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Methamphetamine-induced dopamine terminal deficits in the nucleus accumbens are exacerbated by reward-associated cues and attenuated by CB1 receptor antagonism

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ABSTRACT

Methamphetamine (METH) exposure is primarily associated with deleterious effects to dopaminergic neurons. While several studies have implicated the endocannabinoid system in METH's locomotor, rewarding and neurochemical effects, a role for this signaling system in METH's effects on dopamine terminal dynamics has not been elucidated. Given that CB1 receptor blockade reduces the acute potentiation of phasic extracellular dopamine release from other psychomotor stimulant drugs and that the degree of acute METH-induced increases in extracellular dopamine levels is related to the severity of dopamine depletion, we predicted that pretreatment with the CB1 receptor antagonist rimonabant would reduce METH-induced alterations at dopamine terminals. Furthermore, we hypothesized that administration of METH in environments where reward associated-cues were present would potentiate METH's acute effects on dopamine release in the nucleus accumbens and exacerbate changes in dopamine terminal activity. Fast-scan cyclic voltammetry was used to measure electrically-evoked dopamine release in the nucleus accumbens and revealed markers of compromised dopamine terminal integrity nine days after a single dose of METH. These were exacerbated in animals that received METH in the presence of reward-associated cues, and attenuated in rimonabant-pretreated animals. While these deficits in dopamine dynamics were associated with reduced operant responding on days following METH administration in animals treated with only METH, rimonabant-pretreated animals exhibited levels of operant responding comparable to control. Moreover, dopamine release correlated significantly with changes in lever pressing behavior that occurred on days following METH administration. Together these data suggest that the endocannabinoid system is involved in the subsecond dopaminergic response to METH.

1. Introduction

In 2008, methamphetamine use was associated with over an estimated 66,000 emergency room visits (SAMHSA, 2010). Repeated use and high doses of METH are associated with cognitive (Gonzalez et al., 2004; Scott et al., 2007), structural and neurochemical abnormalities (Chang et al., 2007; Wilson et al., 1996). The negative behavioral and cognitive effects of chronic use are attributed to enduring changes in brain structure, function and chemistry (Chang et al., 2007; Scott et al., 2007), and thus elucidation of the neural correlates of METH-induced alterations have profound implications for developing therapeutics to treat METH-abuse. METH exerts significant dopaminergic effects through blockade and reversal of the dopamine transporter (DAT) (Sulzer et al., 1995), leading to increased levels of extracellular dopamine, and subsequent free radical production (Yamamoto and Zhu, 1998). The resulting oxidative stress likely underlies dopamine alterations from METH, with the degree of acute METH-induced aberrant dopamine overflow predicting the severity of enduring dopamine depletions (Yamamoto and Zhu, 1998).

Neurochemical depletions associated with chronic METH exposure are particularly pronounced in the striatum (Chang et al.,

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2007). Amphetamines exert significant action on DAT in the nucleus accumbens (NAc, Wise and Bozarth, 1985), an area in which chronic METH users exhibit deficits in glucose metabolism (Wang et al., 2004), DAT density, dopamine content and activity of the rate-limiting enzyme, tyrosine hydroxylase (Wilson et al., 1996). We therefore hypothesized that any manipulation of dopaminergic activity in this brain region during METH's acute effects would alter METH-induced dopaminergic effects. As drug users take substances in a wide range of environments, research into the impact of environmental stimuli (present during drug administration) on the effects of METH is warranted. As cues that predict access to food-maintained responding contribute to increased phasic dopamine release in the NAc (Roitman et al., 2004), we hypothesized that administration of the drug in environments with such cues would augment acute METH-induced raises in dopamine in this brain region and thus exacerbate METH-induced neurochemical depletion.

The endocannabinoid system, comprised of its neurotransmitters, receptors and enzymes (Alger and Kim, 2011), has been implicated in the stereotypy and rewarding effects of METH, yet no work has investigated its role in METH-induced dopaminergic alterations. Studies have demonstrated that CB1 receptor blockade decreases METH self-administration systemically (Schindler et al., 2010) and into the NAc core (Rodriguez et al., 2011). In addition, local administration of the CB1 receptor antagonists rimonabant or AM251 into the NAc reduces stereotypy from METH (Morra et al., 2010). Importantly, CB1 receptors are densely expressed in the NAc (Julian et al., 2003), and their activity modulates the mesolimbic dopamine system, the rostral dopaminergic projection from the ventral tegmental area (VTA) to the NAc (Cheer et al., 2003; Hungund et al., 2003; Wenger and Fürst, 2004). Indeed, rats treated with rimonabant exhibit markedly attenuated cocaine-induced increases in frequency and amplitude of NAc dopamine transients (Cheer et al., 2007b). We therefore hypothesized that rimonabant pretreatment would be associated with a reduction in the rise in extracellular dopamine caused by METH and thus a decrease in the effects of METH in the NAc.

2. Matierals and methods

2.1. Subjects

Male Sprague-Dawley rats ($n = 31$; 275–350 g and 65 days old upon arrival) were ordered from Charles River (Charles River, Wilmington, MA), housed individually in a temperature-controlled environment on a 12-h light/dark schedule (on at 7:00 am, off at 7 pm). Upon arrival, animals were allowed two days of rest and were provided food and water *ad libitum*. They were then weighed and brought down to 85% body-weight over 8 days.

