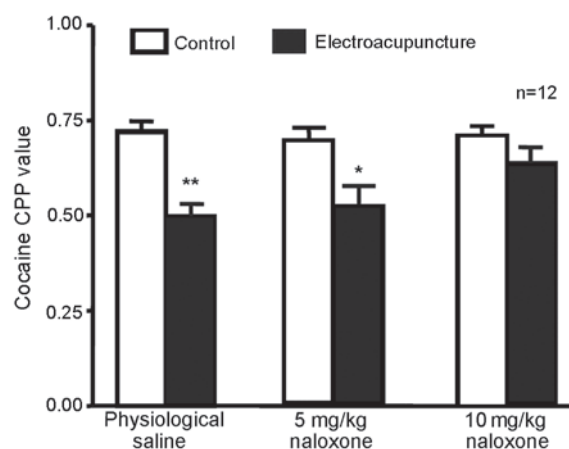


Figure 3. Effect of naloxone on 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. In the naloxone groups, naloxone was injected 24 h after the final conditioning trial. CPP was again tested 24 h after naloxone treatment. In the naloxone + electroacupuncture group, 100 Hz electroacupuncture was delivered 24 h after the final conditioning trial, and naloxone was injected 20 min prior to the electroacupuncture was delivered, while CPP was tested 24 h after the electroacupuncture treatment. n=12 in each group. *P<0.05 and **P<0.01 vs. saline alone. CPP, conditioned place preference.

China) for 15 min, observed using a Gel Doc 2000 imaging system (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), and analyzed semi-quantitatively. Results are expressed as the intensity ratio to GAPDH products.

Statistical analysis. All data are expressed as the mean \pm standard error and were analyzed by one-way analysis of variance followed by the Student-Newman-Keuls test. P<0.05 was considered to indicate a statistically significant difference. Data were analyzed by GraphPad software, version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Cocaine at 5 or 10 mg/kg induces the expression of CPP in rats. The natural preference of rats was measured in each group prior to training and the results revealed no natural preference following cocaine treatment compared with physiological saline treatment (Fig. 1). Rats were administered 5 or 10 mg/kg cocaine or physiological saline followed by CPP training. The CPP value was measured 24 h after the final training (Fig. 1). CPP values in rats that received 5 or 10 mg/kg cocaine were significantly increased (P=0.0008 for 5 mg/kg and P=0.0006 for 10 mg/kg) compared with their respective natural preference values, indicating that CPP was established in the 5 and 10 mg/kg cocaine treatment groups.

Administration of 100 Hz electroacupuncture inhibited 5 and 10 mg/kg cocaine-induced CPP. A total of 48 rats were randomly assigned to 2 groups of CPP training (5 and 10 mg/kg cocaine; n=24 in each group). CPP training was conducted and CPP values were measured 24 h after the final training. Rats (n=12 from each group) received 100 Hz electroacupuncture 24 h prior to CPP measurement. The remaining rats from each group (n=12) served as controls. CPP values were significantly decreased in the 5 mg/kg (P=0.006) and 10 mg/kg (P=0.022)

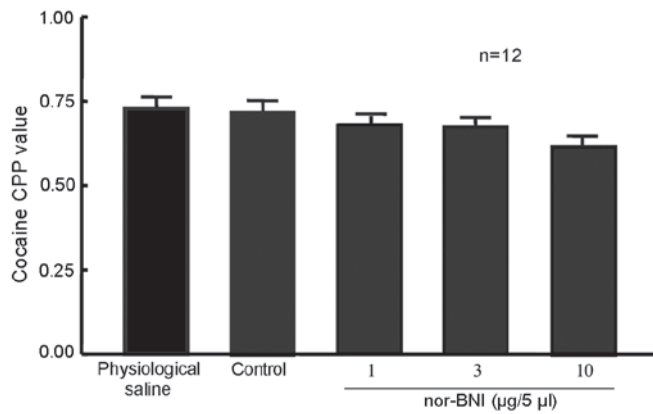


Figure 4. Effect of nor-BNI on cocaine-induced CPP. After receiving 10 mg/kg cocaine CPP training, rats were administered 1, 3 or 10 $\mu\text{g}/5 \mu\text{l}$ nor-BNI or physiological saline via the lateral ventricle. $n=12$ in each group. $P>0.05$, nor-BNI (all concentrations) vs. saline and control. CPP, conditioned place preference. nor-BNI, nor-binaltorphimine.

