

Original Article

Suppression of Cocaine Intake by Acupuncture at HT7

Bong-Hyo Lee¹, Sung-Hwan Kim¹, Sung-Chul Lim¹, Jae-Su Kim¹, Yun-Kyu Lee¹,
Ji-Hye Lee¹, Tae-Young Jung², Chae-Ha Yang³, Seong-Shoon Yoon³,
Chang-Hyun Han⁴, Kyung-Min Lee¹

¹Dept. of Acupuncture, Moxibustion, & Acupoint,

²Diagnostics,

³Physiology, College of Oriental Medicine, Daegu Haany University,

⁴TKM Information Research Division, Korea Institute of Oriental Medicine

Objectives: Cocaine has been well known as a representative drug of abuse for its strong reinforcing effect linked to the mesolimbic reward system including dopamine, and such reinforcement leads to the addiction. Acupuncture has been used widely in eastern Asia for the treatment of various diseases including mental disorders and psychiatric problems, and is gathering more interest as one of the complementary and alternative medicines in western countries. In a previous study, we demonstrated that acupuncture at HT7 could affect dopamine release in the mesolimbic system of rats sensitized to alcohol and morphine. This study was designed to investigate whether acupuncture at HT7 could attenuate cocaine intake or not.

Material & Methods: Male Sprague-Dawley rats weighing 270-300 g at the start of experiment were trained to self-administer food pellets under a fixed ratio 1 schedule. After the success of acquisition of 100 pellets within 3 h for 3 consecutive days, animals were subjected to surgery whereby Silastic tubing was implanted into right jugular vein and secured with mesh under the anesthetization using pentobarbital injection (50 mg/kg, i.p.). Following recovery, rats were trained to self-administer cocaine (0.25 mg/kg) in daily 2 h sessions under fixed ratio 1 schedule over 10 days. Each treatment was performed on the next day of each establishment of baseline.

Results: Results show that acupuncture at HT7, but not at control points, reduced cocaine intake significantly. Acupuncture at HT7 decreased selectively active lever response from 63.15 ± 3.35 to 51.46 ± 3.99 corresponding $82.12 \pm 5.31\%$ compared to basal level. Also, it was demonstrated that the effect of acupuncture was mainly occurred at the half period. Nevertheless, acupuncture at HT7 did not influence the food taking behavior.

Conclusions: From the results of this study, it may be suggested that acupuncture at HT7, at least in part, could contribute to the treatment of cocaine abuse.

Key Words : Acupuncture, HT7; Cocaine; Self-administration; Drug abuse; Rat.

Introduction

Cocaine has been well known as a representative drug of abuse. Frequent exposure to cocaine drives people to dependence through tolerance¹⁾ and eventually leads to addiction. According to many studies, such

a severe problem like abuse is related with the reinforcing effect of cocaine, and this reinforcing effect occurs by dopamine (DA) playing key role in the mesolimbic reward system including ventral tegmental area (VTA) and nucleus accumbens (NAc)²⁻⁶⁾. As well, some studies have shown that cocaine

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• Correspondence to : Kyung-Min Lee

Dept. of Acupuncture & Moxibustion, Daegu Oriental Hospital of Daegu Haany University, 165 Sang-dong, Suseong-gu, Daegu, Korea.

Tel : +82-53-770-2112, Fax : +82-53-768-6340, E-mail : leehanamp@hanmail.net

exhibits its reinforcement maintaining self-administration through the inhibition of DA reuptake in synapse^{7,8)}. Thus, it is believed that DA plays very important role in cocaine abuse.

Acupuncture has been widely used in eastern Asia for a variety of diseases, including mental disorder and substance abuse, and is gathering more interest as a complementary and alternative medicine in western countries^{9,10)}. What is more, NIH has accepted that acupuncture might be helpful in the treatment of pain, including drug addiction¹¹⁾. Supporting this trend, many studies have revealed that acupuncture is useful for the treatment of drug abuse. Ren *et al.* revealed that acupuncture attenuated the expression of conditioned place preference induced by cocaine in rats¹²⁾, and Shi *et al.* demonstrated that high frequency electroacupuncture inhibited the conditioned place preference caused by morphine¹³⁾. In addition, acupuncture suppressed c-Fos expression in the NAC and striatum as well as behavioural sensitization induced by repeated nicotine exposure in rats¹⁴⁾.

Although more research is needed to confirm the function and define the exact mechanism, acupuncture has become a useful method in the treatment of drug abuse.