2.2. Drugs

Methamphetamine HCL was obtained from Sigma-Aldrich and dissolved in saline at a concentration of 15 mg/mL. Rimonabant (SR141716A) (Research Triangle Institute—National Institute on Drug Abuse, Raleigh, NC) was freshly mixed in a 1:1:18 ratio of ethanol, emulphor (Alkamuls EL-620; Rhodia, Cran-bury, NJ), and saline (0.9%) at a concentration of 3 mg/mL. All drugs were injected intraperitoneally (IP).

2.3. Training boxes

Plexiglas chambers of 43 × 43 × 53 cm dimensions (Med. Associates, Inc., St Albans, VT, USA) were used for training. Chambers were individually housed in sound-attenuating cubicles. The beginning of training sessions was indicated by illumination of a cue light positioned above the right lever, a house light positioned at the top of the box opposite to the lever, lever extension of both the right and left levers and white noise. Pressing the right, but not the left lever induced 1) release of one chocolate pellet (F05984, 45 mg Chocolate flavored, Bio-Serv, Frenchtown, NJ) into a food cup positioned between the two levers, 2) withdrawal of both levers, 3) initiation of a tone and 4) termination of both the house and cue lights. After an inter- trial interval of 10 s, the levers extended, the lights re-illuminated and the tone was terminated. Rats were permitted to press the lever during daily one hour sessions. White noise remained on throughout the session.

2.4. Experimental design

2.4.1. Behavioral experiment

A between subjects design ($n = 31$) was used to assess how METH administration in an environment with reward-associated cues and rimonabant pretreatment modulates dopaminergic deficits that follow METH administration (Fig. 1). Four groups, FOOD + Saline, FOOD + METH, NoFOOD + METH and FOOD + RIMO + METH were randomly created upon arrival. Chocolate pellets (F05984, 45 mg Chocolate flavored, Bio-serv, Frenchtown, NJ) were placed in animal home cages 2 days prior to shaping to reduce neophobia on shaping days. Experimental groups (FOOD + Saline, FOOD + METH and FOOD + RIMO + METH) were shaped on a discrete 30 min FR1 food-maintained responding task for 4 days (Inter-Trial Interval (ITI) = 1 s), at which point all subjects displayed robust operant responding. An additional group (NoFOOD + METH) was not shaped and did not receive any food in the training box, but was placed in the same environment to control for environmental variables. In order to closely replicate the training environment of experimental animals, cues associated with food delivery were delivered non-contingently to NoFOOD + METH animals at a frequency similar to the rate of responding of trained rats. Following removal from training boxes, this group was given food in their home cages to maintain 85% weight. Post-shaping, subjects of the experimental groups (FOOD + Saline, FOOD + METH, FOOD + RIMO + METH) continued for seven days on daily discrete FR1 food-maintained responding (ITI = 10s) sessions for one hour. The NoFOOD + METH group was placed in the same environment, including non-contingent administration of cues associated with reward, but pellets were never dispensed. The NoFOOD + METH group did not engage in the operant behavior. All rats received IP injections of saline immediately preceding placement in training boxes, during shaping and training. Animals therefore did not experience an unusual amount of injection-related discomfort on days when they were administered drugs. On the eighth day of food-maintained responding, subjects were administered drugs before placement in their relative training environments. The FOOD + Saline ($n = 12$) group received behavioral training, vehicle pretreatment followed by saline injection on the day of drug administration. The FOOD + METH ($n = 6$) group received behavioral training,

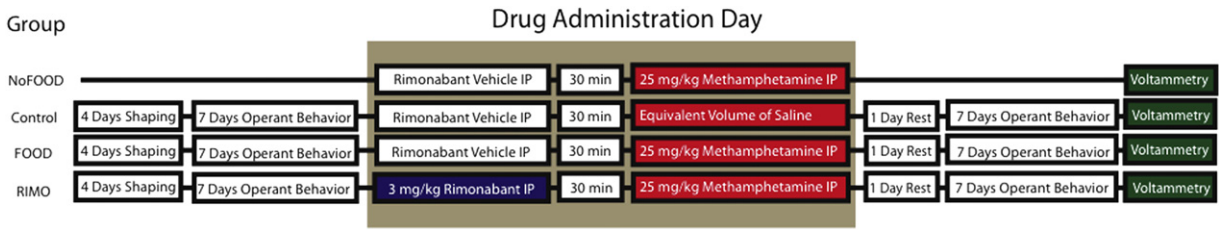

Fig. 1. Visual depiction of experimental design. A between subjects design ($n = 31$) was used to assess how the presence of reward-associated cues and rimonabant pretreatment impacts the dopaminergic deficits that follow METH administration. Animals in the different groups, FOOD + METH, FOOD + RIMO + METH, Food + Saline were shaped for 4 days, then maintained on hourly food-maintained responding sessions for seven days. On the following day animals were administered drugs, given one day of rest and returned to hourly sessions for seven days. Voltammetric experiments were then performed. The NoFOOD + METH group was not conditioned to lever press for food, was given food only in home cages and received vehicle pretreatment and METH administration; FOOD + Saline group was conditioned to lever press for food and received vehicle pretreatment and saline administration. FOOD + METH group was conditioned to lever press for food and received vehicle pretreatment and METH administration (25 mg/kg IP); FOOD + RIMO + METH group was conditioned to lever press for food and received rimonabant (3 mg/kg) pretreatment followed by METH. NoFOOD + METH group received equal exposure to the same environment with cues associated with reward, but lever press did not dispense food.