Integrative Medicine Research 13 (2024) 101052

Contents lists available at ScienceDirect

Integrative Medicine Research

journal homepage: www.elsevier.com/locate/imr

Original Article

A synergistic effect of herb and acupuncture on the methamphetamine

Jin Gyeom Lee [a,b], Yuchi Li [c], Nam Jun Kim [a,b], Han Byeol Jang [a,b], Chae Ha Yang [d], Hee Young Kim [e], Seong Shoon Yoon [d], Suchan Chang [d], Seon-Ju Jeong [d], Sang Chan Kim [b,f], Bok Suk Sa [g], Bong Hyo Lee [a,b,*]

[a] Department of Acupuncture, Moxibustion and Acupoint, College of Korean Medicine, Daegu Haany University, Daegu, Republic of Korea
[b] Research Center for Herbal Convergence on Liver Disease, Daegu Haany University, Gyeongsan, Republic of Korea
[c] China Novartis Institutes for BioMedical Research, PR China
[d] Department of Physiology, College of Korean Medicine, Daegu Haany University, Daegu, Republic of Korea
[e] Department of Physiology, Yonsei University College of Medicine, Seoul, Republic of Korea
[f] Department of Herbal Formula, College of Biomedical Science, Daegu Haany University, Daegu, Republic of Korea
[g] Chung Shin Herbal Medicine, Daegu, Republic of Korea

ARTICLE INFO

Keywords:
Acupuncture-herb synergy
Methamphetamine
Psychostimulant

ABSTRACT

Background: Herbal medicine Ja-Geum-Jeong (JGJ) has been used for the treatment of detoxification in Eastern Asia. However, the mechanisms involved are not clearly defined. The purpose of the present study was to investigate if herb medication inhibits Methamphetamine (METH)'s reinforcing effect and also examined if a combination of herb medication and acupuncture produces a synergistic effect on METH.

Methods: Male Sprague-Dawley rats were given acute METH intraperitoneally and the locomotor activity and ultrasonic vocalization (USV) calls were measured. Rats were administered JGJ orally and acupuncture was given at HT7 or SI5. Monosodium glutamate (MSG) and gamma-aminobutyric acid (GABA) agonists were injected into the Central amygdala (CeA) to investigate a possible neuroscientific mechanism. Tyrosine hydroxylase (TH) and fast scan cyclic voltammetry (FSCV) were measured to immunohistochemically and electrically confirm the behavioral data.

Results: Locomotor activity and USV calls were increased by METH ($P < 0.05$) and these increases were inhibited by JGJ ($P < 0.05$). Also, JGJ had no effect on the normal group given saline, and acupuncture at SI5 acupoint, but not at HT7 acupoint, produced a synergistic effect when combined with JGJ ($P < 0.05$). The JGJ's inhibition was blocked by the inactivation of CeA ($P < 0.05$), and MSG mimicked JGJ ($P < 0.05$). TH and FSCV measures showed the same pattern with the behavioral data ($P < 0.05$).

Conclusion: Results of the present study suggest that JGJ had inhibitory effects on the METH which was mediated through the activation of CeA and that combination of acupuncture and herb produced synergistic effect.

1. Introduction

Drug addiction is a representative obstinate disease and methamphetamine (METH) is one of the drugs worldwide abused.[1] Also in Asia, methamphetamine addiction has reached a serious level.[2] METH decreases appetite and increases motor activity, respiration, heart rate and body temperature. Repeated use of METH results in not only functional but also structural changes in brain,[3,4] and when withdrawn, diverse emotional, neuropsychiatric, and physical problems can occur.[5]

METH exerts the psychostimulant effects by enhancing the activity of dopamine (DA) neurons, the representative excitatory neurons, projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and known to play a key role in the mesolimbic system of midbrain, so-called reward center. Therefore, development of a therapy has been focused on regulating DA activity.[6]

Although many studies have been performed to develop an optimal treatment, METH addiction remains difficult to treat.[7-9] Therefore, various approaches including complementary and alternative medicine (CAM) have been tried.

Oriental medicine, a representative CAM developed in Eastern Asia, has been gaining interest in many fields, including drug addiction.[10,11] It seeks for functionally harmonizing or balancing rather than activates or inhibits in one-way.[12] Herb medication, one of the major therapies of the Oriental medicine, has been used to treat diverse diseases for

* Corresponding author at: Department of Acupuncture, Moxibustion and Acupoint, College of Korean Medicine, Daegu Haany University, 136 Shincheondong-ro, Suseong-Gu, Daegu 42158, Republic of Korea.
E-mail address: dlqhdgy@dhu.ac.kr (B.H. Lee).

https://doi.org/10.1016/j.imr.2024.101052
Received 6 February 2024; Received in revised form 20 May 2024; Accepted 30 May 2024
Available online 1 June 2024

thousands of years in Eastern Asia. Scientific researches have demonstrated that herb medication has therapeutic effects for many health problems,[13-15] and even neuropsychiatric disease has been shown to be improved.[16,17] However, there have been few studies showing an inhibitory effect of herb medication on drug addiction, in particular METH.

Ja-Geum-Jeong (JGJ, also known as Zi Jin Ding in Chinese), a famous herb formula for detoxification in Eastern Asia, has also been used for cancer and inflammatory diseases.[18] Cho et al. reported JGJ's inhibitions of nitric oxide (NO) and reactive oxygen species (ROS) in hepatoma cell and regulation of cell cycle,[19-21] and recently an animal study has demonstrated JGJ's amelioration of an atopic dermatitis-like skin lesion through regulation of the inflammation-related chemokines.[22] Thus, JGJ's effects of detoxification and anti-inflammation were anticipated to be effective for the drug addiction and we examined if JGJ could inhibit the METH's psychostimulant effects, and a possible neuroscientific mechanism was investigated with focusing on the DA neuron activity.

In the neuroscience research, tyrosine hydroxylase (TH) expression serves as an immunohistochemical evidence for the activation of a neuron such as DA.[23] And the fast scan cyclic voltammetry (FSCV) is usually used to electrically examine a neuronal activation.[24] Therefore, we measured the TH expression and FSCV to confirm the behavioral data in another way.

2. Methods

2.1. Animals

Eight-week-old male Sprague-Dawley rats were obtained from Daehan Animal (Seoul, Korea) and reared under controlled-conditions for temperature (23 ± 2 ˚C), humidity (55 ± 5 %), and a 12-hour light/dark cycle (lights on at 7:00 a.m.) with ad libitum food and water. Experimental procedures involving animals were performed under protocols approved by the Daegu Haany University Institutional Animal Care and Use Committee (DHU2021–032).

2.2. Chemicals and reagents

METH hydrochloride, Mono Sodium Glutamate (MSG), Muscimol (MUS; Gamma-aminobutyric acid$_A$ ($GABA_A$) receptor agonist), and Baclofen (BAC; $GABA_B$ receptor agonist) were purchased from Sigma Aldrich (St. Louis, MO, USA). JGJ, provided by Chung Shin Herbal Medicine (Daegu, Korea), was dissolved in 0.05 % carboxymethyl cellulose (CMC) and water for oral administration following a recipe described previously.[20,22]

2.3. Stereotaxic surgery and microinjection

Rats were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg, i.p.). For the intracerebroventricular (i.c.v.) injection, 26 gauge guide cannula (Plastics One, Roanoke, VA) was implanted into the central amygdala (CeA) (AP −2.5, ML ±4.5, DV −7.5) according to an atlas[25] and was fixed to the skull with dental cement. The animals were allowed 7 days before the test to recover from surgery. The CeA was infused using an internal cannula, connected by polyethylene tubing to a 1 μl Hamilton syringe. The inner cannula was left in place for an additional 60 s to allow diffusion of the solution and to prevent reflux. CeA injections were performed 5 min before testing.

