Cessation of cocaine intake following chronic drug exposure is typically characterized by a negative emotional state (e.g., anxiety, irritability) (Koob and Le Moal, 1997, 2008). Importantly, anxiety induced during cocaine abstinence is often a provoking factor leading to relapse to cocaine use in human addicts (Gawin and Kleber, 1986; Koob and Bloom, 1988). Also, in a state of withdrawal from METH, addicts commonly experience heightened states of anxiety (Cruickshank and Dyer, 2009). However, it remains obscure whether an increase of anxiety-like behavior would be observed during METH withdrawal in animal models of relapse using METH self-administration. Secondly, we examined the possibility that an anxiety-like behavior changes during withdrawal after METH self-administration.

2. Material and methods

2.1. Animals

The subjects were 69 male Wistar rats (250–350 g, 10 weeks old, Nippon SLC, Hamamatsu), weighing 280–300 g at arrival. Subjects were housed in a temperature- and humidity-controlled environment under a 12-h light/dark cycle (lights on at 7:00 a.m.).

Before surgery, the animals had unlimited access to food and water and were housed at 3–4 animals/cage. Each rat was housed individually after surgery and, after 5 days for recovery, food was limited to 15–20 g/day/body. Procedures for animal treatments were conducted in accordance with the Guide for the care and use of Laboratory Animals as adopted and promulgated by the Declaration of Helsinki and Faculty of Pharmaceutical Science, Nagasaki International University Publication, enacted 2009.

2.2. Drugs

Methamphetamine HCl (METH; Dainippon Sumitomo Pharma, Osaka) was dissolved in saline. For self-administration, METH (0.02 mg/0.1 ml/infusion) was delivered intravenously (i.v.). α-Helical CRF_{9-41}, a non-selective CRF receptor antagonist (10 and 30 μg/rat, i.c.v.; Sigma, St Louis, MO), was dissolved in distilled water. NBI27914, a selective CRF_1 receptor antagonist (32 and 100 μg/rat, i.c.v.; Sigma, Louis, MO), was dissolved in a mixture of ethanol, cremophor EL and saline (1:1:9). Both these antagonists were injected intracerebroventricularly (i.c.v.) 10 min before the test of reinstatement. Metyrapone (2-Methyl-1,2-di-3-pyridinyl-1-propanone; Tocris Bioscience, Park Ellisville, MO), an inhibitor of corticosterone synthesis, was dissolved in distilled water containing 2% Tween 80. Metyrapone was injected subcutaneously (s.c.) at a dose of 100 mg/kg 3 h before the test of reinstatement (Shaham et al., 1997).

2.3. Procedure

2.3.1. Surgery

2.3.1.1. Intravenous catheterization. After the completion of food training, rats were anesthetized with isoflurane (Mylan Pharmaceut, Osaka), prior to the surgical implantation of indwelling i.v. catheters. Catheters were constructed from Silastic laboratory grade tubing (0.5 mm i.d., 1.0 mm o.d., Kaneka Medix, Osaka). The catheter was implanted into the right jugular vein and secured in place with silk suture around the silicon nodule. The Silastic tubing ran under the skin to an exit point in the mid-scapular region. Rats were infused i.v. with 0.1 ml of heparinized saline (30 U/ml) daily during the experiment in order to prevent blockage of the catheter.

2.3.1.2. Intraventricular cannulation. During surgery, each rat was implanted with a 21 gage guide cannula from which the injector extended 1 mm to end in the right or left lateral ventricle. Stereotaxic coordinates used were as follows: −0.8 mm from bregma, +1.4 mm lateral from the midline, and −2.5 mm from dura (Paxinos and Watson, 1986) measured from the tip of the injector.

2.3.2. METH self-administration

2.3.2.1. Food training. Rats were restricted to approximately 90% of their normal weight for 2 days prior to the start of food training and trained to press levers for 45-mg food pellets (Bio-Serv, Frenchtown, NJ). The training took place on a fixed ratio 1 (FR1) schedule during which no stimuli were presented. Lever-pressing training ceased when rats could obtain 30 pellets within 250 s for three consecutive sessions.

2.3.2.2. Intravenous METH self-administration. METH self-administration training was conducted in standard operant chambers (30×20×24 cm; Neuroscience, Tokyo) with two fixed levers (5 cm above the chamber floor). White circular stimulus lights were located 4 cm above the levers, and a house light was located on the wall on the same side and top of the chamber. One end of the swivel was connected via polyethylene tubing (Kaneka Medix, Osaka) encased in a protective stainless steel spring tether (Instech Laboratories, Plymouth Meeting, PA) to the animal's catheter while the other end of the swivel was connected via polyethylene tubing to the infusion pump. The self administration apparatus was enclosed in a ventilated, sound-attenuating chamber (Neuroscience, Tokyo). METH was delivered using a computer-controlled infusion pump located inside the sound attenuating chamber. The entire system was computer integrated using MED PC 4 (Actimetrics, Wilmette, IL). Rats were self-administered METH on 10 consecutive days during 2-h sessions. The animals were connected to the drug infusion line and the session was initiated. Each session began with illumination of the house light that remained lit for the entire session. Responses on the active lever resulted in delivery of METH (0.02 mg/0.1 ml) infusion over 6 s; this training was accomplished using an FR1 schedule. Responses on the left lever had no programmed consequences, but were recorded. Each infusion was paired with a 26-s compound stimulus presentation, consisting of the white stimulus light (200 lx) over the right lever and a tone (2.9 kHz, 85 dB) delivered via a programmable audio generator (Neuroscience, Tokyo). Following drug delivery and stimulus presentation, responses on the active lever had no programmed consequences (no drug or stimulus delivery) for 20 s, but lever responses were recorded. Following each self administration session, rats were administered 0.2 ml of heparinized saline (30 U/ml) i.v., and catheter ports were closed to maintain patency.

2.3.2.3. Extinction. After the self-administration sessions, at least five extinction sessions (1-h) were conducted daily during which active lever responding resulted in an infusion of saline instead of METH without presentation of the METH-associated cues. Rats conducted this extinction session until they achieved the extinction criterion of less than 10 responses per session on the previously active lever.

2.3.2.4. Reinstatement. A stress-induced reinstatement test was conducted every 10 days under a FR-1 schedule. The footshock testing session was preceded by 15 min of intermittent footshock. During the test, the levers were extended and rats were not connected to the infusion system to avoid damage resulting from the jumping behavior induced by footshock stress.

2.3.3. Electrical footshock

Footshocks (current intensity 0.8 mA; 1.0-s trains) were delivered through a scrambler (Random Shocker version 7.1; Neuroscience, Tokyo) to the grid floor of the operant chamber. They were administered during a period 15 min in an intermittent manner according to a variable interval schedule (mean interval: 40 s; range: 10–70 s).

2.3.4. Elevated plus maze test

The elevated-plus maze (Neuroscience, Tokyo) was constructed from black plastic and consisted of two open arms (50×10 cm) and two enclosed arms (50×10×50 cm) that extended from a central platform (10×10 cm). The maze was elevated 50 cm above the floor. Experiments began by placing a single rat on the central platform facing a closed arm. During the 10 min of free exploration, time spent in open arms (defined as the animal placing its forepaws onto an arm) and the number of crossings between closed arms were recorded manually. The maze was cleaned thoroughly between animals using water.

2.3.5. Measurement of brain/plasma CRF and plasma corticosterone levels

Blood samples were obtained from the orbital vein with a heparinized capillary (Hirschmann Laborgerate, Germany) under inhalation anesthetic. The blood was collected into tubes containing EDTA-2Na on ice and centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was removed and stored at −80 °C until assayed. For the brain CRF assay, the entire brain was removed from the skull immediately after decapitation and placed on ice, then the amygdala, nucleus accumbens and hypothalamus were removed using a stainless-steel brain slicer (RBSC-0.5S, Neuroscience, Tokyo) according to the brain map (16). These tissues were immediately homogenized in an extraction buffer (PBS with 0.2% Nonidet P-40) and centrifuged at 15,000 rpm for 20 min at 4 °C. The sample was stored at −80 °C until assayed. Both blood and brain samples were collected immediately after the self-administration sessions. Brain/plasma CRF levels were determined with a Mouse/Rat CRF-HS ELISA assay kit (Yanaihara Institute, Shizuoka). Plasma corticosterone levels were determined using a Corticosterone EIA kit (Cayman Chemical, Ann Arbor, MI).

2.4. Statistical analysis

Data represent the mean ± SEM number of lever responses or CRF/corticosterone concentrations. Response totals were analyzed by ANOVA (a within-subjects design). A one-way ANOVA was used to compare means, and Bonferroni–Dunn tests were used for post hoc analyses. Differences were considered significant at $p<0.05$. All statistical analyses were performed by using the Stat View software program (v. 5.0; SAS Institute, Cary, NC).

3. Results

3.1. Effects of CRF receptor antagonist on reinstatement of footshock-induced METH-seeking behavior

The number of METH infusions in the last training session (day 10) was 22.0 ± 0.9 and the total number of METH infusions during the

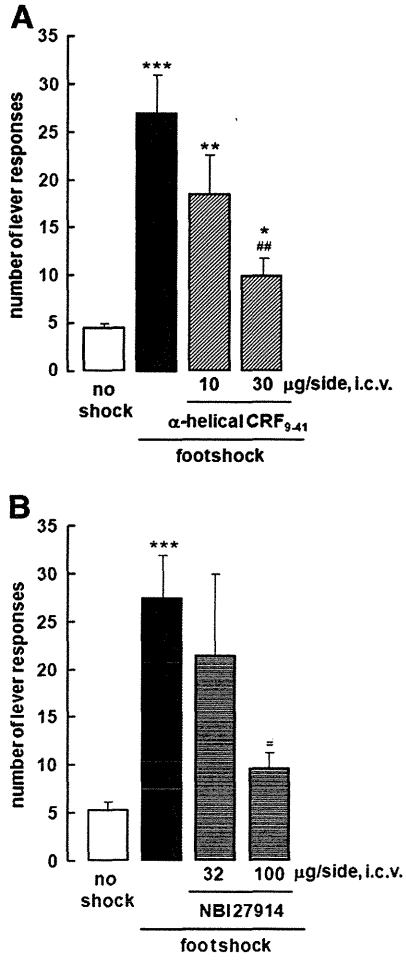

Fig. 1. Effects of CRF receptor antagonists on reinstatement of METH-seeking behavior induced by footshock.Each bar represents the mean (±SEM) number of presses on the previously active lever during the 15-min exposure to footshock stress (or no-shock). A. Rats were pretreated with a non-selective CRF receptor antagonist, α-helical CRF_{9-41} (10, 30 µg, i.c.v.), 10 min before the start of the session (n=8). B. Rats were pretreated with a selective CRF_1 receptor antagonist, NBI27914 (32, 100 µg, i.c.v.), 10 min before the start of the session (n=5). *Different from the no-shock condition (no shock), $p<0.05$; **, $p<0.01$; ***, $p<0.001$. #Different from the footshock condition, $p<0.05$; ##, $p<0.01$.

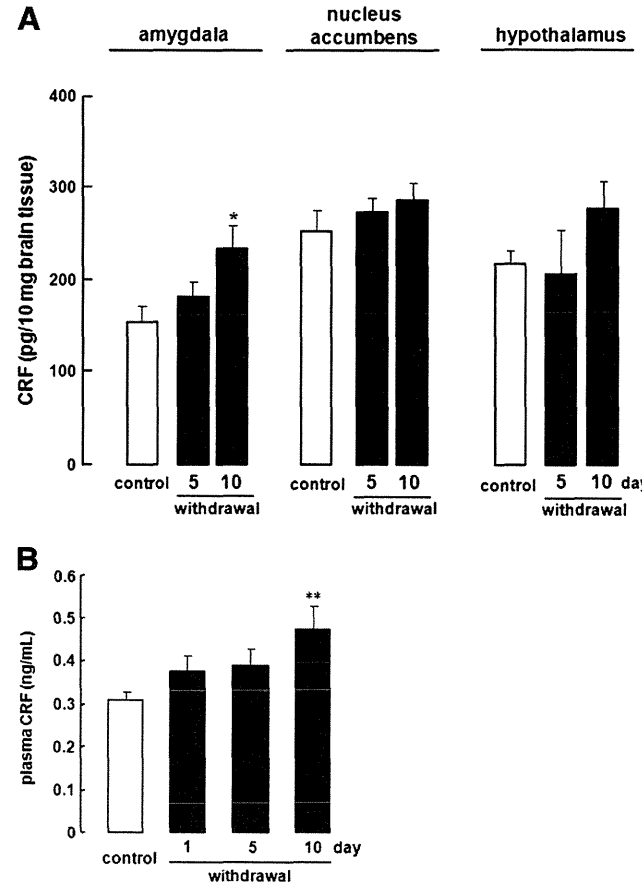

Fig. 2. CRF levels in METH self-administered rats during withdrawal. A. Each bar represents the mean (±SEM) CRF concentration (pg/10 mg brain tissue) in the amygdala, nucleus accumbens and hypothalamus (withdrawal day 10: n=6). *different from the control (naïve animals: n=4), $p<0.05$. B. Bars represent mean (±SEM) CRF (n=13–18) concentrations (ng/ml) in plasma. Blood was taken from the orbital vein under inhalation anesthesia immediately after the end of each session. **Different from the control, $p<0.01$. Control: before the start of METH self-administration acquisition training.

training session was 192.9 ± 8.9. When the drug was replaced with saline, the lever press responses gradually decreased from 17.4 ± 2.7 on the first day, reaching 7.6 ± 2.4 lever presses on day 5 of extinction. Under the extinction condition, footshock stress significantly reinstated lever-pressing behavior, so called METH-seeking behavior (5.4 ± 0.7 to 26.9 ± 4.1; F [1, 25] = 24.7, $p<0.001$), compared with the no-shock condition (no shock). Pretreatment with the CRF receptor antagonist α-helical CRF_{9-41} (10 and 30 μg/rat, i.c.v.) attenuated this METH-seeking behavior in a dose-dependent manner and significantly suppressed the lever presses at 30 μg (26.9 ± 4.1 to 10.0 ± 1.8, F [1, 20] = 8.9, $p<0.01$; Fig. 1A). Furthermore, the METH-seeking behavior was also suppressed by the selective CRF_1 receptor antagonist NBI27914 (100 μg/rat, i.c.v.) (27.6 ± 4.5 to 9.6 ± 1.7; F [1, 19] = 4.7, $p<0.05$; Fig. 1B).

