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MEDIODORSAL NUCLEUS OF THE THALAMUS IS CRITICAL FOR THE EXPRESSION OF MEMORY OF METHAMPHETAMINE-PRODUCED CONDITIONED PLACE PREFERENCE IN RATS

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Abstract—Methamphetamine (MA) is a powerful and highly addictive psychostimulant. However, the neural substrate mediating MA-induced conditioned effects, an essential part of addiction, remain unclear. The present study investigated the involvement of the anterior cingulate cortex (ACC), the lateral nucleus of amygdala (LNA), and the mediodorsal nucleus of the thalamus (MD) in MA-conditioned place preference (CPP). Rats underwent bilateral radio-frequency lesions of the ACC, LNA, or MD followed by MA CPP training. Lesions of the MD, but not the ACC or LNA, disrupted MA CPP learning. To clarify the role of the MD on the different stages of the MA CPP memory process, bilateral microinfusions of lidocaine into the MD were performed 5 min prior to each conditioning trial, immediately after the conditioning trial, or 5 min before the testing phase. Pretesting, but not pre- or post-conditioning, infusions of lidocaine into the MD impaired MA CPP. Furthermore, a clear preference for the previously conditioned MA paired cues was expressed when the rats were tested again 24 h after infusions of lidocaine. These results are interpreted as indicating that the MD is specifically involved in the memory retrieval process of MA associated memory which suggests the MD could have an important role in relapse in individuals suffering from MA addiction.

Key words: methamphetamine, mediodorsal nucleus of thalamus, medial prefrontal cortex, the lateral nucleus of amygdala, conditioned place preference.

Cues associated with the use of abused substances can elicit drug-craving and drug-seeking (Weiss, 2005). This powerful, long lasting associative learning process, and the resulting salience attributed to drug-associated cues, can be studied in laboratory animals using conditioned place preference (CPP). CPP is based on the fact that pairing an environment with neutral distinctive stimuli with an abused drug (unconditioned stimuli) results in an acquired preference for that environment (conditioned stimuli) (Tzschentke, 2007). Methamphetamine (MA) is a commonly used addictive drug and it is more potent than other forms of amphetamines. The powerful addictive property has been attributed to its high lipophilicity (Gulaboski et al., 2007) and long elimination half-life (Perez-Reyes et al., 1991). MA has been shown to produce robust CPP in rats and mice (Mizoguchi et al., 2004; Kawamura et al., 2005; Nakagawa et al., 2005; Yang et al., 2006; Niwa et al., 2007). Extensive efforts have been devoted to identify brain areas that control the conditioned effect of cues associated with abused drugs including cocaine (Isaac et al., 1989; Brown and Fibiger, 1993; Fuchs et al., 2002; Zavala et al., 2003; Sellings et al., 2006), morphine (Spyraki et al., 1983; Bechara and van der Kooy, 1992; Olmstead and Franklin, 1997a,b; White et al., 2005), alcohol (Bechtholt and Cunningham, 2005; Gremel and Cunningham, 2008), and amphetamine (Clarke et al., 1990; Hiroi and White, 1991, 1993; Olmstead and Franklin, 1996; Leri and Franklin, 2000; Liao, 2008). In contrast, very few studies have addressed the neural circuitry underlying MA CPP learning.

The involvement of the mesolimbic dopamine pathway in the acquisition and expression of CPP has been established for most drugs of abuse. Investigations of other brain areas have indicated a more selective or less conclusive involvement in CPP to commonly abused drugs. For example, lesions of the lateral nucleus of amygdala (LNA) have been shown to impair acquisition of the food CPP (White and McDonald, 1993) Similarly, animals failed to express amphetamine CPP when the LNA was damaged after conditioning (Hiroi and White, 1991). In contrast, lesions of the LNA had no effect on morphine CPP (Olmstead and Franklin, 1997a) or impaired morphine CPP depending on the configuration of the apparatus (White et al., 2005). Lesions of the fornix facilitated acquisition on food CPP, but impaired morphine CPP (Olmstead and Franklin, 1997a). Even though the dopamine transporter is a common target of MA, amphetamine and cocaine, the development of CPPs to these stimulants may not involve identical neural substrates.

The anterior cingulate cortex (ACC), a part of the brain's limbic system, plays a key role in cognitive, motor, and emotional processing (Bush et al., 2000). It has been strongly activated by cocaine and morphine associated cues (Childress et al., 1999; Sell et al., 1999) and is implicated in encoding and retrieval of drug-related memory that leads to drug craving and drug use (Robbins et al., 2008). The amygdala has been considered as a critical

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Abbreviations: ACC, anterior cingulate cortex; AP, anteroposterior; CPP, conditioned place preference; DV, dorsal-ventral; LNA, lateral nucleus of amygdale; MA, methamphetamine; MD, mediodorsal nucleus of the thalamus; ML, mediolateral.

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neural substrate underlying the formation of stimulus-reward associations (Baxter and Murray, 2002). Lesions of the LNA, but not the central or basolateral nucleus of amygdala, attenuated expression of amphetamine CPP (Hiroi and White, 1991), suggesting the LNA is critical for expression of amphetamine CPP. The MD is part of the limbic-cortical circuitry shown to be involved in motor, cognitive, and motivational aspects of behavior (Baxter and Murray, 2002). Bilateral excitotoxin-induced lesions of the MD produced a marked attenuation in the acquisition of cocaine self-administration (Weissenborn et al., 1998). Moreover, it has been reported that approximately 10% of MD neurons showed strong responses during the presentation of cues associated with a reward (Kawagoe et al., 2007). Based on the involvement of the ACC, LNA, and MD in rewarding behaviors, the effects of radio-frequency lesions of these brain regions on MA CPP were examined.