2.4. Locomotor activity and ultrasonic vocalization (USV)[26]

Locomotor activity and USV calls (50-kHz) were measured simultaneously in a sound-attenuated chamber consisting of two black acrylic boxes to minimize exterior noise (inside box: 54 × 38 × 35 cm, outside box: 68 × 50 × 51 cm). A condenser ultrasonic microphone (Ultramic 250 K, Dodotronic, Castel Gandolfo, Italy) and a digital camera were suspended at the center of the ceiling of the chamber. As performed in our previous study, locomotor activity was recorded with a video-tracking system (Ethovision XT; Noldus Information Technology BV, Wageningen, Netherlands) and 50-kHz USVs were recorded using an Ultra Sound Gate 416H data acquisition device (Avisoft Bioacoustics, Glienicke, Germany) and analyzed by Avisoft-SAS Lab Pro (version 4.2, Avisoft Bioacoustics). After recording the baselines of locomotor activity and USVs for 30 min, i.p. injection of METH (1 mg/kg)[27,28] and acupuncture treatment were performed, and then monitored up to 60 min. GABA mixture (MUS 0.06 nmol[29,30] + BAC 0.6 nmol[31,32]; 0.5 μl/side, i.c.v.) and MSG (400 μg/1 μl; 0.5 μl/side, i.c.v.)[33] was injected into the CeA before METH administration. Data were expressed as the distance traveled (cm) for locomotor activity and numbers of 50-kHz calls for USV, during each 10-min period.

2.5. FSCV

Electrically stimulated DA release in the NAc was measured by FSCV in vivo as described previously.[34,35] A 7.0-μm diameter carbon fiber was inserted into borosilicate glass capillary tubing (1.2 mm o.d.; A-M Systems, WA, USA) under negative pressure and was subsequently pulled on a pipette puller (Sutter Instrument, Model P-97, CA, USA). The carbon fiber electrode (CFE) was then cut under microscopic control with 150 to 200 μm of bare fiber protruding from the end of the glass micropipette and was back-filled with 3 M KCl. The CFE potential was linearly scanned with a triangular waveform from −0.4 to 1.3 V and back to −0.4 V vs. Ag/AgCl (scan rate 400 V/s). Cyclic voltammograms were recorded at the CFE every 100 milliseconds (10 Hz) using a Chem Clamp voltage clamp potentiometers (Dagan Corporation, MN, USA). Recordings were performed and analyzed using LabVIEW-based (National Instruments, TX, USA) customized software (Demon Voltammetry) .[34] For FSCV recording of DA signals, rats were anesthetized with urethane (1.5 mg/kg, i.p.) and placed at a stereotaxic frame. Bipolar coated stainless steel electrodes and a capillary glass-based CFE were stereotaxically positioned into the medial forebrain bundle (MFB; AP −2.5, ML ±1.9, DV 8.0 to 8.3) and the NAc (AP −1.6, ML ±0.8 to 1.9, DV 6.5 to 8.0), respectively, according to the rat brain atlas.[36]

The MFB was stimulated with 60 monophasic pulses at 60 Hz (4 millisecond pulse) with 2-min intervals. Once the stimulated DA response was stable for at least five consecutive collections (variation less than 10 % of mean), baseline measurements were taken. After a stable baseline establishment, rat received an injection of METH. The DA signals were recorded for 60 min after treatment.

JGJ (100 mg/kg) was administered orally.[20,22] GABA mixture (MUS 1 mg/kg/ml[37-39] + BAC 3 mg/kg/ml[40-43]) and MSG (4 mg/kg/ml)[44,45] was injected intravenously.

2.6. Immunohistochemistry for TH expression[23]

Brains were prepared to measure TH immunoreactivity levels in the VTA (AP −5.8 to −6.3, ML ±0.8, DV −8.2 to −8.5). Brains were taken out after perfusion with 4 % paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4), post-fixed, and cryo-sectioned into 30-μm-thick.[46] The brain sections were incubated with rabbit polyclonal antibodies, anti-TH (1:500; Millipore, USA), followed by 120 min of incubation at room temperature with a biotinylated donkey anti-rabbit Alexa Fluor 594 (red; 1:500; Sigma Aldrich, USA) and were mounted onto gelatin-coated slides. The slides were photographed and quantified using a confocal laser scanning microscope (LSM700, Carl Zeiss, Germany). The level of TH immunoreactivity in each VTA brain section was counted and averaged from collected three sections.

2.7. Acupuncture treatment

Acupuncture was performed at each acupoint bilaterally for 1 min without anesthetization. Small Intestine meridian 5 acupoint (SI5) is located on the posteromedial aspect of the wrist, in the depression between the triquetral bone and the ulnar styloid process.[47] Heart meridian 7 acupoint (HT7) is located on the anteromedial aspect of the wrist, radial to the flexor carpi ulnaris tendon, on the palmar wrist crease.[47,48] Locations of acupoints anatomically corresponded to the points in human.[49]

Acupuncture needle (0.18 mm diameter and 8 mm length, Dongbang Medical Co., Qingdao, China) was inserted vertically to a depth of 2–3 mm into each acupoint and stimulation was delivered by twisting the needle at a frequency of 2 times/*sec* for 10 s when inserted and withdrawn immediately after an intraperitoneal injection of METH. To minimize stress during acupuncture, daily handling was given for 2–3 min. The normal group received the same treatment with acupuncture group without needle stimulation.

2.8. Statistical analysis

Data were parametric and were analyzed by one- or two-way repeated measurement analysis of variance (ANOVA) followed by post hoc Tukey test or unpaired *t*-test using Prism 7 program (GraphPad). Data were presented as mean ± standard error of the mean (SEM) and P values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Effects of combination with JGJ and acupuncture at SI5 on the METH's reinforcement: locomotor activity

Systemic injection of METH (1 mg/kg) increased locomotor activity, which lasted up to about 60 min compared to the normal group (Fig. 1B, $^{*}P < 0.05$). However METH-induced increase of the locomotor activity was significantly inhibited by JGJ (Fig. 1B, $^{\#}P < 0.05$, two-way ANOVA, $F_{(24,\ 120)} = 15.42$). Also, the JGJ itself did not affect the locomotor activity in the saline-treated normal rats (Fig. 1B–D).

In addition, acupuncture at SI5, but not at HT7, enhanced inhibitory effect of JGJ on the increase of locomotor activity by METH (Fig. 1E–G, $^{*}P < 0.05$, two-way ANOVA, $F_{(16,\ 80)} = 5.456$), showing a synergistic effect.

3.2. Effects of combination of JGJ and SI5 acupuncture on the METH's reinforcement: USVs

To investigate the effects of JGJ on the METH-induced excitatory affective states, the 50-kHz USV call was measured. Acute administration of METH significantly increased the number of 50-kHz USV calls for 60 min, compared to normal group (Fig. 2C, $^{*}P < 0.05$). However the increase was significantly inhibited by the JGJ (Fig. 2B, $^{\#}P < 0.05$, two-way ANOVA, $F_{(24,120)}=5.354$). And this inhibitory effect of JGJ was not shown in the normal group (Fig. 2B and C).

Also, the inhibitory effect of JGJ was enhanced by the acupuncture at SI5, (Fig. 2D, two-way ANOVA, $F_{(16,\ 80)}=2.259$; Fig. 2E, one-way ANOVA, $F_{(5,\ 10)}=0.062$, $P < 0.05$).

3.3. Involvement of CeA in the inhibitory effects of the JGJ on the METH's reinforcing effect

Locomotor activity and USVs were measured in the same manner with the previous experiment to investigate whether CeA was involved in the inhibitory effect of JGJ. METH significantly increased locomotor activity (Fig. 3B, $^{*}P < 0.05$), which lasted for 60 min, and JGJ suppressed this increase. However, this inhibitory effect of JGJ was reversed by the pretreatment with the GABA mixture into the CeA, implying that activation of the CeA underlied JGJ's effect (Fig. 3B; two-way ANOVA, $F_{(40,477)}=4.527$, $^{\#}P < 0.05$, METH+JGJ vs. METH; $^{\$}P < 0.05$, GABA+METH+JGJ vs. METH+JGJ). The GABA only treatment produced no change from the normal group.

The MSG+METH group was assigned to exam if activation of the CeA could mimic the JGJ's inhibitory effect on the METH's reinforcement. MSG showed a similar effect with the JGJ (Fig. 3B–D).

Likewise, number of the 50-kHz USV calls increased by METH and was suppressed by JGJ. And microinjection of GABA mixture into the CeA reversed the inhibitory effect of JGJ (Fig. 3E and F; two-way ANOVA, $F_{(40,477)}=1.31$, $^{*}P < 0.05$, METH vs. Normal; $^{\#}P < 0.05$, METH+JGJ vs. METH; $^{\$}P < 0.05$, GABA+METH+JGJ vs. METH+JGJ). Unlikely locomotion, MSG did not show an inhibition pattern against METH (Fig. 3E and F).