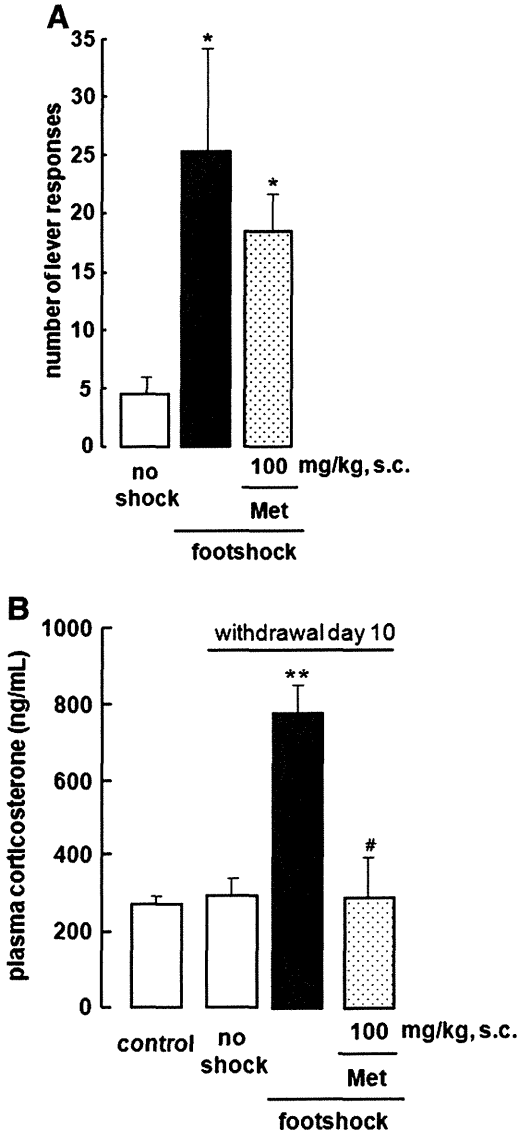

Fig. 3. Effects of an inhibitor of corticosterone synthesis on reinstatement of footshock-induced METH-seeking behavior. A. Each bar represents the mean (±SEM) number of presses on the previously active lever during the 15-min exposure to footshock stress (or no-shock). Rats were pretreated with an inhibitor of corticosterone synthesis, metyrapone (Met, 100 mg, s.c.), 3 h before the start of the session (n = 5). *Different from the no-shock condition (no shock), $p<0.05$. B. Bars represent mean (±SEM) corticosterone (n = 3–5) concentrations (ng/ml) in plasma. Blood was taken from the orbital vein under inhalation anesthesia immediately after the end of each session. **Different from the control, $p<0.01$. #Different from the footshock condition, $p<0.05$. Control: before the start of METH self-administration acquisition training.

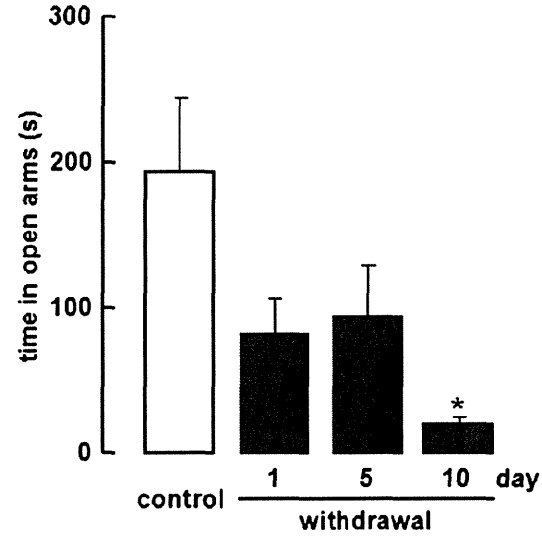

Fig. 4. Anxiety-like behavior in METH self-administered rats during withdrawal.Anxiety-like behavior during withdrawal after METH self-administration in the elevated plus maze test. Each bar represents the mean (±SEM) total time spent in open arms in the 10-min test session (n = 5–7). *Different from the control, $p<0.05$. Control: before the start of METH self-administration acquisition training.

3.2. CRF levels in METH self-administered rats during withdrawal

Fig. 2A shows CRF concentrations (pg/10 mg brain tissue) in the amygdala, NAc, and hypothalamus on days 5 and 10 of withdrawal from METH self-administration. The level of CRF in the amygdala gradually increased after withdrawal and showed a significant increase on day 10 (F [1, 8] = 5.5, $p<0.05$; Fig. 2A). In the nucleus accumbens and hypothalamus, however, the level showed a slight increase, though the change was not significant in either region (Fig. 2A).

Fig. 2B shows the levels of CRF in plasma. The plasma CRF level (ng/ml) increased with no significance on day 1 and day 5 of withdrawal. Then, it significantly increased on day 10 (0.30 ± 0.02 to 0.47 ± 0.06, F [1, 28] = 9.9, $p<0.01$; Fig. 2B).

3.3. Effects of an inhibitor of corticosterone synthesis on reinstatement of footshock-induced METH-seeking behavior

A corticosterone synthesis inhibitor, metyrapone (100 mg/kg, s.c.), did not block the footshock-induced METH-seeking behavior (Fig. 3A). Although the plasma corticosterone concentration (ng/ml) showed no change on day 10 of withdrawal ($p = 0.75$), it increased significantly immediately after the exposure to footshock (275.1 ± 22.1 to 747.8 ± 69.8, F [1, 6] = 29.2, $p<0.01$). Metyrapone completely reversed this increase (747.8 ± 69.81 to 279.1 ± 104.6, F [1, 5] = 15.2, $p<0.05$; Fig. 3B).

3.4. Anxiety-like behavior in METH self-administered rats during withdrawal

In the elevated plus maze test, control rats spent 193.2 ± 52.2 s in the open arms during the 10-min test session, whereas METH self-administered rats on day 10 of withdrawal spent just 20.8 ± 4.9 s in the open arms (F [1, 9] = 8.9, $p<0.05$; Fig. 4). However, the number of crossings between closed arms was not affected in the METH self-administered rats on withdrawal day 10 (Table 1).

Table 1
Number of crossings in the elevated plus maze test.

		Withdrawal after METH self-administration		
	Control	Day 1	Day 5	Day 10
Number of crossings	21.3 ± 3.8	10.7 ± 4.0	12.0 ± 4.4	16.0 ± 4.5

4. Discussion

The METH-seeking behavior induced by footshock was attenuated by both non-selective CRF and selective CRF_1 receptor antagonists. CRF is a primary activator of the HPA axis and an essential mediator of behavioral and autonomic outcomes of stress. It causes the release of adrenocorticotropic hormone (ACTH) from the pituitary, which in turn induces the secretion of glucocorticoids such as corticosterone from the adrenal gland. Administration of METH is known to increase plasma corticosterone levels in mice (Ago et al., 2009). Therefore, we investigated the effect of an inhibitor of corticosterone synthesis, metyrapone, as well as CRF receptor antagonists on METH-seeking behavior to determine the involvement of corticosterone in the reinstatement. However, metyrapone did not elicit a suppressing effect on the reinstatement of METH-seeking behavior. Hence, CRF but not corticosterone is likely to have a facilitatory role in footshock-induced METH-seeking behavior. This idea is supported by the present finding that levels of CRF in the amygdala were significantly increased on day 10 of withdrawal after METH self-administration without an accompanying increase in plasma corticosterone levels. The present study is the first to reveal a facilitating role of CRF/CRF_1 receptors in footshock-induced METH-seeking behavior with increases of CRF levels in both the amygdala and plasma during withdrawal from METH in METH self-administered rats. The role of CRF in reinstatement of cocaine-seeking behavior has been demonstrated previously. Treatment with a non-selective CRF receptor antagonist, D-Phe CRF_{12-41}, has been shown to block footshock-induced reinstatement in cocaine-trained rats (Erb et al., 1998). An attenuating effect on METH-seeking behavior by a CRF_1 receptor antagonist has also been shown in a cue-and METH priming-induced reinstatement model (Moffett and Goeders, 2007). Therefore, activation of the CRF receptor is likely to modulate psychostimulant-induced reinstatement and CRF_1 receptors mediate METH-seeking behavior in common with the three main stimuli described above.

Neurons in the amygdala provide an excitatory input, such as glutamate (Glu), to the prefrontal cortex (PFC) and NAc (Kalivas and Volkow, 2005). We previously revealed critical roles of the PFC and NAc in the reinstatement of METH-seeking behavior using lidocaine (Hiranita et al., 2006). Footshock elicits a significant increase in Glu in the PFC and the blockade of this increase attenuated the reinstatement of cocaine-seeking behavior (McFarland et al., 2004). Interestingly, as the administration of CRF significantly increased amygdala-PFC EPSC amplitude in chronic cocaine-treated animals, CRF appears to help facilitate amygdala-PFC glutamatergic transmission in cocaine-treated animals (Orozco-Cabal et al., 2008). There is a possibility that CRF in the amygdala also modulates glutamatergic transmission in the NAc taking into account neural projections from the amygdala into the NAc. Taken together, the increase of CRF levels in the amygdala in METH self-administered rats may be involved in facilitating the transmission of Glu in the PFC or NAc during reinstatement.

The plasma CRF level was significantly increased on day 10 of withdrawal similar to that in the amygdala. This may reflect the level of CRF in the brain because a large amount of CRF injected i.c.v. can diffuse via the specific unidirectional brain-to-blood transport system for CRF (Martins et al., 1996).

In the absence of METH administration, abusers commonly experience heightened states of anxiety (Cruickshank and Dyer, 2009). Similarly, METH self-administered rats in withdrawal showed an increase in anxiety-like behavior in the elevated plus maze test. Spiga et al. (2006) reported that the injection of a CRF receptor agonist, urocortin 1, into the amygdala produced anxiogenic effects in social interaction tests, reducing total interaction time without affecting locomotor activity or exploratory behavior. This report suggests that CRF in the amygdala has a facilitatory role in anxiety-like behaviors. Indeed, an increase in CRF is likely to parallel a heightening of negative emotions such as anxiety-like behavior. Consequently, the activation of CRF in the amygdala during withdrawal from METH may be attributable to the increase of anxiety-like behaviors in METH self-administered rats. Recent clinical study reveals that abstinent cocaine abusers exhibit significantly higher and more persistent stress- and cue-induced drug-craving and negative emotions such as increased anxiety, anger, fear and sadness (Fox et al., 2008). Based on this finding, enhanced sensitivity to stress and negative emotions may result in a decrease in the threshold to reinstate drug craving.

In summary, the present findings indicate that increased CRF levels in the amygdala may, at least in part, play a facilitating role in the reinstatement of METH-seeking behaviors following footshock and the increase in anxiety-like behavior without the participation of corticosterone. Therefore, CRF/CRF_1 receptor antagonists may be useful as an anti-craving agent. Moreover, although further study is required, plasma CRF levels may have a potential as a diagnostic biomarker for measuring the craving risk in METH abusers.

Acknowledgments

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References

Ago Y, Arikawa S, Yata M, Yano K, Abe M, Takuma K, et al. Role of prefrontal dopaminergic neurotransmission in glucocorticoid receptor-mediated modulation of methamphetamine-induced hyperactivity. Synapse 2009;63:7-14.
Anggadiredja K, Nakamichi M, Hiranita T, Tanaka H, Shoyama Y, Watanabe S, et al. Endocannabinoid system modulates relapse to methamphetamine seeking: possible mediation by the arachidonic acid cascade. Neuropsychopharmacology 2004;29:1470–8.
Carrasco GA, Van de Kar LD. Neuroendocrine pharmacology of stress. Eur J Pharmacol 2003;463:235–72.
Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. Corticotrophin-releasing factor receptors: from molecular biology to drug design. Trends Pharmacol Sci 1996;17:166–72.
Cruickshank CC, Dyer KR. A review of the clinical pharmacology of methamphetamine. Addiction 2009;104:1085–99.
de Wit H. Priming effects with drugs and other reinforcers. Exp Clin Psychopharmacol 1996;4:5-10.
Erb S, Shaham Y, Stewart J. The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. J Neurosci 1998;18:5529–36.
Fox HC, Hong KI, Siedlarz K, Sinha R. Enhanced sensitivity to stress and drug/alcohol craving in abstinent cocaine-dependent individuals compared to social drinkers. Neuropsychopharmacology 2008;33:796–805.
Gawin FH, Kleber HD. Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations. Arch Gen Psychiatry 1986;43:107–13.
Griebel G, Simiand J, Steinberg R, Jung M, Gully D, Roger P, et al. 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3- fluoro-4-methylp henyl) ethyl]5-methyl-N-(2-propynyl)-1, 3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. J Pharmacol Exp Ther 2002;301:333–45.
Hiranita T, Nawata Y, Sakimura K, Anggadiredja K, Yamamoto T. Suppression of methamphetamine-seeking behavior by nicotinic agonists. Proc Natl Acad Sci U S A 2006;103:8523–7.
Hiranita T, Nawata Y, Sakimura K, Yamamoto T. Methamphetamine-seeking behavior is due to inhibition of nicotinic cholinergic transmission by activation of cannabinoid CB1 receptors. Neuropharmacology 2008;55:1300–6.
Ising M, Zimmermann US, Künzel HE, Uhr M, Foster AC, Learned-Coughlin SM, et al. High-affinity CRF_1 receptor antagonist NBI-34041: preclinical and clinical data suggest safety and efficacy in attenuating elevated stress response. Neuropsychopharmacology 2007;32:1941–9.
Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 2005;162:1403–13.
Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. Science 1988;242:715–23.
Koob GF, Le Moal M. Drug abuse: hedonic homeostatic dysregulation. Science 1997;278:52–8.
Koob GF, Le Moal M. Addiction and the brain antireward system. Annu Rev Psychol 2008;59:29–53.
Martins JM, Kastin AJ, Banks WA. Unidirectional specific and modulated brain to blood transport of corticotropin-releasing hormone. Neuroendocrinology 1996;63:338–48.
McFarland K, Davidge SB, Lapish CC, Kalivas PW. Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. J Neurosci 2004;24:1551–60.

Moffett MC, Goeders NE. Neither non-contingent electric footshock nor administered corticosterone facilitate the acquisition of methamphetamine self-administration. Pharmacol Biochem Behav 2005;80:333–9.

Moffett MC, Goeders NE. CP-154,526, a CRF type-1 receptor antagonist, attenuates the cue-and methamphetamine-induced reinstatement of extinguished methamphetamine-seeking behavior in rats. Psychopharmacology 2007;190: 171–80.

Orozco-Cabal L, Liu J, Pollandt S, Schmidt K, Shinnick-Gallagher P, Gallagher JP. Dopamine and corticotropin-releasing factor synergistically alter basolateral amygdala-to-medial prefrontal cortex synaptic transmission: functional switch after chronic cocaine administration. J Neurosci 2008;28:529–42.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic; 1986.

Peltier RL, Li DH, Lytle D, Taylor CM, Emmett-Oglesby MW. Chronic d-amphetamine or methamphetamine produces cross-tolerance to the discriminative and reinforcing stimulus effects of cocaine. J Pharmacol Exp Ther 1996;277:212–8.

Richter RM, Weiss F. In vivo CRF release in rat amygdala is increased during cocaine withdrawal in self-administering rats. Synapse 1999;32:254–61.

Shaham Y, Rajabi H, Stewart J. Relapse to heroin-seeking in rats under opioid maintenance: the effects of stress, heroin priming, and withdrawal. J Neurosci 1996;16: 1957–63.

Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J. Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. J Neurosci 1997;17:2605–14.

Shepard JD, Bossert JM, Liu SY, Shaham Y. The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. Biol Psychiatry 2004;55:1082–9.

Shimosato K, Ohkuma S. Simultaneous monitoring of conditioned place preference and locomotor sensitization following repeated administration of cocaine and methamphetamine. Pharmacol Biochem Behav 2000;66:285–92.

Sinha R, Talih M, Malison R, Cooney N, Anderson GM, Kreek MJ. Hypothalamic–pituitary–adrenal axis and sympatho-adreno-medullary responses during stress-induced and drug cue-induced cocaine craving states. Psychopharmacology 2003;170:62–72.

Skutella T, Criswell H, Moy S, Probst JC, Breese GR, Jirikowski GF, et al. Corticotropin-releasing hormone (CRH) antisense oligodeoxynucleotide induces anxiolytic effects in rat. Neuroreport 1994;2:2181–5.

Spiga F, Lightman SL, Shekhar A, Lowry CA. Injections of urocortin 1 into the basolateral amygdala induce anxiety-like behavior and c-Fos expression in brainstem serotonergic neurons. Neuroscience 2006;138:1265–76.

Stenzel-Poore MP, Heinrichs SC, Rivest S, Koob GF, Vale WW. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. J Neurosci 1994;14:2579–84.

No.7
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A Cannabinoid CB_1 Receptor Antagonist Ameliorates Impairment of Recognition Memory on Withdrawal from MDMA (Ecstasy)

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(+/−)-3,4-Methylenedioxymethamphetamine (MDMA, 'Ecstasy') abusers have persistent neuropsychiatric deficits including memory impairments after the cessation of abuse. On the other hand, cannabinoid CB_1 receptors have been implicated in learning/memory, and are highly expressed in the hippocampus, a region of the brain believed to have an important function in certain forms of learning and memory. In this study, we clarified the mechanism underlying the cognitive impairment that develops during MDMA withdrawal from the standpoint of the cannabinoid CB_1 receptors. Mice were administered MDMA (10 mg/kg, i.p.) once a day for 7 days. On the 7th day of withdrawal, a novel object recognition task was performed and the amount of cannabinoid CB_1 receptor protein was measured with western blotting. Recognition performance was impaired on the 7th day of withdrawal. This impairment was blocked by AM251, a cannabinoid CB_1 receptor antagonist, administered 30 min before the training trial or co-administered with MDMA. At this time, the level of cannabinoid CB_1 receptor protein increased significantly in the hippocampus but not the prefrontal cortex or striatum. This increase of CB_1 receptor protein in the hippocampus was also blocked by the co-administration of AM251. Furthermore, CB_1 receptor knockout mice showed no impairment of recognition performance on the withdrawal from MDMA. The impairment of recognition memory during withdrawal from MDMA may result from the activation of cannabinoid CB_1 receptors in the hippocampus.
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Keywords: MDMA; cannabinoid CB_1 receptors; recognition memory; novel object recognition task; withdrawal; hippocampus

INTRODUCTION

(+/−)-3,4-Methylenedioxymethamphetamine (MDMA) is widely abused throughout the world. MDMA abusers have neuropsychiatric deficits including memory impairments (McCardle *et al*, 2004; Wareing *et al*, 2007). Recent studies suggest that this neuropsychiatric deficit persists after the cessation of abuse (Ward *et al*, 2006). In addition, cocaine, amphetamine, or opiate abusers also show cognitive impairment during long-term drug abstinence (Ersche *et al*, 2006; Pace-Schott *et al*, 2008).

Cannabis usage causes deficits in attention, executive functioning, and short-term memory (O'Leary *et al*, 2002; Medina *et al*, 2007). We showed earlier that repeated treatment with Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient of marijuana (*cannabis*), impaired delayed matching-to-sample performance even 24 h after the administration (Miyamoto *et al*, 1995). An other study has also found that Δ^9-THC impairs spatial memory (Lichtman and Martin, 1996). These reports suggest that the activation of the brain cannabinoid system impairs working memory. Furthermore, it has been revealed that the cannabinoid system is involved in drug dependence (Yamamoto and Takada, 2000; Yamamoto *et al*, 2004). A cannabinoid CB_1 receptor antagonist, SR141716A, attenuated the reinstatement of methamphetamine-seeking behavior (Anggadiredja *et al*, 2004; Hiranita *et al*, 2008). Moreover, cannabinoid CB_1 receptor knockout mice failed to establish cocaine, morphine, and ethanol self-administration (Cossu *et al*, 2001; Soria *et al*, 2005; Thanos *et al*, 2005). In a biochemical study, Gonzalez *et al* (2002) reported that chronic exposure to morphine increased levels of cannabinoid CB_1 receptor mRNA and CB_1 receptor binding in the brain. In addition, the hippocampal cannabinoid system seems to be activated during withdrawal from ethanol, because both endogenous cannabinoids and CB_1 receptors levels increased (Mitrirattanakul *et al*, 2007). Despite the close involvement of the cannabinoid system in

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the reward system, it is still unclear whether the system is involved in cognitive impairment on withdrawal from chronic exposure to drugs of abuse.

Here, we clarified the role of the cannabinoid system in cognitive impairment during withdrawal from MDMA using the novel object recognition task. We also investigated that the effect of MDMA on the level of cannabinoid CB_1 receptor protein correlated with a behavioral test.

MATERIALS AND METHODS

Animals

Male CD1 (wild-type) mice (Charles River, Yokohama, Japan) and cannabinoid CB_1 receptor knockout (CB_1 KO) mice on a CD1 background, provided by Dr Catherine Ledent (Institut de Recherches en Biologie Humaine et Moléculaire, Université Libre de Bruxelles), and weighing 30–35 g, were used in the present experiment. There were 117 wild-type mice and 29 CB_1 KO mice used in all experiment. We conducted each experiment with a small control group of 2–3 mice each and these control group data were combined together in the end to represent the control values. The animals were housed in plastic cages and kept in a regulated environment (23 ± 1°C), with a 12/12 h light–dark cycle (lights on at 7:00 am). Food and water were available *ad libitum*. Procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the Declaration of Helsinki and Nagasaki International University Publication, enacted in 2006.

Drugs

(+/−)-3,4-Methylenedioxymethamphetamine HCl (MDMA; provided by Dr Tatsunori Iwamura, Matsuyama University) was dissolved in saline. AM251 (Sigma, St Louis, MO) was dissolved in a mixture of DMSO, Tween-80 and saline (1 : 1 : 18, respectively). All drugs were administered intraperitoneally (i.p.), and injected at a volume of 0.1 ml per 10 g of body weight. Saline or MDMA (10 mg/kg) was administered once or once daily for 7 days. AM251 (1.0 or 3.2 mg/kg) was co-administered with MDMA or singly administered 30 min before the training trial on the 7th day of withdrawal after the repeated administration of MDMA.

Behavioral Testing

Object recognition test. The object recognition test was carried out on the 1st or 7th day after the repeated administration of MDMA in separate groups. This test was performed in a Plexiglas open-field box (in cm 70 wide × 70 deep × 40 high) with black vertical walls and a floor. The objects to be discriminated were silver cone-shaped and bulb-shaped. Mice were habituated to the open field for 1 h (habituation trial). The next day, in the training trial, each mouse was placed in the open field and allowed to explore two identical objects for 10 min. The test trial was performed 3 h after the training trial. One familiar object and one novel object were placed in the same location as in the training trial. For the measurement on the 1st day of withdrawal, the habituation trial was conducted just before the last drug injection. The time spent exploring each object and the total amount of time spent exploring both objects were recorded. Exploration of an object was defined as placing the nose or a forepaw at or beyond marks put on the open-field at a distance of 1 cm from each object. A discrimination ratio was calculated as the difference in time spent exploring the novel and familiar object, expressed as a ratio of the total time spent exploring both objects in the test trial. Mice showing a total exploration time of <10 s during the training trial were excluded. The ambulation during the trial was measured with a digital tracking and computerized scoring system (LimeLight, Actimetrics). To determine whether the mice discriminated between novel and familiar objects, the discrimination ratios obtained under each condition were compared with those that would be expected by chance (ie, a ratio of 0.0), using one-sample *t* tests.

Biochemical Testing

Western blot analysis. Immediately after decapitation, the whole brain was removed from the skull, placed on ice, and the hippocampus, prefrontal cortex, and striatum were removed as described earlier (Yamaguchi *et al*, 2004). These tissues were immediately homogenized in a lysis buffer (10 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 5 mM EDTA, 250 mM Sucrose, 1 mM Dithiothreitol, 1% Triton X-100, 1% sodium cholate, and protease inhibitor cocktail). All samples were subjected to a BCA assay to adjust the amount of protein loaded before the sample buffer was added. The sample (10 μg) was applied to a 10% polyacrylamide gel (BioRad, Hercules, CA), and the proteins were transferred electrophoretically to nitrocellulose membranes (Bio Rad). The membrane was blocked with TBS-Tween 20 (0.1%) and 5% nonfat dry milk and incubated with the primary antibodies [anti-cannabinoid CB_1 (1 : 1000, Calbiochem, US and Canada) overnight at 4°C and anti-β-actin (1 : 2000, Sigma)] 1 h at room temperature. The antibodies were detected using HRP-conjugated anti-rabbit and anti-mouse IgG (GE Healthcare, Tokyo, Japan, 1 : 1000) secondary antibodies. The blots were detected using a chemiluminescence method (ECL system; GE Healthcare).

Data Analysis

Data are expressed as mean ± SE. A one-way ANOVA was used to compare means, and Bonferroni–Dunn tests were used for *post hoc* analysis. $p<0.05$ was accepted as statistically significant.

RESULTS

Novel Object Recognition Performance During MDMA Withdrawal

In the training trial, vehicle, single MDMA, and repeated (for 7 days) MDMA-treated mice on the 1st day after the last treatment spent 23.1 ± 1.8, 22.0 ± 5.6, and 21.3 ± 2.0 s exploring objects, respectively. Meanwhile, on the 7th day after treatment, the time spent exploring objects in the vehicle, single MDMA, and repeated MDMA-treated groups was 19.3 ± 2.7, 26.6 ± 5.2, and 21.4 ± 3.1 s, respectively.

Hence, the time spent exploring objects on the 1st day of withdrawal in the training trial in single MDMA- and repeated MDMA-treated groups was not significantly different from that of vehicle-treated group ($p=0.06$ and $p=0.55$ *vs* vehicle-treated group, respectively). In addition, on the 7th day of withdrawal, there was no significant difference in the time spent exploring objects among the three groups in the training trial ($p=0.28$, $p=0.94$ *vs* vehicle-treated group, respectively). In the test trial, the vehicle-treated mice spent significantly longer exploring the novel object (21.3 ± 2.3 s) than the familiar object (5.4 ± 1.0 s) ($F[1,28]=30.8$, $p<0.0001$ *vs* exploration time for the familiar object). On the 1st and 7th day after a single administration of MDMA, there was no significant change in the discrimination ratio ($p=0.47$ and $p=0.13$ *vs* control group on the 1st and 7th day, respectively). However, the discrimination ratio significantly decreased on the 1st and 7th days of withdrawal from repeated administration of MDMA (0.597 ± 0.071–0.26 ± 0.106%: $F[1,19]=5.3$, $p<0.05$ and 0.633 ± 0.048–0.048 ± 0.049%: $F[1,27]=70.2$, $p<0.001$ *vs* control group on the 1st and 7th days, respectively) (Figure 1). Discrimination ratios were significantly above chance in all groups except for mice on the 7th days of withdrawal from repeated MDMA ($p<0.001$; control group on the 1st and 7th day, $p<0.01$; on the 1st and 7th day of withdrawal from single MDMA, $p<0.05$; on the 1st day of withdrawal from repeated MDMA). In this test trial, ambulation did not differ between the MDMA-treated and vehicle-treated groups ($p=0.21$ and $p=0.61$ on the 1st and 7th days, respectively). The decrease in the discrimination ratio on the 7th day of withdrawal was prevented by the co-administration of AM251, a cannabinoid CB_1 receptor antagonist, with MDMA in a dose-dependent manner (0.048 ± 0.049 to 0.592 ± 0.067%: $F[1,22]=48.5$, $p<0.001$ *vs* MDMA group) (Figure 2a). However, ambulation in AM251 co-administered group (4269 ± 269 cm) did not differ from ambulation in vehicle (4578 ± 354 cm) or MDMA (4334 ± 310 cm) groups ($p=0.51$ and $p=0.55$ *vs* vehicle and MDMA alone, respectively). On the other hand, a single administration of AM251 30 min before the training trial on the 7th day of withdrawal from repeated MDMA treatment stopped the reduction in the discrimination ratio in a dose-dependent manner (0.048 ± 0.049 to 0.661 ± 0.074%: $F[1,18]=54.7$, $p<0.001$ *vs* MDMA group) (Figure 2b). A single administration of AM251 on 7th day of withdrawal from repeated MDMA had no effect on ambulation ($p=0.19$ and $p=0.3$ *vs* vehicle and MDMA alone, respectively). Discrimination ratios were significantly above chance in mice co-administered and singly administered AM251 ($p<0.001$). While, there was no significant difference in the time spent exploring objects and the discrimination ratio between vehicle-treated wild-type and CB_1 KO mice in the test trial. However, CB_1 KO mice did not exhibit a reduction in the discrimination ratio on both 1st and 7th day of withdrawal from repeated MDMA treatment (Figure 3). Discrimination ratios were significantly above chance in all groups of CB_1 KO mice ($p<0.001$).

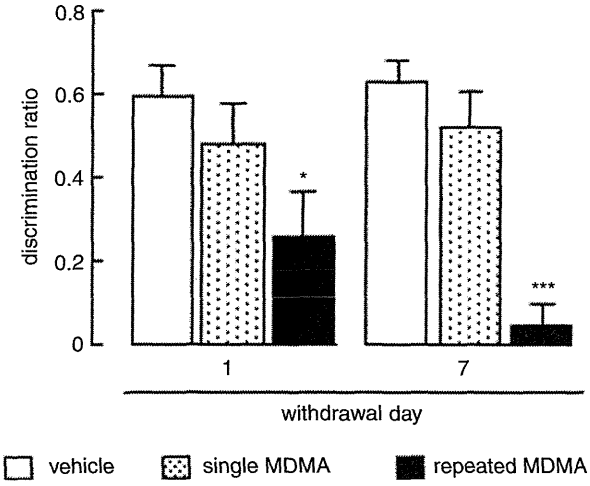

Figure 1 Novel object recognition performance in wild-type mice on the 1st or 7th day of withdrawal after single or repeated (daily for 7 day) MDMA treatment (10 mg/kg, i.p.). Each graph shows the discrimination ratio in the test trial. Data represent the mean ± SEM ($n=5$–15). $^{*}p<0.05$, $^{***}p<0.001$ *vs* vehicle-treated mice. Vehicle includes results for mice administered saline once or repeatedly for 7 days.