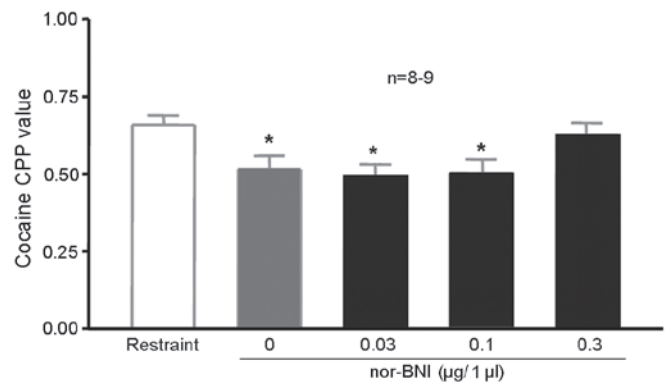


Figure 6. Administration of nor-BNI in the NAc reverses 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. Following 10 mg/kg cocaine CPP training, rats were administered 0.03, 0.1 or 0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI or physiological saline to the bilateral NAc 15 min prior to 100 Hz electroacupuncture. * $P<0.05$ vs. simple restraint group. CPP, conditioned place preference. nor-BNI, nor-binaltorphimine; NAc, nucleus accumbens.

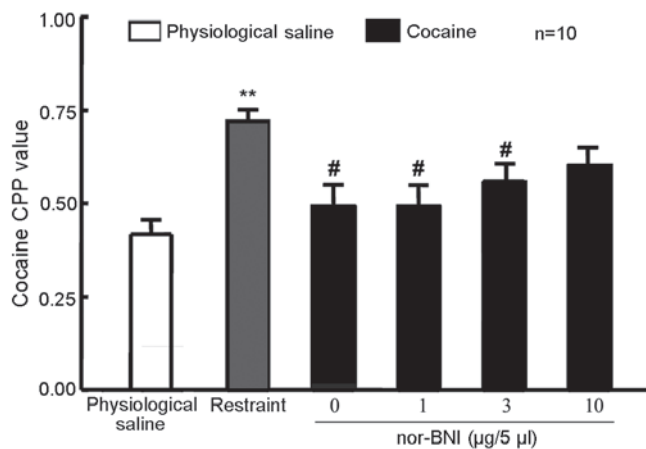


Figure 5. Effect of nor-BNI on 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. Rats received 10 mg/kg cocaine CPP training, then were then given 1, 3 or 10 $\mu\text{g}/5 \mu\text{l}$ nor-BNI or physiological saline via the lateral ventricle. 100 Hz electroacupuncture was conducted 15 min later. $n=10$ in each group. ** $P<0.01$ vs. physiological saline group; # $P<0.05$ vs. simple restraint group. CPP, conditioned place preference nor-BNI, nor-binaltorphimine.

cocaine-treated rats exposed to 100 Hz electroacupuncture compared with the respective controls (Fig. 2). These present results indicate that 100 Hz electroacupuncture inhibits 5 and 10 mg/kg cocaine-induced CPP.

High-dose (10 mg/kg) naloxone blocks 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. A total of 72 rats were randomly divided into 3 groups ($n=24$ in each) receiving 5 mg/kg cocaine and CPP training. The rats were intraperitoneally injected with 5 or 10 mg/kg naloxone or physiological saline. Next, 12 rats in each group underwent 100 Hz single electroacupuncture 20 min after injection of physiological saline or naloxone; CPP values were measured 24 h after the final training. Compared with the physiological saline + 100 Hz electroacupuncture group, no significant difference in CPP value was observed in the 5 mg/kg naloxone + 100 Hz electroacupuncture group (Fig. 3).