3.4. Immunohistochemical analysis; TH expression in the VTA

In the immunofluorescence examination of the VTA slices, JGJ inhibited the increase of TH expression by METH, and MSG mimicked the JGJ effect. Also, microinjection with GABA reversed JGJ's inhibition, in parallel with the behavioral data.

In addition, the combination with SI5 acupuncture showed a synergistic effect compared to the JGJ only.

On the other hand, the GABA only and the JGJ+SI5 combination did not result in a significant difference from the normal group (Fig. 4B; one-way ANOVA, $F_{(7,57)}=232.6$, $^{*}P < 0.05$, METH vs. Normal ;$^{\#}P < 0.05$, METH+JGJ vs. METH; $^{\$}P < 0.05$, GABA+METH+JGJ vs. METH+JGJ; $^{@}P < 0.05$, METH+JGJ+SI5 vs. METH+JGJ).

3.5. Effect of JGJ on the electrically stimulated DA release in the NAc

To confirm the data of behaviors and TH expression electrically using measurement of DA neuron activation in the mesolimbic system, the FSCV was applied to the NAc.

METH increased the peak amplitude of DA release in the NAc by 207.6 ± 21.4 % at 16 min compared to the baseline and remained elevated for up to 50 min. This increase of DA release was attenuated by 124.8 ± 4.8 % by JGJ (Fig. 5D, two-way ANOVA, $F_{(192,\ 1152)}=1.998$, $^{*}P < 0.05$, METH vs. Normal; $^{\#}P < 0.05$, METH+JGJ vs. METH).

Also, MSG mimicked JGJ's inhibition of the METH's reinforcement, and the GABA mixture blocked JGJ's inhibition but not statistically significant.

The combination with SI5 acupuncture enhanced the JGJ's inhibition in the later period but the difference was not statistically significant.

4. Discussion

The present study found that JGJ inhibited the increases of locomotor activity and USV calls by METH and that SI5 acupuncture enhanced these inhibitory effects when combined with JGJ. Also, JGJ's inhibition was mediated, at least in part, through the activation of CeA, as shown in our previous study of acupuncture.[50]

At first, we investigated whether JGJ had inhibitory effects on METH. METH increased the locomotor activity and the number of USV calls (Figs. 1A-C and 2C and D), in parallel with the previous studies,[35,51] and JGJ significantly attenuated these increases. The pattern of increase of the 50-kHz USV calls was similar with that of the locomotor activity, as shown in our previous study.[26] These results suggest that JGJ has inhibitory effects on the METH's reinforcement. JGJ was recently reported to be effective for cancers and atopic disease.[19,22] Most of all, JGJ showed detoxifying effect on the alcohol-induced liver damage.[52] Therefore, we speculated it could be effective for the METH, and indeed, JGJ inhibited METH. And interestingly, JGJ did not show an inhibitory effect on the locomotor activity and USV calls in the normal group which was treated with saline, implying that JGJ selectively

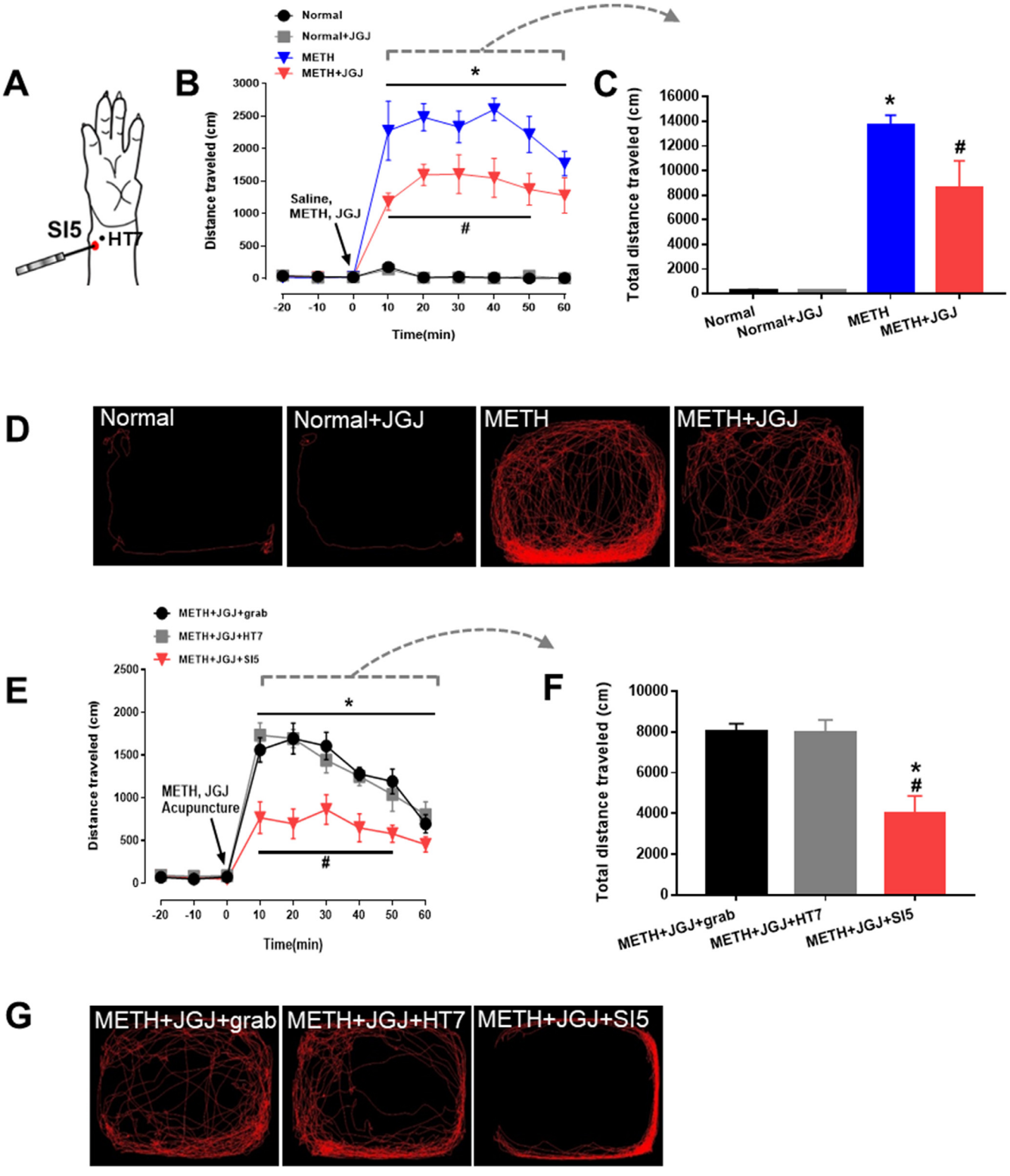

Fig. 1. Effect of JGJ and acupuncture on METH-induced increase of locomotor activity. After basal activity for 20 min, rats were orally given saline or JGJ prior to systemic injection of saline or METH and acupuncture treatment. (A) Schematic of the locations of SI5 and HT7 acupoints in rat; (B-D) Inhibitory effects of JGJ on METH-induced increase of locomotor activity: (B) Traveled-distance according to the time course, (C) Total traveled-distance ($^{*}P < 0.05$, METH vs. Normal; $^{\#}P < 0.05$, JGJ vs. METH), (D) Representative moving trace during 60 min after METH; (E-G) Synergistic effects of the combination of JGJ and acupuncture on METH-induced increase of locomotor activity: (E) Traveled-distance according to the time course, (F) Total traveled-distance ($^{*}P < 0.05$, METH+JGJ+SI5 vs. METH+JGJ+HT7; $^{\#}P < 0.05$, METH+JGJ+SI5 vs. METH+JGJ+grab), (G) Representative moving trace during 60 min after METH. Each group $n = 6$.

