

Original Article

Duration Pattern of the Effect of Acupuncture at HT₇ in Morphine Self-administration

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국문초록

모르핀 자가투여에서 신문혈 자침효과의 지속 양상

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목적 : 기존 연구에서 신문혈 자침은 알코올 및 코카인을 자가섭취하는 동물 모델에서 효과적이라는 점이 밝혀졌으며, 또한 모르핀 자가섭취를 억제할 수 있음이 밝혀졌다. 이러한 결과를 바탕으로 본 연구에서는 자침의 효과가 얼마나 지속될 수 있는지 알아보았다.

재료 및 방법 : 체중 270~300g의 수컷 SD계 흰쥐를 이용하였다. 먹이섭취 훈련을 통과한 후 오른쪽 경정맥에 관을 삽입하는 수술을 거쳐, 0.1mg/kg의 모르핀을 매일 1시간, 총 3주 동안 자가섭취 하도록 하였다. 모르핀을 일정하게 섭취한 동물에게는 다음날 침술을 시행하였다. 두 번째 실험에서는 GABA_A 및 GABA_B 길항제를 자침 30분 전에 투여하여 침술의 효능과 GABA 수용체계 사이의 관계를 검증하였다.

결과 : 모르핀 자가섭취를 억제하는 신문혈 자침의 효과는 매일 비슷하게 나타나지 않았으며, 4일째에는 유의한 효과가 없는 것으로 나타났다. 그러나 5일째와 6일째에는 다시 유의한 효과가 나타나 뒤집어진 U 자형 곡선을 나타내었다. 또한 GABA 수용체의 길항제들은 자침의 효과가 유의하게 나타났을 때 이를 차단하는 결과를 보였다.

* This research was supported by a grant from Daegu Haany University Ky · lin Foundation(2008-901-14)
· Acceptance : 2010. 6. 28. · Adjustment : 2010. 7. 13. · Adoption : 2010. 7. 19.
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결론 : 모르핀 중독 치료에서 침술의 효과는 항상 비슷하게 발휘되는 것이 아니므로 침 치료는 적어도 1 주일에 2회 이상 받을 필요가 있다. 아울러 보다 심도 있는 연구를 위하여 뇌신경 체계에 대한 연구가 이루어질 필요가 있는 것으로 사료된다.

주제어 : 침, 신문, 모르핀, 지속, 자가투여, 가바

I. Introduction

Morphine, one of the most widely abused drugs in the world, is usually used for the care of a number of pain diseases. However, repeated exposure to morphine results in tolerance driving psychiatric and physical dependence characterized by withdrawal symptoms¹⁾.

The mesolimbic dopamine(DA) system is known to be an important pathway for the reinforcement of morphine²⁾. Morphine increases DA release in the nucleus accumbens(NAc) and it causes drugseeking and drug maintenance behaviors³⁾. Drugs increasing DA levels when delivered directly into NAc maintain self-administration and conditioned place preference in animals⁴⁾. These results suggest that DA plays an important role in the morphine addiction.

Acupuncture, a representative therapy in the oriental medicine, is gaining more and more the population as a useful complementary and alternative therapy for the treatment of drug abuse in western countries^{5,6)}. Most of all, acupuncture is famous for the advance that produce speed and satisfactory results with little side effect. However, such a fine result does not always have the same duration. As well, in spite the duration of effect takes important part in the acupuncture treatment, little has been known about it.

HT₇ located on the transverse crease of fore paw in the heart meridian, has shown that could decrease or increase the DA release in the NAc in ethanol-sensitized or withdrawan rats respectively^{7,8)}. Also, it has demonstrated that suppressed with-

drawal signs in ethanol withdrawan rats. In addition, it attenuated cocaine taking behavior in self-administering rats as well as attenuated cocaine-induced sensitization⁹⁻¹¹⁾. And HT₇ has shown that ameliorated behavioral hyperactivity and DA release in the NAc in rats sensitized to morphine¹²⁾.

In the previous study¹³⁾, Yoon et al. has shown that acupuncture at HT₇ could attenuate morphine intake using animal model of self-administration. Also, there are a lot of studies have demonstrated that acupuncture could be effective in the morphine abuse¹⁴⁻¹⁶⁾. However, most of them just have revealed the effectiveness of acupuncture, and there is little study that has investigated the duration of effectiveness. Based on this, it was investigated how long the effect of acupuncture to suppress morphine taking would last.

II. Materials and methods

1. Animals

Male Sprague-Dawley rats(Daehan Animal, Seoul, Korea) weighing 270~300g at the beginning of the experiment, were used. Rats were housed in home cage of *ad libitum* food and water and a 12h light-dark cycle except the 1st day of the food training on which animals were kept under overnight schedule. Animals had passed 3days of adaptation period to home cage and experimental environment before the beginning of study. Measures were performed with minimized stress under NIH Guidelines for the Care and Use of Laboratory Ani-

mals(NIH publication no. 80-23).

