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Mephedrone (4-methylmethcathinone), a principal constituent of psychoactive bath salts, produces behavioral sensitization in rats

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Abstract

Background—The present study tested the hypothesis that mephedrone (MEPH) produces behavioral sensitization (i.e., a progressive increase in motor response during repeated psychostimulant exposure) in rats.

Methods—MEPH was administered in two paradigms: 1) a 7-day variable-dosing paradigm (15 mg/kg on the first day, 30 mg/kg for 5 days, 15 mg/kg on the last day) and 2) a 5-day constant-dosing paradigm (15 mg/kg for 5 days). Following 10 days of drug absence, rats were challenged with MEPH (15 mg/kg).

Results—MEPH challenge produced enhancement of repetitive movement compared to acute MEPH exposure in both paradigms. Sensitization of repetitive movements to MEPH was also detected following a shorter (2-day) absence interval, before initiation of an absence interval (i.e., following repeated daily exposure), and across context-independent and –dependent dosing schedules. A lower dose of MEPH (5 mg/kg) did not produce sensitization of repetitive movement. Sensitization of ambulatory activity was not detected in any experimental paradigm.

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Contributors: Authors Scott M. Rawls, Ryan Gregg, and Chris Tallarida designed the behavioral studies. Authors Allen B. Reitz and Christopher McCurdy supervised the synthesis of mephedrone. Author Christophe Mesangeau carried out the synthesis of mephedrone. Authors Ryan Gregg and Chris Tallarida conducted the behavioral sensitization studies. Authors Ryan Gregg and Scott Rawls conducted statistical analyses and wrote the manuscript. Drafts of the manuscript were subsequently circulated to all authors for their comments, critiques and suggestions. All authors contributed to and have approved the final manuscript and its submission to *Drug and Alcohol Dependence*.

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Conclusion—These results suggest that repeated MEPH exposure produces preferential sensitization to repetitive movement produced by acute MEPH challenge. Our findings suggest that MEPH is a unique stimulant displaying weak sensitizing properties with overlapping, but distinctive, features relative to established psychostimulant drugs.

Keywords

mephedrone; bath salts; behavioral sensitization; cathinone; stereotypy; MEPH

1. Introduction

Mephedrone (4-methylmethcathinone; MEPH) is a constituent of psychoactive bath salts that continues to gain a foothold in the illicit drug market; it was the sixth most frequently used drug of abuse in a survey of drug users in the United Kingdom (Winstock et al., 2011). MEPH is a substrate of plasma membrane monoamine transporters that increases acute locomotor activity in rats, with a weaker locomotor stimulus than methamphetamine (Baumann et al., 2013; Motbey et al., 2012; Huang et al., 2012). It increases extracellular dopamine (DA) and serotonin (5-HT) in the nucleus accumbens, with preferential effects on 5-HT (Baumann et al., 2012; Kehr et al., 2011). MEPH produces inward currents (depolarizing) at the dopamine transporter similar to dopamine-releasing agents (e.g. amphetamine and methamphetamine; Cameron et al., 2012). Consistent with its reported psychoactive properties, MEPH produces conditioned place preference (CPP) in rats and mice and is self-administered by rats maintained on a fixed-ratio schedule of reinforcement (Hadlock et al., 2011; Lisek et al., 2012). The ability of repeated, intermittent MEPH administration to produce sensitization to stimulant effects of acute MEPH challenge has not been extensively investigated (Lisek et al., 2012; Shortall et al., 2012). Behavioral sensitization is produced by psychostimulants and involves repeated administration of the drug, an absence interval in which the drug is not administered, and reintroduction to the drug (Steketee and Kalivas, 2011). Sensitization is present when motor activity produced by repeated drug exposure exceeds that produced by initial exposure (Robinson and Camp, 1987). On the basis of its structural and pharmacological similarities to commonly abused amphetamines, we hypothesized that repeated exposure to MEPH would elicit behavioral sensitization in rats.

