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Intravenous self-administration of entactogen-class stimulants in male rats

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ABSTRACT

The intravenous self-administration (IVSA) of 3,4-methylenedioxymethamphetamine (MDMA) is inconsistent in rats, with up to half of subjects failing to acquire reliable drug intake. It is unknown if this changes under long-access conditions (6 h sessions) under which the IVSA of cocaine and methamphetamine escalates. The entactogen class cathinone stimulants which exhibit MDMA-like monoamine effects in the nucleus accumbens, mephedrone (4-methylmethcathinone) and methylone (3,4-methylenedioxymethcathinone), may support more reliable IVSA but results have been mixed. This study was designed to directly compare the IVSA of these three compounds. Groups of male Wistar rats were trained to self-administer mephedrone, methylone or MDMA (0.5 mg/kg/inf) under a Fixed-Ratio (FR) 1 schedule of reinforcement for 14 sessions. Following the acquisition interval, animals were evaluated in FR (0.0, 0.125, 0.25, 0.5, 1.0, 2.5 mg/kg/inf) and Progressive Ratio (PR; 0.125, 1.0 mg/kg/inf) dose-substitution procedures. Long access conditions escalated MDMA intake over the 6 h session but not in the first 2 h. In short access, drug intake was significantly higher in mephedrone-trained rats compared with either the methylone-trained or MDMA-trained groups during acquisition. Mephedrone resulted in the highest intakes during FR and PR dose-substitution in MDMA- and mephedrone-trained groups. Overall it was found that mephedrone is a more effective reinforcer than methylone or MDMA and represents a higher risk for compulsive use.

1. Introduction

Recreational use of cathinone derivative drugs has increased substantially since 2009 and continues to expand worldwide and in the USA. Use of these substances continues despite legal control efforts internationally, at the US federal level, within multiple US states and even the local US jurisdictions. In particular, the earliest appearing entities such as 4-methylmethcathinone (4-MMC; **Mephedrone**) and 3,4-methylenedioxymethcathinone (**Methylone**; beta keto-MDMA) remain highly popular (De Paoli et al., 2011; Deluca et al., 2012; Winstock et al., 2011) and appear to have joined established drugs such as 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine or cocaine, rather than replacing them, in user populations (Moore et al., 2013). Although studies of dependence on these novel drugs are not available yet, a compulsive-use pattern is clear in many Case Reports of morbidity and user surveys point to use patterns and symptoms consistent with high potential for addiction and dependence (Dargan et al., 2010; Winstock et al., 2011). In addition, case reports of fatalities involving mephedrone or methylone are reminiscent of similar deaths attributed to MDMA (Lusthof et al., 2011; Patel et al., 2004; Pearson et al., 2012; Schifano, 2004; Torrance and Cooper, 2010). This motivates investigation of addiction liability in controlled animal models to determine the relative risks compared with more established recreational drugs.

The intravenous self-administration (IVSA) of MDMA has proven variable in rats (De La Garza et al., 2007; Feduccia et al., 2010), exhibits greater inter-subject variability than amphetamine (Dalley et al., 2007) and 40–50% of rats fail to meet acquisition criteria in some studies (Colussi-Mas et al., 2010; Oakly et al., 2014; Schenk et al., 2007). Furthermore, while long access (6 h daily sessions) to cocaine (Ahmed and Koob, 1998; Larson et al., 2007) or methamphetamine (Kitamura et al., 2006; Schwendt et al., 2009) leads to escalation of drug intake relative to animals trained only in 1–2 h sessions, a report of MDMA IVSA using 6 h sessions reported

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no difference in *total session intake* between 6 h and 2 h groups over the first 11 sessions (Schenk et al., 2003). That lab also reported that only 60% of rats met *a priori* acquisition criteria for MDMA IVSA even when trained in 6 h sessions for 15 days (Schenk et al., 2007) and that escalated intake is a function of the cumulative number of sessions (>20) more so than the duration of those sessions (Schenk et al., 2008). The studies by Schenk et al. were conducted in the rats' inactive period of the day which may explain why 6 h access did not result in many infusions past the first few hours and in addition, a relatively high per-infusion dose (1.0 mg/kg/inf) was used. One major goal of the present study was therefore to determine if 2 h and 6 h MDMA IVSA sessions result in identical intakes when rats are trained in their active part of the day, using a more moderate per-infusion dose (0.5 mg/kg/inf) as a reinforcer.

Pharmacologically, mephedrone and methylone have been found to serve as monoamine transporter substrates and monoamine releasers with enhanced effect on serotonin over dopamine systems (Baumann et al., 2012; Eshleman et al., 2013; Hadlock et al., 2011; Simmler et al., 2013), making them most similar to 3,4-methylenedioxymethamphetamine (MDMA) within the familiar amphetamine class substances. Mephedrone and methylone also both produce greater relative increases in nucleus accumbens serotonin compared with dopamine *in vivo* (Baumann et al., 2012; Kehr et al., 2011; Wright et al., 2012), which is a profile produced by MDMA (Baumann et al., 2008) but not amphetamine or methamphetamine. Thus it might be predicted that mephedrone and methylone would produce inconsistent IVSA such as has been described for MDMA. Evidence suggests, however, that mephedrone supports more consistent IVSA (Aarde et al., 2013a; Hadlock et al., 2011; Motbey et al., 2013) and methylone has been reported to be readily self-administered by male Sprague–Dawley rats (Watterson et al., 2012) in one study. A prior report from this laboratory found the IVSA of methylone to be similar to MDMA and dissimilar to mephedrone in *female* Wistar rats (Creehan et al., 2015), therefore another major goal was to determine if the relative reinforcer efficacies of these three drugs was similar in male Wistar rats under short access conditions.