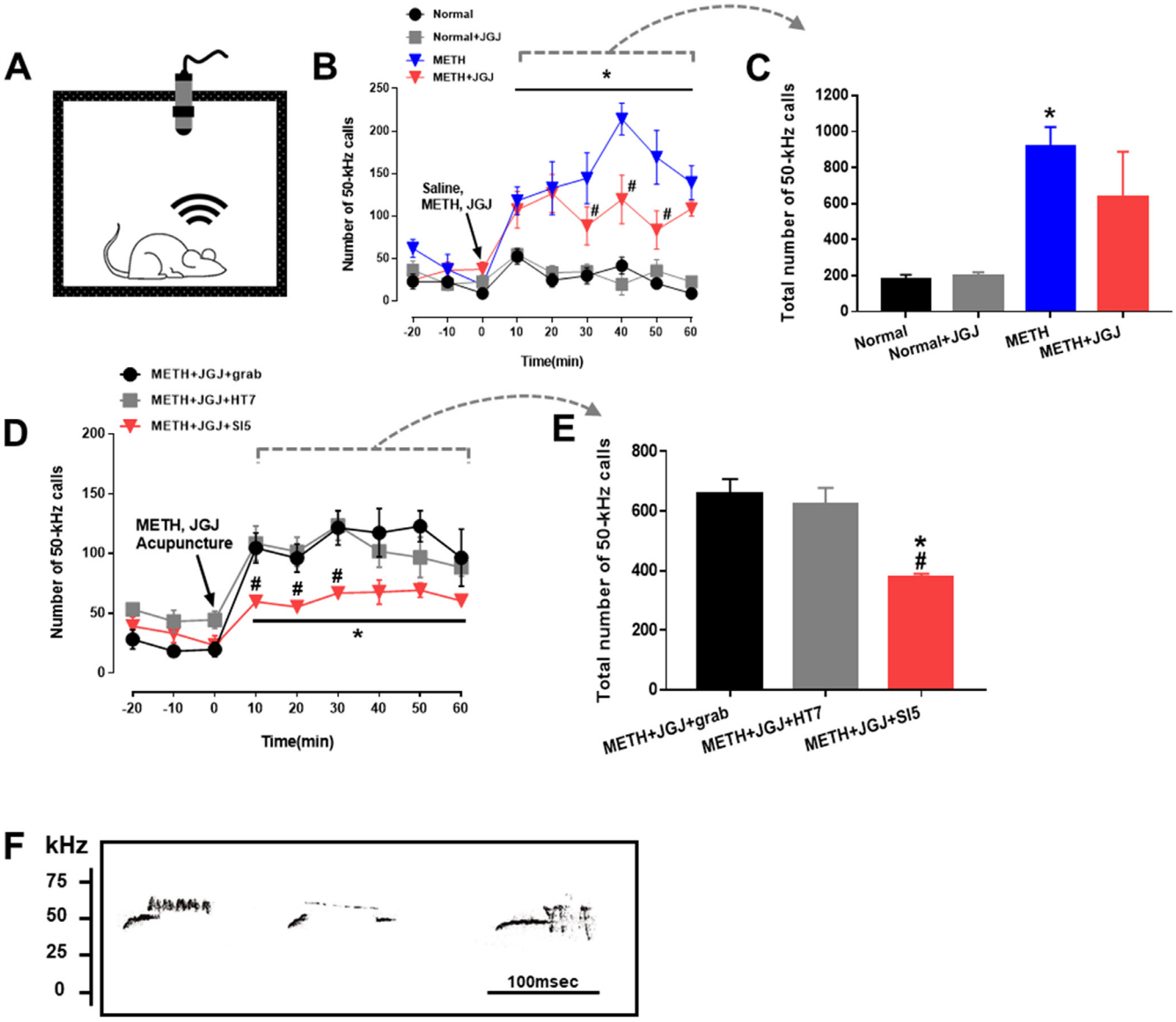

Fig. 2. Effect of JGJ and acupuncture on METH-induced increase of USV calls. After basal activity for 20 min, rats were orally given saline or JGJ prior to systemic injection of saline or METH and acupuncture treatment. (A) Schematic illustration showing paradigm of USV recording in a freely moving rat; (B-C) Effect of JGJ on METH-induced increase of USV call numbers: (B) 10 min time course, (C) Total number for 60 min ($^{*}P < 0.05$ METH vs. Normal, $^{\#}P < 0.05$ METH+JGJ vs. METH); (D-E) Synergistic effects of the combination of JGJ and acupuncture on METH-induced increase of USV calls: (D) 10 min time course, (E) Total number for 60 min ($^{*}P < 0.05$, METH+JGJ+SI5 vs. METH+JGJ+grab; $^{\#}P < 0.05$ METH+JGJ+SI5 vs. METH+JGJ+HT7); (F) Representative spectrogram of 50 kHz USV call following METH injection. Each group $n = 6$.

acted on the METH (Figs. 1B–D and 2B and C). These results of selective effectiveness are similar with those of previous studies[53,54] and this phenomena is thought to be because those Oriental Medicine therapies produce effects by normalizing an abnormal status,[49] not by acting in one way of activation or inhibition, i.e. Oriental Medicine seeks for the harmony or homeostasis.[12] Therefore, its treatments may not effective in normal status.

More importantly, JGJ produced a synergistic effect when combined with acupuncture. Acupuncture at SI5 enhanced the JGJ's inhibition of locomotor activity and USV calls (Figs. 1E–G and 2D–F). In other study also, a combination of acupuncture and herb medication has shown synergistic effects.[55] Actually, these two treatments are often administered together in Oriental Medicine clinics of Eastern Asia.[56] However, few studies have demonstrated scientifically the synergistic effects of the combination, and thus this study is meaningful.

Also, acupuncture at SI5 but not at HT7 showed the synergistic effects, indicating that this acupuncture effect was not from mechanical needle stimulation. SI5 has been used to control mental unease or emotion-related diseases.[47,57] It reduces cravings for drug, locomotor activity, and c-Fos expression induced by abused drugs such as morphine. .[58,59,60] This point specificity has been shown frequently in our previous studies.[53,58,59,61] And, this study has demonstrated for the first time that the point specificity works also in the synergistic effect which is produced by the combination with herb.

The CeA has been focused in many studies about drug addiction and has been revealed to play an important role in the METH's reinforcement, although the role in detail is controversial.[50,62,63] In our previous study also, the acupuncture's inhibition of METH's reinforcement was mediated through the activation of CeA[50] and therefore we investigated whether the CeA was involved in the JGJ's inhibition of METH's rein-