2. Materials and Methods

2.1. Animals and drugs

Male Sprague-Dawley rats (260-290 g; Harlan Laboratories, Indianapolis, IN) were housed 2 per cage and maintained on a 12-hour light-dark cycle. Food and water were provided *ad libitum*. Animal use procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and institutional Guidelines for the Care of Animals. MEPH was synthesized by Drs. Allen Reitz and Christopher McCurdy and dissolved in physiological saline and injected intraperitoneally (ip).

2.2. Dosing schedules and behavioral experiments

Two sensitization paradigms were used. One was a variable-dose paradigm adapted from Kalivas and Duffy (1998) as follows: day 1 (15 mg/kg MEPH or saline); days 2-6 (30 mg/kg MEPH or saline); day 7 (15 mg/kg MEPH or saline); and 15 mg/kg MEPH after 2 or 10 days of drug absence. Activity was measured following acute MEPH exposure on day 1, after 7 days of repeated MEPH exposure, and following MEPH challenge after 2 and 10 days of drug absence. The second paradigm was a constant-dose model in which rats were injected with 15 mg/kg MEPH or saline for 5 days and then challenged with 15 mg/kg MEPH after 10 days of drug absence (Rasmussen et al., 2011). Activity was measured following MEPH challenge. Injections were conducted in home cages except for days on which activity was measured. For behavioral experimentation, rats were placed individually into activity chambers and allowed to acclimate for 60 min. Basal activity was recorded for 60 min prior to MEPH or saline injection, followed by recording of activity for 90 min post-injection. The Digiscan DMicro system (Accuscan, Inc., Columbus, OH) was used to measure movement (Lisek et al., 2012; Rasmussen et al., 2011). The chambers consisted of transparent plastic boxes (45 cm × 20 cm × 20 cm) set inside metal frames equipped with 16 infrared light emitters and detectors. The beam height was 4.5 cm, and the space between beams was 2.5 cm. The number of photocell beam breaks was recorded by a computer interface. Ambulatory activity was recorded as consecutive beam breaks resulting from horizontal movement. Non-ambulatory activity was recorded by repetitive-beam breaks. Movement resulting from repetitive-beam breaks does not identify specific stereotypic behavior, rather repeated breaks of the same beam are indicative of a stationary animal engaged in a repetitive behavior. Thus, we designated horizontal-beam breaks as ambulatory activity and repetitive-beam breaks as repetitive movement.

Two additional experiments were conducted for comparative purposes. One experiment tested a lower dose of MEPH (5 mg/kg) using a constant-dosing schedule (*i.e.*, 5 mg/kg MEPH injected for 57 days and challenged with 5 mg/kg MEPH after 10 days of no injections). The second experiment combined the variable-dosing schedule with a context-dependent design in which all injections were performed in the activity chambers.

2.3. Statistical Analysis

Eight rats were used per experimental group. Data were analyzed using repeated-measures ANOVA (within-subjects, Figure 1) or two-way ANOVA with pretreatment and treatment as variables (between-subjects, Figs. 2 and 3). In cases of a significant ANOVA, a Bonferroni test was used to identify differences between groups. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Repeated MEPH exposure produces sensitization of repetitive movement

Activities produced by acute (day 1) and repeated (day 7) MEPH exposure are presented in Figure 1. Repetitive movement is presented in Figure 1A as the cumulative number of repetitive-beam breaks in the 90 min following MEPH (or saline) injection (time-course data are shown in box). Significant effects on treatment [$F(1, 14) = 66.69, p < 0.0001$], time [$F(1,$

14) = 10.19, $p < 0.01$], and interaction [$F(1, 14) = 5.60$, $p < 0.01$] were identified. Post-hoc analysis indicated that 7 days of repeated MEPH produced greater repetitive movement than acute MEPH exposure (day 1) ($p < 0.001$). For ambulatory activity (Figure 1B), an effect of treatment [$F(1, 14) = 84.89$, $p < 0.0001$], but not time [$F(1, 14) = 0.77$, $p > 0.05$] or interaction [$F(1, 14) = 3.48$, $p > 0.05$], was identified.