This suggests that at this dose, naloxone did not inhibit the 100 Hz electroacupuncture-induced CPP. However, CPP values were significantly higher in the 10 mg/kg naloxone + 100 Hz electroacupuncture group compared with the saline + electroacupuncture group ($P=0.043$), indicating that a higher dose (10 mg/kg) of naloxone was able to reverse the inhibition of 100 Hz electroacupuncture on cocaine-induced CPP.

nor-BNI did not affect 10 mg/kg cocaine-induced CPP. A total of 60 rats were randomly divided into 5 groups ($n=12$ in each group). In one group, rats that were left intact served as the control. Rats in the remaining 4 groups received lateral ventricle cannulation. After 5 days, the rats received 10 mg/kg cocaine and CPP training, which occurred concurrent to that of the unoperated group. Rats were then given 1, 3 or 10 $\mu\text{g}/5 \mu\text{l}$ nor-BNI or physiological saline via the lateral ventricle. Control rats were not administered any treatment 24 h prior to the CPP measurement. No significant difference in the CPP value was detected in rats exposed to the three doses of nor-BNI compared with physiological saline and control groups (Fig. 4), suggesting that the selected dose of nor-BNI in the present study produced no effect on 10 mg/kg cocaine-induced CPP.

Injection of nor-BNI in the lateral ventricle affects the inhibition of 100 Hz electroacupuncture on cocaine CPP. A total of 60 rats were randomly divided into 6 groups ($n=10$ in each group). Two groups were unoperated, one without cocaine CPP (saline-only), the other fixed in the stereotaxic apparatus with 10 mg/kg cocaine CPP training (simple restraint group), without electroacupuncture stimulation. The rats in the remaining four groups received lateral ventricle cannulation. After 5 days, the rats received 10 mg/kg cocaine CPP training alongside the second group. The rats in the 4 groups were given 1, 3 or 10 $\mu\text{g}/5 \mu\text{l}$ nor-BNI or physiological saline via the lateral ventricle. CPP values were significantly higher in the simple restraint group compared with the physiological saline training group, indicating the induction of cocaine CPP ($P=0.043$; Fig. 5). CPP values were significantly lower in the 0 $\mu\text{g}/5 \mu\text{l}$ nor-BNI group (with 100 Hz electroacupuncture)

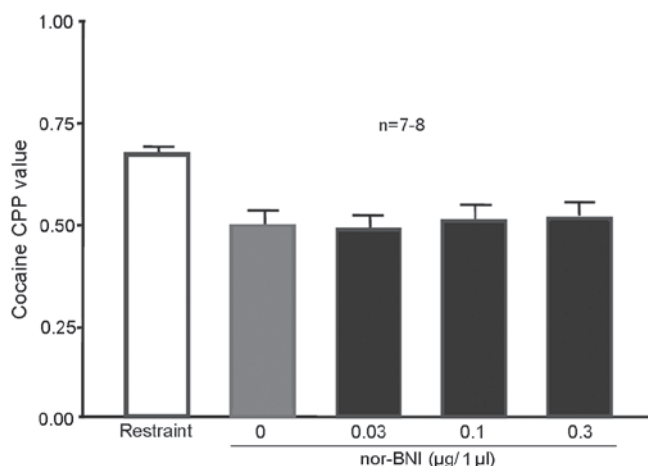


Figure 7. Effect of nor-BNI administration to the ventral tegmental area on 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. Following the administration of 10 mg/kg cocaine CPP training, rats were administered 0.03, 0.1 or 0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI or physiological saline in the ventral tegmental area for 15 min prior to 100 Hz electroacupuncture. $P<0.05$ vs. simple restraint group. CPP, conditioned place preference; nor-BNI, nor-binaltorphimine.

compared with those in the simple restraint group ($P=0.003$; Fig. 5), indicating that 100 Hz electroacupuncture was able to suppress cocaine-induced CPP, as indicated by the preceding experiments. The CPP values for rats treated with 10 $\mu\text{g}/5 \mu\text{l}$ nor-BNI did not significantly differ compared with the simple restraint group (Fig. 5), suggesting that 10 mg/kg reversed the inhibitory effects of 100 Hz electroacupuncture on cocaine-induced CPP.