2. Apparatus

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

3. Food training

Animals that adapted to the home cage and experimental environment for 3 days were trained to press the active lever for 45mg sucrose pellets (Bio-serve, Frenchtown, NJ, USA) under a daily FR 1 schedule. Rats were required to achieve 100 sucrose pellets within 3h except the first day on which animals tried over night without limitation of time. The house light was turned off and cue light was illuminated across all of the food training. Food training was performed once a day under food restriction to promote the learning of active lever-press. When animals had succeeded in achievement of 100 sucrose pellets for 3 consecutive days, they were subjected to the next phase of surgery.

4. Surgery

Animals that had passed the food training(100 sucrose pellets within 3h for three consecutive daily tests) were allowed free access to food and water for at least 1 day for surgery. Then, they were anesthetized with sodium pentobarbital(50mg/kg, i.p.). Chronic silastic jugular vein catheters(Dow Corning, Midland, MI, USA; 0.02" ID×0.037" OD) coated with tridodecylmethyl ammonium chloride (TDMAC) heparin(Polysciences Inc., Warrington, PA, USA),

were surgically implanted and fixed with mersilene surgical mesh(Ethicon Inc., Somerville, NJ, USA). The catheters were exteriorized in the back of rats using 22gauge guide cannulae(Palstics One, Roanoke, VA, USA) after skin incision. Silastic tubing and guide cannulae were embedded in dental cement and secured with Prolene surgical mesh. The patency of the catheters during recovery period was maintained by daily infusion of 0.2mℓ of saline containing heparin.

5. Morphine training

After recovery of at least 1week, animals were trained to self-administer morphine hydrochloride(JEIL Pharmaceutical CO. LTD, DAEGU, Korea) dissolved in saline, and 0.2mℓ of heparin was delivered into the guide cannula of jugular vein immediately before and after daily session to maintain the patency of the catheters. When animals press the active lever, the house light was extinguished and the cue light was illuminated simultaneously for 5sec, and also 0.1mℓ of morphine solution was delivered through intravenous catheters. Five sec of morphine delivery was followed by 10sec of "time-out"(TO) period in which both of house light and cue light were extinguished and no result was produced. If the TO period end, the house light was turned on again. The responses of inactive lever were recorded producing no result. Animals were trained to self-administer morphine solution under daily 1h schedule except the 1st day on which they were exposed during 4h to facilitate lever press for morphine taking. After 3weeks of training, the responses of active lever were checked, and animals who had taken stably(establishment of baseline : variation of active lever responses for 3 consecutive sessions less than 20% of the mean) received test.

6. Test

In the 1st experiment, animals who had established the baseline received test for 7 consecutive days. After the establishment of baseline, animals

were divided randomly into following 3 groups. Rats of HT₇ group(*n*=8) were given acupuncture at bilateral HT₇ points of heart channel, and LI₅ group (*n*=10) received at LI₅ points of large intestine channel. Rats of control group(*n*=10) received the same treatment with acupuncture group without needle stimulation. The anatomical locations of stimulated acupoints in rats were determined according to the acupoints in animal acupuncture atlas¹⁷⁾.

In the 2nd experiment, animals of HT₇ group(*n*=6) were given the same acupuncture treatment with HT₇ group of 1st experiment. HT₇ + bicuculline group(*n*=6) received acupuncture at HT₇ and the selective GABA_A receptor antagonist, bicuculline (Tocris, Ellisville, MO, USA ; 1mg/kg) injected intravenously 30min before acupuncture treatment. HT₇ + SCH 50911 group(*n*=6) received acupuncture at HT₇ and the selective GABA_B receptor antagonist, SCH 50911(Tocris, Ellisville, MO, USA ; 2mg/kg) injected intravenously 30min before acupuncture treatment. Animals of bicuculline group(*n*=6) and SCH 50911 group(*n*=6) were given bicuculline and SCH 50911 injection respectively, as well as the same treatment with control group.

Acupuncture was given bilaterally immediate before the start of session by oriental medical doctor, and stainless-steel needles(Dongbang Acupuncture INC, Chingdao, China) with a diameter of 0.18mm and a length of 8mm were inserted vertically into a depth of 2~3mm on each acupoints for 1min. The acupuncture stimulation was given by twisting needles bi-directionally at a frequency of twice per sec for a total of 2sec 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 2min during training period to minimize the stress from restriction.

7. Statistical analysis

Schedule manager program(Med Associates, St. Albans, VT) collected data of lever responses, and SPSS statistics program analyzed data using one-

way analysis of variance(ANOVA) and post hoc test with Tukey for the 1st experiment and the least significant difference(LSD) for the 2nd experiment. The statistical significance was regarded with the *p* value less than 0.05.

III. Results

1. The 1st experiment : effect of acupuncture

1) Basal level of active lever response in morphine intake

The results of present study show that the basal level of active lever response was 16.13±1.27 for control group, 12.63±0.96 for HT₇ group, 12.84±0.60 for LI₅ group. And, statistical analysis has shown that the basal levels of active lever-presses for 3days were similar among the groups and that there was no significant difference between groups(Fig. 1).

