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Evaluation of α-Pyrrolidindovalerophenone (α-PVP) and its Isomers in Male and Female Sprague-Dawley Rats Trained to Discriminate MDPV

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by

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Recreational use of synthetic cathinones, 3,4-methylenedioxypyrovalerone (MDPV) and α-pyrrolidinovalerophenone (α-PVP), has become increasingly popular, thus prompting characterization of their behavioral and neurochemical effects. MDPV has been studied for several years now, though there is still much unknown about α-PVP, and its isomers. The primary objective of this study was to characterize the discriminative effects of α-PVP and its isomers in comparison to MDPV. Male and female Sprague-Dawley rats were trained to discriminate 0.5mg/kg MDPV from saline injections under a fixed ratio 20 (FR 20) schedule of food reinforcement. Substitution tests were conducted with MDPV (0.05-0.5mg/kg), α-PVP (0.05-0.5mg/kg), (S)-α-PVP (0.05-1mg/kg) and (R)-α-PVP (0.05-10mg/kg). All substances produced a dose-dependent increase in MDPV-lever responses and dose response curves were similar between males and females. Full substitution was observed in females at the highest dose of all test compounds and all except (R)-α-PVP in males. These results illustrate that α-PVP, and its isomers produce similar discriminative effects to those of MDPV with few differences between males and females. Although the current findings add to the established psychopharmacology of α-PVP further investigations into its precise mechanisms of action are critical to advancing medical treatments for substance abuse disorders involving this and related synthetic cathinones.

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Kaley Cargile

TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

Synthetic cathinones are a class of popular recreational "designer drugs" that became prevalent in the early 2000's. Cathinone is a naturally occurring amphetamine-like alkaloid found in *Catha edulis* (khat), a native plant in the Middle East and Africa. The extracts in khat leaves have been used for centuries for their psychostimulant effects, though a variety of synthetic cathinones have been named and sold deceptively as "bath salts", "plant fertilizer", "jewelry cleaner", among many other names. Synthetic cathinone constituents mephedrone, 3,4-methylenedioxypyrovalerone (MDPV), and methylone were permanently scheduled as Schedule I drugs in 2012 and 2013 (Drug Enforcement Agency, 2014). Although these substances are now Schedule I drugs, illicit use still remains a significant public health concern.

α-pyrrolidinovalerophenone (α-PVP) is another synthetic cathinone frequently used and abused, commonly sold under the name "Flakka" or "gravel." First synthesized in 1960, this chemical does not have an extensive history of cultural use. More recently, α-PVP has become an extremely popular "designer drug." It gained popularity, particularly among recreational users attempting to circumvent regulations of structurally related psychostimulants. Due to its high potency and desired effects among recreational users, α-PVP is considered a dangerous drug, reported to produce agitated delirium (Patocka et al., 2020). Agitated delirium is "characterized by agitation, aggression, acute distress and sudden death" (Takeuchi, Ahern, and Henderson, 2011, p.77). Agitated delirium is sometimes caused by drugs that can also produce hypothermia, cardiopulmonary arrest, and altered dopamine signaling. (Takeuchi, Ahern, & Henderson, 2011). Psychopharmacological studies of α-PVP are limited and the effects of α-PVP on the central nervous system (CNS) are still not understood. A few recent studies have examined the

behavioral effects of α-PVP on spontaneous locomotor activity, conditioned place preference, and conditioned taste avoidance with rats, self-administration and activity wheel running with mice, and self-administration and drug discrimination in rhesus monkeys (Gianotti et al., 2017; Nelson et al., 2017; Nelson et al., 2019; Nelson et al., 2019; Javadi-Paydar et al., 2018; Smith et al., 2017; Collins et al., 2019). Only one of these studies examined α-PVP enantiomers in comparison to the racemate. Additional pharmacological, pharmacogenetic, and pharmacogenomic studies are ongoing to better characterize its behavioral and neurochemical effects (Kolesnikova et al., 2019).

Drug discrimination is widely accepted preclinical behavioral assay commonly used to compare novel chemicals to drugs with known neurochemical mechanisms related to their abuse liability. MDPV and a variety of synthetic cathinones have been studied in this behavioral paradigm for nearly a decade, though no published studies to date directly compared the discriminative effects of MDPV and α-PVP. Stimulus substitution has been observed with other psychostimulants in mice (Fantegrossi et al., 2013) or rats (Berquist et al., 2017; Risca & Baker, 2019) trained to discriminate MDPV. Conversely, MDPV substitutes in rats trained to discriminate amphetamine (Harvey et al., 2017) or mice trained to discriminate cocaine (Gannon et al., 2016). Additionally, α-PVP substituted in male rhesus monkeys trained to discriminate cocaine (Smith et al., 2017) and rats trained to discriminate cocaine or methamphetamine (Gatch et al., 2015; Naylor et al., 2015; Xu et al., 2021). Since MDPV and α-PVP are structurally and pharmacologically similar and both substances exhibit comparable discriminative stimulus effects to other psychostimulants, it was predicted that α-PVP will produce stimulus substitution in rats trained to discriminate MDPV. With respect to predictive validity drug discrimination is among the best behavioral paradigms to compare these substances.