2. Methods

2.1. Subjects

Male Wistar rats (Charles River, New York) were used for these investigations. Animals were housed in a humidity and temperature-controlled (23 ± 1 °C) vivarium on 12:12 h light:dark cycles. Animals entered the laboratory at 13–14 weeks of age and weighed an average of 383.1 (SEM: 6.3) grams at the start of the self-administration study. Animals had *ad libitum* access to food and water in their home cages. All procedures were conducted in the dark cycle, under protocols approved by the Institutional Care and Use Committees of The Scripps Research Institute and consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Intravenous catheterization

Rats (MDMA Short Access, N = 18; MDMA Long Access, N = 12; mephedrone Short Access, N = 18; methylone Short Access, N = 18) were anesthetized with an isoflurane/oxygen vapor mixture (isoflurane 5% induction, 1–3% maintenance) and prepared with chronic intravenous catheters as described previously (Aarde et al., 2013b, 2015a; Miller et al., 2012). Briefly, the catheters consisted of an 14-cm length of polyurethane based tubing (Micro-Renathane®, Braintree Scientific, Inc, Braintree MA, USA) fitted to a guide cannula (Plastics One, Roanoke, VA) curved at an angle and encased in dental cement anchored to an ~3 cm circle of durable mesh. Catheter tubing was passed subcutaneously from the animal's back to the right jugular vein. Catheter tubing was inserted into the vein and tied gently with suture thread. A liquid tissue adhesive was used to close the incisions (3M™ Vetbond™ Tissue Adhesive; 1469SB).

A minimum of 4 days was allowed for surgical recovery prior to starting an experiment. For the first three days of the recovery period, an antibiotic (cephazolin) and an analgesic (flunixin) were administered daily. During testing and training, intravenous catheters were flushed with ~0.2–0.3 ml heparinized (166.7 USP/ml) saline before sessions and ~0.2–0.3 ml heparinized saline containing cefazolan (100 mg/mL) after sessions.

Catheter patency was assessed nearly once a week after the last session of the week via administration through the catheter of ~0.2 ml (10 mg/ml) of the ultra-short-acting barbiturate anesthetic Brevital sodium (1% methohexital sodium; Eli Lilly, Indianapolis, IN). Animals with patent catheters exhibit prominent signs of anesthesia (pronounced loss of muscle tone) within 3 s after infusion. Animals that failed to display these signs were considered to have faulty catheters and were discontinued from the study. Data that was taken prior to failing this test and after the previous passing of this test were excluded from analysis.

2.3. Drugs

The racemic 4-methylmethcathinone (mephedrone) HCl used for this study was obtained from Fox Chase Chemical Diversity Center (Doylestown, PA). Racemic 3,4-methylenedio xymethamphetamine (MDMA) HCl was provided by the National Institute on Drug Abuse's Drug Supply Program. Racemic 3,4-methylenedioxymethcathinone (methylone) HCl was obtained from Cayman Chemical. All doses are expressed as the salt and were dissolved in physiological saline for injection.

2.4. Self-administration procedure

2.4.1. Acquisition

Drug self-administration was conducted in operant boxes (Med Associates) located inside sound-attenuating chambers located in an experimental room (ambient temperature 23 ± 1 °C; illuminated by red light) outside of the housing vivarium as in prior studies (Aarde et al., 2015b; Miller et al., 2015). To begin a session, the catheter fittings on the animals' backs were connected to polyethylene tubing contained inside a protective spring suspended into the operant chamber from a liquid swivel attached to a balance arm. Each operant session started with the extension of two retractable levers into the chamber. Following each completion of the response requirement (response ratio), a white stimulus light (located above the reinforced lever) signaled delivery of the reinforcer and remained on during a 20-sec post-infusion timeout, during which responses were recorded but had no scheduled consequences. Drug infusions were delivered via syringe pump. The training dose for all three drugs (0.5 mg/kg/infusion; ~0.1 ml/infusion) was selected from prior self-administration studies (Aarde et al., 2013a; Hadlock et al., 2011; Watterson et al., 2012) and comparison of mephedrone vs MDMA potency in locomotor and thermoregulatory studies (Aarde et al., 2013a; Huang et al., 2012; Miller et al., 2013; Wright et al., 2012) and confirmed in a prior study of IVSA in female rats (Creehan et al., 2015). Session duration for the normal (Short Access; ShA) acquisition and Fixed-Ratio dose-substitution sessions was 2 h, up to 3 h sessions were conducted for Progressive-Ratio dose substitution and the Long Access (LgA) training sessions were 6 h in duration.