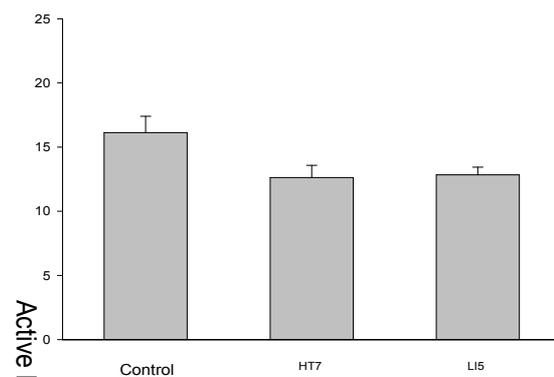


Fig. 1. The basal level of infusion of morphine solution

Results are mean±S.E.M. of infusions. One-way ANOVA and post hoc Tukey test. Rats of HT₇ group(*n*=8) were given acupuncture at bilateral HT₇ points of heart channel, and LI₅ group(*n*=10) received at LI₅ points of large intestine channel. Rats of control group(*n*=10) received the same treatment with acupuncture group without needle stimulation.

2) Effect of acupuncture on the active lever response

The results of the present study show that the

active lever response was 15.30±1.47 for control group, 7.50±1.64 for HT₇ group, 11.80±1.47 for LI₅ group on the 1st test day, and 15.40±1.35 for control group, 8.38±1.51 for HT₇ group, 11.90±1.35 for LI₅ group on the 2nd test day, and 16.60±1.51 for control group, 9.00±1.69 for HT₇ group, 12.50±1.51 for LI₅ group on the 3rd test day, and 17.60±2.15 for control group, 11.00±2.41 for HT₇ group, 12.90±2.15 for LI₅ group on the 4th test day, and 16.80±2.25 for control group, 8.13±2.51 for HT₇ group, 12.70±2.25 for LI₅ group on the 5th test day, and 16.90±1.96 for control group, 8.38±2.19 for HT₇ group, 12.20±1.96 for LI₅ group on the 6th test day, and 15.50±1.30 for control group, 9.25±1.45 for HT₇ group, 11.40±1.30 for LI₅ group on the 7th test day. According to the results of the present study, the effect of acupuncture at HT₇ to attenuate morphine taking behavior was not constant and it was more apparent on 1-3 day than behind (** : $p < 0.01$, * : $p < 0.05$, HT₇ group vs. control group. Fig. 2).

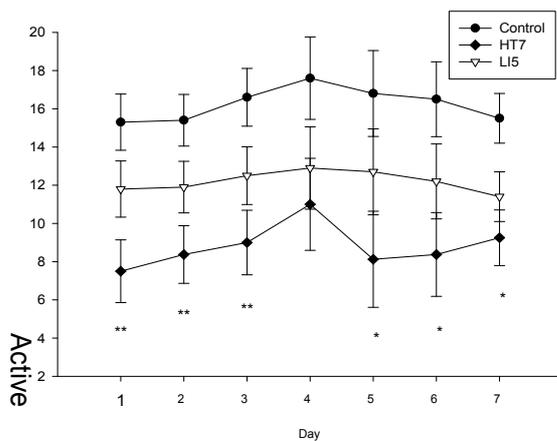


Fig. 2. The effect of acupuncture on the active lever response

Results are mean±S.E.M. of responses. One-way ANOVA and post hoc Tukey test. ** : $p < 0.01$, * : $p < 0.05$, HT₇ group vs. control group. Rats of HT₇ group ($n=8$) were given acupuncture at bilateral HT₇ points of heart channel, and LI₅ group ($n=10$) received at LI₅ points of large intestine channel. Rats of control group ($n=10$) received the same treatment with acupuncture group without needle stimulation.

3) Effect of acupuncture on the inactive lever response

The inactive lever response was 2.56±1.18 for

control group, 3.88±1.25 for HT₇ group, 5.40±1.12 for LI₅ group on the 1st test day, and 3.22±1.22 for control group, 3.25±1.29 for HT₇ group, 3.40±1.15 for LI₅ group on the 2nd test day, and 3.11±0.85 for control group, 2.50±0.91 for HT₇ group, 2.90±0.81 for LI₅ group on the 3rd test day, and 4.67±1.35 for control group, 3.25±1.44 for HT₇ group, 4.40±1.28 for LI₅ group on the 4th test day, and 1.33±1.04 for control group, 3.88±1.11 for HT₇ group, 3.50±0.99 for LI₅ group on the 5th test day, and 4.22±1.49 for control group, 5.63±1.58 for HT₇ group, 2.00±1.42 for LI₅ group on the 6th test day, and 2.00±1.44 for control group, 2.50±1.53 for HT₇ group, 3.80±1.37 for LI₅ group on the 7th test day. There was no significant difference between groups on every treatment day (Fig. 3).