Among the meridians, the heart channel is related with heart disease, circulatory disease, mental disorder, and psychiatric problems, and HT7 on the heart channel has been especially used for the mental and psychiatric disorders¹⁵⁻¹⁷⁾.

In previous study, it was demonstrated that acupuncture at HT7 modulated DA level in the NAC in alcohol sensitization and withdrawal through gamma aminobutyric acid (GABA) receptor system^{18,19)}. In addition, acupuncture at HT7 ameliorated the locomotor activity and c-Fos gene expression in morphine sensitized rats²⁰⁾. Furthermore, acupuncture at HT7 has shown that locomotor activity and tyrosine hydroxylase expression can be modulated by acupuncture in cocaine sensitized rats²¹⁾. Thus, acupuncture at HT7 has been shown to be able to

take part in the normalization of the neurochemical changes and behavioral disorders resulted from drug abuse.

Based on these results, the question of whether acupuncture at HT7 could influence cocaine intake or not was investigated using self-administration fixed ratio (FR) 1 schedule.

Materials and Methods

1. Animals

Male Sprague-Dawley rats (Daehan Animal, Seoul, Korea), weighing 270-300 g at the start of the experiment, were used. Rats were housed on *ad libitum* food and water and maintained on a 12 h light-dark cycle except the 1st day of the food training, on which animals were kept under overnight schedule. Measures were performed to minimize stress following NIH Guidelines for the Care and Use of Laboratory Animals (NIH publication no. 80-23).

2. Apparatus

Self-administration of cocaine and food was carried out in the same operant chambers housed in sound-attenuated wood cubicles (Med Associates, St. Albans, VT). Chambers have two kinds of lights: the house lights on a wall, and cue lights above the active lever on the opposite wall. The signal for active lever-press was delivered to a computer installed with a schedule manager program and the motor equipped with the syringe of cocaine solution was operated according to experiment design.

3. Food training

Animals that had been adapted to the home cage and experimental environment for 3 days were subjected to the experiments. Animals were trained to press the active lever for 45 mg sucrose pellets (Bio-serv, Frenchtown, NJ, USA) under a daily FR 1 schedule. Rats were required to achieve 100 sucrose

pellets within 3 h except the first day, on which animals tried overnight without limitation of time. The house light was extinguished and cue light was illuminated across the food training. Food training was performed once a day under food restriction to promote the acquisition of active lever-press. When the animal had succeeded to acquire 100 sucrose pellets for 3 consecutive days, the next phase of surgery was expanded.

4. Surgery

Animals that had passed the food training (100 sucrose pellets within 3 h for three consecutive tests) were allowed free access to food and water for at least 1 day. Then, sodium pentobarbital (50 mg/kg, i.p.) was injected for anesthetization of the rat. Chronic Silastic catheters for jugular vein (Dow Corning, Midland, MI, USA; 0.02 "ID \times 0.037" OD) treated with tridodecylmethyl ammonium chloride (TDMAC) heparin (Polysciences Inc., Warrington, PA), were surgically implanted and fixed with Mersilene surgical mesh (Ethicon Inc.). The catheters were exteriorized in the back of animals through 22 gauge guide cannulae (Plastics One, Roanoke, VA, USA) after skin incision. Silastic tubing and guide cannulae were embedded in dental cement and secured with Prolene surgical mesh. Daily infusion of 0.2 mL of saline containing heparin maintained the patency of the catheters during the recovery period.

5. Cocaine training

After a recovery period of at least 10 days, animals were trained to self-administer cocaine hydrochloride dissolved in saline, and 0.2 mL of heparin was infused into the guide cannula of the jugular vein immediately before and after the session to maintain the patency of the catheters. When animals pressed the active lever, the house light was extinguished for 5 sec and the cue light was illuminated simultaneously, as well 0.1 mL of cocaine solution was delivered

through intravenous catheters. Five sec of cocaine injection was followed by 10 sec of "time-out" (TO) period in which both the house light and cue light were extinguished and no result was produced. After TO period, the house light was illuminated again. The responses of inactive lever were recorded but produced no result. Initially, animals were trained to self-administer 1.0 mg/kg of cocaine to facilitate the cocaine intake using 4 h session FR 1 schedule for 3 days. On the 4th day, cocaine dose was decreased to 0.25 mg/kg. After 10 days of training, the active lever responses were checked, and animals who had established a stable baseline (variation of active lever responses for 3 consecutive sessions less than 10% of the mean) proceeded to the next phase of the test.

