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Effects of acupuncture on stress-induced relapse to cocaine-seeking in rats

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Abstract

Rationale—Cocaine addiction is associated with high rates of relapse, and stress has been identified as a major risk factor. We have previously demonstrated that acupuncture reduces drug self-administration and dopamine release in the nucleus accumbens (NAc), a brain structure implicated in stress-induced reinstatement of drug-seeking behavior.

Objective—This study was conducted to investigate the effects of acupuncture on footshock-induced reinstatement of cocaine-seeking and the expression of c-Fos and the transcription factor cAMP response element-binding protein (CREB) in the NAc, used as markers of neuronal activation in conditions of stress-induced reinstatement to cocaine.

Methods—Male Sprague–Dawley rats were trained to self-administer cocaine (1.0 mg/kg) for 14 days, followed by extinction and then footshock stress. Acupuncture was applied at bilateral Shenmen (HT7) points for 1 min after footshock stress.

Results and conclusions—Acute footshock stress reinstated cocaine-seeking behavior and enhanced c-Fos expression and phosphorylated CREB (pCREB) activation in the NAc shell in cocaine pre-exposed rats. On the other hand, acupuncture at HT7, but not at control point (LI5), markedly reduced reinstatement of cocaine-seeking (86.5 % inhibition vs. control value), c-Fos expression (81.7% inhibition), and pCREB activation (79.3% inhibition) in the NAc shell. These results suggest that acupuncture attenuates stress-induced relapse by regulating neuronal activation in the NAc shell.

Keywords

Cocaine reinstatement; Footshock; Stress; Acupuncture; Nucleus accumbens; c-Fos; pCREB

Introduction

Cocaine addiction is a chronic, relapsing disease, which causes serious societal and economic problems. One potential risk factor for relapse in humans is exposure to a stressful condition (Goeders 2003). Stress is a potent trigger to reinstate cocaine-seeking behavior in animals (Sinha 2007). Previous findings indicate considerable overlap in neural circuits and substrates involved in stress and drug addiction (Sinha 2001, 2007). First, it has been suggested that the corticotropin-releasing factor (CRF)-containing pathway from the central nucleus of the amygdala to the bed nucleus of the stria terminalis critically contributes to stress-induced relapse to cocaine-seeking (Erb et al. 2001). Second, the neural substrates associated with stress have a significant impact on the function of the mesolimbic dopamine (DA) system. CRF released in the ventral tegmental area (VTA) in response to footshock stress triggers glutamate release in the VTA and reinstates cocaine-seeking behavior (Wang et al. 2005). Stress increases DA release in the nucleus accumbens (NAc), an important nexus of DA projections from the VTA, and reinstates drug-seeking behavior (Shaham et al. 2000), which is reversed by local injection of DA receptor antagonists into the NAc (Xi et al. 2004), suggesting that stress affects NAc DA release leading to relapse. Notwithstanding the promise of these preclinical studies, no current satisfactory medical intervention is available to prevent cocaine relapse.

Acupuncture has proven to be an effective treatment for reducing nausea, pain (for review, see Jindal et al. 2008), and drug abuse (Cui et al. 2008). Acupuncture activates enkephalinergic and β -endorphinergic neurons in the arcuate nucleus of the hypothalamus (Wang et al. 1990a, b), and endorphinergic fibers projecting from the arcuate nucleus can in turn activate opioid receptors (ORs) expressed on GABA neurons in the VTA and NAc (Mansour et al. 1988). Our previous studies have provided strong evidence that acupuncture

at Shenmen (HT7) produces potent suppression on the reinforcing effects of abused drugs, by suppressing DA release via modulation of VTA GABA neurons. For example, HT7 acupuncture suppresses ethanol or morphine self-administration and NAc DA release through modulation of GABA neuro-transmission in morphine- and ethanol-administered rats (Kim et al. 2005; Yang et al. 2010; Yoon et al. 2004, 2010). It also inhibits locomotor activity, DA release, and tyrosine hydroxylase in the NAc associated with acute cocaine challenge (Lee et al. 2009). In addition, we have demonstrated that acupuncture can attenuate stress responses including increases in blood pressure, heart rate, and plasma catecholamine in rats challenged with immobilization stress (Yang et al. 2002). A pilot clinical study reported that bilateral acupuncture at HT7 is effective in reducing psychological stress in patients (Chan et al. 2002). Thus, these findings have led to the hypothesis that acupuncture can suppress stress-induced reinstatement of cocaine-seeking and modulate neuronal activation in the NAc associated with stress-induced relapse.