Injection of 0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI in the nucleus accumbens blocked 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. A total of 50 rats were randomly assigned to 5 groups ($n=10$ in each group). One of the groups served as the simple restraint group, and remained intact. The nucleus accumbens of rats in the remaining 4 groups received cannulation prior to training. After 5 days, all rats received 10 mg/kg cocaine CPP training. The restraint group was subjected to simple restraint 24 h prior to CPP measurement. Rats in the remaining 4 groups were given 0.03, 0.1 or 0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI or physiological saline to the bilateral nucleus accumbens 15 min prior to the administration of 100 Hz electroacupuncture. Rats were decapitated following CPP value measurement. The entire brain was then obtained and sliced into sections for observation. Rats with their cannula at the designated place ($P:1.2 \text{ mm}$, $L:\pm 0.8 \text{ mm}$, $H:5.5 \text{ mm}$) ($n=9$ for the 0 and 0.1 $\mu\text{g}/1 \mu\text{l}$ nor-BNI group; $n=8$ for the 0.03 and the 0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI group) were selected for statistical analysis. CPP values were significantly lower in rats injected with physiological saline (0 $\mu\text{g}/1 \mu\text{l}$ nor-BNI group) in the nucleus accumbens compared with the simple restraint group [$P=0.045$ (NS), 0.037 (0.03 $\mu\text{g}/1 \mu\text{l}$ nor-BNI) and 0.043 (0.1 $\mu\text{g}/1 \mu\text{l}$ nor-BNI); Fig. 6], indicating that 100 Hz electroacupuncture inhibited cocaine-induced CPP. CPP values of rats injected with 0.3 $\mu\text{g}/1 \mu\text{l}$ in the nucleus accumbens did not significantly differ compared with the simple restraint group, suggesting that the addition of nor-BNI at this dose in the

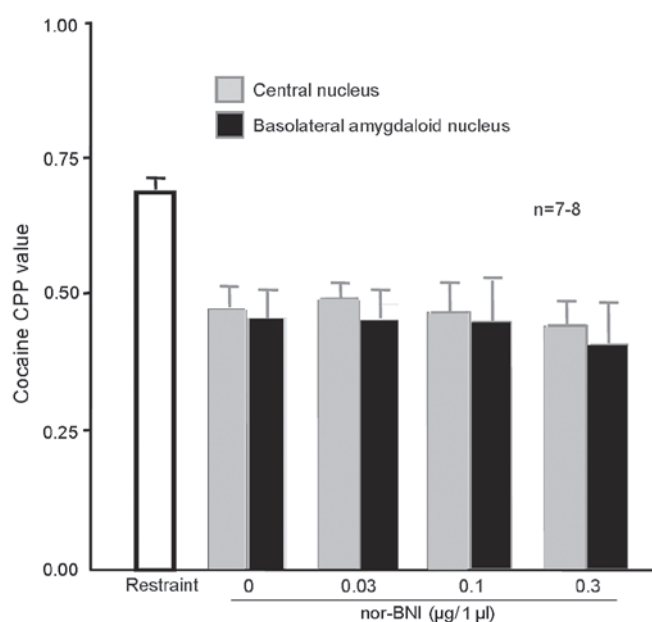


Figure 8. Effect of nor-binaltorphimine administration in the amygdala on 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. Received 10 mg/kg cocaine CPP training, rats were given 0.1, 0.3, or 1 $\mu\text{g}/1 \mu\text{l}$ nor-binaltorphimine or physiological saline in the bilateral amygdala central nucleus or bilateral basolateral amygdaloid nucleus 15 min prior to 100 Hz electroacupuncture. $P<0.05$ vs. simple restraint group. CPP, conditioned place preference; nor-BNI, nor-binaltorphimine.

nucleus accumbens reversed the inhibitory effects of 100 Hz electroacupuncture on cocaine-induced CPP.