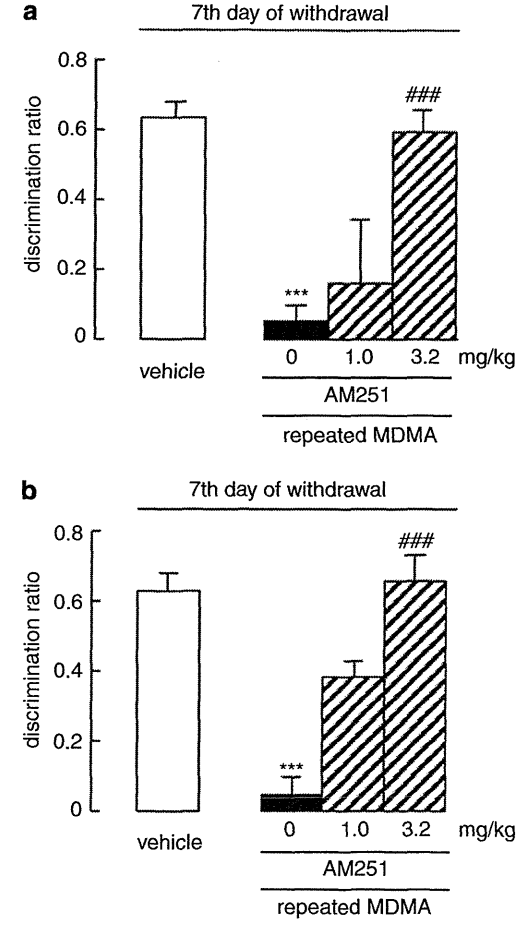

Figure 2 Effect of a cannabinoid CB_1 receptor antagonist, AM251, on cognitive impairment on the 7th day of MDMA withdrawal (10 mg/kg, i.p., daily for 7 days) in wild-type mice. (a) AM251 (1.0 or 3.2 mg/kg, i.p.) was co-administered with MDMA. Data represent the mean ± SEM ($n=8$–15). $^{***}p<0.001$ *vs* vehicle-treated mice; $^{###}p<0.001$ *vs* MDMA (10 mg/kg)-treated mice. Vehicle means results for mice administered saline. (b) AM251 (1.0 or 3.2 mg/kg, i.p.) was administered 30 min before the training trial. Data represent the mean ± SEM ($n=5$–15). $^{***}p<0.001$ *vs* vehicle-treated mice; $^{###}p<0.001$ *vs* MDMA-treated mice. Vehicle means results for mice administered saline.

Alteration of the Level of Cannabinoid CB_1 Receptor Protein During Withdrawal from Repeated MDMA Treatment

The level of cannabinoid CB_1 receptor protein did not change on the 1st day of withdrawal from repeated administration of MDMA in the hippocampus. On the 7th day of withdrawal, the level of CB_1 receptor protein in the hippocampus was significantly increased (0.48 ± 0.06–0.96 ± 0.07, $F[1,13] = 28.1$, $p < 0.001$ *vs* vehicle-treated group) (Figure 4a). This increase was prevented by co-administration of AM251 with MDMA (0.96 ± 0.07–0.60 ± 0.09, $F[1,14] = 9.6$, $p < 0.01$ *vs* MDMA-treated group) (Figure 4b). There was no significant change in the prefrontal cortex or striatum on both 1st and 7th day of withdrawal (Figure 4a).

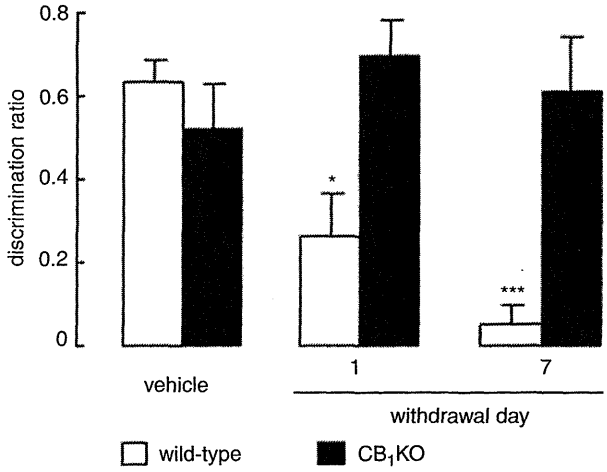

Figure 3 Comparison of novel object recognition performance in wild-type and CB_1 receptor knockout mice on the 1st and 7th day of MDMA withdrawal (10 mg/kg, i.p., daily for 7 days). Each graph shows the discrimination ratio in the test trial. Data represent the mean ± SEM ($n = 8$–15). $^{*}p < 0.05$, $^{***}p < 0.001$ *vs* vehicle-treated mice. Open and closed bars indicate wild-type and CB_1 receptor knockout mice, respectively. Vehicle means results for mice administered saline.

DISCUSSION

Object recognition memory in mice was impaired on withdrawal from repeated MDMA. This impairment was prevented by the co-administration of AM251, a cannabinoid CB_1 receptor antagonist, with MDMA in wild-type mice. In addition, a single treatment of AM251 on the 7th day of MDMA withdrawal ameliorated this recognition memory impairment. In CB_1 KO mice, recognition memory was not impaired on withdrawal from MDMA. These results suggest that the activation of cannabinoid CB_1 receptors is involved in the appearance of cognitive impairment on withdrawal from MDMA. In rats, it was also reported that object recognition memory is also impaired on withdrawal from MDMA similar to our present findings in mice (Morley *et al*, 2001; McGregor *et al*, 2003; Piper and Meyer, 2004). However, this is the first report to show the involvement of cannabinoid CB_1 receptors in the appearance of cognitive impairment on withdrawal from abusive drugs. Recently, Touriño *et al* (2008) indicated that CB_1 KO mice did not show the performance of MDMA self-administration. This finding suggests that the activation of CB_1 receptors is involved in the MDMA reinforcing effect.

The level of cannabinoid CB_1 receptor protein in the hippocampus was significantly increased on the 7th day of withdrawal but not on the 1st day while recognition

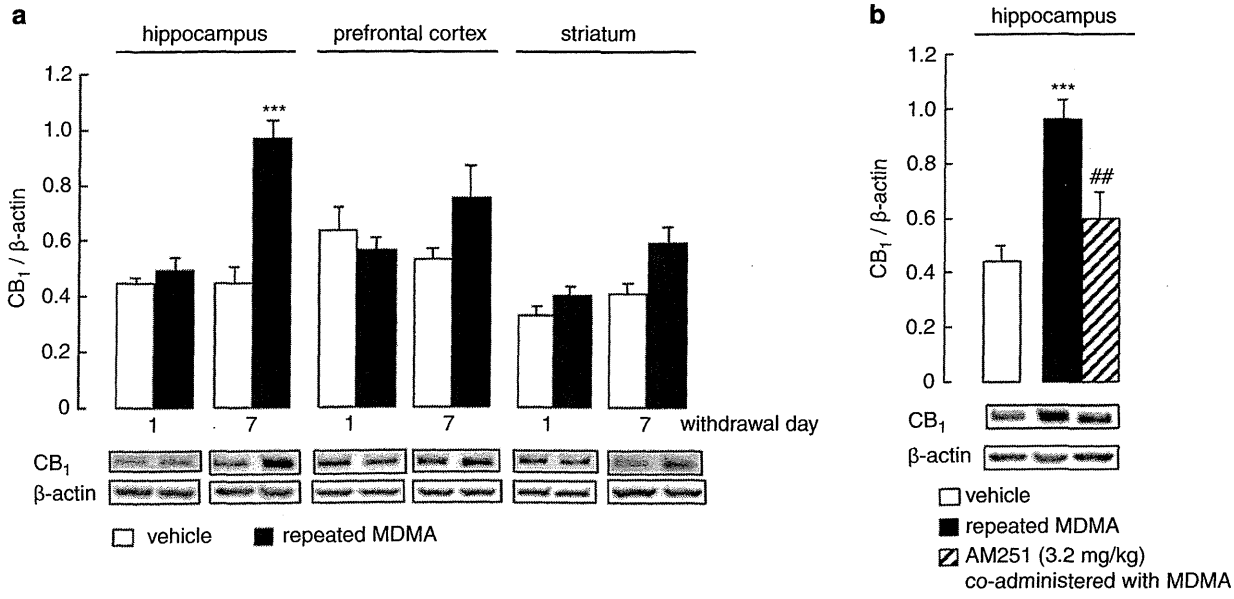

Figure 4 Effect of repeated administration of MDMA on the levels of CB_1 receptor protein in the brain in wild-type mice on the 1st and 7th day of MDMA withdrawal (10 mg/kg, i.p., daily for 7 days). (a) The levels of CB_1 receptor protein in the hippocampus, prefrontal cortex, and striatum were measured. Data represent the mean ± SEM ($n = 8$–11). (b) Effect of repeated administration of AM251 (3.2 mg/kg) with MDMA on MDMA-induced up-regulation of CB_1 receptor protein expression in the hippocampus. Open and closed bars indicate vehicle- and repeated MDMA-treated group. Data represent the mean ± SEM ($n = 5$–11). $^{***}p < 0.001$ *vs* vehicle-treated mice; $^{\#\#}p < 0.01$ *vs* MDMA-treated mice. Vehicle means results for mice administered saline.

memory was impaired on both the 1st day and 7th day of MDMA withdrawal. In this regard, cognitive impairment on the 1st day of MDMA withdrawal may be due to the increase in hippocampal endocannabinoid. Mitrirattanakul *et al* (2007) reported that the amount of endocannabinoid 2-AG in the hippocampus significantly increased in the early phase of ethanol withdrawal without any increase in CB_1 receptor expression. Our findings may be supported by this result. On the other hand, a single treatment with CB_1 receptor antagonist for MDMA withdrawal significantly ameliorated the recognition memory impairment on 7th day of MDMA withdrawal. Accordingly, CB_1 receptors may be activated with the increase in their expression at this time.

In addition, the activation of the brain cannabinoid system causes deficits in attention, executive functioning, and short-term memory (Lichtman and Martin, 1996; O'Leary *et al*, 2002; Medina *et al*, 2007). It is also demonstrated that object recognition memory was impaired by both endogenous cannabinoid Δ^9-THC and synthetic CB_1 receptor agonist WIN 55,212-2 (Schneider and Koch, 2002; Quinn *et al*, 2008). Our findings may be supported by the literatures above.

The important function of the hippocampus in cognitive functions including recognition memory is well established by earlier findings. Hippocampal damage from ibotenic acid disrupted recognition memory in the novel object recognition task and the visual paired comparison task (Clark *et al*, 2000; Broadbent *et al*, 2004). Furthermore, an intrahippocampal WIN 55,212-2, also impaired performance of the novel object recognition task (Kosiorek *et al*, 2003; Suenaga and Ichitani, 2008).

Hampson and Deadwyler (2000) found that Δ^9-THC and WIN 55,212-2 act selectively to disrupt the encoding of events in the hippocampus during memory processing, on measuring the combined simultaneous multineuron firing rate. Recently, it was also suggested that the cannabinoids Δ^9-THC and a cannabinoid CB_1 receptor agonist CP55940 disrupted the temporal coordination of hippocampal neurons, and that this effect may correlate with memory deficits in individuals (Robbe *et al*, 2006).

Enocannabinoids are known to participate in forms of synaptic plasticity (Mackie, 2008). This phenomenon associated with endocannabinoids may help explain the mechanism by which cannabinoids impair memory. Long-term potentiation (LTP) is a form of synaptic plasticity thought to have functional roles in learning and memory processes. The importance of the hippocampal LTP in learning and memory has also been shown that hippocampal LTP is facilitated after following exposure to a novel environment but not by exposure to a familiar environment (Li *et al*, 2003). In addition, it has been shown that the cannabinoid system affects the hippocampal LTP by chronic Δ^9-THC blocking hippocampal LTP via CB_1 receptors after withdrawal (Hoffman *et al*, 2007). It has been shown that cannabinoids inhibit neurotransmitter release via presynaptic cannabinoid CB_1 receptors (Schlicker and Kathmann, 2001). Additionally, LTP disruption in the hippocampus by WIN 55,212-2 may be associated with an inhibition of hippocampal glutamatergic transmission (Misner and Sullivan, 1999). Accordingly, the appearance of cognitive impairment during MDMA withdrawal may result in dysfunction of hippocampal LTP via inhibition of glutamate release induced by an activation of CB_1 receptors. These reports strongly support our present finding that the activation of the hippocampal cannabinoid system disrupts recognition memory during MDMA withdrawal.

In conclusion, our results suggest the impairment of recognition memory during withdrawal from repeated administration of MDMA to be due to the activation of cannabinoid CB_1 receptors in the hippocampus. Moreover, these findings suggest that cannabinoid CB_1 receptor antagonists would have a therapeutic effect on cognitive dysfunction in MDMA abusers.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

Anggadiredja K, Nakamichi M, Hiranita T, Tanaka H, Shoyama Y, Watanabe S *et al* (2004). Endocannabinoid system modulates relapse to methamphetamine seeking: possible mediation by the arachidonic acid cascade. *Neuropsychopharmacology* **29**: 1470–1478.

Broadbent NJ, Squire LR, Clark RE (2004). Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci USA* **101**: 14515–14520.

Clark RE, Zola SM, Squire LR (2000). Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci* **20**: 8853–8860.

Cossu G, Ledent C, Fattore L, Imperato A, Bohme GA, Parmentier M *et al* (2001). Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behav Brain Res* **118**: 61–65.

Ersche KD, Clark L, London M, Robbins TW, Sahakian BJ (2006). Profile of executive and memory function associated with amphetamine and opiate dependence. *Neuropsychopharmacology* **31**: 1036–1047.

Gonzalez S, Fernandez-Ruiz J, Sparpaglione V, Parolaro D, Ramos JA (2002). Chronic exposure to morphine, cocaine or ethanol in rats produced different effects in brain cannabinoid CB(1) receptor binding and mRNA levels. *Drug Alcohol Depend* **66**: 77–84.

Hampson RE, Deadwyler SA (2000). Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *J Neurosci* **20**: 8932–8942.

Hiranita T, Nawata Y, Sakimura K, Yamamoto T (2008). Methamphetamine-seeking behavior is due to inhibition of nicotinic cholinergic transmission by activation of cannabinoid CB1 receptors. *Neuropharmacology* **55**: 1300–1306.

Hoffman AF, Oz M, Yang R, Lichtman AH, Lupica CR (2007). Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. *Learn Mem* **14**: 63–74.

Kosiorek P, Hryniewicz A, Bialuk I, Zawadzka A, Winnicka MM (2003). Cannabinoids alter recognition memory in rats. *Pol J Pharmacol* **55**: 903–910.

Li S, Cullen WK, Anwyl R, Rowan MJ (2003). Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. *Nat Neurosci* **6**: 526–531.

Lichtman AH, Martin BR (1996). Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. *Psychopharmacology* **126**: 125–131.