Here, we investigated whether acupuncture could prevent stress-induced relapse of cocaine-seeking. We examined the effect of acupuncture on stress-induced cocaine-seeking behavior and neuronal activation in the NAc that we would expect to underlie the acupuncture effect on reinstatement responding. After cocaine self-administration, expression of c-Fos and phosphorylated cAMP response element-binding protein (pCREB) activation, used as generalized markers of neuronal activation (Brown et al. 2010; Chen et al. 2006; Erb et al. 2004), were observed in the NAc by immunohistochemistry or in situ hybridization. To our knowledge, this is the first report that acupuncture can attenuate stress-induced cocaine relapse.

Materials and methods

Animals

Male Sprague–Dawley rats (Daehan animal, Seoul, South Korea), weighing 270–320 g at the start of the experiment, were used. Animals were kept on ad libitum food and water and maintained on a 12-h light/dark cycle, except during the food-training period. Experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at the Daegu Haany University.

Self-administration and surgery

Cocaine self-administration training and reinstatement testing were performed in operant conditioning chambers equipped with two response levers (active and inactive levers), as well as house and cue lights (Med Associates, Georgia, VT, USA). Rats were food-restricted (approximately 16 g of lab chow per day) to facilitate the acquisition of operant responding and trained to press a lever for 45 mg food pellets on a continuous fixed ratio 1 reinforcement schedule (FR1) until a criterion level was obtained (100 food pellets for three consecutive days) in the daily 3-h session, as described previously (Bachtell et al. 2005; Boutrel et al. 2005; McFarland et al. 2004). Rats then had ad libitum food access for the remainder of the experiment. One day after food training, a chronic indwelling jugular catheter (Dow Corning, Midland, MI, USA) was surgically implanted into the right jugular vein under intraperitoneal pentobarbital anesthesia (50 mg/kg). After surgery, the catheter

was flushed daily with 0.2 ml of heparinized (30 U/ml) saline containing gentamycin sulfate (0.33 mg/ml) to prevent clogging and infection. The rats were allowed to recover for at least 6 days and then subjected to cocaine self-administration in the daily 3-h session for 7 days per week. Each response on the active lever delivered cocaine intravenously (1.0 mg/kg in 0.1 ml over 5 s) and illumination of a cue light located above the active lever followed by an additional 10-s “timeout” period (TO). During the TO period, house cue lights were turned off and responses on the active and inactive levers were recorded. Once stable self-administration behavior under FR1 schedule was achieved, the response requirement was gradually increased to FR5. Rats remained in training until the establishment of a stable response pattern under baseline conditions (less than 10% variation in total number of responding for three consecutive sessions). Typically, this required 12 to 14 sessions following initiation of cocaine self-administration.

Extinction and reinstatement testing

After the final cocaine self-administration session, the rats were returned to their home cage for 5 days until reinstatement testing, similar to what others have reported previously (Erb et al. 2001; McFarland et al. 2004). Rats received four 60-min extinction sessions with 30-min intervening periods. On the next day, the reinstatement test was begun after two or three 60-min extinction sessions with 30-min intervening periods until they made 15 or fewer responses on the active lever.

During extinction and reinstatement, the same conditions as those of the cocaine self-administration were maintained, except that cocaine was not infused with responses on the active lever. To examine the effects of acupuncture on stress-induced reinstatement to cocaine-seeking, the rats were subjected to 15 min of intermittent (random interval 40 s) exposure to footshock (0.5 mA, 0.5 s duration) and received acupuncture treatment for 1 min after termination of foot-shock. Non-reinforced responses on each lever were recorded for 3 h after exposure to footshock stress and acupuncture.

