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Effects of Low Dose Mixtures of 3,4-Methylenedioxypyrovalerone and Cocaine on Locomotor Activity and Brain Monoamine Content in Sprague-Dawley Rats

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EFFECTS OF LOW DOSE MIXTURES OF 3,4-METHYLENEDIOXYPYROVALERONE AND COCAINE ON LOCOMOTOR ACTIVITY AND BRAIN MONOAMINE CONTENT IN SPRAGUE-DAWLEY RATS

by

Robert J. Kohler

A thesis submitted to the Graduate College in partial fulfillment of the requirements for the degree of Master of Arts Psychology Western Michigan University June 2017

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EFFECTS OF LOW DOSE MIXTURES OF 3,4-METHYLENEDIOXYPYROVALERONE AND COCAINE ON LOCOMOTOR ACTIVITY AND BRAIN MONOAMINE CONTENT IN SPRAGUE-DAWLEY RATS

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Synthetic cathinones, known as “bath salts” on the illicit drug market, pose a significant and growing public health concern. 3,4-Methylenedioxypyrovalerone (MDPV), one of several popular constituents of the illicit bath salts, produces similar pharmacological actions to cocaine, albeit with greater potency. The present study sought to characterize behavioral and neurochemical effects of repeated exposure to MDPV alone and in combination with cocaine. Male Sprague-Dawley rats were randomly assigned to one of four treatments: 1 mg/kg MDPV, 5 mg/kg cocaine, 1 mg/kg MDPV + 5 mg/kg cocaine, or saline. Locomotor activity was assessed for one hour immediately before and one hour immediately after injections on days 1 and 6. Brains were harvested 20 minutes after the final injection on day 7. Total monoamine content within the anterior striatum, medial prefrontal cortex, and nucleus accumbens was determined with High-Performance Liquid Chromatography (HPLC). Drug-induced increases in horizontal activity were significantly greater on treatment day 6 compared to treatment day 1 in all three drug treatment groups in comparison to the saline control group. Moreover, MDPV produced significantly higher increases in activity compared to either saline or cocaine. Neurochemical analyses provided no evidence of alterations in total monoamine content following repeated administration of MDPV or cocaine. Further investigations targeting possible changes in DA receptor sensitivity following repeated exposure to MDPV may help elucidate the mechanistic changes responsible for MDPV-induced behavioral sensitization.
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Robert J. Kohler
TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....................................................................................................................ii

LIST OF FIGURES............................................................................................................................v

CHAPTER

I. INTRODUCTION..............................................................................................................................1

   Substance Use..............................................................................................................................1

   Synthetic Cathinones...................................................................................................................1

   Dopamine and Substance Abuse.................................................................................................3

   Pharmacology of Cocaine and MDPV.........................................................................................4

   Psychopharmacology Studies of MDPV.....................................................................................5

   Research Objective.......................................................................................................................5

II. METHODS......................................................................................................................................7

   Animals.........................................................................................................................................7

   Apparatus......................................................................................................................................7

   Drugs............................................................................................................................................7

   Dosing and Locomotor Assessment..............................................................................................7
LIST OF FIGURES

1. Locomotor Activity Across Time.................................................................11

2. Horizontal Activity Day 1 vs. Day 6..............................................................12

3. Monoamine Concentrations.........................................................................13
CHAPTER I
INTRODUCTION

Substance Abuse

Substance Use Disorder (SUD) is characterized by the severity with which a drug negatively impacts an individual’s ability to maintain important aspects of one’s life including in school, the workplace, or among social circles (American Psychological Association, 2013). Unfortunately, many individuals may go undiagnosed or succumb to a fatal overdose. The U.S Department of Justice estimates 11 billion dollars is spent on health care related to substance abuse each year with emergency department spending accounting for 161 million (National Drug Intelligence Center, 2011). The Center for Disease Control estimates the number of drug overdoses has increased 137% between the years of 2000-2016 amounting to 14.7 out of 100,000 persons in the 2014 (Center for Disease Control and Prevention, 2014). Individually, significant increases were seen between both sexes, individuals 25-44, > 55, and non-Hispanic whites and blacks. This increase in total overdoses is often attributed to an increase in prescriptions written for the management of pain and psychological disorders (Paulozzi et al., 2011) but is also associated with increases in drug potency. Increases in drug potency often occur because of attempts to circumvent the legal system through the manufacturing of drugs analogous to illegal equivalents, which may then be sold legally. In recent years, synthetic cathinones, often referred to by the street name “Bath Salts”, have provided blueprints for this process.