Mackie K (2008). Signaling via CNS cannabinoid receptors. *Mol Cell Endocrinol* **286**: S60–S65.

McCardle K, Luebbers S, Carter JD, Croft RJ, Stough C (2004). Chronic MDMA (ecstasy) use, cognition and mood. *Psychopharmacology* **173**: 434–439.

McGregor IS, Gurtman CG, Morley KC, Clemens KJ, Blokland A, Li KM *et al* (2003). Increased anxiety and 'depressive' symptoms months after MDMA ('ecstasy') in rats: drug-induced hyperthermia does not predict long-term outcomes. *Psychopharmacology* **168**: 465–474.

Medina KL, Hanson KL, Schweinsburg AD, Cohen-Zion M, Nagel BJ, Tapert SF (2007). Neuropsychological functioning in adolescent marijuana users: subtle deficits detectable after a month of abstinence. *J Int Neuropsychol Soc* **13**: 807–820.

Misner DL, Sullivan JM (1999). Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. *J Neurosci* **19**: 6795–6805.

Mitrirattanakul S, Lopez-Valdes HE, Liang J, Matsuka Y, Mackie K, Faull KF *et al* (2007). Bidirectional alterations of hippocampal cannabinoid 1 receptors and their endogenous ligands in a rat model of alcohol withdrawal and dependence. *Alcohol Clin Exp Res* **31**: 855–867.

Miyamoto A, Yamamoto T, Watanabe S (1995). Effect of repeated administration of delta 9-tetrahydrocannabinol on delayed matching-to-sample performance in rats. *Neurosci Lett* **201**: 139–142.

Morley KC, Gallate JE, Hunt GE, Mallet PE, McGregor IS (2001). Increased anxiety and impaired memory in rats 3 months after administration of 3,4-methylenedioxymethamphetamine ('ecstasy'). *Eur J Pharmacol* **433**: 91–99.

O'Leary DS, Block RI, Koeppel JA, Flaum M, Schultz SK, Andreasen NC *et al* (2002). Effects of smoking marijuana on brain perfusion and cognition. *Neuropsychopharmacology* **26**: 802–816.

Pace-Schott EF, Morgan PT, Malison RT, Hart CL, Edgar C, Walker M *et al* (2008). Cocaine users differ from normals on cognitive tasks which show poorer performance during drug abstinence. *Am J Drug Alcohol Abuse* **34**: 109–121.

Piper BJ, Meyer JS (2004). Memory deficit and reduced anxiety in young adult rats given repeated intermittent MDMA treatment during the periadolescent period. *Pharmacol Biochem Behav* **79**: 723–731.

Quinn HR, Matsumoto I, Callaghan PD, Long LE, Arnold JC, Gunasekaran N *et al* (2008). Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology* **33**: 1113–1126.

Robbe D, Montgomery SM, Thome A, Rueda-Orozco PE, McNaughton BL, Buzsaki G (2006). Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nat Neurosci* **9**: 1526–1533.

Schlicker E, Kathmann M (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci* **22**: 565–572.

Schneider M, Koch M (2002). The cannabinoid agonist WIN 55,212-2 reduces sensorimotor gating and recognition memory in rats. *Behav Pharmacol* **13**: 29–37.

Soria G, Mendizabal V, Tourino C, Robledo P, Ledent C, Parmentier M *et al* (2005). Lack of CB1 cannabinoid receptor impairs cocaine self-administration. *Neuropsychopharmacology* **30**: 1670–1680.

Suenaga T, Ichitani Y (2008). Effects of hippocampal administration of a cannabinoid receptor agonist WIN 55,212-2 on spontaneous object and place recognition in rats. *Behav Brain Res* **190**: 248–252.

Thanos PK, Dimitrakakis ES, Rice O, Gifford A, Volkow ND (2005). Ethanol self-administration and ethanol conditioned place preference are reduced in mice lacking cannabinoid CB1 receptors. *Behav Brain Res* **164**: 206–213.

Touriño C, Ledent C, Maldonado R, Valverde O (2008). CB1 cannabinoid receptor modulates 3,4-methylenedioxymethamphetamine acute responses and reinforcement. *Biol Psychiatry* **63**: 1030–1038.

Ward J, Hall K, Haslam C (2006). Patterns of memory dysfunction in current and 2-year abstinent MDMA users. *J Clin Exp Neuropsychol* **28**: 306–324.

Wareing M, Fisk JE, Montgomery C, Murphy PN, Chandler MD (2007). Information processing speed in ecstasy (MDMA) users. *Hum Psychopharmacol* **22**: 81–88.

Yamaguchi T, Kubota T, Watanabe S, Yamamoto T (2004). Activation of brain prostanoid EP3 receptors via arachidonic acid cascade during behavioral suppression induced by Delta8-tetrahydrocannabinol. *J Neurochem* **88**: 148–154.

Yamamoto T, Takada K (2000). Role of cannabinoid receptor in the brain as it relates to drug reward. *Jpn J Pharmacol* **84**: 229–236.

Yamamoto T, Anggadiredja K, Hiranita T (2004). New perspectives in the studies on endocannabinoid and cannabis: a role for the endocannabinoid-arachidonic acid pathway in drug reward and long-lasting relapse to drug taking. *J Pharmacol Sci* **96**: 382–388.

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A TRYPTAMINE-DERIVED CATECHOLAMINERGIC ENHANCER, (−)-1-(BENZOFURAN-2-YL)-2-PROPYLAMINOPENTANE [(−)-BPAP], ATTENUATES REINSTATEMENT OF METHAMPHETAMINE-SEEKING BEHAVIOR IN RATS

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Abstract—Relapse to drug craving is problematic in treatment for drug abuse. Evidence suggests inactivation of dopaminergic neurotransmission during drug withdrawal. Meanwhile, a tryptamine analogue, (−)-1-(benzofuran-2-yl)-2-propylaminopentane [(−)-BPAP], has been reported to enhance electrical stimulation of monoamine release. This study examined the effect of (−)-BPAP on reinstatement of methamphetamine-seeking behavior in an animal model of relapse to drug abuse. Rats were trained to i.v. self-administer methamphetamine paired with a light and tone (methamphetamine-associated cues) under a fixed-ratio 1 schedule of reinforcement for 10 days. After extinction session under saline infusions without cues, a reinstatement test under saline infusions was begun. Reinstatement induced by methamphetamine-associated cues or methamphetamine-priming injections was attenuated by repeated administration of (−)-BPAP during the extinction phase. Acute administration of (−)-BPAP on test day dose-dependently attenuated both reinstatements. Acute administration of (−)-BPAP neither reinstated methamphetamine-seeking behavior alone nor affected methamphetamine self-administration. Pretreatment with either *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH-23390), a dopamine D_1-like receptor antagonist, or amisulpride, a dopamine D_2-like receptor antagonist, did not appreciably affected the acute effect of (−)-BPAP on both reinstatements. Co-pretreatment with the dopamine receptor antagonists failed to alter the effects of (−)-BPAP. Meanwhile, pretreatment with a dopamine D_1-like receptor agonist, (+/−)-6-chloro-7,8-dihydroxy-l-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide (SKF-81297), dose-dependently attenuated reinstatement induced by the cues or methamphetamine-priming injections. In contrast to (−)-BPAP, pretreatment with SCH-23390 reversed the effects of SKF-81297. Our findings suggest activation of dopamine D_1-like receptors results in attenuation of the reinstatement of methamphetamine-seeking behavior. Additionally, our findings provide evidence to develop (−)-BPAP and dopamine D_1-like receptor agonists as an anti-relapse medication for methamphetamine abusers.

Key words: craving, dopamine, methamphetamine, reinstatement, relapse, self-administration.

Abuse of the psychostimulant methamphetamine is a growing problem worldwide. Due to the prevalence of its abuse and lack of effective treatment in methamphetamine abuse, a clear need exists in clarifying the mechanisms underlying methamphetamine dependence. Among symptoms in drug dependence, relapse to craving is a main hurdle of treatment. In human and animal models of relapse, three different kinds of stimuli are capable of eliciting relapse: stress, cues predicting drug availability, and re-exposure to a previously self-administered drug (Shalev et al., 2002). Medications that reduce the ability of these stimuli to induce relapse may be effective in treatment of drug dependence. So far, we have reported (1) important roles of cannabinoid CB_1, nicotinic acetylcholine, and opioid receptors in reinstatement of methamphetamine-seeking behavior and (2) brain regions responsible for the reinstatement in rats (Anggadiredja et al., 2004a,b; Hiranita et al., 2004, 2006, 2008). We have reported agonists for $\alpha4\beta2$ nicotinic acetylcholine receptors and antagonists for cannabinoid CB_1 and opioid receptors as anti-relapse agents.

Evidence suggests inactivation of central dopaminergic neurotransmission during methamphetamine withdrawal. For example, clinical studies demonstrated loss of dopamine transporters in methamphetamine abusers during withdrawal (Volkow et al., 2001a,b). A post-mortem study also found reduced levels of dopamine nerve terminal markers such as dopamine, dopamine transporter, and tyrosine hydroxylase, an enzyme responsible for dopamine synthesis, in the striatum of methamphetamine abusers (Wilson et al., 1996). Recently, we demonstrated involvement of the nucleus accumbens, one of the main terminals of dopaminergic neurons, in the reinstatement of methamphetamine-seeking behavior in rats (Hiranita et al., 2006, 2008). Available reports on psychostimulants other than methamphetamine also suggest the inactivation of dopaminergic neurotransmission during drug withdrawal.

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Abbreviations: BD 1063, *N*-[2-(3, 4-dichlorophenyl) ethyl]-4-methylpiperazine; (−)-BPAP, (−)-1-(benzofuran-2-yl)-2-propylaminopentane; CHO cells, Chinese hamster ovary cells; SCH-23390, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; SKF-81297, (+/−)-6-chloro-7,8-dihydroxy-l-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide; σ1-R, σ1 receptor.

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Thus, a decrease in basal extracellular dopamine levels in the nucleus accumbens during withdrawal from cocaine self-administration has been shown (Weiss et al., 1992). Furthermore, decreased striatal 6-fluorodopa uptake, an index of dopaminergic presynaptic activity, was associated with increased duration of cocaine withdrawal (Wu et al., 1997). In the reinstatement model, dopamine D_1-like receptor agonists have been reported to block reinstatement of cocaine-induced cocaine-seeking behavior, whereas dopamine D_2-like receptor agonists enhance this behavior in rats (Self et al., 1996, 2000). Additionally, high levels of cocaine use have been reported to be subsensitive to the ability of the dopamine D_1-like receptor agonist (+/−)-6-chloro-7,8-dihydroxy-l-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide (SKF-81297) to inhibit cocaine-induced reinstatement of cocaine-seeking behavior, but supersensitive to the dopamine D_2-like receptor agonist quinpirole-induced reinstatement of cocaine seeking behavior in rats (Edwards et al., 2007). These findings suggest that inactivation of dopaminergic neurotransmission through dopamine D_1-like receptors during drug withdrawal might be pre-requisite to reinstatement of drug-seeking behavior. However, the involvement of the dopaminergic inactivation in relapse to methamphetamine-seeking behavior is not well understood.

Endogenous biogenic amines such as β-phenylethylamine and tryptamine have been found to enhance electrically stimulated release of [^{3}H] monoamines from the rat brainstem (Knoll et al., 1996). Based on the structure of these amines, (−)-1-(benzofuran-2-yl)-2-propylaminopentane [(−)-BPAP] has been synthesized and reported as a highly potent enhancer (Yoneda et al., 2001) of the electrically stimulated monoamine release (Miklya and Knoll, 2003). Dissimilar to methamphetamine (Yoneda et al., 2001) and tyramine (Shimazu et al., 2003b), however, (−)-BPAP alone does not release catecholamines. Furthermore, (−)-BPAP also has been reported to inhibit monoamine uptake (IC_{50} values: [3H] dopamine, [3H] noradrenaline, and [3H] serotonin; 42, 52, and 640 nM, respectively) (Shimazu et al., 2003b). However, none of the standard monoamine uptake inhibitors has the enhancing effect of electrically stimulated monoamine release (Miklya and Knoll, 2003). In addition, (−)-BPAP blocked tyramine-induced monoamine release from rat brain synaptosomes (Shimazu et al., 2003b). These findings suggest (−)-BPAP as an atypical monoamine uptake inhibitor. Behavioral studies demonstrated that (−)-BPAP stimulated locomotor activity in rats (Shimazu et al., 2003a) and that (−)-BPAP-induced hyperlocomotion was attenuated by pre-treatment with *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH-23390), a dopamine D_1-like receptor antagonist (Shimazu et al., 2001). Additionally, chronic administration of (−)-BPAP ameliorated impairment of social interaction behavior following forced swimming and the ameliorating effect was blocked by pre-treatment with either SCH-23390 or sulpiride, a dopamine D_2-like receptor antagonist (Tsunekawa et al., 2008). These findings suggest that (−)-BPAP functions as the activator of dopaminergic neurotransmission. Considering reports on inhibitory action of dopamine D_1-like receptor agonists on reinstatement of cocaine-seeking behavior (Self et al., 1996, 2000), we examined whether pretreatment with (−)-BPAP would block reinstatement of methamphetamine-seeking behavior in rats in comparison with SKF-81297, a dopamine D_1-like receptor agonist.

EXPERIMENTAL PROCEDURES

Drugs

The drugs used were methamphetamine hydrochloride (Dainippon Pharmaceutical Co., LTD, Osaka, Japan), [(−)-BPAP] (gift from Fujimoto Pharmaceutical Corporation), R-(+)-SCH-23390 (a dopamine D_1-like receptor antagonist, Sigma, St. Louis, MO, USA), amisulpride (a dopamine D_2-like receptor antagonist, Sigma), and SKF-81297 (a dopamine D_1-like receptor agonist, Sigma). All of the drugs were dissolved in 0.9% saline. Methamphetamine was delivered i.v. for self-administration (0.02 mg/0.1 ml/infusion) and i.p. for priming injections (1.0 mg/kg) 30 min before test sessions. R-(+)-SCH-23390 and SKF-81297 were administered s.c. 30 and 15 min before test sessions, respectively, while (−)-BPAP and amisulpride were administered i.p. 30 min and 1 h before test sessions, respectively. Repeated administration of (−)-BPAP or saline was administered daily, i.p. 30 min after the extinction sessions for 5 days.

Subjects

At the beginning of this study, 192 male Wistar rats (250–350 g, 10 weeks old, Nippon SLC Co., Hamamatsu, Japan) were individually housed in a temperature- and humidity-controlled environment under a 12-h light/dark cycle (lights on at 7:00 AM). Food and water were available *ad libitum* in the home cage except when daily food intake was limited to 15–20 g after the catheter implantation to fix the distance between the proximal position of a catheter in the vein and the surface of the atrial auricle. Procedures for animal handling were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the Declaration of Helsinki and Faculty of Pharmaceutical Sciences, Kyushu University Publication, 1988.

