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Sensitization to the locomotor stimulant effects of "bath salt" constituents, 4-methylmethcathinone (4-MMC) and 3,4-methylenedioxypyrovalerone (MDPV), in male Sprague-Dawley rats

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ABSTRACT

Background: Synthetic cathinones, 4-methylmethcathinone (4-MMC) and 3,4-methylenedioxypyrovalerone (MDPV), serve as a substrate or blocker at monoaminergic transporters, respectively, and produce locomotor stimulant effects in rodents. The present study investigated in rats the effects of repeated exposure to 4-MMC, MDPV, or mixtures of the two on the induction of locomotor sensitization and expression of cross-sensitization to cocaine.

Methods: Seventy-two male Sprague-Dawley rats received daily intraperitoneal injections of saline, MDPV (0.5 mg/kg), 4-MMC (0.5, 1.0, or 2.0 mg/kg) or mixtures of 0.5 mg/kg MDPV + 4-MMC (0.5, 1.0, or 2.0 mg/kg) for seven consecutive days. Locomotor activity was recorded on days 1 and 7 and again after an acute injection of 5 mg/kg cocaine following a 10 day drug washout period.

Results: Rats injected with 0.5 mg/kg MDPV, 0.5, 1.0, or 2.0 mg/kg 4-MMC, or 2.0 mg/kg 4-MMC + 0.5 mg/kg MDPV displayed time-dependent increases in horizontal activity that were augmented on day 7 compared to day 1. In addition, rats pretreated with 0.5 mg/kg MDPV, 2.0 mg/kg 4-MMC, or mixtures of 4-MMC + MDPV displayed an enhanced response to cocaine.

Conclusions: Locomotor responses sensitize to MDPV and to certain mixtures of MDPV and 4-MMC following repeated dosing. Furthermore, previous exposure to these substances may produce cross-sensitization to the locomotor stimulant effects of cocaine. Considered together with recent findings that 4-MMC and MDPV have different sites of action, but both influence monoaminergic functioning, further investigations utilizing a variety of behavioral assays may prove informative regarding the abuse liability of synthetic cathinone mixtures.

1. Introduction

Experimental investigations of the physiological and behavioral effects of synthetic cathinones (cathinone derivatives) have increased in direct response to their international popularity among recreational drug users at the turn of the 21st century. Since their emergence into the public domain, widespread media attention and toxicology reports have detailed numerous instances of untoward side effects and fatalities associated with the consumption of these drugs (*e.g.*, Ross et al., 2012; Wood et al., 2010a, 2010b; Torrance and Cooper, 2010). For example, in 2011, synthetic cathinones were involved in over 20,000 emergency room visits in the United States (The DAWN Report, 2013). Amid these reports, three of the cathinone derivatives, 4-methylmethcathinone (4-MMC, mephedrone), 3,4-methylenedioxypyrovalerone (MDPV), and methylone, were placed on the Schedule I list of controlled substances on October 21, 2011, and an additional 10 derivatives were temporarily added March 7, 2014 (Drug Enforcement Agency (DEA), 2011, 2016). Despite legislative efforts devoted to criminalizing the sale, possession, and recreational use of certain synthetic cathinones, these drugs are the third most frequently identified (following synthetic cannabinoids and phenethylamines) new psychoactive substances reported to the United Nations Office on Drugs and Crime (UNODC), 2014.

Recreational users of 4-MMC and MDPV have reported their psychological effects to be similar to those of MDMA and cocaine (Winstock et al., 2010, 2011; Ross et al., 2012; Johnson and Johnson, 2014). Also similar to the amphetamines, untoward

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psychological effects of frequent heavy use include paranoia, hallucinations, aggressive/violent behavior, excited delirium, and psychosis (Wood et al., 2010a, 2010b; Ross et al., 2012; German et al., 2014). Psychoactive "bath salt" use is associated with increased risk-taking behaviors, such as unprotected sex, putting individuals at risk for human immunodeficiency virus and other sexually-transmitted diseases (Johnson and Johnson, 2014). Further, a majority of synthetic cathinone users have reported poly-substance abuse with cocaine, MDMA, alcohol, tobacco, and/or cannabis (for review, Prosser and Nelson 2012; Johnson and Johnson, 2014), which may further increase risks to health and safety.

Despite the prevalence of poly-substance use among recreational "bath salt" users, the majority of preclinical investigations to date have only assessed the effects of single constituents. Undoubtedly, such investigations are a necessary first step to explicating the psychopharmacology of these substances. There is now sufficient evidence regarding the neurochemical and behavioral effects of individual synthetic cathinones to warrant studying their combined effects. The current study represents the first known attempt to characterize the behavioral effects of mixtures containing MDPV with variable doses of 4-MMC. These two chemicals were selected for the current study because there is already substantial published research on the behavioral effects of each individual substance.

The popularity of 4-MMC and MDPV among users may be partly attributable to their neuropharmacological effects. Previous research has demonstrated that 4-MMC shares similarities with MDMA in its potency and selectivity at membrane monoamine transporters (Baumann et al., 2012; Kehr et al., 2011; Rickli et al., 2015). Electrophysiological studies revealed that 4-MMC produces dopamine-releasing effects at hDAT (Cameron et al., 2013a, 2013b; Rickli et al., 2015). Furthermore, MDPV produces neurochemical actions similar to cocaine (*i.e.*, ineffective as a monoamine releaser), inhibiting dopamine transporter activity by blocking reuptake (Cameron et al., 2013a, 2013b). Therefore, consistent with the aforementioned reports of comparable psychological effects, current evidence indicates 4-MMC and MDPV possess neurochemical profiles similar to MDMA and cocaine, respectively.