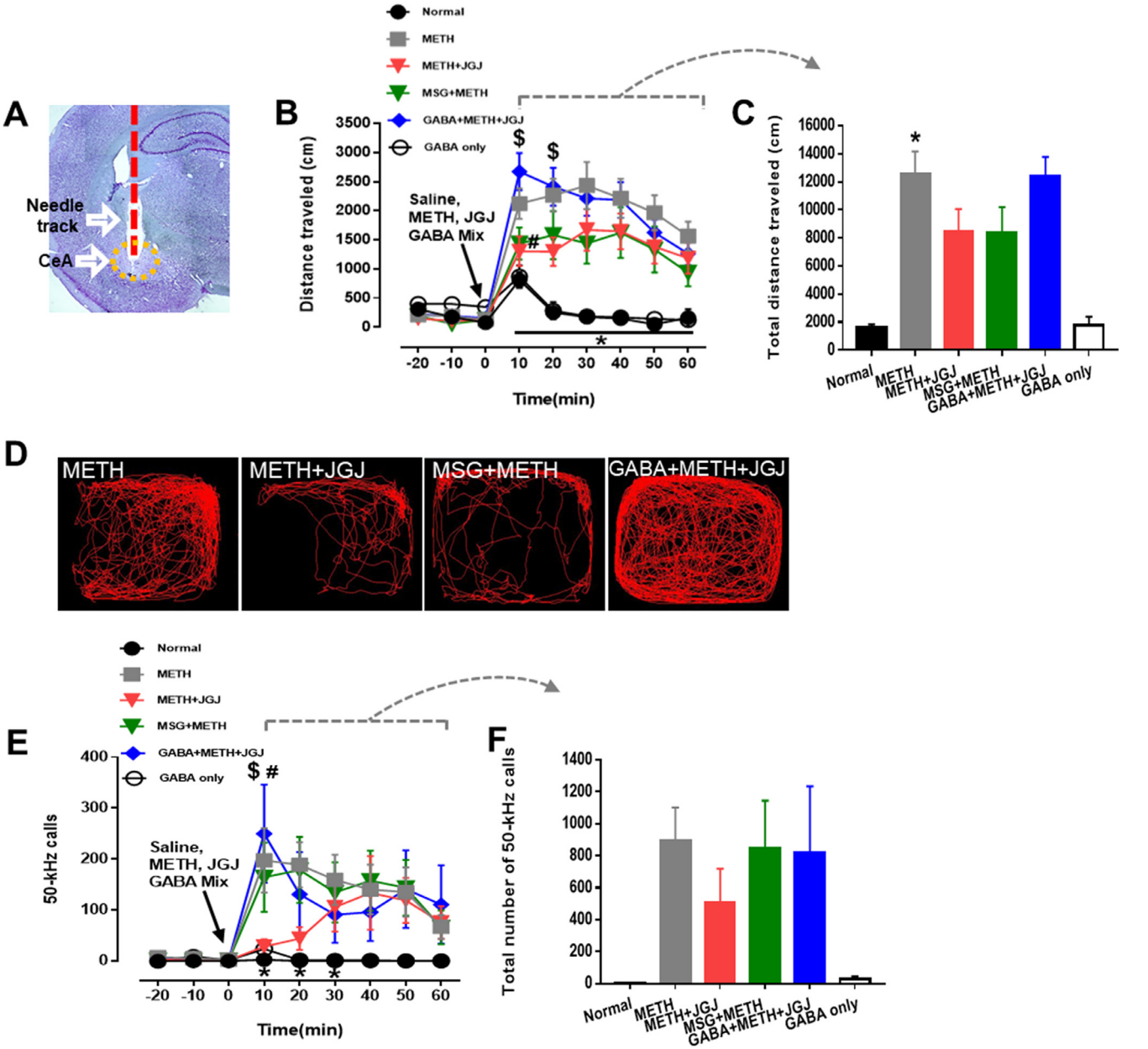

Fig. 3. Involvement of the CeA in the inhibitory effects of JGJ on METH's reinforcement. (A) Track of inserted Guide cannula in CeA stained with Toluidine blue; (B-C) Involvement of the CeA in the inhibitory effects of JGJ on the increase of locomotor activity by METH: (B) Time course, (C) Total moving distance; (D) Representative moving patterns; (E-F) Involvement of the CeA in the inhibitory effects of JGJ on the increase of 50-kHz USV calls by METH: (E) Time course, (F) Total numbers. $^{*}P < 0.05$, METH vs. Normal; $^{\#}P < 0.05$, METH+JGJ vs. METH; $^{\$}P < 0.05$ GABA+METH+JGJ vs. METH+JGJ. Normal group, $n = 10$; METH group, $n = 10$; METH+JGJ group, $n = 8$; MSG+METH group, $n = 11$; GABA+METH+JGJ group, $n = 10$; GABA only group, $n = 10$.

forcement. Our results showed that inactivation of CeA by pretreatment with GABA agonists mixture[62] blocked the JGJ's inhibition of METH (Fig. 3). This indicates that JGJ's inhibition of METH was mediated, at least in part, through the activation of CeA. In addition, we microinjected the MSG to activate CeA and MSG mimicked JGJ, suggesting that inhibition of the METH requires activation of the CeA. These results suggest that the CeA is involved in the METH's reinforcement and that JGJ's inhibitory effects are mediated through the activation of CeA, as shown in our previous study that showed acupuncture's inhibition of METH was mediated via activation of CeA.

CeA, belonging to the extended amygdala, serves as an integrative hub for behavioral and physiological responses.[64,65] It is related to not only emotional and motivational problems but also substance abuse.[66] The major neuron of CeA is GABAergic[64] and it modulates mesolimbic DA system serving as the reward center in the drug abuse[67,68,69] through the GABAergic projection to the VTA.[70] Taken together, our results of the JGJ's inhibition of METH may be because JGJ activated the CeA and the activated-GABAergic inhibitory input to the VTA DA pathway attenuated the METH's reinforcing effect.

To immunohistochemically confirm the behavioral data, TH expression was examined as evidence of DA neuron activity.[23] In the mesolimbic system, the DA neuron projecting from the VTA to the NAc is known to play a key role for the psychostimulants' reinforcing effects. As shown

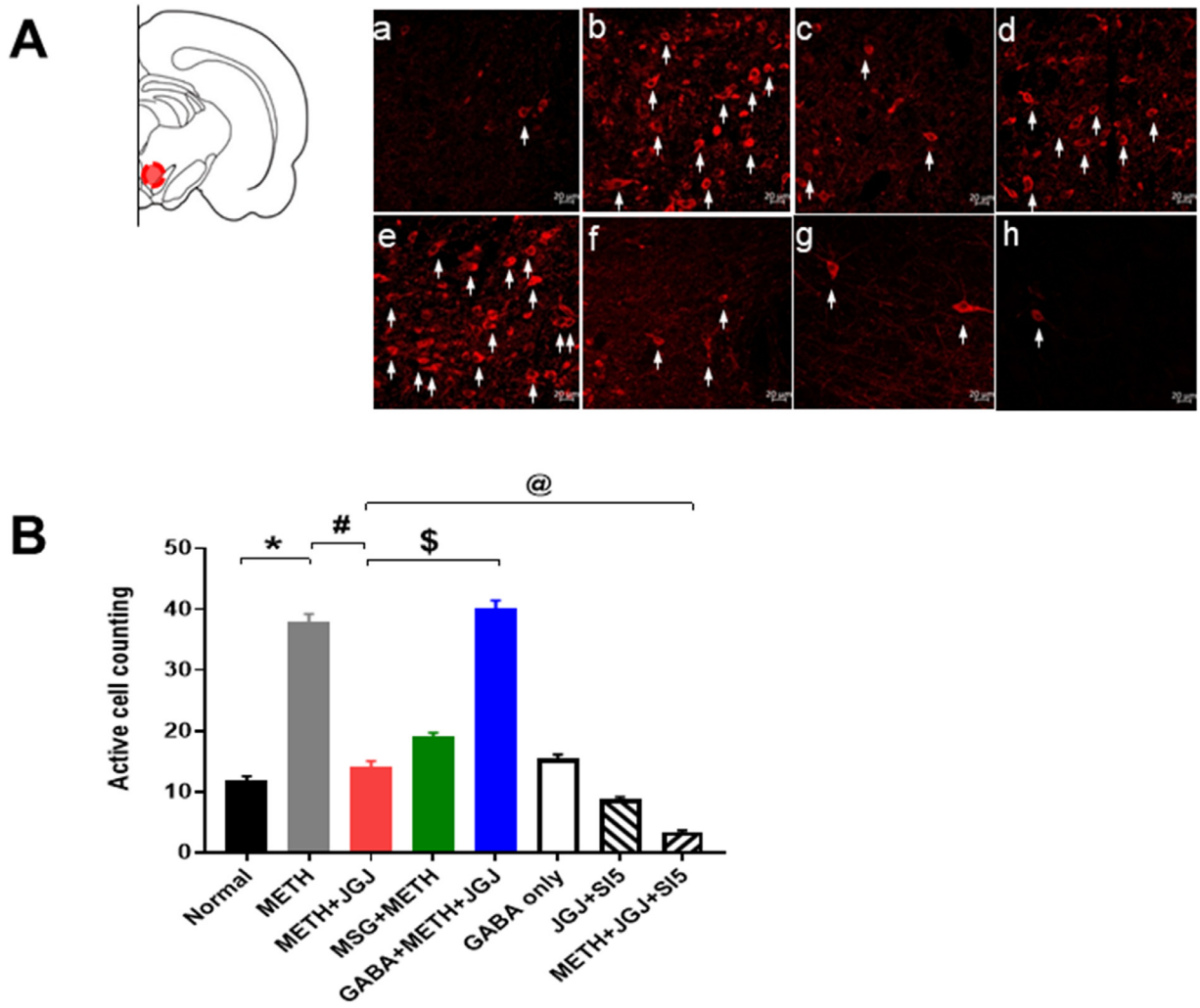

Fig. 4. TH expression in the VTA. (A) Representative TH expression images. a: Normal, b: METH, c: METH+JGJ, d: MSG+METH, e: GABA+METH+JGJ, f: GABA only, g: JGJ+SI5, h: METH+JGJ+SI5. Scale bar=20 μm; (B) Number of TH reactive cells ($P < 0.05$). $^{*}P < 0.05$, METH vs. Normal; $^{\#}P < 0.05$, METH+JGJ vs. METH; $^{\$}P < 0.05$, GABA+METH+JGJ vs. METH+JGJ; $^{@}P < 0.05$, METH+JGJ+SI5 vs. METH+JGJ. Each group $n = 8$.

in the locomotor activity and 50-kHz USV calls, TH expression was increased by METH and reduced by JGJ. And this reduction by JGJ was enhanced by the combination with SI5 acupuncture, suggesting the synergistic effect. In addition, MSG mimicked JGJ and the GABA mixture blocked the JGJ's inhibition (Fig. 4), supporting the behavioral data.