3.2. Repeated, intermittent MEPH exposure elicits sensitization of repetitive movement across different dosing schedules and absence intervals

Repetitive and ambulatory activities produced by MEPH challenge in rats exposed to chronic MEPH exposure under variable- and constant-dosing designs are presented in Fig. 2. The MEPH absence interval and injection contexts were also varied. For clarity, Fig. 2 data were obtained from rats exposed to the following parameters (Fig. parameters): (2A-B: variable dose, context-independent, 2-day withdrawal); (2C-D: variable dose, context-independent, 10-day withdrawal); (2E-F: variable dose, context-dependent, 10-day withdrawal); and (2G-H: constant dose, context-independent, 10-day withdrawal).

For the variable-dosing design (Figs. 2A-F), the MEPH absence interval (2 or 10 days) and context in which MEPH injections were administered (*i.e.*, independent or dependent) were varied. For repetitive movement in the 2-day absence group (Fig. 2A), significant effects on treatment [$F(1, 14) = 5.24$, $p < 0.05$] and time [$F(18, 266) = 9.48$, $p < 0.0001$] were identified, and a significant interaction was detected [$F(18, 266) = 3.59$, $p < 0.001$]. Post-hoc analysis indicated that MEPH challenge produced greater repetitive movement in MEPH-pretreated rats than in previously MEPH-naïve rats ($p < 0.001$, 10 min post-injection and $p < 0.01$, 15 min post-injection). For repetitive movement in the 10-day absence group (Fig. 2C), significant effects on treatment [$F(1, 14) = 14.08$, $p < 0.001$] and time [$F(18, 266) = 12.62$, $p < 0.0001$] were identified, but a significant interaction was not detected [$F(18, 266) = 0.74$, $p > 0.05$]. Post-hoc analysis indicated that repetitive activity following MEPH challenge was greater in MEPH-pretreated rats than in rats previously naïve to MEPH ($p < 0.05$, 5 min post-injection). For context-dependent experiments (*i.e.*, all MEPH injections were given in the activity chambers) (Fig. 2E), significant effects on treatment [$F(1, 14) = 37.42$, $p < 0.0001$] and time [$F(18, 266) = 10.72$, $p < 0.0001$] (but not interaction [$F(18, 266) = 1.33$, $p > 0.05$]) were identified. Post-hoc analysis indicated that MEPH challenge produced greater repetitive movement in MEPH-pretreated rats than in previously MEPH-naïve rats ($p < 0.05$, 20 min post-injection). For ambulatory activity (Figs. 2B, 2D, 2E), significant treatment effects were not observed under any of the conditions for the variable-dosing design ($p > 0.05$).

For the constant-dose paradigm (10 days of MEPH absence, context-independent) (Fig. 2G), effects on treatment [$F(1, 14) = 33.19$, $p < 0.0001$], time [$F(18, 266) = 16.76$, $p < 0.0001$], and interaction [$F(18, 266) = 2.85$, $p < 0.0001$] were identified. MEPH challenge produced greater stereotypical activity in MEPH-pretreated rats than in previously MEPH-naïve rats [20 min ($p < 0.05$), 25 min ($p < 0.001$), 30 min ($p < 0.05$), 35 min ($p < 0.05$)]. Ambulatory sensitization was not detected ($p > 0.05$) (Fig. 2H). In rats treated with a lower dose of MEPH (5 mg/kg $\times$ 5 days) or saline (5 days) under the same conditions (*i.e.*, constant-dose, 10 days of MEPH absence, context-independent), repetitive and ambulatory activities

produced by MEPH (5 mg/kg) challenge following 10 days of drug absence were not significantly different ($p > 0.05$) (data not shown).

4. Discussion

Repeated MEPH exposure produced sensitization to repetitive movement caused by acute MEPH. The effect was consistent across multiple paradigms (*i.e.*, constant-dosing and variable dosing schedules, 2- and 10-day MEPH absence intervals, and context-independent and context-dependent designs). Commonalities between sensitization produced by MEPH and established psychostimulant drugs (e.g., cocaine, methamphetamine, MDMA) included the presence of sensitization before and after a fixed period of drug absence and following short (2 day) and longer (10 day) drug absence periods (Ball et al., 2006, 2011; Kalivas et al., 1998).