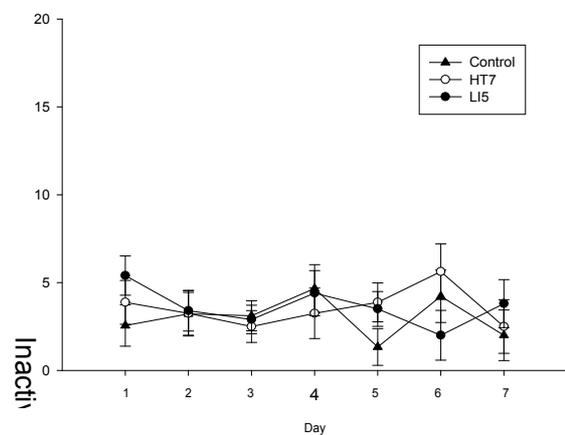


Fig. 3. The effect of acupuncture on the inactive lever response

Results are mean±S.E.M. of responses. One-way ANOVA and post hoc Tukey test. Rats of HT₇ group ($n=8$) were given acupuncture at bilateral HT₇ points of heart channel, and LI₅ group ($n=10$) received at LI₅ points of large intestine channel. Rats of control group ($n=10$) received the same treatment with acupuncture group without needle stimulation.

4) Effect of acupuncture on morphine intake

The results of the present study show that the percent of active lever compared to basal level was 97.51±7.06% for control group, 50.73±9.07% for HT₇ group, 92.74±6.55% for LI₅ group on the 1st day, and 95.01±10.49% for control group, 62.66±8.02% for HT₇ group, 93.23±5.69% for LI₅ group on the 2nd

day, and 102.71±5.96% for control group, 73.40±8.84% for HT₇ group, 93.71±11.13% for LI₅ group on the 3rd day, and 104.51±10.56% for control group, 84.95±14.42% for HT₇ group, 99.66±9.69% for LI₅ group on the 4th day, and 100.35±8.62% for control group, 65.78±12.10% for HT₇ group, 105.12±11.86% for LI₅ group on the 5th day, and 97.18±7.69% for control group, 65.23±11.69% for HT₇ group, 92.64±7.97% for LI₅ group on the 6th day, and 96.33±4.48% for control group, 70.54±15.86% for HT₇ group, 89.22±6.58% for LI₅ group on the 7th day. There were significant differences between HT₇ group and both of control group and LI₅ group. The effect of acupuncture at HT₇ to attenuate morphine taking behavior was the best on the 1st day, and the effect was weak on the 2nd, 3rd, 5th, and the 6th day. However, the effect was not significant on the 4th and 7th day (** : $p < 0.01$, * : $p < 0.05$, HT₇ group vs. control group. ## : $p < 0.01$, # : $p < 0.05$, HT₇ group vs. LI₅ group. Fig. 4).

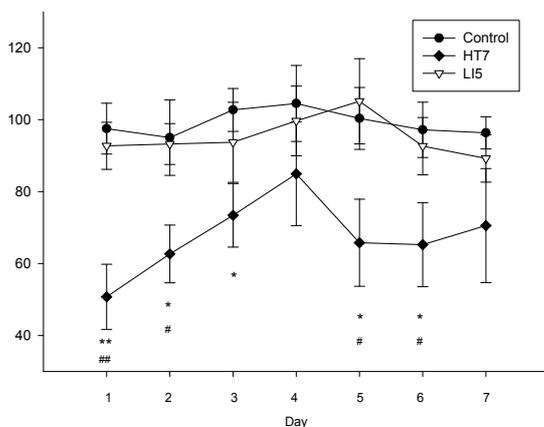


Fig. 4. The effect of acupuncture on morphine taking behavior

Results are mean±S.E.M. of percents compared to basal level. One-way ANOVA and post hoc Tukey test. ** : $p < 0.01$, * : $p < 0.05$, HT₇ group vs. control group ; ## : $p < 0.01$, # : $p < 0.05$, HT₇ group vs. LI₅ group. Rats of HT₇ group ($n=8$) were given acupuncture at bilateral HT₇ points of heart channel, and LI₅ group ($n=10$) received at LI₅ points of large intestine channel. Rats of control group ($n=10$) received the same treatment with acupuncture group without needle stimulation.

2. The 2nd experiment: neuronal involvement

1) Basal level of active lever response in morphine intake

The basal level of active lever response was 14.72±5.36 for HT₇, 11.77±1.15 for HT₇ + bicuculline group, 13.77±1.23 for HT₇ + SCH 50911 group, 15.03±1.07 for bicuculline alone group, 14.75±2.44 for SCH 50911 alone group. The basal levels of active leverpresses were similar among the groups and there was no significant difference between groups (Fig. 5).