Thereafter, we electrically measured DA neuron activity using FSCV[24] for additional confirmation. Likewise, METH increased DA release in NAc, and JGJ significantly reduced this increase by METH. These results suggest that JGJ attenuated METH-induced increases of locomotor activity and 50-kHz USV calls by reducing DA release in the NAc. Taken together with the TH expression, it is thought that the decrease of DA neuron activity in the mesolimbic system underlies JGJ's inhibition of METH.

In addition, MSG mimicked JGJ's effect, suggesting that inhibition of METH requires the activation of CeA. GABA reversed JGJ's inhibition, and SI5 was synergistic with JGJ, showing similar pattern with the TH expression and behavioral data, although it is not that all of the differences in the FSCV were statistically significant.

One more interesting thing is, Zarrindast et al.[71] showed that intra-CeA injection of nicotine resulted in the anxiogenic-like behavior and the authors questioned whether activation of CeA by nicotine reduced VTA DA impulse flow. Here our TH and FSCV data showed that METH-induced increase of DA neuron activity measured in the VTA and NAc was inhibited by the activation of CeA with JGJ or MSG.

In summary, the present study demonstrated that JGJ attenuated the METH's reinforcement, and that this inhibition was mediated through the activation of CeA, in parallel with our previous study on acupuncture. Also, the combination with acupuncture at SI5 enhanced JGJ's inhibition, suggesting that a combination of herb medication and acupuncture produced synergistic effect compared to the herb alone.

This study has some limitations. First, it would be better if the effects of JGJ was studied in more detail i.e. the most effective single compound was investigated. Also, the neuronal network in the brain is very complex, therefore other neuroscientific mechanisms in addition to the CeA should be further studied, regarding to the synergistic inhibitory effects of JGJ and SI5.

In conclusion, this study suggests that JGJ and SI5 acupuncture can be considered as useful treatments for METH addiction and that their combination is better than alone. Further researches should follow this study to confirm the usefulness of the combination of herb and acupuncture for the treatment of drug addiction.

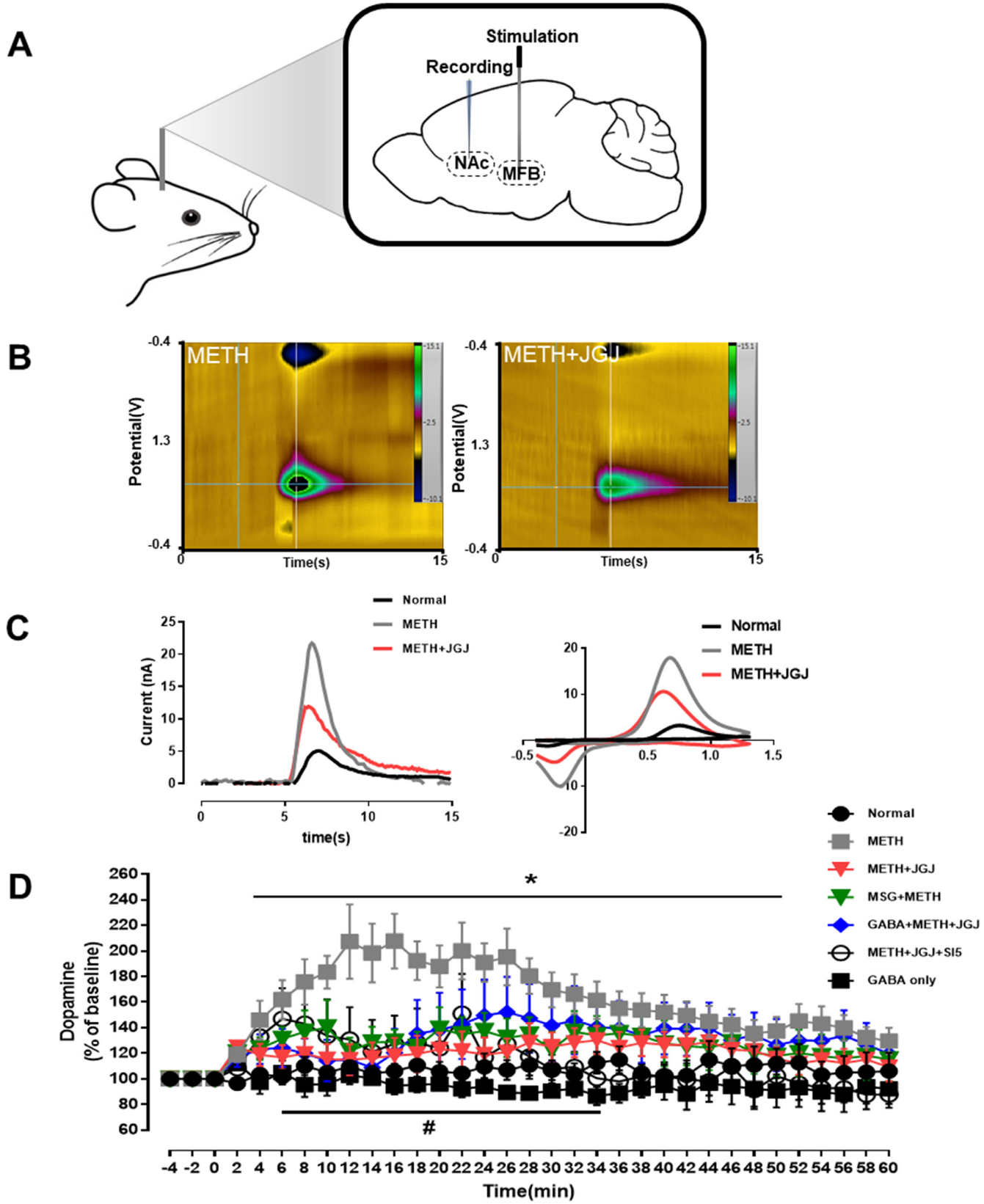

Fig. 5. Effect of JGJ and acupuncture on the electrically stimulated-DA releases in the NAc. (A) Schematic of FSCV for recording of NAc DA release; (B) Pseudo-color plots by electrical stimulation in the NAc; (C) Current vs. time (Lt.) and representative superimposed cyclic voltammogram (Rt.) of DA release plots; (D) Effect of JGJ on the electrically stimulated DA release in the NAc. ($^{*}P < 0.05$, METH vs. Normal, $^{\#}P < 0.05$, METH+JGJ vs. METH). Each group $n = 7$.

Declaration of competing interest

The authors declare no competing interests.

CRediT authorship contribution statement

Jin Gyeom Lee: Data curation, Formal analysis, Investigation, Writing – review & editing. **Yuchi Li:** Data curation, Formal analysis, Investigation. **Nam Jun Kim:** Data curation, Formal analysis, Investigation. **Han Byeol Jang:** Data curation, Formal analysis, Investigation. **Chae Ha Yang:** Data curation, Formal analysis, Investigation. **Hee Young Kim:** Data curation, Formal analysis, Investigation. **Seong Shoon Yoon:** Data curation, Formal analysis, Investigation. **Suchan Chang:** Data curation, Formal analysis, Investigation. **Seon-Ju Jeong:** Data curation, Formal analysis, Investigation. **Sang Chan Kim:** Data curation, Formal analysis, Investigation. **Bok Suk Sa:** Data curation, Formal analysis, Investigation. **Bong Hyo Lee:** Conceptualization, Writing – review & editing, Funding acquisition.

Funding

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2018R1A5A2025272, 2020R1A2C1103154), and the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HF22C0081).