Sensitization produced by MEPH was preferentially expressed as an increase in repetitive movement; sensitization of ambulatory activity was not detected in any paradigm. The preferential sensitization of repetitive movement may have been related to multiple factors including magnitude of dose, frequency of dosing, and duration of MEPH absence. For example, in the case of cocaine and commonly abused amphetamine derivatives, extension of the drug absence interval following repeated exposure results in more robust behavioral sensitization (Kalivas and Duffy, 1993). In cases of higher doses or more frequent dosing, detectable sensitization is even more dependent on longer drug absence periods (Paulson et al., 1991; Kalivas and Duffy, 1993). We speculated that the relatively high doses of MEPH (15, 30 mg/kg) used here contributed to the preferential sensitization of repetitive movement. Since these doses of MEPH fall on the high-end of the dose-response curve, they might be more apt to stimulate repetitive movement rather than ambulation. Lower doses of MEPH (e.g., between 3 and 10 mg/kg) do enhance ambulation (Lisek et al., 2012; Kehr et al., 2011). When a lower dose of 5 mg/kg was tested here, the sensitization of repetitive movement that was observed at higher dosing schedules was not detected. Furthermore, sensitization of ambulatory activity was still not detected with the lower dose. We also speculated that the context of MEPH administration impacted the quality (repetitive- versus horizontal-beam breaks) and magnitude of behavioral sensitization (Ball et al., 2011). For MDMA, sensitization in paired rats (*i.e.*, those injected in activity chambers) was expressed as an increased repetitive movement whereas sensitization in unpaired rats (*i.e.*, those injected in home cages) was expressed as enhanced ambulation (Ball et al., 2011). To control for effects of context, we conducted an additional experiment in which MEPH injections were always paired with the activity chamber. Results from the context-dependent paradigm were similar to context-independent findings; that is, rats displayed a weak, transient sensitization of repetitive movement.

In summary, the major finding of the present study is that MEPH produces relatively weak behavioral sensitization. A consistent finding across multiple dosing schedules and patterns was preferential sensitization of repetitive movement; sensitization of ambulatory activity was not detected in the paradigms used here. While it is evident that considerable overlap exists between sensitization produced by cocaine and commonly abused amphetamine

derivatives, it is equally apparent that the behavioral stimulant profile of MEPH cannot be simply extrapolated from previous findings with established psychostimulant drugs.

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Figure 1. Effects of repeated MEPH exposure

Rats were treated with 15 mg/kg MEPH or saline (SAL) on day 1, 30 mg/kg MEPH or SAL on days 2-6, and 15 mg/kg MEPH or SAL on day seven. Repetitive movement (1A) and ambulatory activity (1B) were measured on days 1 and 7. Data are expressed as cumulative counts $\pm$ S.E.M. for the 90 min after MEPH or SAL injection or as a time course (box). N=8 rats/group. *** $p < 0.001$ compared to day 1 exposure.

Fig. 2. Effects of repeated, intermittent MEPH exposure across different absence intervals, dosing patterns, and injection contexts

(2A-G) Variable Dose: Rats were treated with 15 mg/kg MEPH on day 1, 30 mg/kg MEPH on days 2-6, 15 mg/kg MEPH on day seven, and 15 mg/kg MEPH in context-independent (2A-D) or context-dependent (2E-F) designs. Following 2 days (2A-B) or 10 days (2C-F) of no injections, rats were challenged with MEPH and activity was measured for 90 min. **(2H-I) Constant Dose:** Rats were treated with 15 mg/kg MEPH for 5 days and challenged with MEPH (15 mg/kg) 10 days later. In all cases, time-course data following MEPH challenge are presented as counts + S.E.M. N=8 rats/group. * $p < 0.05$, ** $p < 0.01$, or *** $p < 0.001$ compared to respective Acute MEPH group.