Food reinforcement

To examine the effects of acupuncture on extinction responding in food-trained rats, the responses on each lever during the extinction condition were measured in separate groups of rats. Rats were initially food-restricted to maintain 85% of initial body weight and trained to self-administer 45 mg food pellets on a FR1 schedule of reinforcement. Food availability was indicated by the illumination of a cue light above the active lever. Food-trained rats self-administered food pellets for 3 h or until a maximum of 100 pellets for 3 h were consumed over three consecutive sessions. Rats reached this criterion around 6–7 days after the start of food training. Following the establishment of the stable responses, the rats were given acupuncture treatment immediately before extinction testing. Responding at both levers was recorded for 3 h. Extinction sessions were identical to food-training sessions, except that food was not available.

Immunohistochemistry for pCREB and c-Fos

In a separate group of animals, pCREB or c-Fos expression was measured in the NAc. Fifteen minutes after footshock stress and acupuncture treatment, brains were removed

under anesthesia, post-fixed with 4% formaldehyde, cryoprotected in 30% sucrose, and cryosectioned at 30 μ m in thickness. Sections were incubated with rabbit anti-pCREB antibody (Ser133; 1:1,000; Cell Signaling Technology, CA, USA) or rabbit anti-c-Fos antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). They were then incubated in goat anti-rabbit secondary antiserum (Vector Labs, CA, USA) followed by avidin–biotin–peroxidase reagents (Vector Labs, CA, USA) and diaminobenzidine (Sigma). Photographs were taken in both core and shell of NAc (between 1.0 and 1.6 mm anterior to bregma) for histological analysis under $\times 100$ objective with a LABOMED light microscope (LABO America Inc., Fremont, CA, USA) equipped with a digital camera (iCAM3000).

Acupuncture treatment

Acupuncture needles (size 0.10 mm in diameter and 7 mm in length) were inserted with manual twisting for 2 s (a rate of two times per second) at a depth of 3 mm, maintained for 1 min and withdrawn for 2 s. This paradigm was successfully used to produce acupuncture effects in our previous studies (Yang et al. 2010; Yoon et al. 2004, 2010). Positioning of acupoints HT7 or LI5 was based on the transpositional method, which locates animal acupoints on the surface of their skin corresponding to the anatomic site of human acupoints (Stux and Pomeranz 1998). The anatomical location of acupuncture points stimulated in rats corresponded to the acupoints in humans as described previously (Stux and Pomeranz 1998) and in animal acupuncture atlas (Schoen 2001). HT7 is located on the transverse crease of the wrist of the forepaw, radial to the tendon of the flexor carpi ulnaris muscle (Stux and Pomeranz 1998). Yangxi (LI5) is located at the distal end of the radius between the tendons of the palmaris longus muscle and flexor carpi radialis muscle proximal to the transverse crease of the wrist of the forepaw. LI5 was tested as the corresponding control point to HT7 on the opposite side of the forelimb, about 5 mm apart from HT7 (Yoon et al. 2010). Acupuncture was applied bilaterally at the acupoints of rats that were lightly restrained by handling for 1 min after foot-shock stress in the self-administration chamber. The animals in the non-acupuncture groups were lightly restrained in the same manner as the acupuncture treatment, but without needle insertion.

Data analysis

A minimum of six rats were used per group. Data are presented as means $\pm$ SEM (standard error of the mean) and analyzed by one-way ANOVA, followed by Tukey post hoc testing.

Results

Acupuncture at HT7 blocks footshock-induced reinstatement of cocaine-seeking

The following groups of rats were studied in cocaine self-administration experiments: (1) no footshock (NFS); no foot-shock and HT7 (NFS + HT7); (2) footshock (FS); (3) footshock and HT7 acupuncture (FS + HT7); and (4) footshock and LI5 acupuncture (FS + LI5). There were no significant differences among groups for cocaine responding during the acquisition period of self-administration ($p=0.920$). The mean number of cocaine infusions averaged over the last 3 days of training in each group was NFS=33.0 $\pm$ 2.1 ($n=6$), FS=36.0 $\pm$ 3.1 ($n=6$), FS+HT7=33.9 $\pm$ 8.7 ($n=6$), and FS + LI5=31.2 $\pm$ 0.8 ($n=6$). During the last 1-h extinction session, the mean number of active lever responses (infusions+timeout responses) in each