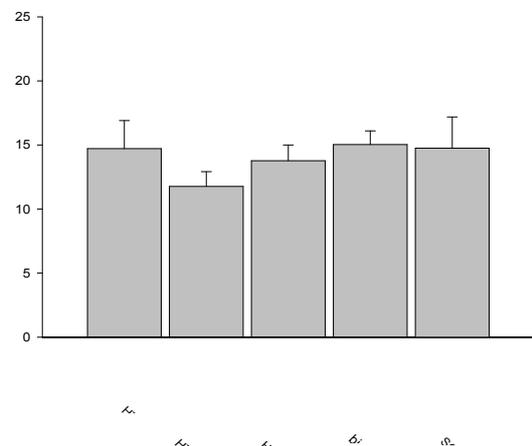


Fig. 5. The basal level of infusion of morphine solution

Results are mean±S.E.M. of infusions. One-way ANOVA and post hoc LSD test. Rats of HT₇ group ($n=6$) were given the same acupuncture treatment with HT₇ group of 1st experiment. HT₇ + bicuculline group ($n=6$) received acupuncture at HT₇ and the selective GABA_A receptor antagonist, bicuculline (1mg/kg) injected intravenously 30min before acupuncture treatment. HT₇ + SCH 50911 group ($n=6$) received acupuncture at HT₇ and the selective GABA_B receptor antagonist, SCH 50911 (2mg/kg) injected intravenously 30min before acupuncture treatment. Animals of bicuculline group ($n=6$) and SCH 50911 group ($n=6$) were given bicuculline and SCH 50911 injection respectively, as well as the same treatment with control group.

2) Neuronal involvement in the effect of acupuncture on the active lever

The active lever response was 8.83±1.40 for HT₇ group, 13.33±0.88 for HT₇ + bicuculline group, 13.83±1.85 for HT₇ + SCH 50911 group, 16.33±2.36 for bicuculline alone group, 13.00±2.89 for SCH 50911 alone

group on the 1st test day, and 13.00±3.46 for HT₇ group, 12.50±0.81 for HT₇ + bicuculline group, 16.33±2.42 for HT₇ + SCH 50911 group, 12.17±2.32 for bicuculline alone group, 14.67±2.54 for SCH 50911 alone group on the 2nd test day, and 12.50±2.43 for HT₇ group, 14.00±1.88 for HT₇ + bicuculline group, 17.00±2.63 for HT₇ + SCH 50911 group, 14.33±2.36 for bicuculline alone group, 13.33±2.89 for SCH 50911 alone group on the 3rd test day, and 12.33±2.11 for HT₇ group, 13.83±4.28 for HT₇ + bicuculline group, 14.83±2.54 for HT₇ + SCH 50911 group, 16.50±2.29 for bicuculline alone group, 16.17±3.70 for SCH 50911 alone group on the 4th test day, and 11.50±2.67 for HT₇ group, 18.00±4.81 for HT₇ + bicuculline group, 13.50±2.01 for HT₇ + SCH 50911 group, 14.83±2.24 for bicuculline alone group, 16.00±3.36 for SCH 50911 alone group on the 5th test day, and 10.33±2.62 for HT₇ group, 14.50±5.13 for HT₇ + bicuculline group, 14.83±2.95 for HT₇ + SCH 50911 group, 10.83±1.89 for bicuculline alone group, 14.33±3.04 for SCH 50911 alone group on the 6th test day, and 10.17±

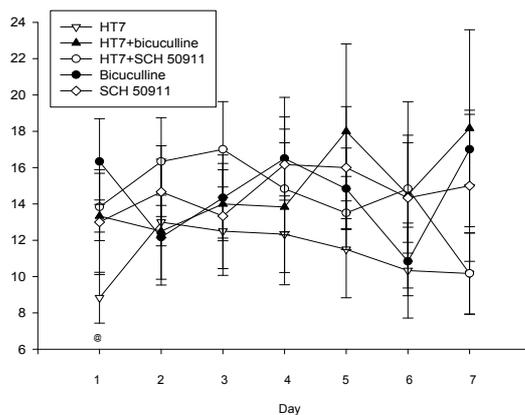


Fig. 6. The effect of acupuncture on the active lever response through GABA receptor system

Results are mean±S.E.M. of responses. One-way ANOVA and post hoc LSD test. @ : $p < 0.05$, HT₇ group vs. bicuculline group. Rats of HT₇ group ($n=6$) were given the same acupuncture treatment with HT₇ group of 1st experiment. HT₇ + bicuculline group ($n=6$) received acupuncture at HT₇ and the selective GABA_A receptor antagonist, bicuculline (1mg/kg) injected intravenously 30min before acupuncture treatment. HT₇ + SCH 50911 group ($n=6$) received acupuncture at HT₇ and the selective GABA_B receptor antagonist, SCH 50911 (2mg/kg) injected intravenously 30min before acupuncture treatment. Animals of bicuculline group ($n=6$) and SCH 50911 group ($n=6$) were given bicuculline and SCH 50911 injection respectively, as well as the same treatment with control group.

2.24 for HT₇ group, 18.17±5.42 for HT₇ + bicuculline group, 10.17±2.23 for HT₇ + SCH 50911 group, 17.00±1.93 for bicuculline alone group, 15.00±4.16 for SCH 50911 alone group on the 7th test day. The significant difference was observed only on the 1st day between HT₇ group and bicuculline group (@ : $p < 0.05$. Fig. 6).