Injection of nor-BNI in the ventral tegmental area of the midbrain did not affect 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. A total of 50 rats were randomly assigned to 5 groups ($n=10$ in each group). The first group was the simple restraint group and was used as a control. The ventral tegmental area of rats in the remaining 4 groups received cannulation prior to training. After 5 days, all rats received 10 mg/kg cocaine CPP training. Rats in the restraint group were subjected to simple restraint 24 h prior to CPP measurement, whilst rats in the remaining four groups were administered 0.03, 0.1 or 0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI or physiological saline in the ventral tegmental area 15 min prior to 100 Hz electroacupuncture. Rats were decapitated after measurement, and the entire brain was dissected and sliced into sections for observation. Rats with their cannula at the designated place ($n=7$ for the 0 and 0.03 $\mu\text{g}/1 \mu\text{l}$ nor-BNI group; $n=8$ for the 0.1 and 0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI group) were selected for statistical analysis. CPP values were significantly lower in rats administered physiological saline (0 $\mu\text{g}/1 \mu\text{l}$ group) in the ventral tegmental area compared with the simple restraint group ($P=0.024$ (NS), 0.023 (0.03 $\mu\text{g}/1 \mu\text{l}$ nor-BNI), 0.034 (0.1 $\mu\text{g}/1 \mu\text{l}$ nor-BNI) and 0.039 (0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI); Fig. 7), indicating that 100 Hz electroacupuncture inhibited cocaine-induced CPP. CPP values were significantly lower in rats injected with different doses of nor-BNI in the ventral tegmental area than those in the simple restraint group ($P<0.05$), indicating that the doses administered did not affect the inhibitory effects of 100 Hz electroacupuncture on cocaine CPP expression.

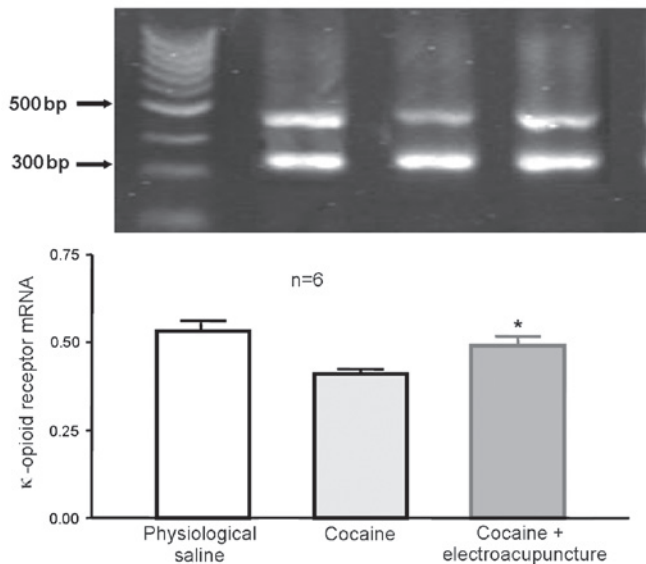


Figure 9. Electroacupuncture (100 Hz) significantly increases the mRNA expression levels of κ -opioid receptor in the nucleus accumbens of rats with cocaine-induced CPP. After receiving 10 mg/kg cocaine CPP training, rats were administered 0.1, 0.3 or 1 $\mu\text{g}/1 \mu\text{l}$ nor-BNI or physiological saline in the bilateral amygdala central nucleus or bilateral basolateral amygdaloid nucleus 15 min prior to 100 Hz electroacupuncture. * $P < 0.05$ vs. cocaine group. CPP, conditioned place preference; nor-BNI, nor-binaltorphimine.