group was $NFS=2.6\pm1.8$, $FS=5.0\pm3.1$, $FS + HT7=0.2\pm0.2$, and $FS + LI5=4.2\pm3.0$. The rats were then subjected to foot-shock before cocaine reinstatement testing. In the NFS group, rats were left undisturbed in the operant conditioning chambers for 15 min without footshock after the same cocaine self-administration and extinction sessions. The number of lever presses on the active and inactive lever during each 3-h test for reinstatement following footshock stress and/or acupuncture treatment is shown in Fig. 1. When 15 min of intermittent (random interval 40 s, 0.5 mA) footshock was applied, the rats exhibited significant increases in responses on the active lever (40.8 ± 1.62 ; Fig. 1a), but not on the inactive lever (Fig. 1b), compared to the NFS group (17.2 ± 1.99 ; Fig. 1a). To test the effects of acupuncture on the footshock-induced reinstatement to cocaine-seeking, the rats received acupuncture treatment at HT7 or LI5 for 1 min after exposure to footshock stress and acupuncture and then lever presses were recorded for 3 h. Acupuncture at HT7 (FS+HT7 group) significantly reduced footshock-induced reinstatement, compared to the FS + LI5 group (Tukey's post hoc test, $F_{(3, 20)}=21.72$, $p<0.001$; Fig. 1a). Significant reduction in responses on the inactive lever was also observed in the FS + HT7 group, compared to the FS group (Tukey's post hoc test, $F_{(3, 20)}=5.55$, $p<0.01$; Fig. 1b).

We performed food self-administration study to examine the possibility that acupuncture affects the motivation to natural reward (i.e., food pellets). Additionally, in order to test the non-selective effects of acupuncture on cocaine-seeking, we also investigated the effects of acupuncture on cocaine-seeking under the non-stressed control condition. Acupuncture was applied at HT7 acupoints without footshock stress in a separate group of rats. The mean number of cocaine infusions averaged over the last 3 days of training was $NFS + HT7=33.6\pm6.6$ ($n=6$) and $NFS=28.9\pm4.8$ ($n=6$). During the last 1-h extinction session, the mean number of active lever responses (infusions+timeout responses) was $NFS + HT7=3.8\pm3.4$ and $NFS=8.8\pm3.2$. HT7 acupuncture affected neither the reinstatement of cocaine-seeking under non-stress conditions (Fig. 1c) nor food reinstatement (Fig. 1d), suggesting that HT7 acupuncture selectively reduces stress-induced cocaine reinstatement.

Acupuncture at HT7 reduces the increase of c-Fos and pCREB in NAc shell by footshock

Figure 2 shows the expression of c-Fos (Fig. 2a, b) and pCREB (Fig. 2c, d) in the NAc shell 15 min after footshock stress with or without HT7 acupuncture stimulation. Enhanced expression of c-Fos and pCREB in the NAc shell was observed following footshock stress, compared to the NFS group (Tukey's post hoc test, $p<0.05$). More importantly, acupuncture treatment at HT7, but not LI5, significantly reduced the increase of c-Fos and pCREB in NAc shell following foot-shock stress (Fig. 2; $F_{(3, 20)}=9.08$, $p=0.001$).

On the other hand, enhanced expression of c-Fos or pCREB following footshock was not seen in the NAc core ($p>0.05$, NFS vs. FS). The average numbers of c-Fos positive nuclei for NFS and FS after exposure to footshock stress were 4.8 ± 1.1 and 7.1 ± 1.5 , respectively. On the other hand, the mean numbers of pCREB-positive nuclei for NFS and FS after exposure to footshock stress were 42.4 ± 15.9 and 41.3 ± 12.8 , respectively. There was no difference in the expression of c-Fos or pCREB between the NFS and FS groups in the NAc core.

Discussion

Our findings demonstrate that footshock stress reinstates cocaine-seeking behavior, supporting previous studies, and enhances neuronal activation, as measured by the expression of c-Fos and pCREB in the NAc shell in cocaine-exposed rats. These results agree with other studies showing that the pharmacological stressor yohimbine induces reinstatement of ethanol-seeking behavior and c-fos expression in the NAc (Cippitelli et al. 2010), and forced swim stress precipitates reinstatement of cocaine-seeking accompanied by pCREB activation in the NAc (Kreibich and Blendy 2004). In addition, we found that reinstatement of cocaine self-administration is almost completely blocked by acupuncture at HT7, but not LI5, indicating site-specific efficacy and suggesting that acupuncture can prevent stress-induced cocaine relapse by regulating neuronal activation within the NAc shell, either directly or indirectly.