3) Neuronal involvement in the effect of acupuncture on the inactive lever

The inactive lever response was 3.50±0.89 for HT₇ group, 1.50±1.15 for HT₇ + bicuculline group, 2.00±1.06 for HT₇ + SCH 50911 group, 4.00±1.21 for bicuculline alone group, 5.00±0.97 for SCH 50911 alone group on the 1st test day, and 3.50±1.12 for HT₇ group, 1.00±0.63 for HT₇ + bicuculline group, 3.50±1.45 for HT₇ + SCH 50911 group, 6.17±2.40 for bicuculline alone group, 1.67±0.67 for SCH 50911 alone group on the 2nd test day, and 2.00±0.68 for HT₇ group, 3.33±1.99 for HT₇ + bicuculline group, 3.00±1.03 for HT₇ + SCH 50911 group, 8.50±1.89 for bicuculline alone group, 8.50±3.80 for SCH 50911 alone group on the 3rd test day, and 2.17±1.17 for HT₇ group, 1.33±0.88 for HT₇ + bicuculline group, 2.00±1.44 for HT₇ + SCH 50911 group, 9.33±1.15 for bicuculline alone group, 4.67±1.87 for SCH 50911 alone group on the 4th test day, and 1.83±1.05 for HT₇ group, 1.33±0.80 for HT₇ + bicuculline group, 1.67±1.12 for HT₇ + SCH 50911 group, 5.67±1.05 for bicuculline alone group, 3.50±1.34 for SCH 50911 alone group on the 5th test day, and 3.50±1.93 for HT₇ group, 1.17±0.54 for HT₇ + bicuculline group, 3.33±2.17 for HT₇ + SCH 50911 group, 3.50±0.89 for bicuculline alone group, 3.67±1.76 for SCH 50911 alone group on the 6th test day, and 2.50±0.72 for HT₇ group, 3.67±1.84 for HT₇ + bicuculline group, 2.33±0.80 for HT₇ + SCH 50911 group, 12.50±4.29 for bicuculline alone group, 5.17±1.42 for SCH 50911 alone group on the 7th test day. The significant differences were HT₇ + bicuculline group vs. SCH 50911 group (+ : $p < 0.05$) on the 1st day, HT₇ + bicuculline group vs. bicuculline group (‡ : $p < 0.05$), bicuculline alone group vs. SCH 50911 alone group (& : $p < 0.05$).

$p < 0.05$) on the 2nd day, HT₇ group vs. bicuculline group(@ : $p < 0.05$), HT₇ group vs. SCH 50911 group(\$: $p < 0.05$) on the 3rd day, HT₇ group vs. bicuculline group(@@ : $p < 0.01$), HT₇ + bicuculline group vs. bicuculline group(### : $p < 0.001$), bicuculline group vs. SCH 50911 group(& : $p < 0.05$), HT₇ + SCH 50911 group vs. bicuculline group(%% : $p < 0.01$) on the 4th day, HT₇ group vs. HT₇ + bicuculline group(* : $p < 0.05$), HT₇ + bicuculline group vs. bicuculline group(## : $p < 0.01$), HT₇ + SCH 50911 group vs. bicuculline group(% : $p < 0.05$) on the 5th day, HT₇ group vs. bicuculline group(@@ : $p < 0.01$), HT₇ + bicuculline group vs. bicuculline group(‡ : $p < 0.05$), bicuculline group vs. SCH 50911 group(& : $p < 0.05$), HT₇ + SCH 50911 group vs. bicuculline group(%% : $p < 0.01$) on the 7th day(Fig. 7).

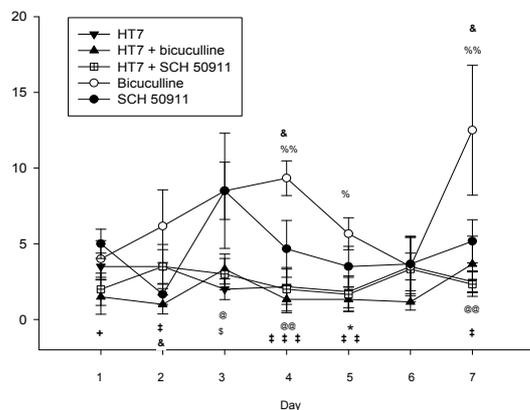


Fig. 7. The effect of acupuncture on the inactive lever response through GABA receptor system

Results are mean±S.E.M. of responses. One-way ANOVA and post hoc LSD test. + : $p < 0.05$, HT₇ + bicuculline group vs. SCH 50911 group ; ‡ : $p < 0.05$, ## : $p < 0.01$, ### : $p < 0.001$, HT₇ + bicuculline group vs. bicuculline group ; & : $p < 0.05$, bicuculline alone group vs. SCH 50911 alone group ; @ : $p < 0.05$, @@ : $p < 0.01$, HT₇ group vs. bicuculline group ; \$: $p < 0.05$, HT₇ group vs. SCH 50911 group ; % : $p < 0.05$, %% : $p < 0.01$, HT₇ + SCH 50911 group vs. bicuculline group. Rats of HT₇ group($n=6$) were given the same acupuncture treatment with HT₇ group of 1st experiment. HT₇ + bicuculline group($n=6$) received acupuncture at HT₇ and the selective GABA_A receptor antagonist, bicuculline (1mg/kg) injected intravenously 30 min before acupuncture treatment. HT₇ + SCH 50911 group($n=6$) received acupuncture at HT₇ and the selective GABA_B receptor antagonist, SCH 50911(2mg/kg) injected intravenously 30min before acupuncture treatment. Animals of bicuculline group ($n=6$) and SCH 50911 group($n=6$) were given bicuculline and SCH 50911 injection respectively, as well as the same treatment with control group.