Synthetic Cathinones

Cathinone, in its natural form, is found in leaves of the khat plant (Catha edulis Forsk) and is typically cultivated in areas of the Middle East. The psychoactive effects of the khat plant have been documented for centuries as producing stimulant-like effects when the leaves are
chewed and the active ingredient is absorbed orally (Katz et al., 2014). In the late 1990s, it was estimated around six million khat leaves were consumed each day (Kalix, 1996). Unfortunately, more current estimates of daily consummation are not reported. In areas of East Africa, where Khat is found growing naturally, 80% of adult males report daily use. (Odenwald et al., 2005). The first synthetic cathinone, mephedrone, was synthesized in the 1920s but did not receive attention until the advent of internet drug markets in the 2000s (German et al., 2014). In the United States, synthetic formulations of cathinone accounted for 22,904 emergency room visits in 2011, with users presenting symptoms of tachycardia, hallucinations, agitation, and psychotic episodes often requiring sedation (Spiller et al., 2011; The DAWN Report, 2013). As a result, the DEA classified three of the primarily abused synthetic cathinones (mephedrone, 3,4-methylenedioxypyrovalerone (MDPV), and methylone) as schedule I controlled substances in 2011 (DEA Report, 2012).

MDPV is the most commonly abused synthetic cathinone in the United States (German et al., 2014) and is typically administered orally, intravenously, or insufflated with effects lasting between three and four hours (Wyman et al., 2013). MDPV was first patented in 1967 but, like mephedrone, did not receive attention for abuse until the 2000s (German et al., 2014). Users report effects similar to the classic psychostimulant cocaine (Spiller et al., 2011) and even supplement its use with MDPV, leading to fatal overdoses (Murray et al., 2012). These overdoses may be a result of the user’s ignorance to the difference in potency between the two drugs, leading to MDPV dosing that is akin to cocaine. Further research is necessary to understand the extent to which MDPV affects the central nervous system at clinically relevant doses. Several recent preclinical studies have characterized the neurochemical and behavioral effects of MDPV. A brief review of the findings relevant to the current study is addressed below.
**Dopamine and Substance Abuse**

The role of dopamine in the neurobiological underpinnings of substance abuse has been elucidated with the help of animal models. Most CNS DA is synthesized in the substantia nigra and ventral tegmental area (VTA), where neurons from these regions project to forebrain areas, including the prefrontal cortex, limbic structures, and the basal ganglia (Tritsch and Sabatini, 2012). DA released from these nerve terminals subsequently activate G-protein coupled receptors (GPCR) that modulate post-synaptic neurotransmission through effects on metabotropic receptor function on one of five receptor subtypes (D1-5), the first being the most abundant (Tritsch and Sabatini, 2012). Activation of these DA GPCRs in the VTA is associated with the presentation of rewarding, or salient stimuli in rats (Kim et al., 2012). Seminal work by Kim et al. (2012) provided direct evidence of this association. Using optogenetic techniques to stimulate DA neurons within the rat VTA that were genetically engineered to activate with the presentation of blue light, this study demonstrated the release of DA in the VTA is a sufficient reinforcer for the maintenance of operant behaviors. Interestingly, drugs that are frequently abused in humans modulate release in a similar manner.