6. Test

After the establishment of the baseline at the end of the cocaine training period, the daily session time was changed to 2 h, and animals that had reestablished a stable baseline were subjected to acupuncture treatment on the next day of baseline. Each treatment was performed randomly without fixed order after each establishment of baseline, and each animal received all treatments. Animals of the HT7 group (n = 13) were given acupuncture at bilateral HT7 points of the heart channel, and the PC6 group (n = 12) at PC6 points of pericardium channel. Rats of the tail group (n = 13) received acupuncture at the tail to determine whether the mechanical stimulation at non-acupoints had influence or not. Rats of the control group (n = 13) received the same treatment without acupuncture stimulation.

7. Acupuncture treatment

The anatomical location of HT7 of the heart meridian is on the transverse crease of the wrist of the forepaw, radial to the tendon of the flexor carpi ulnaris muscle. That of PC6 of the pericardium meridian is between the tendons of the palmaris longus

muscle and flexor carpi radialis muscle, 4 mm proximal to the transverse crease of the wrist of the forepaw. For the tail points, needles were inserted apart into nonacupoints one fifth of tail length from the proximal region of the tail to avoid the two tail acupoints (proximal tail and tip of the tail). These nonacupoints are distal to the proximal tail acupoints. The anatomical locations of stimulated acupoints in rats were determined according to the acupoints in the animal acupuncture atlas²².

For the acupuncture treatment, stainless-steel needles (Dongbang Acupuncture INC, Chingdao, China) with a diameter of 0.18 mm and a length of 8 mm were inserted vertically into a depth of 2-3 mm on each point for 1 min by the same oriental medical doctor whose major is meridians and acupoints. The manual treatment of acupuncture stimulation was delivered by twisting needles bidirectionally at a frequency of twice per sec for a total of 2 sec of stimulation while needles were inserted and withdrawn from acupoints. Animals were given a slight movement restriction for the acupuncture treatment by the other researcher and were given daily handling for 2 min to minimize the stress from restriction.

8. Food latency

Another food experiment was performed to examine whether acupuncture influenced food-taking or not. Rats were trained to press the active lever for sucrose

pellets under daily FR 1 schedule receiving food restriction in similarity with food training and required to achieve 100 pellets within 5 min. When animals succeeded for 3 consecutive days, the time (sec) spent for 100 pellets was checked. Rats of the acupuncture group were given acupuncture stimulation on HT7 while rats of the control group were given the same treatment as the acupuncture group except for needle stimulation. Inactive lever responses were recorded, producing no result.

9. Statistical analysis

Data of lever responses were collected by schedule manager program (Med Associates, St. Albans, VT) and then analyzed with one-way analysis of variance (ANOVA) and post hoc least significant difference (LSD) test using SPSS statistics program. The statistical significance was regarded with the P value less than 0.05.

Results

1. Basal level of active lever responses in cocaine intake

Animals received randomized treatments and the results of the present study show that the basal level of active lever response was 63.02 ± 4.95 for the control group, 63.15 ± 3.35 for the HT7 group, 57.71 ± 4.33 for the PC6 group, and 59.25 ± 4.89



Picture 1. Apparatus (Operant Chamber)

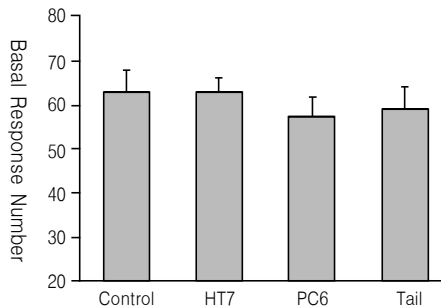


Fig. 1. The basal level of active lever responses in cocaine intake. Results are mean ± S.E.M. of responses. One-way ANOVA and post hoc LSD test. Control group n = 13, HT7 group n = 13, PC6 group n = 12, tail group n = 13.

for the tail group, so the basal levels of active lever-presses for 3 days before treatments were very similar among the groups. Although the basal level of the PC6 group was a little less than that of the control and HT7 groups, there was no significant difference between the groups (Fig. 1).

2. Effect of acupuncture on the active lever response

The results of the present study show that the active lever response was 60.54 ± 4.30 for the control group, 51.46 ± 3.99 for the HT7 group, 56.00 ± 4.62 for the PC6 group, and 60.15 ± 5.06 for the tail group. Thus, acupuncture at HT7 reduced cocaine

intake to 85.00% of control, and the representative pattern of cocaine taking behavior in each group was exhibited using a hatch mark (Fig. 2).