4) Neuronal involvement in the effect of acupuncture on morphine intake

The percent of active lever compared to basal level was 59.95±3.83% for HT₇ group, 116.37±9.29% for HT₇ + bicuculline group, 98.87±4.53% for HT₇ + SCH 50911 group, 107.07±10.94% for bicuculline group, 85.45±10.56% for SCH 50911 group on the 1st day, and 84.45±9.92% for HT₇ group, 107.88±4.92% for HT₇ + bicuculline group, 116.23±10.69% for HT₇ + SCH 50911 group, 82.90±14.83% for bicuculline group, 100.60±7.08% for SCH 50911 group on the 2nd day, and 83.20±5.14% for HT₇ group, 117.72±7.22% for HT₇ + bicuculline group, 121.40±10.22% for HT₇ + SCH 50911 group, 98.07±17.31% for bicuculline group, 87.20±8.01% for SCH 50911 group on the 3rd day, and 87.07±15.51% for HT₇ group, 132.92±16.62% for HT₇ + bicuculline group, 105.43±11.66% for HT₇ + SCH 50911 group, 114.12±18.86% for bicuculline group, 105.40±12.48% for SCH 50911 group on the 4th day, and 74.55±9.33% for HT₇ group, 143.93±20.04% for HT₇ + bicuculline group, 98.15±14.00% for HT₇ + SCH 50911 group, 97.62±11.24% for bicuculline group, 105.32±8.90% for SCH 50911 group on the 5th day, and 66.13±8.33% for HT₇ group, 112.90±24.70% for HT₇ + bicuculline group, 103.95±15.86% for HT₇ + SCH 50911 group, 73.58±12.75% for bicuculline group, 93.67±6.17% for SCH 50911 group on the 6th day, and 68.00±11.96% for HT₇ group, 143.10±24.03% for HT₇ + bicuculline group, 87.72±11.64% for HT₇ + SCH 50911 group, 113.23±10.21% for bicuculline group, 93.65±16.69% for SCH 50911 group on the 7th day. There were significant differences between HT₇ group and both of control group and LL₅ group. The significant differences were HT₇ group vs. HT₇ + bicuculline group(*** : $p < 0.001$), HT₇ group vs. HT₇ + SCH 50911 group(## : $p < 0.01$), HT₇ group vs. bicuculline group(@@ : $p < 0.01$), HT₇ group vs. SCH 50911 group(\$: $p < 0.05$) on the 1st day, HT₇ group vs. HT₇ + SCH 50911 group(# : $p < 0.05$) on the 2nd day, HT₇ group vs. HT₇ + bicuculline group(* : $p < 0.05$), HT₇ group vs. HT₇ + SCH 50911 group(# : $p < 0.05$) on the 3rd day, HT₇ group vs. HT₇ + bicuculline

group(** : $p < 0.01$) on the 5th day, HT₇ group vs. HT₇ + bicuculline group(* : $p < 0.05$) on the 6th day, HT₇ group vs. HT₇ + bicuculline group(** : $p < 0.01$) on the 7th day, and on the 4th day, there was not any significant difference(Fig. 8).

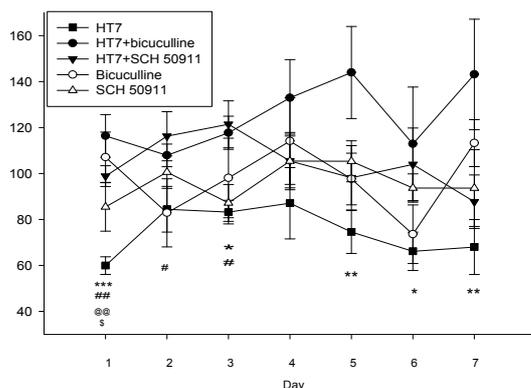


Fig. 8. The effect of acupuncture on morphine taking behavior through GABA receptor system

Results are mean±S.E.M. of responses. One-way ANOVA and post hoc LSD test. * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$, HT₇ group vs. HT₇ + bicuculline group ; # : $p < 0.05$, ## : $p < 0.01$, HT₇ group vs. HT₇ + SCH 50911 group ; @ : $p < 0.01$, HT₇ group vs. bicuculline group ; \$: $p < 0.05$, HT₇ group vs. SCH 50911 group. Rats of HT₇ group($n=6$) were given the same acupuncture treatment with HT₇ group of 1st experiment. HT₇ + bicuculline group($n=6$) received acupuncture at HT₇ and the selective GABA_A receptor antagonist, bicuculline(1mg/kg) injected intravenously 30min before acupuncture treatment. HT₇ + SCH 50911 group($n=6$) received acupuncture at HT₇ and the selective GABA_B receptor antagonist, SCH 50911(2mg/kg) injected intravenously 30min before acupuncture treatment. Animals of bicuculline group($n=6$) and SCH 50911 group($n=6$) were given bicuculline and SCH 50911 injection respectively, as well as the same treatment with control group.