Preclinical studies using in vivo microdialysis techniques reveal that many drugs of abuse, including the synthetic cathinones, preferentially increase DA levels in NAc of freely moving rats after a single injection (Di Chiara 1988b; Schindler et al., 2016). Additionally, increases in DA release have been shown in the VTA following administration of cocaine (Bradberry, 1989), morphine (Bozarth, 1981) and nicotine (Corrigall et al., 1994). Further evidence supporting the role of DA in the addiction process comes from self-administration studies in which animals receive drug infusions contingent on lever presses in an operant chamber. In animals trained to self-administer cocaine or heroin, chemical disruption of
dopamine neurons within the NAc was shown to disrupt self-administration behaviors with a high correlation ($r=0.88$) between amount of NAc disruption and deficits in maintaining the behavior, specifically in animals receiving cocaine infusions (Zito et al., 1985). Moreover, this correlation may be related to cocaine’s specific action on DA within the mesolimbic region. Cocaine is a hallmark psychostimulant with effects characterized by euphoria, enhanced moods, and intensified, non-distorted environments (Gawin, 1991). The addictive potential of cocaine is so severe that rats given ad libitum access to intracranial infusions typically die within 14 days (Gawin, 1991).

**Pharmacology of Cocaine and MDPV**

Cocaine’s pharmacodynamic actions have been well documented since the 1970s when researchers chemically ablated dopamine nerve terminals in the NAc of rats, preventing increases in locomotion typical of the psychostimulant (Kelly, 1976). These actions were produced through cocaine’s ability to block dopamine (DAT) and serotonin transporters (SERT), increasing extracellular levels of both neurotransmitters (Hall et al., 2014). Mice bred to express a form DAT that is insensitive to the effects of cocaine displayed no evidence for cocaine-induced place preference or changes in basal DA levels after repeated exposure (Chen et al., 2006), further detailing the role of DAT in the mediation of cocaine’s mechanism of action. In vitro transporter assays indicate the synthetic cathinone MDPV is mechanistically similar to cocaine in its inhibition of DAT (Marusich et al., 2014). Analysis of extracellular dopamine content in the NAc of rats administered cocaine or MDPV revealed that MDPV is approximately 10-fold more potent than cocaine (Baumann et al., 2013) with longer lasting effects on DAT (Cameron et al., 2013). MDPV produces weak inhibition at SERT and the norepinephrine
Transporter (NET) (Glennon and Young, 2016) in a manner similar to cocaine (Sora et al., 2001).

**Psychopharmacology Studies of MDPV**

MDPV is a potent reinforcer in self-administration assays (Aarde et al., 2013; Watterson et al., 2014; Schindler et al., 2016) and produces conditioned place preference at a range of doses (1.0, 1.8, 3.2 mg/kg) (King et al., 2015) in rats and in mice (Karlsson et al., 2014). Sensitization to the locomotor stimulant effects of MDPV has been established with doses of 0.5 mg/kg administered repeatedly for seven days (Berquist II et al., 2016) and 1.0 mg/kg when administered in five, 48-hour intervals (Watterson et al., 2016). Locomotor sensitization reflects enhancements in the stimulus effects produced by repeated exposure to psychostimulants (Robinson and Berridge., 1993) and may contribute to stronger learned associations between environmental cues and drug injections (Pierce and Kalivas, 1997). These enhancements are mediated by dopaminergic neurons in the NAc and striatum (Henry et al., 1998) and glutamate receptor trafficking in the NAc (Boudreau and Wolf, 2005).

**Research Objective**

The aim of the current study was to extend the characterization of sensitized locomotor responses and the neurochemical effects of MDPV when administered concurrently with a low dose of cocaine. No current research is available describing the potential additive effects of these two psychostimulant drugs on monoamine release or locomotor sensitization. Additionally, no research has been published describing the neurochemical effects of MDPV alone after repeated exposure and data describing locomotor effects is limited by dose (Berquist II et al., 2016) or protocol (Watterson et al., 2016). Repeated administration of cocaine has been shown to produce robust locomotor sensitization at doses of 15 and 30 mg/kg (Kalivas and Duffy., 1993),
diminished increases in medial prefrontal cortex (mPFC) DA after repeated, systemic injections (Sorg et al., 1997), and increases in NAc DA (Weiss et al., 1992). However, few studies have investigated the behavioral effects of lower cocaine doses. Data from at least one study suggest rats administered 5.0 mg/kg of cocaine display significant place preference to the drug paired side of the apparatus (Gong et al., 1997) and develop sensitized locomotor responses after repeated administration (Drouin et al., 2001). Taken together, it is hypothesized that the combination of MDPV (1.0 mg/kg) and cocaine (5.0 mg/kg) will produce additive effects in locomotor activity and total DA content in the NAc and striatum and lower DA concentrations in the mPFC.
CHAPTER II