After demonstrating that lesions of MD impaired MA CPP, the critical role of the MD in information processing in the different stages of CPP was evaluated by a reversible inactivation procedure using lidocaine microinfusions. Lidocaine is a local anesthetic agent that produces reversible inactivation of neural tissue via blockade of voltage-gated sodium channels (Cahalan, 1978). Infusions were made either before or immediately after conditioning to focus on acquisition (encoding) or consolidation of memory processes, or before the testing phase to act specifically on retrieval of CPP memory.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (National Laboratory Animal Center, Taiwan) weighing 250–300 g were used in this study. They arrived at the animal facilities at least 5 days prior to the start of the experiments. Rats were housed in groups of three on a 12/12 light-dark cycle (lights on 0700 h) at 22 °C. Food and water were available *ad libitum* during the time the animals were in their home cages. The experimental protocol was approved by Review Committee of the Tzu Chi University for the Use of Animal Subjects.

Drugs

Methamphetamine (MA) hydrochloride was purchased from National Bureau of Control Drug, Department of Health, Taiwan. All other chemicals were obtained from Sigma (St. Louis, MO, USA).

Surgery

Radiofrequency lesion procedure. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and positioned in the stereotaxic apparatus. Coordinates for bilateral lesions were based on the atlas of Paxinos and Watson (Paxinos and Watson, 1998) and adjusted according to pilot studies to the following positions from bregma; ACC (anteroposterior (AP): +1.5 and +2.5; mediolateral (ML): ±0.5; dorsal-ventral (DV): 1.5), LNA (AP: 3.5; ML: ±5.5; DV: 8.4), MD (AP: 2.5; ML: ±0.5; DV: 5.4), and MD Control (AP: 2.5; ML: ±0.5; DV: 5). Lesions were made using enamel-insulated nichrome electrode (0.25 mm in diameter) with a Lesion Generator System (Model RFG-4A, Radionics Inc., Burlington, MA, USA) at least 1 week prior to CPP training. The lesion parameters for the ACC and LNA were 1.5 mA for 30 s; those for the MD were 3 mA for 30 s.

Microinfusion of lidocaine

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and positioned in the stereotaxic apparatus in a flat skull position with the incisor bar set at −3.5 to −4.0 mm. Bilateral burr holes were drilled 2.5 mm posterior and 0.8 mm lateral to bregma. Rats were implanted with two 15 mm 22 gauge stainless guide cannulae to a depth of 4.4 mm from the surface of the skull to target the area 1 mm dorsal to the MD. The cannulae were fixed with dental acrylic cement and secured by three skull screws. Stylets were inserted into the guide cannulae to prevent clogging. The rats had at least 1 week to recover prior to testing in the CPP procedure. Rats received bilateral microinfusions of lidocaine 1 μl (20 μg/μl, dissolved in saline) at an infusion rate of 1 μl/min 5 min prior to each MA conditioning trial, immediate after conditioning trial, or 5 min before testing phase. The effect of lidocaine as a reversible Na^+-channel blocker may last for 30 min after infusion (Tehovnik and Sommer, 1997). The 28-gauge stainless steel infusion cannula extended 1 mm beyond the tip of the guide cannula targeting the MD and was subsequently left in place for 1 min following the infusion.

CPP apparatus

The apparatus and procedure for CPP were adopted according to the method of White et al. (White et al., 2005). It consisted of a large box made of wood, except for the front wall, which was Plexiglas. The box was divided into two compartments (A and B) of equal size (45×45×30 cm^3) by a wooden partition. One compartment was painted grey. The other compartment was painted with black and white vertical stripes on the walls and its floor and ceiling were painted white. An unpainted compartment C (36×18×20 cm^3), protruding from the rear of the compartments A and B, connected the two entrances (7×9 cm^2). The doors had removable wooden partitions that when lowered, would confine the rat to one of the larger compartments. When the partitions were removed, the rat could freely move between the two compartments via compartment C. The apparatus was situated in a brightly lit room about 60 cm from a one-way vision window, preventing the rats from seeing any of the cues in the room. The rats were observed from a darkened area on the other side of the window.

CPP procedure sessions

Behavioral testing and conditioning began after four to six daily handling. The procedure was divided into three consecutive phases.

Pre-exposure

On the first day of the experiment the partitions were not used allowing the rats to freely explore all three chambers of the apparatus for 10 min. To start the trial the rats were placed in compartment C. This phase of testing was performed in all experiments to allow habituation to the apparatus, and to verify that the rats did not exhibit any spontaneous preference for a given compartment.

Conditioning

During the conditioning phase, the partitions between the compartments were in place confining the rat to one chamber for the entire 30-min duration of the trial. This phase lasted a total of 4 days with each rat having one trial per day. Each rat was injected with MA (2 mg/kg, dissolved in saline, 1 ml/kg, i.p.) on 1 day and with the saline on the alternate day. Confinement to compartments occurred immediately after injections.