IV. Discussion

Based on the previous study, we anticipated that the effect would hold out until the last test day or may be stronger in the latter part than fore part of the test period and investigated through the 1st experiment. The basal level of each group was similar among groups(Fig. 1), and there was no significant difference between groups in the inactive lever through the test(Fig. 3). Therefore, it may be

said that animals had taken morphine solution spontaneously. And, acupuncture at HT₇ decreased morphine taking on the 1st test day as the result of the previous study has shown¹³). However, the result of the present study has demonstrated that the effect of acupuncture was not constant rather than diminished steadily up to the 4th day showing the best on the 1st day(Fig. 4). Although the effect appeared again on the 5th and 6th day, it has gone again on the 7th day. So, the anticipation that acupuncture might produce similar effectiveness throughout the test period was proved not true. In a similar pattern with the percent compared to the basal level, the active lever press of HT₇ group was attenuated more in the fore part than the latter part, and was not significantly reduced compared to control group in the middle part(Fig. 2). It is interesting that the effect of acupuncture at HT₇ disappeared on the 4th day, being weakened steadily. Even if lots of things must be considered, authors propose a hypothesis that it is a kind of ‘tolerance’. The specific acupoint HT₇ of subjects might have experienced the specific stimulation by needle for the first time on the 1st day. However, the same stimulation with the 1st day might not be enough to give the same charge to the acupoint HT₇. Another unexpected and interesting thing is that the effect appeared again on the 5th and 6th day. So, it is possible that the tolerance of the effect of acupuncture is not long lasting but short.

Although many things still remain to be studied in the changing pattern of the effect of acupuncture, the results of the present study might suggest that acupuncture’s suppression of morphine taking does not appear constantly every day and therefore, it is needed to take acupuncture treatment at least twice per a week changing acupoints or stimulation technique for the treatment of morphine abuse.

In 2nd experiment, we investigated the neuronal involvement in the duration of the effect of acupuncture using GABA receptor antagonists. GABA receptor system has been revealed to be involved in the effects of acupuncture at HT₇ in our previous study about alcohol and morphine addiction^{7,13,18}). In

the present study, the selective GABA_A receptor antagonist, bicuculline inhibited the effect of acupuncture at HT₇ significantly on the 1st, 3rd, 5th, 6th, 7th day, and the selective GABA_B receptor antagonist, SCH 50911 inhibited the effect of acupuncture at HT₇ significantly on the 1st, 2nd, 3rd day. This result is parallel with the previous study¹³⁾, and expected to be another evidence to account for the involvement of GABA receptor system in the effect of acupuncture at HT₇ in drug addiction. According to several studies, the ventral tegmental area(VTA) is known to play an important role in morphine addiction¹⁹⁻²¹⁾. So, for the more certain find, the neurochemical and histological study investigating the function of VTA in the effect of acupuncture would be needed.

In this study, both antagonists reversed the effect of acupuncture in the front or the rear showing the best on the 1st day, and there was no reverse on the 4th day(Fig. 8). This pattern of reverse is thought to be due to the unique duration pattern of the effect of acupuncture, i.e. the difference of the lasting aspect between HT₇ group and both groups of HT₇ + bicuculline and HT₇ + SCH 50911. In other words, both groups of HT₇ + bicuculline and HT₇ + SCH 50911 did not show 'overturned-U shape' while HT₇ group did. So, it might be said that the unique lasting pattern of the effect of acupuncture at HT₇ produced this phenomenon.

In addition, one more surprising thing is embedded in the results of this study. The reverse of the effect of acupuncture by both of bicuculline or SCH 50911 is occurred in the front or the rear, and this pattern is very similar with the aspect of duration of the effect acupuncture at HT₇. Therefore, it has been found that the regents antagonized the effect of acupuncture only when it was significant, and did not affect when it was not. Eventually, it might be said that the action of regents appeared selectively according to whether acupuncture function or not. From this result, it seems that there is a relation between the function of acupuncture at HT₇ and the action of regents.

V. Conclusion

As a conclusion, the results of this study suggest that the effect of acupuncture at HT₇ to suppress morphine taking is not constant, and may be weakened or disappear after some treatments. Therefore it is needed to take acupuncture treatment at least twice per a week. As well, GABA receptor system is involved in the effect of acupuncture at HT₇ and also, there may be a relation between the action of antagonists and the function of acupuncture at HT₇.

VI. References

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