METHODS

Animals

Seventy-two male Sprague-Dawley rats (Charles River) weighing between 250g-300g were pair-housed in temperature (20 °C) and humidity (50%) controlled rooms on 12:12 light/dark cycle. Food and water were provided *ad libitum* in polycarbonate cages consisting of corncob bedding (Harlan Teklad, Conrad, Iowa). All procedures were reviewed and approved by the Western Michigan University Institutional Animal Care and Use Committee and were in accordance with the *Guide for the Care and use of Laboratory Animals* (National Research Council, 2010).

Apparatus

Six custom built acrylic chambers (40.5 x 40.5 x 40.5 cm) were equipped with horizontal and vertical mounted infrared sensors to track locomotor activity (Accuscan Instruments Inc., Columbus, OH). Infrared beam breaks were recorded and analyzed using Versamax® software (Accuscan Instruments Inc., Columbus, OH).

Drugs

Methylenedioxypyrovalerone hydrochloride and cocaine hydrochloride (National Institute on Drug Abuse Drug Control Supply, Bethesda, Maryland) were dissolved in 0.9% bacteriostaticsodium chloride and administered at a volume of 1 ml/kg via interperitoneal injection.

Dosing and Locomotor Assessment

Animals were randomly assigned to one of the following drug treatment group: 1.0mg/kg MDPV, 5.0mg/kg COC, 1.0mg/kg MDPV + 5.0mg/kg COC, or Saline. Animals received a total
of seven injections, once per day over the course of seven days. On treatment days 1 and 6, injections were administered immediately following 60 min habituation to the behavioral test apparatus. Rats were injected and placed back into the test apparatus and activity was monitored for an additional 60 min. On treatment days 2 through 5, animals were injected and immediately placed back in their home cages. On day 7, animals were injected and brains were removed 20 min later. All injections were administered at the same time of day on each treatment day. During the behavioral testing period, white noise was generated at 70dB to prevent outside disturbances. Animals were tested in cohorts of six, with at least three treatment groups represented in each cohort. Test chambers were cleaned with a 35% isopropyl alcohol between cohorts.

**Neurochemical Analysis**

On day 7, brains were harvested via rapid decapitation 20 minutes after an animal’s final injection. Once removed, brains were immediately sliced utilizing a chilled stainless steel rat brain matrix and placed directly on a flat surface of dry ice for dissection. A 1.5mm biopsy punch was then used to extract tissue from the medial prefrontal cortex, anterior striatum, and nucleus accumbens in accordance with the Paxinos and Watson Rat Brain Atlas (Paxinos and Watson, 2007). Punches for each tissue region were placed into micro-centrifuge tubes and tissue weight was obtained by subtracting the initial micro-centrifuge tube weight from the final weight of the tube with the tissue. After a weight was obtained, tissue was frozen and stored for subsequent neurochemical analysis. Monoamine analysis was performed on a Dionex Ultimate 3000 UHPLC system (Thermoscientific, Waltham, MA). This system is equipped with an auto-sampler maintained at 4 degrees Celsius, a 100 uL sample loop, and a C18-RP (2uL diameter) column maintained at 25°C. TEST Mobile Phase (Thermoscientific) containing acetonitrile,
phosphate buffer, and an ion-pairing reagent was utilized. Coulometric electrochemical detection was achieved with a dual electrode cell set at -175 mV (reference) and 300 mV (working). Monoamine levels (i.e., dopamine, serotonin, and their metabolites) were expressed as absolute tissue values (ng neurochemical / mg tissue weight).