3. Effect of acupuncture on the inactive lever response

The results of the present study show that the inactive lever response was 0.23 ± 0.23 for the control group, 0.38 ± 0.21 for the HT7 group, 0.09 ± 0.09 for the PC6 group, and 0.31 ± 0.24 for the tail group. There was no significant difference between the four groups, demonstrating that the treatments had little effect on inactive lever response (Fig. 3).

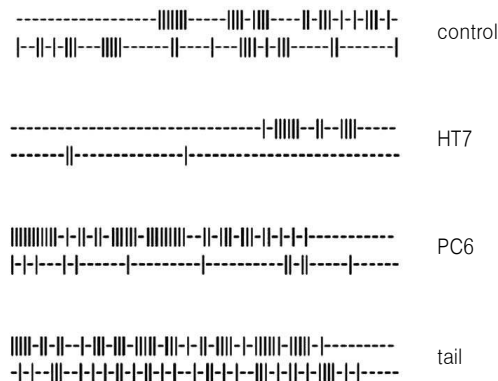


Fig. 2. The representative hatch marks of active lever presses during 2 h session time in each group. A hatch means the moment at which animal acquired cocaine by pressing active lever.

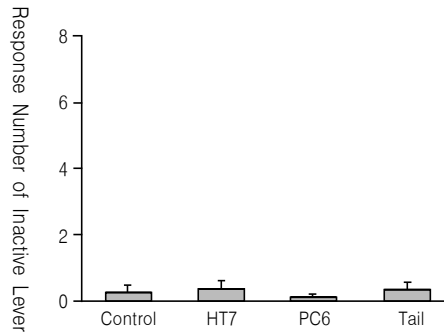


Fig. 3. The effect of acupuncture on the inactive lever response. Results are mean \pm S.E.M. of responses. One-way ANOVA and post hoc LSD test. Control group n = 13, HT7 group n = 13, PC6 group n = 11, tail group n = 13.

4. Effect of acupuncture on cocaine intake

The results of the present study show that the percent of active lever compared to basal level was $97.17 \pm 2.61\%$ for the control group, $82.12 \pm 5.31\%$ for the HT7 group, $96.82 \pm 4.11\%$ for the PC6 group, and $101.49 \pm 2.24\%$ for the tail group. There were significant differences between the HT7 group and each of the control, PC6, and tail groups ($P < 0.01$, Fig. 4).

5. Analysis of the effect of acupuncture on cocaine intake using the time course

According to the analysis of active lever responses using time course of 5 min interval, some significant differences between each group were shown. There were significant differences between the HT7 and control groups with 1.62 ± 0.24 vs. 2.67 ± 0.26 at 65 min, 1.85 ± 0.32 vs. 2.75 ± 0.22 at 95min, 1.77 ± 0.30 vs. 2.58 ± 0.36 at 100 min, and 1.85 ± 0.27 vs. 2.58 ± 0.23 at 105 min ($P < 0.05$). There were also significant differences between the HT7 and PC6 groups with 2.00 ± 0.38 vs. 3.40 ± 0.31 at 20 min, 1.62 ± 0.24 vs. 2.80 ± 0.66 at 65 min ($P < 0.05$), and there was significant difference between

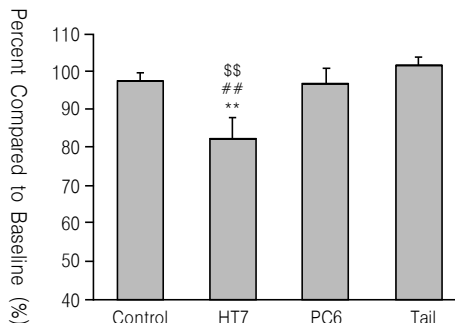


Fig. 4. The effect of acupuncture on cocaine intake. Results are mean \pm S.E.M. of percents compared to basal level. One-way ANOVA and post hoc LSD test.

** $P < 0.01$, HT7 group vs. control group.

$P < 0.01$, HT7 group vs. PC6 group.

\$\$ $P < 0.01$, HT7 group vs. tail group.

Control group n = 13, HT7 group n = 13, PC6 group n = 12, tail group n = 13.

