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Effects of synthetic cathinones contained in "bath salts" on motor behavior and a functional observational battery in mice

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ABSTRACT

Synthetic stimulants commonly sold as "bath salts" are an emerging abuse problem in the U.S. Users have shown paranoia, delusions, and self-injury. Previously published *in vivo* research has been limited to only two components of bath salts (mephedrone and methylone). The purpose of the present study was to evaluate *in vivo* effects of several synthetic cathinones found in bath salts and to compare them to those of cocaine (COC) and methamphetamine (METH). Acute effects of methylenedioxyphyrovalerone (MDPV), mephedrone, methylone, methedrone, 3-fluoromethcathinone (3-FMC), 4-fluoromethcathinone (4-FMC), COC, and METH were examined in male ICR mice on locomotor activity, rotorod, and a functional observational battery (FOB). All drugs increased locomotor activity, with different compounds showing different potencies and time courses in locomotor activity. 3-FMC and methylone decreased performance on the rotorod. The FOB showed that in addition to typical stimulant induced effects, some synthetic cathinones produced ataxia, convulsions, and increased exploration. These results suggest that individual synthetic cathinones differ in their profile of effects, and differ from known stimulants of abuse. Effects of 3-FMC, 4-FMC, and methedrone indicate these synthetic cathinones share major pharmacological properties with the ones that have been banned (mephedrone, MDPV, methylone), suggesting that they may be just as harmful.

1. Introduction

An emerging substance abuse problem is abuse of synthetic research chemicals for their stimulant properties (DEA, 2011; Psychonaut, 2009). These products, commonly labeled as "bath salts" or "plant food," are administered through insufflation, oral, smoking, rectal and intravenous methods (Psychonaut, 2009; Winstock et al., 2011) and can be purchased legally in most states on the internet, at head shops, or at gas stations (DEA, 2011; Karila and Reynaud, 2010; Schifano et al., 2011; Winstock et al., 2010, 2011). The active components contained in bath salts are synthetic cathinone analogs. Within the first 8 months that bath salts were on the U.S. drug market, there were more than 1400 cases of misuse and abuse reported to U.S. poison control centers in 47 of 50 states (Spiller et al., 2011). The number of calls to poison control centers in the U.S. regarding bath salts rose from 303 in 2010 to 6072 in 2011 (American Association of Poison Control Centers, 2012). The growing prevalence of bath salt use makes them a major health concern throughout the U.S. and Europe.

Cathinone is naturally occurring in the leaves of the khat plant (*Catha edulis*), which grows in eastern Africa and southern Arabia where it is used for its amphetamine-like effects (Griffiths et al., 2010). In rats, cathinone produces locomotor increases similar to those produced by amphetamine (Kalix, 1992), and increases extracellular dopamine (Pehek et al., 1990). The synthetic cathinones that have been found in bath salts include, but are not limited to 3-FMC (3-fluoromethcathinone), 4-FMC (4-fluoromethcathinone), buphedrone (α-methylamino-butyrophenone), butylone (beta-keto-N-methyl-3,4-benzodioxyolybutanamine), MDPV (methylenedioxyphyrovalerone), mephedrone (4-methylmethcathinone), methedrone (4-methoxymethcathinone), methylone (3,4-methylenedioxymethcathinone), and naphyrone (naphthylpyrovalerone) (Karila and Reynaud, 2010). MDPV, mephedrone, and methylone are the most commonly found active components worldwide (ACMD, 2010; EMCDDA, 2010; Spiller et al., 2011), with MDPV being the most commonly found component in the U.S. (Spiller et al., 2011).

To the extent that the *in vivo* effects of synthetic cathinones have been examined, they have been found to share pharmacological properties with other abused drugs that increase levels of monoamine neurotransmitters (*e.g.*, stimulants such as cocaine

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and methamphetamine). For example, a low dose of mephedrone (3 mg/kg) produced moderate increases in locomotor activity in rats (Kehr et al., 2011), and higher doses (10 mg/kg and 30 m/kg) produced significant locomotor increases in mice (Angoa-Pérez et al., 2012). Acquisition of mephedrone self-administration also has been demonstrated (Hadlock et al., 2011). Anecdotal and case reports of human use of bath salts suggest these substances produce powerful psychological effects, including psychotic behavior, paranoia, delusions, hallucinations, and self-injury (EMCDDA, 2010; Spiller et al., 2011; Striebel and Pierre, 2011). In addition, since 2010, multiple cases of death while under the influence of bath salts in the U.S. have occurred, including some suicides (CDC, 2011; Spiller et al., 2011). Based on poison control center reports and case studies in the U.S., MDPV in particular tends to produce increased aggression, hallucinations, and paranoia (Antonowicz et al., 2011; Spiller et al., 2011). While these data suggest that the abuse liability and pharmacological effects of synthetic cathinones is likely similar to that of known stimulants of abuse, additional data are needed with a broader range of compounds that have been identified in purchased products.

Although some active components in bath salts have already been made illegal in some states (DEA, 2011; Spiller et al., 2011), and the DEA put an emergency ban on MDPV, mephedrone, and methylone in October 2011, it is likely that manufacturers will continue to make slight alterations in the chemical structure of these compounds in order to avoid detection and allow legal sale (Wohlfarth and Weinmann, 2010). This strategy is not without precedence, as Europe has seen the appearance of new bath salt products containing alternative cathinones following earlier legal restrictions (Camilleri et al., 2010). After legislative bans in Europe on MDPV and mephedrone, naphyrone has become the most commonly used bath salt in Europe. It is expected that once naphyrone becomes a controlled substance, a new compound will be ready for export to replace it (Eastwood, 2010).