**Statistical Analysis**

Locomotor activity measurements were obtained from horizontal beam breaks collected in 1-minute intervals using Versamax Software (Accuscan Instruments Inc., Columbus, OH). Horizontal activity (±S.E.M) for each treatment group was plotted in 1-min intervals for the entire 120-minute session (Figure 1). Total horizontal activity (±S.E.M) for the second 60-minutes following drug injections was plotted to visually compare treatment group differences across each day (Figure 2). A two-factor repeated measures Analysis of Variance (ANOVA) (treatment group x day) was performed on the second 60-minute sum with day as the within-subject repeated factor. When a statistically significant interaction was present, simple main effects were computed to determine significant individual group differences between days 1 and 6 using Holm-Sidak adjustments. Two separate one-factor ANOVAs were also computed to determine differences between among treatment groups on day 1 and on day 6. In the case of a significant treatment effect, Bonferroni multiple comparisons were performed. Total monoamine concentrations (±S.E.M) for DA, 5HT, and NE were plotted for each brain region to compare differences among treatment groups (Figure 3). Monoamine concentrations (ng/mg) were analyzed using separate one-way ANOVAs for each brain region of interest (STR, NAc, mPFC) and each analyte with treatment group as the between-subject factor. GraphPad Prism Version 7.0 software (La Jolla, CA, USA) was used for all statistical analysis pertaining and graphical representation of all data.
CHAPTER III

RESULTS

Induction of Locomotor Sensitization

Figure 2 displays total horizontal activity counts expressed as an average (± S.E.M.) for each treatment. Results from the two-factor repeated measures ANOVA revealed a significant main effect of treatment (F [3, 30] = 52.88, P< .001) and test day (F [1,30] = 30.21, P<.001). A significant interaction (treatment x test day) (F [3,30] = 3.2, P<.05) was obtained and simple main effects were computed for each treatment condition. Analysis of simple effects of treatment by day revealed significant increases in locomotor activity between day 1 and day 6 in MDPV 1.0 mg/kg (F = 11.15, \(\mu_1 - \mu_6 = -12796\)), MDPV 1.0 mg/kg + COC 5 mg/kg (F= 14.75, \(\mu_1 - \mu_6 = -14715\)), and COC 5.0 mg/kg (F = 14.96, \(\mu_1 - \mu_6 = -15715\)). Percent change values were calculated to determine the magnitude of the change in locomotor response (MDPV 1.0 mg/kg = 43%, MDPV 1.0 mg/kg + COC 5.0 mg/kg = 59%, COC 5.0 mg/kg = 100%) between day 1 and 6. A one-factor ANOVA on day 1 activity confirmed a significant effect of treatment (F [3,29] = 15.37, P< .001). Bonferroni multiple comparisons presented significant differences in activity between MDPV 1.0 mg/kg vs. COC 5 mg/kg (\(\mu_1 - \mu_2 = 14344\), P<.01), MDPV 1.0 mg/kg vs. Saline (\(\mu_1 - \mu_2 = 25403\), P<.0001), and MDPV 1.0 mg/kg + COC 5 mg/kg vs. Saline (\(\mu_1 - \mu_6 = 20271\), P<.001). A similar analysis comparing activity among treatment groups on day 6 revealed a significant effect of treatment among groups (F [3,30] = 36.68, P<.0001). Multiple comparison tests found significance differences in day 6 activity among the following groups: MDPV 1.0 mg/kg vs. COC 5 mg/kg (\(\mu_1 - \mu_2 = 11425\), P<.05), MDPV 1.0 mg/kg vs. Saline (\(\mu_1 - \mu_2 = 38132\), P<.0001), MDPV 1.0 mg/kg + COC 5 mg/kg vs. Saline (\(\mu_1 - \mu_6 = 34920\), P<.0001), and COC 5 mg/kg vs. Saline (\(\mu_1 - \mu_6 = 26707\), P<.0001).
Figure 1. Depicted is the entire 120-minute recording session for each treatment group. Sessions contained two distinct 60-minute intervals: habituation and post-injection counts. Each line on represents activity for a given day (Day 1 vs. Day 6) with individual data points representing group means (+/- SEM) at each 1-minute bin. [n=8-9 for each treatment group; two animals’ data was not recorded due to apparatus malfunction and is not represented in the respective treatment groups (MDPV 1.0 mg/kg + COC 5.0 mg/kg and SALINE)]
Figure 3 displays average monoamine concentrations across treatments the STR (a.1,2,3), NAc (b.1,2,3), and mPFC (c.1,2,3). Nine separate one-factor ANOVAs were computed on each brain region of interest to compare monoamine concentrations (DA, 5HT, and NE) among treatment conditions. Results from these analyses revealed no significant effects of drug treatment on monoamine levels in any of the brain regions analyzed.
Figure 3. Monoamine concentrations are expressed as nanograms (ng) of monoamine per milligram (mg) of tissue weight. Monoamine (DA, NE, 5-HT) concentrations (organized by column) are depicted as group averages (+/- SEM) for each tissue region analyzed (organized by row).
CHAPTER IV
DISCUSSION