Ethical statement

This research was approved by the Institutional Animal Care and Use Committee (IACUC) of Daegu Haany University, Daegu, Korea (DHU2021-032).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Brecht ML, Lovinger K, Herbeck DM, Urada D. Patterns of treatment utilization and methamphetamine use during first 10 years after methamphetamine initiation. *J Subst Abuse Treat*. 2013;44(5):548–556.
2. Kim KY, Ekpeghere KI, Jeong HJ, Oh JE. Effects of the summer holiday season on UV filter and illicit drug concentrations in the Korean wastewater system and aquatic environment. *Environ Pollut*. 2017;227:587–595.
3. Coutinho A, Flynn C, Burdo TH, Mervis RF, Fox HS. Chronic methamphetamine induces structural changes in frontal cortex neurons and upregulates type I interferons. *J Neuroimmune Pharmacol*. 2008;3(4):241–245.
4. Chang L, Alicata D, Ernst T, Volkow N. Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. *Addiction*. 2007;102(Suppl 1):16–32.
5. Shoptaw SJ, Kao U, Heinzerling K, Ling W. Treatment for amphetamine withdrawal. *Cochrane Database Syst Rev*. 2009;2009(2):Cd003021.
6. Faure P, Tolu S, Valverde S, Naudé J. Role of nicotinic acetylcholine receptors in regulating dopamine neuron activity. *Neuroscience*. 2014;282:86–100.
7. Stoops WW, Rush CR. Combination pharmacotherapies for stimulant use disorder: a review of clinical findings and recommendations for future research. *Expert Rev Clin Pharmacol*. 2014;7(3):363–374.
8. Forray A, Sofuoglu M. Future pharmacological treatments for substance use disorders. *Br J Clin Pharmacol*. 2014;77(2):382–400.
9. Chan B, Freeman M, Kondo K, Ayers C, Montgomery J, Paynter R, et al. Pharmacotherapy for methamphetamine/amphetamine use disorder-a systematic review and meta-analysis. *Addiction*. 2019;114(12):2122–2136.
10. Ru Q, Xiong Q, Tian X, Xu C, Li C, Chen L, et al. Candidate Chinese herbal medicine alleviates methamphetamine addiction via regulating dopaminergic and serotonergic pathways. *Front Mol Neurosci*. 2022;15:874080.
11. Jang EY, Choe ES, Hwang M, Kim SC, Lee JR, Kim SG, et al. Isoliquiritigenin suppresses cocaine-induced extracellular dopamine release in rat brain through GABA(B) receptor. *Eur J Pharmacol*. 2008;587(1–3):124–128.
12. Ma Y, Zhou K, Fan J, Sun S. Traditional Chinese medicine: potential approaches from modern dynamical complexity theories. *Front Med*. 2016;10(1):28–32.
13. Lee JK, Kim JH, Shin HK. Therapeutic effects of the oriental herbal medicine Sho–saiko-to on liver cirrhosis and carcinoma. *Hepatol Res*. 2011;41(9):825–837.
14. Kang SC, Lee CM, Choi H, Lee JH, Oh JS, Kwak JH, et al. Evaluation of oriental medicinal herbs for estrogenic and antiproliferative activities. *Phytother Res*. 2006;20(11):1017–1019.
15. Jeon SG, Song EJ, Lee D, Park J, Nam Y, Kim JI, et al. Traditional oriental medicines and Alzheimer's disease. *Aging Dis*. 2019;10(2):307–328.
16. Lim JH, Jeong JH, Kim SH, Kim KO, Lee SY, Lee SH, et al. The pilot survey of the perception on the practice pattern, diagnosis, and treatment on korean medicine insomnia: focusing on the difference between Korean Medical Neuropsychiatry Specialists and Korean Medical General Practitioners. *Evid Based Complement Alternat Med*. 2018;2018:9152705.
17. Kim SR, Lee HW, Jun JH, Ko BS. Effects of herbal medicine (Gan Mai Da Zao Decoction) on several types of neuropsychiatric disorders in an animal model: a systematic review: herbal medicine for animal studies of neuropsychiatric diseases. *J Pharmacopuncture*. 2017;20(1):5–9.
18. Kim J-W, Jeon S-H, Jung H-S, Kang K-H, Lee I-S, Lee S-H, et al. HyungSang medical approach to detoxification. *J Physiol Pathol Korea Med*. 2009;23(2):278–287.
19. Park IW, Kim HY, Cheon JH, Shih Y-h, Sah BS, Kim KB. Case series study about the effect of Jageum-Jung on atopic dermatitis. *J Pediatr Korea Med*. 2018;32(1):11–18.
20. Cho Y-k, Jeon J-y, Shin Y-j, Seol J-k, Rhee J-h, Won J-h, et al. Induction of apoptosis and cell cycle arrest by Jageum-Jung in HepG2 hepatoma cells. *J Intern Korea Med*. 2007;28(4):694–708.
21. Park I-d, Son C-g, Cho C-k. A literal study of anti-tumor effects of Jakeumjung. *J Haehwa Med*. 2002;10(2):73–81.
22. Yang JH, Do HJ, Lee E, Yim NH, Cho WK, Park KI, et al. Jageum-Jung improves 2,4-dinitrochlorobenzene-induced atopic dermatitis-like skin lesions in mice and suppresses pro-inflammatory chemokine production by inhibiting TNF-α/IFN-γ-induced STAT-1 and NFκB signaling in HaCaT cells. *J Ethnopharmacol*. 2018;221:48–55.
23. Coyle JT, Axelrod J. Tyrosine hydroxylase in rat brain: developmental characteristics. *J Neurochem*. 1972;19(4):1117–1123.
24. Venton BJ, Cao Q. Fundamentals of fast-scan cyclic voltammetry for dopamine detection. *Analyst*. 2020;145(4):1158–1168.
25. Paxinos G, Watson C, Pennisi M, Topple A. Bregma, lambda and the interaural midpoint in stereotaxic surgery with rats of different sex, strain and weight. *J Neurosci Method*. 1985;13(2):139–143.
26. Kim NJ, Ryu Y, Lee BH, Chang S, Fan Y, Gwak YS, et al. Acupuncture inhibition of methamphetamine-induced behaviors, dopamine release and hyperthermia in the nucleus accumbens: mediation of group II mGluR. *Addict Biol*. 2019;24(2):206–217.
27. Taguchi K, Atobe J, Kato M, et al. The effect of methamphetamine on the release of acetylcholine in the rat striatum. *Eur J Pharmacol*. 1998;360(2–3):131–137.
28. Tamijani SMS, Valian N, Heravi M, Ahmadiani A, Beirami E, Dargahi L. Implication of thyroid hormone receptors in methamphetamine neurocognitive effects. *Neurotoxicology*. 2022;90:130–135.
29. Zaretsky DV, Zaretskaia MV, Samuels BC, Cluxton LK, DiMicco JA. Microinjection of muscimol into raphe pallidus suppresses tachycardia associated with air stress in conscious rats. *J Physiol*. 2003;546(Pt 1):243–250.
30. Rusyniak DE, Zaretskaia MV, Zaretsky DV, DiMicco JA. Microinjection of muscimol into the dorsomedial hypothalamus suppresses MDMA-evoked sympathetic and behavioral responses. *Brain Res.*. 2008;1226:116–123.
31. Landulpho CD, Dias AC, Colombari E. Cardiovascular mechanisms activated by microinjection of baclofen into NTS of conscious rats. *Am J Physiol Heart Circ Physiol*. 2003;284(3):H987–H993.
32. Hammond DL, Nelson V, Thomas DA. Intrathecal methysergide antagonizes the antinociception, but not the hyperalgesia produced by microinjection of baclofen in the ventromedial medulla of the rat. *Neurosci Lett*. 1998;244(2):93–96.
33. Köhler C, Schwarcz R. Monosodium glutamate: increased neurotoxicity after removal of neuronal re-uptake sites. *Brain Res*. 1981;211(2):485–491.
34. Hedges DM, Obray JD, Yorgason JT, Jang EY, Weerasekara VK, Uys JD, et al. Methamphetamine induces dopamine release in the nucleus accumbens through a sigma receptor-mediated pathway. *Neuropsychopharmacology*. 2018;43(6):1405–1414.
35. Jang EY, Yang CH, Hedges DM, Kim SP, Lee JY, Ekins TG, et al. The role of reactive oxygen species in methamphetamine self-administration and dopamine release in the nucleus accumbens. *Addict Biol*. 2017;22(5):1304–1315.
36. Paxinos G, Watson C. *The Rat Brain in Stereotaxic coordinates: Hard Cover Edition*. Elsevier; 2006.
37. Kelly PA, McCulloch J. Effects of the putative GABAergic agonists, muscimol and THIP, upon local cerebral glucose utilisation. *J Neurochem*. 1982;39(3):613–624.
38. Yoon SS, Lee BH, Kim HS, et al. Potential roles of GABA receptors in morphine self--administration in rats. *Neurosci Lett*. 2007;428(1):33–37.
39. Hao XJ, Xu JX, Aldskogius H, Seiger Å, Wiesenfeld-Hallin Z. Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. *Pain*. 1991;45(2):175–185.
40. Yang B, Wang BF, Lai MJ, et al. Differential involvement of GABAA and GABAB receptors in propofol self-administration in rats. *Acta Pharmacol Sin*. 2011;32(12):1460–1465.
41. Domi E, Xu L, Toivainen S, et al. Activation of GABA(B) receptors in central amygdala attenuates activity of PKCdelta + neurons and suppresses punishment-resistant alcohol self-administration in rats. *Neuropsychopharmacology*. 2023;48(9):1386–1395.
42. Filip M, Frankowska M. Effects of GABA(B) receptor agents on cocaine priming, discrete contextual cue and food induced relapses. *Eur J Pharmacol*. 2007;571(2–3):166–173.
43. Vengeliene V, Takahashi TT, Dravolina OA, et al. Efficacy and side effects of baclofen and the novel GABA(B) receptor positive allosteric modulator CMPPE in animal models for alcohol and cocaine addiction. *Psychopharmacol (Berl)*. 2018;235(7):1955–1965.