Apparatus

The injector system contained a fluid swivel (Instech Lab., Inc., PA, USA) mounted on the top of each operant chamber (Neuroscience, Inc., Tokyo, Japan). One end of the swivel was connected via polyethylene tubing (Kaneka Medix Co., Osaka, Japan) encased in a protective stainless steel spring tether (Instech Laboratories, Inc., PA, USA) to the animal's catheter while the other end of the swivel was connected via polyethylene tubing to the infusion pump. The operant chambers were enclosed in ventilated, sound-attenuating cubicles and controlled by computer software (Med Associates Inc., VT, USA). The chamber's light was switched on throughout the session. Lever-pressing responses resulted in methamphetamine infusion (0.02 mg/infusion over 6 s) accompanied by light (mounted 4 cm above the lever, 200 lux) and tone (85 dB/2.9 kHz) for 26 s (methamphetamine associated-cues). The subsequent 20 s was a "time out" period during which lever presses were still recorded but not accompanied with infusions.

Surgery

Silicon catheters (Silascon; inner and outer diameter: 0.5 and 1.0 mm, respectively; Kaneka Medix Co., Osaka, Japan) were surgically implanted into the jugular vein under sodium pentobarbital (40 mg/kg i.p., Kyoritsu Seiyaku Co., Tokyo, Japan) anesthesia as

described previously (Hiranita et al., 2006). After the surgery, catheter patency was maintained by daily infusion of 0.15 ml saline solution containing heparin (30 U/ml) after each session.

Autoshaping

Autoshaping procedures to lever-press for food pellet reinforcement (45 mg; Bioserv, Holton Industries Co., Frenchtown, NJ, USA) in operant chambers under a fixed-ratio 1 schedule of reinforcement (each lever-pressing is reinforced) followed by the surgery for the self-administration training were used. Both the right and left levers were designated as active with a cue (light). The room lamp over the levers was illuminated to indicate that lever-pressing responses resulted in the immediate delivery of a food pellet and activation of the feedback tone for 0.5 s. Each session lasted for 20 min. Cessation of lever-pressing training occurred when the rat was able to deliver 30 pellets within 180 s for three consecutive sessions.

Methamphetamine self-administration, extinction, and reinstatement

Two days after surgery, rats were trained to self-administer methamphetamine under a fixed-ratio 1 schedule of reinforcement in a 2 h daily session for 10 days. Each injection was accompanied by a light and tone (methamphetamine-associated cues). During this time, inactive lever responses had no programmed consequences but were recorded. After the self-administration sessions, at least five extinction sessions (1 h) were conducted daily during which active lever responding resulted in an infusion of saline instead of methamphetamine without presentation of the methamphetamine-associated cues (and until the rats achieved the extinction criterion of less than 10 responses per session on the previously active lever). Reinstatement tests under saline infusions were carried out for 30 min from day six of extinction (or the day after rats achieved the extinction criterion) every 6 days under a fixed-ratio 1 schedule. In the cue-induced reinstatement test, immediately after the onset of the session, rats were re-exposed to the methamphetamine-associated cues and each press on the active lever resulted in presentation of the cues. In the methamphetamine-primed reinstatement test, methamphetamine (1.0 mg/kg i.p.) was injected 30 min before the test. Each response during the test session resulted in an infusion of saline but not the methamphetamine-associated cues. In the present study, subjects were mainly divided to the four following groups. The first group was used for repeated administration of (−)-BPAP (n=15) or saline (n=12) during extinction phase. The second group was used for pre-session treatment with (−)-BPAP before methamphetamine self-administration on the 10th tenth day of methamphetamine self-administration phase (n=6). The third group was used for pre-session treatment with (−)-BPAP, SKF-81297 or SCH-23390 alone after extinction phase (n=16, 18, or 18, respectively). The fourth group was used for pre-session treatment with (−)-BPAP or SKF-81297 on reinstatement test day (n=65 or 42, respectively). In the first group, subjects were further divided to sub-groups depending on treatment (eight for treatment with saline on cue presentation, six for treatment with (−)-BPAP on cue presentation, seven for treatment with saline on methamphetamine-priming injections, or six for treatment with (−)-BPAP on methamphetamine-priming injections). In the fourth group, subjects were also divided to sub-groups depending on reinstatement factors (methamphetamine-associated cues or methamphetamine-priming injections), treatment drugs, or the drug doses (see each figure legend for more detail). Each rat in the fourth group was used for two reinstatement tests first on methamphetamine-associated cues and then methamphetamine-priming injections. The sample sizes of methamphetamine-priming injections in each group were the same as or less than those of methamphetamine-associated cues because data from subjects with problems related to health or catheter issues have been removed. All of the tests were conducted with a mixed order schedule of drug doses. In order to minimize the overall number of subjects, control data were shared in the sub-groups pre-treated with (−)-BPAP or SKF-81297.

Operant task performance for food reinforcement

All subjects had sessions to lever-press for food-pellet reinforcement under a fixed-ratio 1 schedule 5 min after the self-administration or reinstatement session. Each test ended when rats had received 30 pellets or 1200 s had passed.

Data analysis

Data represent the mean±SEM of number of responses or methamphetamine infusions and were analyzed by ANOVA (a between-subjects design). The significance of effects on responding or methamphetamine infusions was assessed by ANOVA, with Dunn or Bonferroni t-test for *post-hoc* analyses as appropriate. To determine if there was a difference in effects of re-exposure to methamphetamine-associated cues or drug priming injections, a two-way (repeated administration of (−)-BPAP) and one-way (others) measures ANOVA was used. A one-way repeated measures ANOVA was used to assess the pre-session treatment effects of a single administration of (−)-BPAP on methamphetamine self-administration. A two-way repeated measures ANOVA was used to assess effects of repeated administration of (−)-BPAP on lever responses during the extinction phase. A two-way measures ANOVA was used to assess the effects of pre-session treatments of the test drugs on reinstatement of methamphetamine-seeking behavior, and food-maintained behavior (drug doses and reinstatement factors). Pearson's correlations were used to analyze correlation between total amount of methamphetamine-intake and number of responses at test sessions. Differences were considered significant at $P<0.05$.

RESULTS

On the first day of methamphetamine self-administration, the numbers of active and inactive lever responses per session were 73.8±7.3 and 70.7±38.4, respectively. During the second and third sessions, both numbers of active and inactive lever responses per session decreased from 46.5±4.0 and 17.7±10.1 to 29.1±2.1 and 4.5±0.8, respectively. During subsequent sessions, both the number of active and inactive lever responses did not alter (data not shown). Compared with lever responses, the total amount of daily methamphetamine intake was less vari-

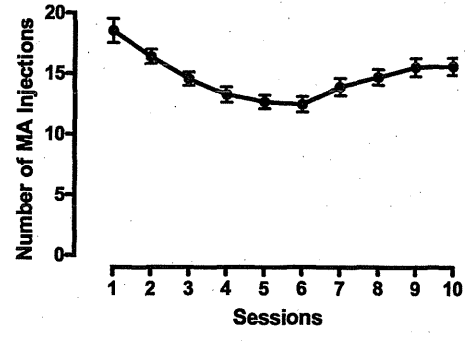

Fig. 1. Methamphetamine self-administration in rats. Rats were allowed to self-administer methamphetamine (0.02 mg/0.1 ml/injection) under a fixed ratio one schedule of reinforcement for a daily 2 h sessions for 10 days (n=180).

able (Fig. 1). On the first day of methamphetamine self-administration, the numbers of methamphetamine infusions per session were 18.5±1.0. In the subsequent sessions, the numbers of methamphetamine infusions per session decreased gradually until the sixth session (12.4±0.6). During the last four sessions, the numbers of methamphetamine infusions per session increased slightly. Total intake of methamphetamine over the course of the self-administration was 3.0±0.1 mg in rats excluding rats used for experiments on the effect of pre-session treatment with (−)-BPAP on methamphetamine self-administration. During the last three sessions of methamphetamine self-administration, both numbers of active and inactive lever responses per session were stable (23.5±1.5 and 3.1±0.1, respectively). On the first day of the extinction session, the numbers of active and inactive lever responses per session were 16.8±1.8 and 4.5±0.6, respectively. Through extinction sessions, the numbers of active lever responses per session decreased, whereas the numbers of inactive lever responses per session were relatively stable (data not shown). On the last day of extinction sessions, the numbers of active and inactive lever responses were 4.9±0.3 and 2.9±0.4, respectively in rats, excluding rats used for experiments on repeated administration of (−)-BPAP on reinstatement with methamphetamine-seeking behavior.

Effect of repeated administration of (−)-BPAP during extinction phase on reinstatement of methamphetamine-seeking behavior

During extinction sessions, the number of lever responses decreased gradually (data not shown). Two-way repeated measures ANOVA indicated significant effects of repeated administration of (−)-BPAP during extinction phase on extinction days ($F(4,100)=13.584$, $P<0.001$ and $F(4,100)=3.732$, $P=0.007$), but not drug treatment ($F(1,100)=3.265$, $P=0.083$ and $F(1,100)=0.0996$, $P=0.755$) or the interaction ($F(4,100)=0.728$, $P=0.575$ and $F(4,100)=0.0478$, $P=0.996$, active and inactive lever responses, respectively). In the saline-pretreated group as the control of (−)-BPAP administration, *post-hoc* comparison indicated significant effects on active lever responses on the first day of extinction compared with those of the third, fourth and fifth day ($t=3.269$, 3.269 and 3.897, $P=0.015$, 0.015 and 0.002, respectively). In the (−)-BPAP-pretreated group, *post-hoc* comparison indicated significant effects on active lever responses on the first day of extinction compared with those of the third, fourth and fifth day ($t=4.328$, 4.474 and 5.497, $P<0.001$, 0.001 and 0.001, respectively). In addition, *post-hoc* comparison indicated significant effects on active lever responses between saline- and (−)-BPAP groups on the first day of extinction ($t=2.036$, $P=0.044$). After extinction sessions, subsequent re-exposure to methamphetamine-associated cues and methamphetamine-priming injections increased active lever responses (Fig. 2). The increases in active lever responses in both were attenuated by repeated administration of (−)-BPAP during the extinction phase (Fig. 2). Two-way measures ANOVA indicated significant effect of (−)-BPAP treatment on the dose ($F(1,23)=122.507$, $P<0.001$), but not reinstatement factor (cues or methamphetamine-priming injections; $F(1,23)=0.133$, $P=0.179$), and significant effect on the interaction ($F(1,23)=16.203$, $P<0.001$). *Post hoc* analysis indicated significant effect of (−)-BPAP treatment on the increases in active lever responses induced by methamphetamine-associated cues ($t=5.056$, $P<0.001$) and methamphetamine-priming injections ($t=10.518$, $P<0.001$). On the other hand, methamphetamine-associated cues and methamphetamine-priming injections did not affect inactive lever responses (Fig. 2, $P\geq0.66$, both). However, two-way measures ANOVA indicated significant effect of (−)-BPAP treatment on the dose ($F(1,23)=7.562$, $P<0.011$), but not reinstatement factor ($F(1,23)=0.817$, $P=0.372$) or the interaction ($F(1,23)=0.817$, $P=0.372$). *Post hoc* analysis indicated significant effect of (−)-BPAP treatment on inactive lever responses induced by methamphetamine-associated cues ($t=2.627$, $P=0.015$) but not by methamphetamine-priming injections ($t=1.282$, $P=0.213$). In addition, the total amount of methamphetamine intake was not correlated with the increase in active lever responses induced by either methamphetamine-associated cues or methamphetamine-priming injections ($r=-0.382$ and -0.276, $P=0.350$ and 0.550, and $n=8$ and 7, respectively).

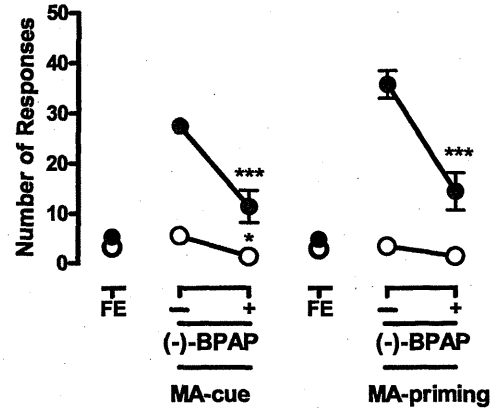

Fig. 2. Effects of repeated administration of (−)-BPAP during extinction phase (1.0 mg/kg i.p., 30 min after the extinction sessions daily for 5 days) on reinstatement of methamphetamine-seeking behavior induced by methamphetamine-associated cues or methamphetamine-priming injections. The reinstatement tests were performed 24 h after the last administration of (−)-BPAP. Closed and open circles indicate responding on active and inactive levers. * $P<0.05$, and *** $P<0.001$ versus a vehicle-treated group challenged with methamphetamine-associated cues or methamphetamine-priming injections. FE; final extinction. The order of test session was first cue presentation and then methamphetamine-priming injections. Data on FE consist of groups challenged with and without (−)-BPAP. In the test on methamphetamine-associated cues, the sample sizes of the FE session, vehicle, and (−)-BPAP pretreatment were 14, eight, and six, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session vehicle, and (−)-BPAP pretreatment were 13, seven, and six, respectively.

Effect of acute administration of (−)-BPAP on reinstatement of methamphetamine-seeking behavior

In an experiment on controls, methamphetamine-associated cues and methamphetamine-priming injections rein-

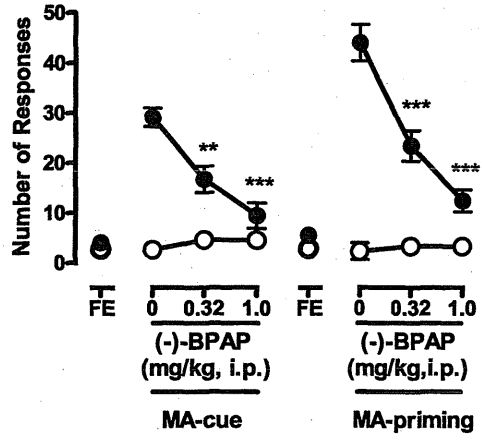

Fig. 3. Effects of acute administration of (−)-BPAP on reinstatement of methamphetamine-seeking behavior. (−)-BPAP was administered i.p. 30 min before the session. Closed and open circles indicate responding on active and inactive levers, respectively. ** $P<0.01$, and *** $P<0.001$ versus a vehicle-treated group challenged with methamphetamine-associated cues or methamphetamine-priming injections. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session and (−)-BPAP pretreatment at the dose of 0, 0.32, and 1.0 mg/kg were 31, 10, 11, and 10, respectively. In the test on methamphetamine-priming the injection, sample sizes of the FE session and (−)-BPAP pretreatment at the dose of 0, 0.32, and 1.0 mg/kg were 23, seven, seven, and nine, respectively.

stated methamphetamine-seeking behavior (Fig. 3). One-way ANOVA indicated significant effects of methamphetamine-associated cues and methamphetamine-priming injections on active lever responses ($P<0.001$, both) but not inactive lever responses ($P=0.792$ and 0.184, respectively). *Post hoc* analysis (Dunn's method) indicated significant effects of methamphetamine-associated cues and methamphetamine-priming injections on active lever responses ($Q=5.060$, $P<0.05$, and $Q=4.208$, $P<0.05$, respectively). The total amount of methamphetamine intake was not correlated with the increase induced by either methamphetamine-associated cues or methamphetamine-priming injections ($r=0.459$ and -0.611, $P=0.182$ and 0.145, $n=10$ and 7, respectively).