Current literature is limited regarding the effects of repeated MDPV administration on locomotor activity. Results of the present study extend previous findings (Berquist et al., 2016) by demonstrating a higher dose of MDPV (1.0 mg/kg vs 0.5 mg/kg) produces enhanced locomotor responses after repeated administration. Furthermore, this effect was demonstrated in animals receiving concurrent treatment with MDPV and cocaine. This is not surprising given both cocaine and MDPV produced significant increases in activity when administered alone. Although MDPV-treated animals displayed higher overall activity on day 6, the MDPV + COC mixture produced a larger change in overall activity between test days than did MDPV alone. This larger percent change may be a result of the stimulus effects related to repeated cocaine exposure, as activity increased by 100% in animals given only cocaine.

Cocaine was the only treatment condition that did not produce significant differences in locomotor activity when compared to saline on day 1. MDPV produced significant differences in activity only when compared to cocaine on both days and produced the most locomotor activity (although not the largest percent change), followed by MDPV + COC and cocaine. The lack of enhanced locomotor stimulation by the MDPV+COC mixture compared to MDPV alone was surprising, but may reflect ceiling effects in activity counts or attenuation produced by cocaine. If animals were given longer access to the apparatus during the post injection interval, the group receiving the mixture may display higher activity because of sustained drug-induced activity after the initial peak. Alternatively, cocaine may compete with MDPV for DAT binding, and thereby attenuate the actions of MDPV. Previous literature indicates MDPV as a more potent blocker than cocaine with longer lasting effects (Baumann et al., 2013) but no research has
described the effects at DAT when both drugs are given in combination. If cocaine preferentially blocks DAT over MDPV, then this may reflect dampened behavioral and neurochemical responses.

No significant differences were obtained from measurements of whole tissue monoamine levels in the NAc, STR, or mPFC of animals administered MDPV, cocaine, or the mixture. Repeated, binge exposure to cocaine (20 mg/kg 3x daily and 10 mg/kg 2x daily) has been shown to decrease DA levels in the NAc as long as 14 days after a final injection (Puig et al., 2012; Imperato et al., 1992). However, research also suggests DA levels in the NAc increase after repeated administration to cocaine (Weiss et al., 1992). These cited studies utilized more precise methodologies to measure DA through microdialysis procedures, whereas the present study determined whole tissue concentrations. With this, it is not surprising that the dose administered in the present study, almost four times as low as reported in the cited literature, did not produce significant alterations in whole tissue DA levels when administered once daily for seven days. Lack of evidence for altered DA levels, despite clear evidence for the induction of locomotor sensitization following repeated exposure to low doses of cocaine and MDPV, begs the question of dopamine’s involvement in this process. Research suggests glutamatergic involvement in the induction of behavioral sensitization to cocaine (Boudreau and Wolf., 2005) through the increased trafficking of \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) in the NAc. AMPA receptor trafficking may be a potential mechanism for the induction of sensitization by MDPV given the results of the present study. Further quantification of this trafficking, and glutamate expression, in rats administered low doses of MDPV and/or cocaine is necessary to build support for this hypothesis.
As described previously, MDPV acts in a similar manner to cocaine at the synapse through its effects on DAT. These similarities at DAT may lead to alterations in DA that are akin to decreases seen after repeated cocaine exposure and may help describe the null results presented herein. No current research details DA concentrations in whole tissue following repeated administration of the synthetic cathinone MDPV. After single injections (Schindler et al., 2016 & Baumann et., 2013), MDPV has been shown to increase extracellular DA. However, these studies may have demonstrated decreases in DA, or conflicting results, had the injections been given repeatedly or if monoamines were analyzed with whole tissue. Interestingly, although highly speculative given non-significant effects, average levels of DA in the NAc of animals given both MDPV and cocaine show close to a 1 ng/mg decrease when compared to the other treatment groups. Given the variability of whole tissue monoamine analyses, more statistical power through the inclusion of more animals may be necessary to obtain significant drug effects between groups. If the hypothesis is accepted that cocaine and MDPV produce decreases in DA following repeated exposure, both drugs given in combination may display additive decreases in DA concentrations in brain areas associated with reward processing. It is important to note, however, that the studies describing decreases in extracellular DA administered cocaine more than once daily and provided a withdrawal period after the last injection (Puig et al., 2012; Imperato et al., 1992)