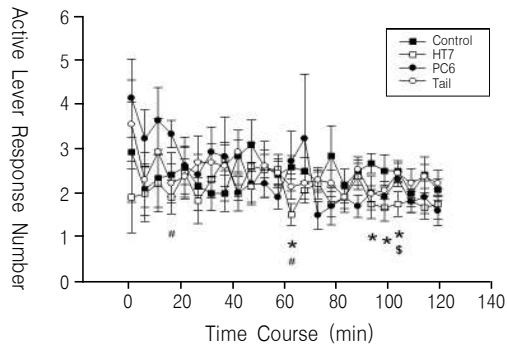


Fig. 5. The analysis of the effect of acupuncture on cocaine intake using the time course. Results are mean \pm S.E.M. of responses. One-way ANOVA and post hoc LSD test.

* $P < 0.05$, HT7 group vs. control group.

$P < 0.05$, HT7 group vs. PC6 group.

\$ $P < 0.05$, HT7 group vs. tail group.

Control group $n = 12$, HT7 group $n = 13$, PC6 group $n = 10$, tail group $n = 13$.

the HT7 and tail groups with 1.85 ± 0.27 vs. 2.54 ± 0.22 at 105 min ($P < 0.05$). From these results, it might be suggested that the effect of acupuncture to reduce cocaine intake mainly occurred in the latter half period (Fig. 5).

6. Effect of order of treatment on cocaine intake

In this study, each treatment was conducted randomly, and the results show that the mean of the

percent compared to the basal level was $95.88 \pm 2.63\%$ for the 1st treatment, $98.63 \pm 3.72\%$ for the 2nd treatment, $88.13 \pm 5.83\%$ for the 3rd treatment, and $94.81 \pm 3.83\%$ for the 4th treatment. So, it seems that there is no relation between the order of treatment and the effect of treatment (Fig. 6).

7. The effect of acupuncture on food intake

These results show that the time spent for 100 sucrose pellets under FR 1 schedule was $145.17 \pm$

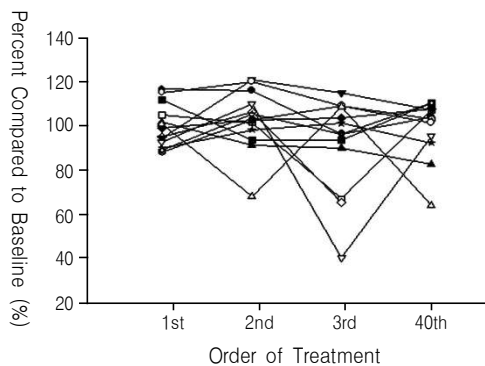


Fig. 6. The effect of order of treatment on cocaine intake. Results are mean \pm S.E.M. of percents compared to basal level. One-way ANOVA and post hoc LSD test. Control group $n = 13$, HT7 group $n = 13$, PC6 group $n = 13$, tail group $n = 12$.

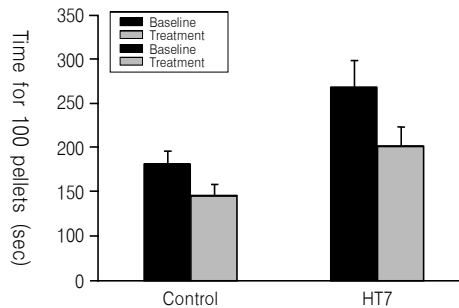


Fig. 7. The effect of acupuncture on food intake (time). Results are mean \pm S.E.M. of time (sec) for 100 pellets. One-way ANOVA and post hoc LSD test. Control group n = 7, HT7 group n = 9.

12.51 min for the control group and 199.75 \pm 22.70 min for the HT7 group. The basal level was 179.45 \pm 15.95 min for the control group and 266.59 \pm 30.30 min for the HT7 group. In both control and HT7 groups, treatments shortened rather than lengthened the time spent for food taking, and there was no significant difference (Fig. 7).

Conversion of time spent for 100 pellets into percent compared to basal level showed that the percent was 82.72 \pm 8.03% for the control group and 77.85 \pm 7.27% for the HT7 group, demonstrating that there was no significant difference between the effects of the two treatments on food-taking (Fig. 8).