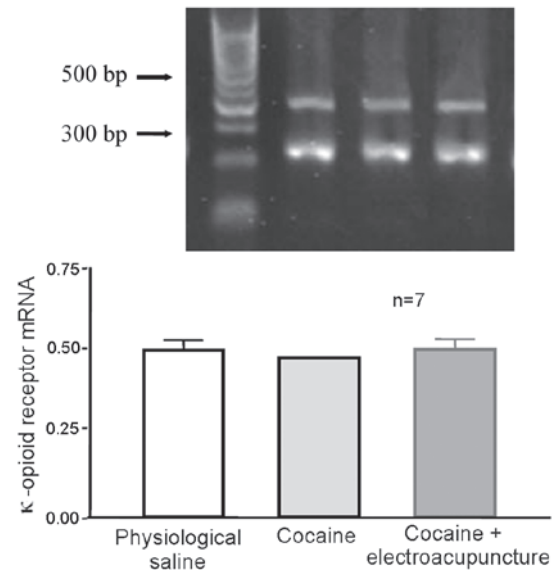


Figure 10. Effect of 100 Hz electroacupuncture on mRNA expression of κ -opioid receptor in different regions of the amygdala in rats with cocaine-induced CPP. Rats received 10 mg/kg cocaine conditioned place preference training, then the electroacupuncture treatment group rats received 100 Hz electroacupuncture stimulation. Rats from each group were decapitated 2 days later and the amygdala was collected for reverse transcription-polymerase chain reaction to measure the mRNA expression of κ -opioid receptor.

Injection of nor-BNI in the amygdala did not affect 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. A total of 108 rats were randomly assigned into 9 groups ($n=12$ in each group). The first group was the simple restraint group. In the other groups, the amygdala central nuclei received cannulation prior to training. The basolateral amygdaloid nuclei in the remaining 4 groups received cannulation prior to training. After 5 days, the 8 groups received 10 mg/kg cocaine CPP training, which was administered simultaneous to that of rats in the first group. The first group was subjected to simple restraint 24 h prior to CPP measurement. The remaining rats were administered 0.1, 0.3 or 1 $\mu\text{g}/1 \mu\text{l}$ nor-BNI or physiological saline in the bilateral amygdala central nuclei or bilateral basolateral amygdaloid nuclei 15 min prior to the administration of 100 Hz electroacupuncture. After measurement, the rats were decapitated and their brains were dissected and sliced into sections for observation. Rats with their cannula at the designated place ($n=8$ for the 0, 0.1 and 0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI and $n=7$ for the 0.03 $\mu\text{g}/1 \mu\text{l}$ nor-BNI group) were selected for statistical analysis. CPP values were significantly lower in the two regions of the brains of the rats injected with physiological saline in the amygdala, compared with the simple restraint group [$P=0.037$ (NS in amygdala central nuclei); Fig. 8], indicating that 100 Hz electroacupuncture inhibited cocaine-induced CPP. CPP values were significantly lower in the brains of the rats injected with different doses of nor-BNI in the two regions of the amygdala, compared with the simple restraint group [0.043 (0.03 $\mu\text{g}/1 \mu\text{l}$ nor-BNI in amygdala central nuclei), 0.032 (0.1 $\mu\text{g}/1 \mu\text{l}$ nor-BNI in amygdala central nuclei) and 0.027 (0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI in amygdala central nuclei); $P=0.031$ (NS in basolateral amygdala nuclei), 0.029 (0.03 $\mu\text{g}/1 \mu\text{l}$ nor-BNI in basolateral amygdala nuclei),

0.024 (0.1 $\mu\text{g}/1 \mu\text{l}$ nor-BNI in basolateral amygdala nuclei) and 0.018 (0.3 $\mu\text{g}/1 \mu\text{l}$ nor-BNI in basolateral amygdala nuclei), indicating that the doses did not alter the inhibitory effects of 100 Hz electroacupuncture on cocaine-induced CPP.