Similar to the repeated administration of (−)-BPAP during the extinction phase, acute administration of (−)-BPAP (0.32 and 1.0 mg/kg i.p.) on test day also attenuated an increase in active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections (Fig. 3). Two-way measures ANOVA indicated significant effects of (−)-BPAP dose ($F(2,48)=47.455$, $P<0.001$) and reinstatement factor ($F(1,48)=14.097$, $P<0.001$), but not the interaction ($F(2,48)=2.620$, $P=0.083$) on active lever responses. The *post hoc* analysis indicated significant effect of (−)-BPAP at the dose of 0.32 and 1.0 mg/kg on active lever responses induced by methamphetamine-associated cues ($t=3.605$, $P=0.002$ and $t=5.584$, $P<0.001$) and methamphetamine-priming injections ($t=4.903$, $P<0.001$ and $t=7.969$, $P<0.001$, respectively). However, acute administration of (−)-BPAP had non-significant effects on inactive lever responses ($P\geq0.25$). Two-way measures ANOVA indicated non-significant effect of (−)-BPAP on (−)-BPAP dose, reinstatement factor, and the interaction ($F(2,48)=0.678$, $P=0.513$; $F(1,48)=0.679$, $P=0.414$; and $F(2,48)=0.074$, $P=0.929$, respectively). As indicated in Fig. 4, pre-session treatment with SCH-23390, a dopamine D_1-like receptor antagonist, (0.1, 1.0, and 10 μg/kg s.c.) did not significantly reverse the attenuating effect of pre-session treatment with (−)-BPAP on the active lever responses induced by methamphetamine-associated cues or methamphetamine-priming injections. Two-way measures ANOVA indicated non-significant effects of SCH-23390 dose, reinstatement factor, and the interaction on the (−)-BPAP-induced decrease in active lever responses ($F(3,38)=1.664$, $P=0.191$; $F(1,38)=1.845$, $P=0.182$; and $F(3,38)=2.720$, $P=0.058$, respectively). When pre-treated with (−)-BPAP, SCH-23390 did not affect inactive lever responses (Fig. 4). Two-way measures ANOVA indicated non-significant effect of the pre-session treatment with SCH-23390 on the drug dose ($F(3,38)=0.261$, $P=0.853$), reinstatement factor ($F(1,38)=2.261$, $P=0.141$), and the interaction ($F(3,38)=0.821$, $P=0.821$). Meanwhile, pretreatment with amisulpride, a dopamine D_2-like receptor antagonist, (3.2 or 10 mg/kg i.p.) failed to affect the attenuating effects of (−)-BPAP on increased active lever responses induced by methamphetamine-associated cues or methamphetamine-priming injections (Fig. 5). Two-way measures ANOVA indicated non-significant effect of amisulpride dose ($F(3,34)=2.166$, $P=0.110$) and reinstatement factor ($F(1,34)=0.576$, $P=0.453$) on the active lever responses. Pre-session treatment with amisulpride (3.2 or 10 mg/kg i.p.) failed to affect inactive lever responses (Fig. 5). Two-way measures ANOVA indicated non-significant effect of amisulpride dose ($F(3,34)=0.570$, $P=0.639$) and reinstatement factor ($F(1,34)=0.254$, $P=0.617$) on the inactive lever responses. Additionally, co-pretreatment with SCH-23390

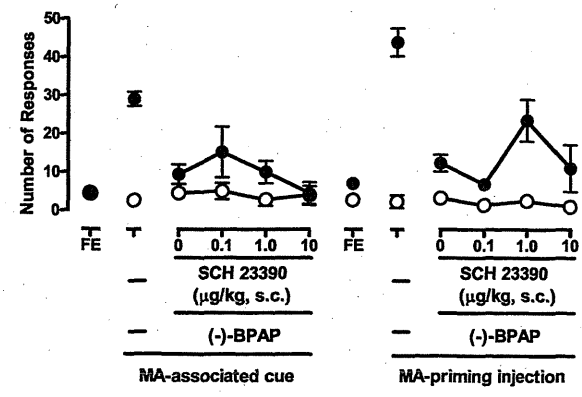

Fig. 4. Effect of acute administration of (−)-BPAP (1.0 mg/kg i.p.) with pre-session treatment with SCH-23390, a dopamine D_1-like receptor antagonist, on reinstatement of methamphetamine-seeking behavior. SCH-23390 was administered s.c. and concurrently with (−)-BPAP 30 min before the session. Closed and open circles indicate responding on active and inactive levers. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with SCH-23390 at the dose of 0, 0.1, 1.0 and 10 mg/kg were 19, 10, five, five, five, and four, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with SCH-23390 at the dose of 0, 0.1, 1.0 and 10 mg/kg were 10, nine, five, four, and three, respectively.

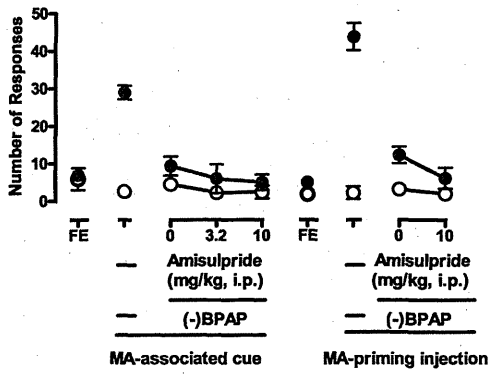

Fig. 5. Effect of acute administration of (−)-BPAP (1.0 mg/kg i.p.) with pre-session treatment of amisulpride, a dopamine D_2-like receptor antagonist, on reinstatement of methamphetamine-seeking behavior. Amisulpride and (−)-BPAP were administered i.p. 1 h and 30 min before the session, respectively. Closed and open circles indicate responding on active and inactive levers. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with amisulpride at the dose of 0, 3.2, and 10 mg/kg were eight, 10, five, four and five, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with amisulpride at the dose of 0, and 10 mg/kg were five, nine, five, and six, respectively.

(10 μg/kg s.c.) and amisulpride (10 mg/kg i.p.) failed to block the attenuating effect of pre-session treatment with (−)-BPAP on the increased active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections (Fig. 6). Two-way measures ANOVA indicated non-significant effect of drug treatment ($F(1,24)=3.138$, $P=0.089$), reinstatement factor ($F(1,24)=0.589$, $P=0.450$), and the interaction ($F(1,24)=0.0616$, $P=0.806$) on the active lever responses. The co-pretreatment with SCH-23390 and amisulpride also failed to alter responses on inactive levers (Fig. 6). Two-way measures ANOVA indicated non-significant effect of drug treatment ($F(1,24)=0.971$, $P=0.334$), reinstatement factor ($F(1,24)=0.0277$, $P=0.869$) and the interaction ($F(1,24)=0.324$, $P=0.574$) on the active lever responses.

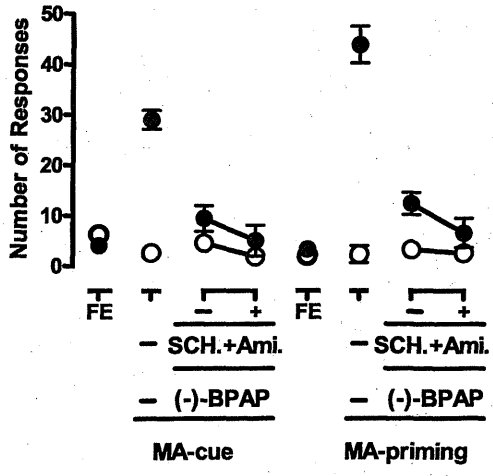

Fig. 6. Effect of pre-session treatment with (−)-BPAP (1.0 mg/kg i.p.) combined with both SCH-23390 (10 μg/kg s.c.) and amisulpride (10 mg/kg i.p.) on reinstatement of methamphetamine-seeking behavior. (−)-BPAP, and SCH-23390 were administered 30 min before the session, whereas amisulpride was administered 1 h before the session. Closed and open circles indicate responding on active and inactive levers. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session, vehicle alone, and pre-session treatment with (−)-BPAP co-pretreated with and without combination of SCH-23390 and amisulpride were five, 10, five, and five, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session, vehicle alone, and pre-treatment with (−)-BPAP co-pretreated with and without combination of SCH-23390 and amisulpride were four, nine, five, and four, respectively.

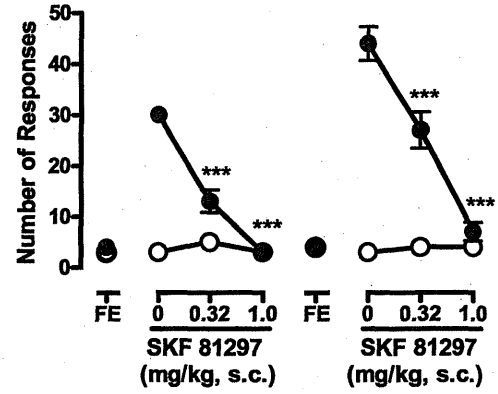

Fig. 7. Effects of pre-session treatment with SKF-81297, a dopamine D_1-like receptor agonist, on reinstatement of methamphetamine-seeking behavior. SKF-81297 was administered s.c. 15 min before the session. Closed and open circles indicate responding on active and inactive levers, respectively. *** $P<0.001$ versus groups challenged with methamphetamine-associated cues or methamphetamine-priming injections alone. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session and SKF-81297 pretreatment at the dose of 0, 0.32, and 1.0 mg/kg were 24, eight, eight, and eight, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session and SKF-81297 pretreatment at the dose of 0, 0.32, and 1.0 mg/kg were 24, eight, eight, and eight, respectively.

Effect of pre-session treatment with SKF-81297, a dopamine D_1-like receptor agonist, on reinstatement of methamphetamine-seeking behavior

In an experiment on controls, methamphetamine-associated cues and methamphetamine-priming injections reinstated methamphetamine-seeking behavior (Fig. 7). One-way ANOVA indicated significant effects of methamphetamine-associated cues on active lever responses ($F(1,30)=579.836$, $P<0.001$) and methamphetamine-priming injections on active and inactive lever responses ($P<0.001$ and $=0.041$). *Post hoc* analysis indicated significant effects of methamphetamine-associated cues on active lever responses ($t=24.080$, $P<0.001$, Bonferroni t-test) and methamphetamine-priming injections on active and inactive lever responses ($Q=4.178$, $P<0.05$, and $Q=2.024$, $P<0.05$, respectively, Dunn's Method). Total amount of methamphetamine intake was not correlated with the increase induced by either methamphetamine-associated cues or methamphetamine-priming injections ($r=-0.681$ and -0.401, $P=0.0628$ and 0.325, $n=8$ and 8, respectively).

Pre-session treatment with SKF-81297 (0.32–1.0 mg/kg s.c.) dose-dependently attenuated an increase in active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections (Fig. 7). Two-way measures ANOVA indicated significant effects of SKF-81297 dose ($F(2,47)=88.858$, $P<0.001$), reinstatement factor ($F(1,42)=30.898$, $P<0.001$), but not the interaction ($F(2,42)=3.163$, $P=0.053$) on increase in active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections. The *post hoc* analysis indicated significant effect of SKF-81297 at the dose of 0.32 and 1.0 mg/kg on an increase in active lever responses induced by methamphetamine-associated cues ($t=5.001$, $P<0.001$, and $t=7.911$, $P<0.001$) and methamphetamine-priming injections ($t=4.889$, $P<0.001$ and $t=10.934$, $P<0.001$, respectively). The pre-session treatment with SKF-81297 failed to affect responses on inactive levers (Fig. 7). Two-way measures ANOVA indicated non-significant effect of SKF-81297 dose ($F(2,47)=1.719$, $P=0.192$), reinstatement factor ($F(1,47)=0.0559$, $P=0.814$) and the interaction ($F(2,47)=0.753$, $P=0.477$) on the inactive lever responses. Pre-session treatment with SCH-23390 (0.1 and 1.0 μg/kg s.c.) dose-dependently blocked the attenuating effect of pre-session treatment SKF-81297 (1.0 mg/kg i.p.) on methamphetamine-associated cues as well as methamphetamine-priming injections (Fig. 8). Two-way measures ANOVA indicated significant effects of SCH-23390 dose ($F(2,30)=91.427$, $P<0.001$), reinstatement factor ($F(1,30)=8.728$, $P<0.001$), and the interaction ($F(2,30)=3.978$, $P=0.0029$) on the active lever responses induced by methamphetamine-associated cues and methamphetamine-priming injections. The *post hoc* analysis indicated significant effect of SCH-23390 at the dose of 0.1 and 1.0 μg/kg on the active lever responses induced by methamphetamine-associated cues ($t=3.049$, $P=0.014$, and $t=7.546$, $P<0.001$) and methamphetamine-priming injections ($t=5.272$, $P<0.001$, and $t=11.526$, $P<0.001$, respectively). However, SCH-23390 did not affect inactive lever responses (Fig. 8). Two-way measures ANOVA indicated significant effects of SCH-23390 dose ($F(2,30)=0.935$, $P=0.404$), reinstatement factor ($F(1,30)=1.555$, $P=0.222$), and the interaction ($F(2,30)=0.004\,31$, $P=0.996$) on the inactive lever responses.

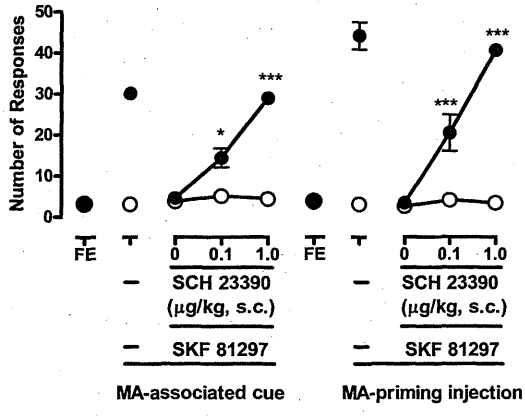

Fig. 8. Effects of pre-session treatment with SKF-81297 (1.0 mg/kg s.c.) combined with SCH-23390 (0, 0.1 and 1.0 μg/kg s.c.) on reinstatement of methamphetamine-seeking behavior. SKF-81297 and SCH-23390 were administered 15 and 30 min before the session. Closed and open circles indicate responding on active and inactive levers. * $P<0.05$ and *** $P<0.001$ versus SKF-81297-pretreated groups challenged with methamphetamine-associated cues or methamphetamine-priming injections alone. FE; final extinction. In the test on methamphetamine-associated cues, the sample sizes of the FE session, vehicle alone, and SKF-81297 co-pretreated with SCH-23390 at the dose of 0, 0.1, and 1.0 mg/kg were 26, eight, six, six, and six, respectively. In the test on methamphetamine-priming injections, the sample sizes of the FE session, vehicle alone, and (−)-BPAP co-pretreated with SKF-81297 at the dose of 0, 0.1, and 1.0 mg/kg were 26, eight, six, six, and six, respectively.