Discussion

Previous studies have demonstrated that the extracellular DA level was normalized and behavioral withdrawal signs were ameliorated by acupuncture at HT7 in ethanol sensitized and withdrawn rats^{18,19,24}. In addition, Lee *et al.* have suggested that acupuncture may be effective in the inhibition of behavior induced by cocaine sensitization through results showing a large increase of locomotor activity and tyrosine hydroxylase expression in the ventral tegmental area of the mesolimbic DA system was suppressed by HT7²¹. Based on these results, the probability of acupuncture at HT7 to influence cocaine intake was examined by self-administration.

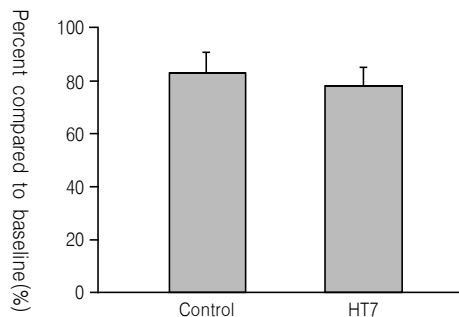


Fig. 8. The effect of acupuncture on food intake (percent). Results are mean \pm S.E.M. of percents compared to basal level. One-way ANOVA and post hoc LSD test. Control group n = 6, HT7 group n = 8.

Animals were sacrificed after intravenous catheter implantation surgery and trained to self-administer cocaine solution, and the basal levels of active lever-presses collected from the data for 3 days before treatments shown in Fig. 1 indicate that the cocaine was taken stably by addicted rats. Even if a little high or low appears, there was no significant difference between groups and animals of each group had taken similar amounts of cocaine stably in daily session time.

However, the animals given acupuncture at HT7 demonstrated that cocaine intake could be decreased by acupuncture treatment. Acupuncture at HT7 attenuated cocaine intake to $82.12 \pm 5.31\%$ of basal level, and one-way ANOVA followed by post hoc LSD test revealed that this mild suppression is significantly different with other treatments. This attenuation of HT7 corresponds to 84.5% compared to that of control, and results show that this effect of HT7 was caused by decrease of active lever press to 85% of control. In contrast, the inactive lever press showed little influence by treatments, so it may be suggested that the reduction of active lever press resulted from the decrease of spontaneous will of animals to take cocaine, and it may be said that acupuncture made animals have patience.

Interestingly, according to the analysis of the result of this study using time course of 5 min, the significant distinction between HT7 and other treatments mainly appeared in the latter half period, although a difference between HT7 and PC6 occurred at 20 min of the first half period. The representative pattern of that is displayed in the hatch mark as shown in fig. 3. So, it may be said that acupuncture at HT7 works later, even if the results of this study could not account for the reason why acupuncture does so in such a manner.

In this study, animals received all treatments unintentionally with counter balance. This random treatment was performed to be free from the possibility that there perhaps is a relation between

the order and the effect. However, the result showed that the effects of treatments did not exhibit a tendency to increase or decrease one way in association with the order. So, it seems that there is no relation between the order and the effect in the treatment of this study.

We know there is a problem needing serious consideration in the therapy for drug abuse. According to Weerts and colleagues, side effects like reduction of appetite are a severe problem with drugs treating cocaine addiction through GABAergic modulation such as baclofen, CGP44532, and tiagabine²⁵. So, a therapy developed to care for addiction needs to prove to be safe from these side effects. As described above, we show that acupuncture at HT7 lowered cocaine-taking behavior. Nevertheless, acupuncture did not influence food-taking behavior. Acupuncture shortened rather than lengthen the time spent for 100 pellets to about 78% of basal level like the control group, as shown in Fig. 9. Based on this result, it seems that the effect of suppression by acupuncture is specific for cocaine, and there is little possibility for acupuncture to make a side effect of reduction of food-taking.

Taken together, it might be suggested from the results of the present study that acupuncture at HT7 could be considered as an assistant in the attenuation of cocaine intake. Also, it is needed as the next step to investigate the mechanism by which acupuncture works.

Conclusion

This study demonstrated that acupuncture at HT7, but not at other points, attenuated cocaine intake significantly by suppressing of active lever press. The results show that the percent of active lever compared to basal level was $97.17 \pm 2.61\%$ for the control group, $82.12 \pm 5.31\%$ for the HT7 group, $96.82 \pm 4.11\%$ for the PC6 group, and $101.49 \pm 2.24\%$ for the tail group. This effect of acupuncture

was shown in the latter half, and it has also been demonstrated that the effect of acupuncture to reduce active lever was specific for cocaine, not food. As a conclusion, it may be said that acupuncture at HT7 could be considered as an assistant in the treatment of cocaine abuse.

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