MDPV demonstrates binding affinity for NE and 5-HT transporters (Baumann et al., 2013) but does not produce significant elevations in extracellular 5-HT concentrations following low dose infusions (Schindler et al., 2016). The results of the present study extend these findings by providing evidence for uninterrupted 5-HT concentrations after 7 days of repeated exposure in areas of reward and provide new evidence for uninterrupted changes in the mPFC. Further
research is necessary to characterize the effects of MDPV on reward-related pathways and the impact of repeated exposure to the drug. Previous findings suggest repeated cocaine treatment (15 mg/kg, 1x for 5 days) attenuates mPFC DA increases after a systemic challenge (Sorg et al., 1997). MDPV may produce similar results in assays more sensitive to extracellular expression because of similarities between cocaine and MDPV’s effects on DAT. Future endeavors should examine a wider range of doses of MDPV and cocaine, when given in combination, to better characterize neurochemical alterations at doses that may be rewarding or neurotoxic.

In conclusion, the results of this study suggest a low dose of MDPV produces robust increases in locomotor activity after repeated exposure that are matched when given in combination with low doses of cocaine. Furthermore, neither MDPV nor the MDPV + COC mixture produced significant alterations in monoamine systems related to reward and reward processing. Finally, this study extends on the literature describing the behavioral and neurochemical effects of polysubstance abuse involving synthetic cathinones. Additional research is necessary to better characterize the effects MDPV may display when given in combination with other common drugs of abuse.
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APPENDIX

Date: February 10, 2016
To: Lisa Baker, Principal Investigator
From: Kathryn Eckler, DVM, Vice Chair
Re: IACUC Protocol No. 13-12-01

This letter will serve as confirmation that the changes to your research project “Evaluation of Drug Combinations for Behavioral Sensitization” requested in your memo received February 3, 2016 (to add decapitation as an alternative form of euthanasia for selected experiments. Specific details of the amended experiments are described below.

The behavioral tests included in this protocol involve administering mephedrone or MDPV alone and in mixtures with another drug (e.g., cocaine, methamphetamine, or MDMA) for seven consecutive days and then an additional dose of one of the above drugs after a 10 day washout period.

For the amended protocol, we propose the following assessments include neurochemical analyses at various endpoints.

1) Mephedrone 1 or 5 mg/kg alone and in combination with 5 mg/kg cocaine. Euthanize subgroups on day 1 and day 7 and after the 10 day washout to harvest brains for neurochemical analysis.
   - Saline, N=18
   - Cocaine 5, N=18
   - Meph 1, N=18
   - Meph 5, N=18
   - Meph 1 + Coc 5, N=18
   - Meph 5 + Coc 5, N=18

2) MDPV 0.5 or 1 mg/kg alone and in combination with 5 mg/kg cocaine. Euthanize subgroups on day 1 and day 7 and after the 10 day washout to harvest brains for neurochemical analysis.
   - Saline, N=18
   - Cocaine 5, N=18
   - MDPV 0.5, N=18
   - MDPV 1, N=18
   - MDPV 0.5 + Coc 5, N=18
   - MDPV 1 + Coc 5, N=18

Six animals from each treatment group will be euthanized immediately after one injection, another six animals will be euthanized immediately after the seventh injection, and the last six will be euthanized immediately after the challenge injection after the 10 day washout.

have been approved by the Institutional Animal Care and Use Committee.

Approval Termination: December 12, 2016