The primary purpose of the present study was to evaluate the *in vivo* effects of synthetic cathinones that have been found in bath salts in the U.S. and to compare these effects to those produced by cocaine and methamphetamine. Cocaine is primarily a monoamine transporter blocker/reuptake inhibitor, and methamphetamine is primarily a monoamine transporter substrate/releaser (Fleckenstein et al., 2000; Rothman et al., 2001). Compounds identified in bath salts have been shown to be either cocaine-like monoamine reuptake inhibitors, methamphetamine-like releasers, or a hybrid of both mechanisms (Baumann et al., 2012; Cozzi et al., 1999; Martinez-Clemente et al., 2012; Meltzer et al., 2006; Nagai et al., 2007; Psychonaut, 2009; Winstock et al., 2010).

2. Materials and methods

2.1. Subjects

Male ICR mice (Harlan) (n = 8/group) were individually housed in clear plastic cages in a temperature-controlled environment (20–24 °C) with a 12 h light–dark cycle (lights on at 6 a.m.). Mice had *ad libitum* access to water and food in their home cages at all times, and were approximately 47–56 days old at the beginning of the study. Research reported in this manuscript was approved by the Institutional Animal Care and Use Committee at RTI International, and followed the principles of laboratory animal care (National Research Council, 2003).

2.2. Apparatus

Locomotor sessions were conducted in open field activity chambers made of clear Plexiglas measuring 33.0 cm × 51.0 cm × 23.0 cm. Beam breaks were recorded by San Diego Instruments Photobeam Activity System software (model SDI: V-71215, San Diego, CA) on a computer located in the experimental room. The apparatus contained two 4-beam infrared arrays that measured horizontal movement. A standard rodent rotorod apparatus was used to measure motor coordination (Stoelting, model 52790). The rotorod revolved at 10 revolutions per min, and reached this speed over the course of the first 30 s of the trial. Trials lasted a maximum of 150 s. Assessment in a functional observational battery (FOB) took place in a clear Plexiglas open field measuring 33.0 cm × 51.0 cm × 23.0 cm, and during handling.

2.3. Procedure

Mice were randomly assigned to a drug group and a particular dose of that drug. In addition, a single saline control group was assessed for comparison to each drug group. Mice were tested for locomotor activity during week 1, trained to walk in the rotorod apparatus during week 2, and tested on the rotorod apparatus, followed by an FOB, in week 3. They were administered two drug injections during the experiment, once during week 1, and once during week 3. Doses examined were saline, 10–42 mg/kg cocaine, 1–10 mg/kg methamphetamine, 1–30 mg/kg MDPV, 10–56 mg/kg of 4-FMC and methedrone, and 3–56 mg/kg 3-FMC, mephedrone, and methylone. All drugs were administered intraperitoneally (i.p.), and doses were chosen based on previous *in vivo* research on mephedrone (Angoa-Pérez et al., 2012; Hadlock et al., 2011; Kehr et al., 2011). Doses administered to each group of mice throughout the experiment are presented in Table 1.

On the first test day, mice were injected with their assigned drug dose and immediately placed into locomotor activity chambers for a 90-min test. Training on the rotorod occurred the following week. Mice were trained to walk on the rotorod on two nonconsecutive days that took place the week before their rotorod test day. For rotorod training day 1, mice were placed on the rotorod. If they fell off, they were placed back on the rotorod immediately. Mice were repeatedly put back on the rotorod if they fell off until they completed two 150-s trials on the rod without falling off, or until 30 min of training was completed, whichever occurred first. Rotorod training day 2 was identical to training day 1 except the maximum training time was 20 min. On the rotorod test day, a 150-s rotorod test was conducted at 15 min post-injection. Rotorod tests were used to evaluate motor coordination, balance (Forster and Lal, 1999; Steinpreis et al., 1999; Walsh and Wagner, 1992), and neurotoxicity (Jatav et al., 2008).

Immediately after the rotorod test, an FOB was used to classify observable effects of the drugs (20 min post-injection). The FOB was modified from a procedure commonly used by the Environmental Protection Agency (U.S. EPA, 1998a,b). This observational battery provides an overall behavioral profile of the effects of each compound with an emphasis on detection of potential safety concerns, and allows assessment of a wide range of drug effects. FOBs were scored by a single trained technician who was not blind to treatment. Mice were observed in pairs (each mouse was observed in a separate chamber), and each FOB took place over 5 min. All measures were scored using an ordinal scale, with 1 = normal/no drug effect, 2 = minor-moderate drug effect, and 3 = major drug effect. The first 1 min was reserved for acclimation, and observations were taken during the remaining 4 min. Observations were taken on ataxia (lacking coordination of muscle movements, as in walking), retropulsion (walking backward), exploration (*e.g.* reorienting the head and sniffing), convulsions (whole body tremors), circling (repeated movement of the animal in a circular manner), excessive grooming (prolonged bouts of grooming, or grooming in a stereotypic manner), flattened body posture (*e.g.*, midsection low to ground, sometimes showing limb