In the present study, acupuncture also reduced responding on the inactive lever. At first glance, the decreased responding on the inactive lever might be interpreted as non-specific motor impairment by HT7 acupuncture. However, the parallel reduction of responding on active and inactive lever responses is a common report during extinction in animal models of drug-seeking. Furthermore, our results are similar to others who showed that the metabotropic glutamate receptor 5 antagonist 2-methyl-6-(phenyl-ethynyl)-pyridine (MPEP) attenuates both active and inactive lever responses, suggesting that decreases in responding on the inactive lever by MPEP could be related to response generalization (Backstrom et al. 2004). In this respect, reduction of inactive lever responses by HT7 acupuncture could be interpreted as response generalization. This interpretation is also supported by the report showing that HT7 acupuncture did not alter locomotor activity in the saline-treated group, although acupuncture treatment suppressed cocaine-induced locomotor stimulation (Lee et al. 2009). Additionally, the present study showed that HT7 acupuncture did not affect food responding and cocaine-seeking behavior under non-stressed conditions. Studying food reinforcement is one way to evaluate the non-selective effects or any side effects of potential medication or treatment on drug self-administration (Mello and Negus 1996). In particular, evaluating active lever responses for food pellets under extinction conditions provides a powerful means of assessing the incentive motivational effects of a reinforcer (Orsini et al. 2002). Thus, acupuncture effects are likely due to reduction of the motivation for cocaine-seeking rather than food-seeking. Taken together, these results suggest that HT7 acupuncture selectively attenuates stress-induced cocaine-seeking behavior by reducing the motivation to seek cocaine rather than performance-related influences.

Relapse is a primary problem of drug addiction treatment and is triggered by various factors. Stress is a well-known risk factor for drug relapse in humans (Sinha 2007). Foot-shock stress is one of the most widely employed methods to study craving and relapse, although it is not equivalent to human stressors (Shaham et al. 2003). In the present study, the brief intermittent footshock, using parameters similar to what has been reported previously (Stewart 2000), reinstated cocaine-seeking behavior after extinction. Moreover, foot-shock increased the expression of c-fos, an identified target gene of CREB (Sheng et al. 1990), and pCREB, a modulator of the gene transcription, in the NAc following footshock stress in rats pre-exposed to cocaine. Interestingly, increased expression of c-Fos and pCREB was found

in the NAc shell (Fig. 2), but not in the NAc core, suggesting that the NAc shell plays a more pivotal role than the core in reinstatement caused by stress. Recent evidence suggests that NAc shell and core are functionally and anatomically distinct (McFarland et al. 2004). The shell is more associated with limbic functions and the core is more involved in motor functions (Brog et al. 1993). Moreover, the shell has more reactive DAergic transmission to stress and drugs than the core, although both the shell and core receive the dense innervations of DA afferents from the VTA (Mogenson et al. 1980). It has been shown that footshock stress increases DA release in the shell only (Kalivas and Duffy 1995). As an identified target signal of CREB, c-fos is also enhanced in the shell by anxiogenic stimuli such as footshock or by administration of yohimbine (Funk et al. 2006). Our finding that pCREB and c-Fos in the NAc shell, but not in the core, were responsive to footshock supports the previous suggestion that the shell is more crucial than the core for mediating motivational cocaine-seeking behaviors elicited by footshock stress (McFarland et al. 2004). Furthermore, activation of the DA system in the NAc shell has been shown to mediate cocaine-seeking behavior (Anderson et al. 2003; Schmidt and Pierce 2006). Thus, it may be suggested that the activational effects of footshock stress on c-Fos and pCREB in the NAc shell are attributable to increased DA release in the shell region.