44. Tunc-Ata M, Altintas F, Senol H, Nizamoglu E, Kucukatay V. Ileal interposition improves metabolic syndrome parameters in a rat model of metabolic syndrome induced by monosodium glutamate. *Life Sci*. 2021;266:118846.
45. Rojas-Castañeda JC, Vigueras-Villaseñor RM, Chávez-Saldaña M, et al. Neonatal exposure to monosodium glutamate induces morphological alterations in suprachiasmatic nucleus of adult rat. *Int J Exp Pathol*. 2016;97(1):18–26.
46. Lee B, Yang CH, Hahm D-H, Choe ES, Lee H-J, Pyun K-H, et al. Inhibitory effects of Coptidis rhizoma and berberine on cocaine-induced sensitization. *Evid-Base Complement Alternat Med*. 2009;6:85–90.
47. Deadman P, Al-Khafaji M, Baker K. A manual of acupuncture. *Hove: J Chin Med*. 2005.
48. Pacific WHOROftW. *WHO Standard Acupuncture Point Locations in the Western Pacific Region*. World Health Organization; 2008.
49. Lee MY, Lee BH, Kim HY, Yang CH. Bidirectional role of acupuncture in the treatment of drug addiction. *Neurosci Biobehav Rev*. 2021;126:382–397.
50. Kim MS, Fan Y, Lee SM, Chang SC, Kim HK, Ryu Y, et al. Role of the central amygdala in acupuncture inhibition of methamphetamine-induced behaviors in rats. *Addict Biol*. 2021;26(1):e12862.
51. Mahler SV, Moorman DE, Feltenstein MW, Cox BM, Ogburn KB, Bachar M, et al. A rodent "self-report" measure of methamphetamine craving? Rat ultrasonic vocalizations during methamphetamine self-administration, extinction, and reinstatement. *Behav Brain Res*. 2013;236(1):78–89.
52. Kim K-Y, Park K-I, Cho W-K, Ma J-Y. Liver protective effects of Jageum-Jung in alcohol-induced liver injury mice model. *Herbal Formul Sci*. 2020;28(2):179–187.
53. Kim YH, Zhao RJ, Lee SM, Kim MS, Lim SC, Kim JS, et al. Acupuncture inhibits reinstatement of intravenous methamphetamine self-administration via gamma aminobutyric acid pathway. *Neuroreport*. 2020;31(4):352–358.
54. Hu X, Sato J, Oshida Y, Xu M, Bajotto G, Sato Y. Effect of Gosha-jinki-gan (Chinese herbal medicine: niu-Che-Sen-Qi-Wan) on insulin resistance in streptozotocin-induced diabetic rats. *Diabetes Res Clin Pract*. 2003;59(2):103–111.
55. Wu X, Zhou Y, Yin F, Dai G, Li L, Xu B, et al. Comparative pharmacokinetics and tissue distribution of schisandrin, deoxyschisandrin and schisandrin B in rats after combining acupuncture and herb medicine (schisandra chinensis). *Biomed Chromatogr*. 2014;28(8):1075–1083.
56. Sun P. *The Treatment of Pain with Chinese Herbs and Acupuncture E-Book*. Elsevier Health Sciences; 2010.
57. Ross J. *Acupuncture Point combinations: The key to Clinical Success*. Churchill Livingstone Edinburgh; 1995.
58. Lee BH, Ma JH, In S, Kim HY, Yoon SS, Jang EY, et al. Acupuncture at SI5 attenuates morphine seeking behavior after extinction. *Neurosci Lett*. 2012;529(1):23–27.
59. Lee BH, Zhao RJ, Lee BG, Kim NJ, Yang CH, Kim HY, et al. Acupuncture suppresses morphine craving in progressive ratio through the GABA system. *J Acupunct Meridian Stud*. 2015;8(4):175–182.
60. Choi S-H, Lee B-H, Park I-S, Choi N-H, Kim K-J, Jang E-Y, et al. The effect of acupuncture on methamphetamineinduced locomotor activity and C-fos expression in rat brain. *J Acupunct Res*. 2009;26(1):39–47.
61. Choi YJ, Kim NJ, Zhao RJ, Kim DH, Yang CH, Kim HY, et al. Acupuncture suppresses intravenous methamphetamine self-administration through GABA receptor's mediation. *Neurosci Lett*. 2018;662:65–70.
62. Li X, Zeric T, Kambhampati S, Bossert JM, Shaham Y. The central amygdala nucleus is critical for incubation of methamphetamine craving. *Neuropsychopharmacology*. 2015;40(5):1297–1306.
63. Cates HM, Li X, Purushothaman I, Kennedy PJ, Shen L, Shaham Y, et al. Genome-wide transcriptional profiling of central amygdala and orbitofrontal cortex during incubation of methamphetamine craving. *Neuropsychopharmacology*. 2018;43(12):2426–2434.
64. Gilpin NW, Herman MA, Roberto M. The central amygdala as an integrative hub for anxiety and alcohol use disorders. *Biol Psychiatry*. 2015;77(10):859–869.
65. Hamann SB, Ely TD, Hoffman JM, Kilts CD. Ecstasy and agony: activation of the human amygdala in positive and negative emotion. *Psychol Sci*. 2002;13(2):135–141.
66. Koob GF. A role for brain stress systems in addiction. *Neuron*. 2008;59(1):11–34.
67. Marek R, Strobel C, Bredy TW, Sah P. The amygdala and medial prefrontal cortex: partners in the fear circuit. *J Physiol*. 2013;591(10):2381–2391.
68. Koob GF. The dark side of emotion: the addiction perspective. *Eur J Pharmacol*. 2015;753:73–87.
69. Ahn S, Phillips AG. Modulation by central and basolateral amygdalar nuclei of dopaminergic correlates of feeding to satiety in the rat nucleus accumbens and medial prefrontal cortex. *J Neurosci*. 2002;22(24):10958–10965.
70. Everitt BJ, Cardinal RN, Hall J, Parkinson J, Robbins T. Differential involvement of amygdala subsystems in appetitive conditioning and drug addiction. *Amygdala: A Funct Analy*. 2000:353–390.
71. Zarrindast MR, Eslahi N, Rezayof A, Rostami P, Zahmatkesh M. Modulation of ventral tegmental area dopamine receptors inhibit nicotine-induced anxiogenic-like behavior in the central amygdala. *Prog Neuropsychopharmacol Biol Psychiatry*. 2013;41:11–17.