Effects of administration of (−)-BPAP, SKF-81297 or SCH-23390 alone under extinction condition in methamphetamine self-administered rats after extinction sessions

(−)-BPAP alone (1.0–3.2 mg/kg i.p.) failed to affect lever responses on active and inactive levers (Fig. 9A). At the higher dose (10 mg/kg i.p.), (−)-BPAP increased in both active and inactive lever responses (Fig. 9A). One-way ANOVA and the subsequent *post hoc* analysis with Dunn's method indicated significant effect of (−)-BPAP at the dose of 10 mg/kg on active lever responses ($Q=3.598$, $P<0.05$). In addition, one-way ANOVA indicated significant effect of (−)-BPAP at 10 mg/kg on inactive lever responses ($P=0.038$); however, the subsequent *post hoc* analysis with Dunn's method indicated non-significant effect of (−)-BPAP at 10 mg/kg on inactive lever responses ($Q=2.071$, $P\geq0.05$) compared with that at 0 mg/kg. Total amount of methamphetamine intake was correlated with responses on neither active nor inactive responses ($r=0.4598$ and 0.2169, $P=0.3589$ and 0.6797, respectively). On the other hand, neither SKF-81297 (0.032–1.0 mg/kg s.c., Fig. 9B) nor SCH-23390 (1–100 μg/kg s.c., Fig. 9C) affected responses on active and inactive levers. One-way ANOVA indicated non-significant effect of SKF-81297 and SCH-23390 on active ($F(3,32)=0.139$, $P=0.936$ and $F(3,32)=0.077$, $P=0.972$) and inactive ($F(3,32)=0.615$, $P=0.611$ and $F(3,32)=0.171$, $P=0.915$, respectively) lever responses.

Effects of pre-session treatment with (−)-BPAP on methamphetamine self-administration

On day 9 of methamphetamine self-administration, the number of methamphetamine infusions was 16.5±2.2 (Fig. 10). On day 10, pre-session treatment with (−)-BPAP (1.0 mg/kg i.p.) did not affect the number of methamphetamine infusions (Fig. 10, 16.8±1.6). Twenty four hours after the (−)-BPAP pretreatment, the number of methamphetamine infusions (Fig. 10, 16.8±2.9) was unchanged from those on the previous 2 days. Similar to the number of methamphetamine infusions, pre-session treatment with (−)-BPAP failed to affect responses on active and inactive levers during these 3 days (date not shown). One-way repeated measures ANOVA indicated non-significant effects of pre-session treatment with (−)-BPAP on total amount of methamphetamine intake ($F(5,10)=2.006$,

P=0.185), and active (F(5,10)=0.0185, P=0.982) and inactive (F(5,10)=0.200, P=0.822) lever responses.

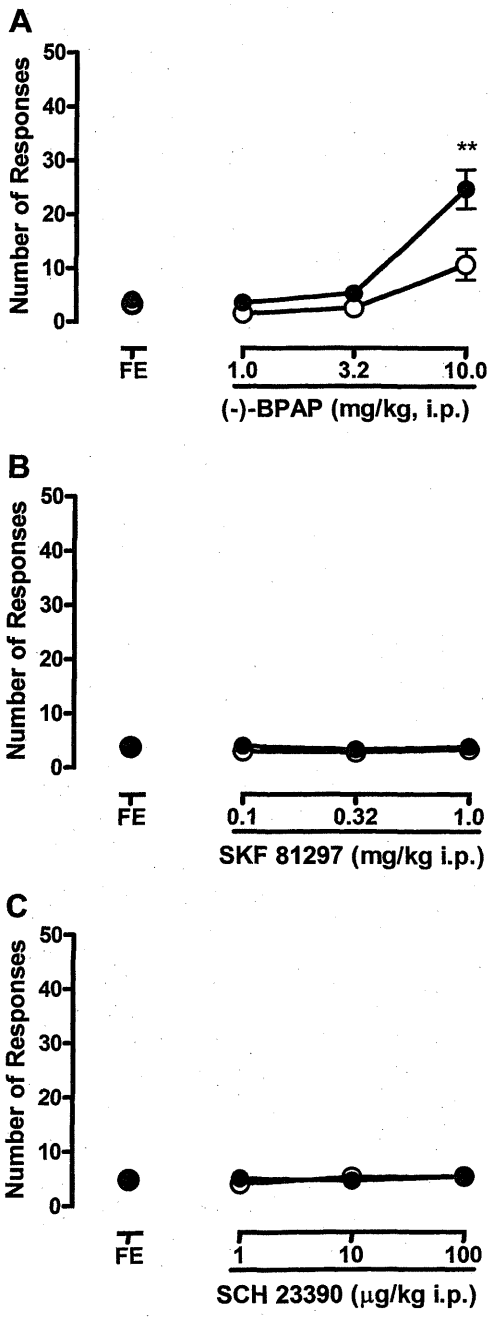

Fig. 9. Priming effects of pre-session treatment with (−)-BPAP, (A) SKF-81297, (B) and SCH-23390, (C) alone under extinction condition in methamphetamine self-administered rats after extinction sessions. (−)-BPAP was administered i.p. 30 min before the sessions, whereas SKF-81297 and SCH-23390 were administered s.c. 15 and 30 min before the session. Closed and open circles indicate responding on active and inactive levers. ** P<0.01 versus responding on active or inactive levers on final extinction (FE) day. (A) The sample sizes of the FE session and (−)-BPAP at the dose of 1.0, 3.2, and 10 mg/kg were 16, six, four, and six, respectively. (B) The sample sizes of the FE session, and SKF-81297 at the dose of 0.1, 0.32, and 1.0 mg/kg were 18, six, six, and six, respectively. (C) The sample sizes of the FE session, and SCH-23390 at the dose of 1.0, 10, and 100 μg/kg were 18, six, six, and six, respectively.

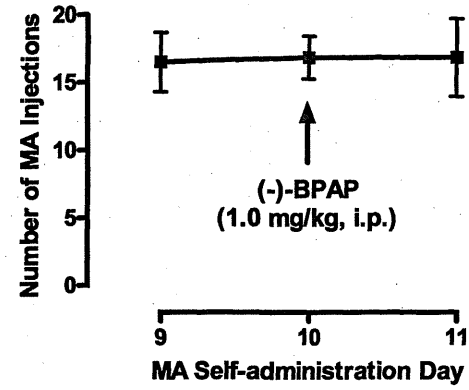

Fig. 10. Effects of pre-session treatment with (−)-BPAP in methamphetamine self-administering rats (n=6). (−)-BPAP was administered i.p. 30 min before the sessions.

Effects of (−)-BPAP and SKF-81297 on food-maintained behavior 5 min after the reinstatement sessions

In contrast to reinstatement of methamphetamine-seeking behavior, no pre-treatment with (−)-BPAP or SKF-81297 across the dose ranges tested had significant effects on food-maintained responses (Table 1). Two-way measures ANOVA indicated non-significant effect of repeated administration of (−)-BPAP on the dose (F(1,23)=2.824, P=0.106), reinstatement factor (F(1,23)=0.192, P=0.665), and the interaction (F(1,23)=0.295, P=0.592). Regarding single administration of (−)-BPAP, two-way measures ANOVA indicated a non-significant effect of (−)-BPAP on the dose (F(2,48)=0.170, P=0.844), reinstatement factor (F(1,48)=0.150, P=0.700), and the interaction (F(2,48)=0.244, P=0.784). In addition, two-way measures ANOVA indicated non-significant effect of SKF-81297 on SKF-81297 dose (F(2,42)=0.162, P=0.851), reinstatement factor (F(1,42)=0.119, P=0.732), and the interaction (F(2,42)=0.0315, P=0.969).

DISCUSSION

Repeated administration of (−)-BPAP during extinction sessions attenuated reinstatement of methamphetamine-seeking behavior induced by methamphetamine-associ-

Table 1. Effect of (−)-BPAP or SKF-81297 on food-maintained behavior 5 min after the reinstatement sessions. Regarding the sample sizes, see Figs. 2, 3 and 7

Treatment	MA-associated cue	MA-priming injection
Repeated (−)-BPAP (mg/kg/day, i.p.)		
0	10.8±1.2	12.0±1.3
1.0	9.5±0.4	9.4±1.2
Single (−)-BPAP (mg/kg, i.p.)		
0	11.4±1.3	11.0±1.6
0.32	10.7±0.9	10.9±1.5
1.0	10.9±1.4	12.4±1.7
SKF-81297 (mg/kg, s.c.)		
0	10.6±0.4	11.1±0.4
0.32	10.9±0.4	10.8±0.4
1.0	10.8±0.5	10.8±0.6

ated cues and methamphetamine-priming injections. This result may suggest a preventive role of (−)-BPAP against the development of relapse to methamphetamine craving. Moreover, even a single pre-session treatment with (−)-BPAP also attenuated the reinstatement induced by methamphetamine-associated cues and methamphetamine-priming injections in a dose-related manner. Surprisingly, neither pre-session treatment with SCH-23390, a dopamine D_1-like receptor antagonist, nor amisulpride, a dopamine D_2-like receptor antagonist, across dose ranges tested appreciably reversed the inhibitory effect of single pre-session treatment with (−)-BPAP. In addition, combined pre-session treatment with SCH-23390 and amisulpride failed to reverse the effect of a single pre-session treatment with (−)-BPAP. This finding suggests that (−)-BPAP blocks the reinstatement of methamphetamine-seeking behavior through mechanisms other than dopamine receptors. On the other hand, pre-session treatment with SKF-81297, a dopamine D_1-like receptor agonist, dose-dependently attenuated the reinstatement of methamphetamine-seeking behavior induced by either methamphetamine-associated cues or methamphetamine-priming injections similar to the result obtained from the single pre-session treatment with (−)-BPAP. In contrast to (−)-BPAP, SCH-23390 dose-dependently reversed the inhibitory effect of SKF-81297. Additionally, SCH-23390 alone failed to reinstate methamphetamine-seeking behavior. Therefore, these results suggest an inhibitory role of dopamine D_1-like receptors to reinstate methamphetamine-seeking behavior in rats. Several studies demonstrated inactivated function of dopamine D_1-like receptors. For example, clinical study demonstrated reduced activity of adenyl cyclase after striatal dopamine D_1-like receptor-stimulation in methamphetamine abusers (Tong et al., 2003). In preclinical study, rats that self-administered methamphetamine exhibited downregulation of dopamine D_1-like receptor protein in the nucleus accumbens during withdrawal (Stefanski et al., 1999). However, inability of SCH-23390 to reinstate methamphetamine-seeking behavior in the present study suggests that blockade of dopamine D_1-like receptors by itself is insufficient to reinstate methamphetamine-seeking behavior.

Decrease in lever responding might result from a general overactivation or suppression of behavioral activity. However, neither repeated nor single pretreatment of (−)-BPAP decreased in responding maintained by food reinforcement. Furthermore, pre-session treatment with SKF-81297 also failed to affect food-maintained responding. Additionally, the half-life of radio-labeled [(−)-BPAP-14C] has been reported to be 5.5 to 5.8 h, which is long enough for (−)-BPAP to continue the action during sessions on food reinforcement (Magyar et al., 2002). Therefore, blocking effects of (−)-BPAP or SKF-81297 on the reinstatement of methamphetamine-seeking behavior do not result from nonspecific behavioral effects.

Pre-session treatment with (−)-BPAP at a dose of 1.0 mg/kg selectively affected reinstatement of methamphetamine-seeking behavior, but not methamphetamine self-administration. Radio-labeled [(−)-BPAP-14C] has been reported to be well-absorbed after the i.p. and s.c. treatment and the peak concentration in the rat brain has been reached at 30 to 60 min following s.c. administration (Magyar et al., 2002). Therefore, during the session on reinstatement and self-administration in the present study, the concentration of (−)-BPAP in the brain appears to reach a peak. Considering these findings, our data suggest that methamphetamine's reinforcing effect might be less sensitive to actions of (−)-BPAP compared to the reinstatement of methamphetamine-seeking behavior. Clinical study also demonstrated that the reinforcing effect of psychostimulants is extremely robust and simply unaltered by even substantial medication effects on drug "craving" or its subjective effects. Thus, maintenance on the antidepressant desipramine in volunteers with a history of cocaine abuse resulted in a 40% decrease in ratings of "I want cocaine," yet had no effect on the amount of cocaine self-administered (Fischman et al., 1990). Therefore, (−)-BPAP may be effective as an anti-relapse therapeutic; however, (−)-BPAP may not work sufficiently as anti-methamphetamine abuse medication.

Pre-session treatment with (−)-BPAP alone at the dose 1.0 and 3.2 mg/kg did not reinstate methamphetamine-seeking behavior, whereas (−)-BPAP at only the highest dose (10.0 mg/kg) demonstrated moderate reinstatement. A tenfold higher dose was needed to reinstate methamphetamine-seeking behavior compared with the dose at which pre-session treatment with (−)-BPAP (1.0 mg/kg) attenuated the reinstatement of methamphetamine-seeking behavior induced by methamphetamine-associated cues and methamphetamine-priming injections. On the other hand, agonist/substitution therapies for opiate abuse with methadone and tobacco addiction with various formulations of nicotine have been reported to be effective (Henningfield, 1995; Kreek, 1996). In addition to the beneficial results, self-administration of methadone (Altshuler et al., 1975; Werner et al., 1976; Oei et al., 1980; Martin et al., 2007) and nicotine (Le Foll and Goldberg, 2005) in humans or experimental animals has been reported. Considering the positive and negative reports on methadone and nicotine, the potential ability of (−)-BPAP at the high dose to reinstate methamphetamine-seeking behavior in clinical situations may not discredit its clinical application as an anti-relapse agent for methamphetamine abusers.

(−)-BPAP has been reported to be an inhibitor of monoamine uptake in HEK cell (IC_{50} values: [^{3}H] dopamine, [^{3}H] noradrenaline, and [^{3}H] serotonin; 42, 52, and 640 nM, respectively) (Shimazu et al., 2003b), suggesting possible involvement of the relatively higher affinity for noradrenaline transporters in inhibitory effects of (−)-BPAP on the reinstatement of methamphetamine-seeking behavior. Moreover, methamphetamine has an at least twofold higher affinity for noradrenaline transporters than dopamine transporters but negligible affinity for serotonin transporters (Rothman and Baumann, 2003). However, dissimilar to methamphetamine (Yoneda et al., 2001) and tyramine (Shimazu et al., 2003b), (−)-BPAP alone does not release catecholamines. In addition, (−)-BPAP blocked tyramine-induced noradrenaline and dopamine