Acupuncture at HT7 suppressed the reinstatement of cocaine-seeking behavior and the expression of c-Fos and pCREB in the NAc shell following footshock in cocaine pre-exposed rats. These results are supported by previous findings that acupuncture at HT7 produces powerful inhibitory effects on drug reinforcement in various addiction models using ethanol, cocaine, and morphine (Kim et al. 2005; Lee et al. 2009; Yang et al. 2010; Yoon et al. 2004, 2010; Zhao et al. 2006). In addition, we have recently reported that VTA GABA neurons play a critical role in mediating the effectiveness of HT7 acupuncture in suppressing ethanol self-administration (Yang et al. 2010). Most importantly, HT7-induced activation of VTA GABA neurons, suppression of VTA GABA neuron firing by ethanol, and reduction of ethanol self-administration were blocked by opioid antagonists. A role for opioids in mediating HT7 modulation of GABA neurons is supported by our previous studies (Yang et al. 2010; Yoon et al. 2010). Acupuncture activates opioidergic neurons in the arcuate nucleus of the hypothalamus (Wang et al. 1990b), and these opioidergic fibers can in turn activate ORs on GABA neurons in the VTA and NAc (Mansour et al. 1988). VTA GABA neurons express μ -ORs (Steffensen et al. 2006), and accumbal GABA terminals to VTA GABA neurons contain δ -ORs (Margolis et al. 2008). Our recent study has shown that δ -ORs on accumbal GABA terminals to VTA GABA neuron play an even more important role than μ -ORs on VTA GABA neurons in ethanol effects (Yang et al. 2010). Although it is well known that systemic blockade of δ -OR by antagonists suppresses alcohol-seeking via complex mechanisms (Le et al. 1999; Liu and Weiss 2002), it should be noted that local infusion of δ -OR agonists into VTA attenuates ethanol intake, due to activation of δ -ORs on accumbal GABA terminals and thus inhibition of accumbal GABA release to VTA GABA (Margolis et al. 2008). Acupuncture at HT7 produces an initial increase of δ -OR-mediated GABA neuron firing (Yang et al. 2010). Previous studies also support that δ -ORs critically contribute to cocaine reward and relapse (Kotlinska et al. 2010; Ward and Roberts 2007). We speculate that acupuncture at HT7 may activate δ -ORs on presynaptic sites of accumbal GABAergic input to VTA GABA neurons, which in turn

increases their activity (Margolis et al. 2008), resulting in a net decrease of mesolimbic DA transmission and suppression of the reinforcing effects of ethanol (Yang et al. 2010). Thus, attenuation of footshock-induced cocaine relapse and neuronal activation in the NAc shell by HT7 acupuncture may be due to the modulation of δ -OR-mediated effects on VTA GABA neurons.

The present study used manual acupuncture at HT7, because the stimulation was sufficient to produce acupuncture effects in our previous addiction experiments (Lee et al. 2011; Yoon et al. 2004, 2010). As most acupuncture studies have been performed by electroacupuncture stimulation rather than manual acupuncture, the question arises whether two modes can be different in stimulation intensity and underlying mechanisms. The majority of studies have shown that electroacupuncture and manual acupuncture recruit the same peripheral afferent pathway and mechanoreceptors, especially in acupuncture-induced analgesia (Yamamoto et al. 2011; Zhao 2008). Similar to those of electroacupuncture studies, the effects produced by manual acupuncture are also blocked by local anesthetic injection or nerve transection (Ulett et al. 1998). A functional magnetic resonance imaging study has revealed that manual acupuncture and electroacupuncture produced similar changes in the hemodynamic response in the human brain (Napadow et al. 2005), providing further evidence that the two methods share common neurological mechanisms to produce acupuncture effects. Another question is how HT7 acupuncture reduces addiction behaviors. We have previously proposed a hypothetical model for how HT7 acupuncture modulates addictive behaviors (Yang et al. 2008). In brief, acupuncture may stimulate the ulnar nerve below HT7 acupoint (Peuker and Cummings 2003). The afferent sensory stimulation activates opioidergic neurons in the arcuate nucleus of the hypothalamus (Wang et al. 1990b), which can in turn modulate ORs in the VTA (Mansour et al. 1988) and suppress addictive behaviors.