Administration of 100 Hz electroacupuncture in cocaine-induced CPP rats led to increased mRNA expression levels of κ -opioid receptor in the nucleus accumbens. A total of 24 rats were randomly assigned to 3 groups ($n=8$ in each group). Some mice died and were excluded from the study. The control group was administered physiological saline during CPP training, whilst the remaining 2 groups (cocaine-induced CPP and 100 Hz electroacupuncture + cocaine-induced CPP) received 10 mg/kg cocaine during CPP training. Subsequent to final training, the electroacupuncture treatment group received 100 Hz electroacupuncture stimulation. After 2 days, rats from each group were then decapitated and the nucleus accumbens was collected for RT-qPCR in order to measure the expression of κ -opioid receptor mRNA expression levels. mRNA expression levels of the κ -opioid receptor were significantly increased in the nucleus accumbens of the cocaine-induced CPP rats previously exposed to 100 Hz electroacupuncture compared with cocaine-induced CPP rats ($P=0.032$; Fig. 9).

Effect of 100 Hz electroacupuncture in cocaine-induced CPP rats did not affect mRNA expression levels of κ -opioid receptor in the amygdala. A total of 24 rats were randomly assigned to 3 groups ($n=8$ per group). Some mice died and were excluded from the study. The control group was administered physiological saline, whilst the remaining 2 groups (cocaine-induced CPP and 100 Hz electroacupuncture on

cocaine-induced CPP) received 10 mg/kg cocaine CPP training. After final training, the electroacupuncture treatment group received 100 Hz electroacupuncture stimulation whilst the remaining rats were left intact. Two days later, rats from each group were decapitated and the amygdala was collected for RT-qPCR to measure the mRNA expression levels of κ -opioid receptor. mRNA expression levels of the κ -opioid receptor were not significantly different in the amygdala of cocaine-induced CPP rats exposed to 100 Hz electroacupuncture and cocaine-induced CPP rats ($P>0.05$; Fig. 10).

Discussion

The results of the present study indicated that 100 Hz single electroacupuncture inhibited 5 and 10 mg/kg cocaine-induced CPP. The present study also revealed that the high-dose (10 mg/kg) naloxone significantly blocked 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. The several types of opioid receptor each display different affinities for naloxone. A low dose (1-5 mg/kg) predominately antagonizes μ and δ receptors, whilst a high dose (10-20 mg/kg) blocks the κ -opioid receptor (14). The present study hypothesized that 100 Hz electroacupuncture exerted its inhibitory effects through the κ -opioid receptor. The present study revealed that 10 μ g/5 μ l nor-BNI blocked 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP, thus confirming the hypothesis.

The κ -opioid receptor is extensively distributed throughout the brain, including the nucleus accumbens, ventral tegmental area and amygdala, which are brain regions strongly associated with reward. Previous studies in numerous animal models have confirmed that the activation of the κ -opioid receptor antagonizes the drug-seeking behavior mediated by cocaine (15-16). κ -Opioid receptor activation in the nucleus accumbens suppresses dopamine release by presynaptic inhibition, thus reducing the dopamine concentration in the synaptic cleft of the nucleus accumbens and resulting in aversion to cocaine (10,17). Results from the present study demonstrated that the injection of 0.3 μ g/1 μ l nor-BNI in the nucleus accumbens blocked 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP. However, the injection of nor-BNI in the ventral tegmental area and amygdala was not observed to produce a significant effect. The results indicate that the κ -opioid receptor in the nucleus accumbens serves an important role in 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP.

Previous studies have demonstrated that 100 Hz electroacupuncture-mediated inhibition of cocaine-induced CPP lasts for a duration of 24 h, and have postulated that electroacupuncture may regulate κ -opioid receptor synthesis at the gene level (9,18). The results of the present study revealed that 100 Hz electroacupuncture significantly increased mRNA expression levels of the κ -opioid receptor in the nucleus accumbens but not in the amygdala of rats with cocaine-induced CPP. The present results demonstrated that 100 Hz electroacupuncture was able to restore the function of the κ -opioid receptor and inhibit cocaine-induced CPP, possibly by regulating the mRNA expression levels of the κ -opioid receptor in the nucleus accumbens.

In conclusion, the results of the present study demonstrated that 100 Hz electroacupuncture inhibits cocaine-induced CPP via the κ -opioid receptor in the nucleus accumbens of rats.

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