In addition to efforts devoted to examining the *in vitro* neurochemical effects of 4-MMC and MDPV, researchers have evaluated the behavioral effects of these compounds. It is well established that repeated and intermittent exposure to certain drugs produces progressive increases in locomotor and stereotyped movements, a phenomenon termed "behavioral sensitization" (see Steketee and Kalivas, 2011). In addition, it is suggested that sensitization is mediated by neuroadaptive changes in dopaminergic mesocorticolimbic and glutamatergic pathways (Vanderschuren and Kalivas, 2000), substrates implicated in drug abuse and chemical dependencies. Recent studies have demonstrated locomotor sensitization in rats following repeated exposure to 4-MMC (Gregg et al., 2013a, 2013b; Shortall et al., 2013; Lisek et al., 2012).

When this study was initiated, there were no published reports demonstrating locomotor sensitization to MDPV. Nonetheless, previous studies have revealed dose-dependent increases in locomotor activity following injections of MDPV in mice (*e.g.*, Fantegrossi et al., 2013; Marusich et al., 2012) and rats (e.g., Aarde et al., 2013; Baumann et al., 2013), and only one known study has assessed locomotor sensitization to a mixture of drugs that included a synthetic cathinone (4-MMC) and *d*-amphetamine (Berquist et al., 2015). The present study investigated in rats the induction of locomotor sensitization with concurrent exposure to 4-MMC and MDPV in comparison to each substance alone, and subsequently assessed cross-sensitization to cocaine. The results are suggestive that MDPV and certain low dose mixtures of the 4-MMC and MDPV produce locomotor sensitization and can enhance locomotor responses to cocaine.

2. Materials and methods

2.1. Subjects, apparatus, and drugs

Seventy-two adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were pair-housed in polycarbonate cages with corncob bedding (Harlan Teklad, Conrad, Iowa) in a temperature and humidity controlled vivarium maintained on a 12:12 h light-dark cycle (lights on at 0700). Animals had *ad libitum* access to standard rodent chow (Purina® 5001, Richmond, Indiana) and deionized water in their home cages. All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (2013) and were approved by the Institutional Animal Care and Use Committee at Western Michigan University.

Locomotor activity was assessed in eight custom-designed, acrylic open field chambers (40.5 cm × 40.5 cm × 40.5 cm). Each chamber was housed within an Accuscan automated activity monitoring system equipped with infrared emitters and detectors connected to a microprocessor with associated Versamax® software programmed to analyze beam breaks and determine various measures of activity (Accuscan Instruments, Inc., Columbus, OH, USA).

(±)-Mephedrone-hydrochloride (4-methylmethcathinone, 4-MMC), 3,4-methylenedioxypyrovalerone-hydrochloride (MDPV), and cocaine-hydrochloride were provided by the National Institute on Drug Abuse (NIDA) drug control supply program (Bethesda, MD). All drugs were prepared in 0.9% bacteriostatic sodium chloride and delivered to rats *via* intraperitoneal injections in a 1 ml/kg volume. Drug mixtures were injected as a single bolus. Drug doses were calculated based on the weights of the salts.

2.2. Experimental procedures

Rats were randomly assigned to one of the following treatment groups: 0.5, 1.0, or 2.0 mg/kg 4-MMC ($n = 8, 8, 8$, respectively), 0.5 mg/kg MDPV ($n = 8$), 0.5, 1.0, or 2.0 mg/kg 4-MMC + 0.5 m/kg MDPV ($n = 8, 8, 8$), or saline ($n = 16$), with housed pairs assigned to the same treatment group. Doses were selected based on previous research demonstrating that in rats 0.5 and 1.0 mg/kg 4-MMC (Lisek et al., 2012), and 0.5 mg/kg MDPV (Aarde et al., 2013), induce increases in locomotor activity. Higher doses of MDPV were not used to avoid potential disruptive effects due to the combined stimulant actions of the drug mixtures tested. The seven-day dosing schedule employed was similar to the variable-dose paradigm used by Gregg et al. (2013a), however, in the present study, all rats received the same dose of their designated treatments on days 1 through 7, and a single dose of cocaine (or saline) after a 10 day drug washout period. All subjects were injected (i.p.) daily over seven consecutive days at approximately the same time of day. Locomotor activity was recorded on day 1 and day 7. On days 2 through 6, rats were injected and placed immediately back into home cages. Following a 10-day washout period, all drug-treated subjects ($n = 56$) and 10 of the saline-treated subjects received cocaine (SAL-COC) (5 mg/kg), while six of the saline-treated controls received saline (SAL-SAL). Locomotor activity was recorded in a similar manner to days 1 and 7, as described below.

All testing occurred during the light phase of the light-dark cycle. Treatment groups and time of day during which locomotor activity was assessed were counterbalanced among animals. On each test day, rats were habituated to the test chambers for a 60 min period prior to injections while activity was recorded. Rats were briefly handled to receive injections and placed back into test chambers for an additional 60 min. Activity recording was turned off during injections and turned back on after all eight animals in each cohort were injected. Test chambers were cleaned with a 35% isopropyl alcohol solution between cohorts. Overhead lights were on during

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