Although the roles of NAc DA or VTA GABA on stress-induced relapse to cocaine-seeking still remain elusive, several studies have shown that blockade of DA input to the NAc inhibits stress-induced reinstatement of cocaine-seeking (Xi et al. 2004) as well as heroin-seeking (Shaham and Stewart 1995). It was reported that the GABA_B receptor agonist baclofen dose-dependently attenuates stress-induced DA release in the NAc and reinstatement of cocaine-seeking behavior (Campbell et al. 1999; Doherty and Gratton 2007; Xi and Stein 1998). Local or systemic injection of GABA receptor agonists reduces cocaine self-administration (Brebner et al. 2000; Di Ciano and Everitt 2004; Shoaib et al. 1998), activity along the hypothalamus–pituitary–adrenocortical axis (Herman et al. 2004), and corticosterone responses to stress (Cullinan et al. 2008; Li et al. 2011), indicating that an increase in DA release in the NAc is, at least in part, responsible for stress-induced reinstatement of cocaine-seeking and is modulated by inhibitory GABAergic neurons in the NAc and VTA (Ashby et al. 1999; Cousins et al. 2002).

Acupuncture suppressed c-Fos expression and pCREB activation in the NAc shell. These findings are consistent with previous studies showing that acupuncture can reduce expression of c-Fos and CREB, as a non-specific marker, in several stress-related structures after exposure to stress (Kim et al. 2009; Liu et al. 2005). Several studies have demonstrated that acupuncture plays an anxiolytic role in stress-induced neurochemical and behavioral responses (Guimaraes et al. 1997; Lee et al. 2004). Moreover, acupuncture attenuates stress-

induced physiological responses via a central GABA pathway (Iwa et al. 2006). Neuronal suppression in the NAc and other stress-related areas is also produced by anxiolytic agents that potentiate inhibitory GABA transmission (Finlay et al. 1995; Giardino et al. 1998). Accordingly, it seems reasonable to propose that acupuncture inhibits stress-induced expression of c-Fos and CREB in the NAc, presumably via activation of inhibitory GABA transmission. Based on our previous studies, acupuncture at HT7 modulates VTA GABA neuron activity resulting in suppression of the reinforcing effects of abused drugs including NAc DA release and addictive behaviors (Kim et al. 2005; Yang et al. 2010; Yoon et al. 2004, 2010; Zhao et al. 2006). Given the established role for acupuncture in potentiating GABA transmission in the VTA, acupuncture-mediated inhibition of c-Fos expression and pCREB activation in the NAc shell following footshock stress is likely to reflect reduced DA activity via activation of inhibitory VTA GABA neurons.

In summary, acupuncture at HT7 prevented the reinstatement to cocaine-seeking elicited by footshock and suppressed c-Fos expression and pCREB activation in the NAc shell in cocaine pre-exposed rats. These results suggest that acupuncture can attenuate stress-induced relapse by regulating neuronal activation within the NAc.

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Fig. 1.

Acupuncture at HT7 reduced footshock-induced reinstatement of cocaine-seeking behavior.

The total number of active lever responses (**a**) and inactive lever responses (**b**) were measured for 3 h after 15 min of footshock in rats that had previously been trained to self-administer cocaine. Footshock increased active and inactive lever responding, which was blocked by acupuncture at HT7, but not at LI5. (**c**) The effects of HT7 on reinstatement of cocaine-seeking without footshock stress. Rats received acupuncture at HT7 or LI5 for 1 min after electrical footshock stress. HT7 acupuncture did not significantly affect active or inactive lever responding in animals not subjected to footshock. (**d**) The effects of acupuncture on extinction responding in food-trained rats. Responding at both levers was recorded for 3 h without food pellet delivery. The rats in the acupuncture group ($n=7$) were given bilateral acupuncture at HT7 for 1 min prior to extinction phase. The control group ($n=6$) received the same handling as the HT7 group, but without acupuncture stimulation

Fig. 2.

The effects of HT7 acupuncture on the expression of c-Fos (**a, b**) or pCREB (**c, d**) in the NAc shell following footshock stress. Representative images of c-Fos (**a**) or pCREB (**c**) staining in the NAc shell 15 min after footshock stress in the NFS, FS, FS + HT7, and FS + LI5 groups. The data for c-Fos and pCREB are summarized in (**b**) and (**d**), respectively. Acupuncture at HT7, but not LI5, significantly reduced both expression of c-Fos and pCREB in the NAc shell by footshock. Bar=100 μ m