The majority of preclinical drug discrimination studies have nearly exclusively used male subjects (Bevins, 2015), limiting the generalization of findings. When this study was initiated, no published drug discrimination studies with MDPV or α-PVP included female subjects. Thus, a secondary aim of the current study was to determine if α-PVP substitution varies between males and females. Though, few published studies have examined the effects of sex on the locomotor stimulant effects and conditioned place preference with MDPV or α-PVP. (King et al., 2015; Nelson et al., 2019; Risca et al., 2020). The results of those studies are reviewed and discussed in comparison to the current findings in the discussion.

EXPERIMENTAL METHODS

Animal Subjects

Eight female Sprague-Dawley rats (Charles River, Inc. Wilmington, MA. USA) weighing 250-300g and eight male Sprague-Dawley rats (Charles River, Inc. Wilmington, MA. USA) weighing 480-510g were used in this study. The subjects were housed individually in polycarbonate cages with corncob bedding (#7097; Envigo, Madison, Wisconsin, USA). The animal facilities were kept at _50 ± 5% humidity and 20 ± 2°C under a 12:12 light/dark cycle (7:00 –19:00). Commercial rodent diet (LabDiet 5001; PMI Nutrition Int. LLC, Brentwood, Missouri, USA) was restricted to once per day feeding to maintain 85%-90% of free feed weights, though the animals had free access to water in the home cage.

Apparatus

Training and testing were conducted in eight standard sound-attenuated operant conditioning chambers equipped with three retractable levers (ENV-001; Med Associates Inc., St. Albans, Vermont, USA). Experiments were controlled with Med-PC software version IV. Reinforcers presented during training sessions were 45mg Dustless Precision Pellets.

Drugs

3,4- methylenedioxypyrovalerone-hydrochloride (MDPV), α-pyrrolidinovalerophenone (α-PVP), and isomers of α-PVP, (S)-α-PVP and (R)-α-PVP were provided by the National Institute of Drug Abuse Drug Control Supply (Bethesda, MD). All compounds were dissolved in sterile 0.9% sodium chloride and injected intraperitoneally 15 min prior to training or test sessions.

Procedures

Preliminary training: Subjects were acclimated to the operant chambers for two 60-minute sessions, once a day for two days. No levers were extended during these sessions and food pellets were delivered under a fixed-time 60 s schedule. This procedure was to familiarize the subjects to the location and sound of the pellet dispenser. Training sessions thereafter were conducted 5-6 days a week and lasted 20 minutes per day. Subjects were initially trained to lever press with the center lever extended; reinforcement was delivered under a fixed-ratio (FR) schedule that was gradually increased from FR 1 to FR 20. Once the subjects were reliably responding on the FR 20 schedule, errorless training sessions commenced with either the right or left lever extended. Half of the subjects were reinforced fore responses on the right lever following 0.5mg/kg MDPV injections and for responses on the left lever following saline injections. Conditions were reversed for the remaining subjects. Animals were injected 15 minutes prior to placement in the operant conditioning chambers. A total of 12 errorless training sessions were conducted in the following order: V, V, D, D, V, D, V, V, D, D, V, D (V=vehicle, D=drug, MDPV). Once schedule control was established on both the drug-paired and vehicle-paired lever, discrimination training started.

Discrimination training Discrimination training sessions lasted 20 minutes and were conducted 5-6 days a week. During discrimination training sessions both right and left levers were extended. Subjects were trained to discriminate 0.5mg/kg MDPV from vehicle on an FR 20 schedule food reinforcement. Like in the preliminary training sessions, subjects' responding was reinforced under a FR 1 schedule that was increased to a FR 20 schedule under both drug and vehicle conditions, independently based on each subject's performance. Drug and

vehicle training sessions were alternated with sessions under the same stimulus conditions occurring no more than two consecutive days. Performance criteria for stimulus control was a minimum of eight out of ten consecutive discrimination training sessions with an 80% or greater correct lever response prior to the delivery of the first reinforcer and for the total session.

Stimulus substitution tests: Test sessions were similar to the discrimination sessions, though the session ended with the completion of the first FR 20 or after 20 minutes, whichever occurred first. No reinforcers were delivered during test sessions. Subjects were required to complete at least one drug discrimination session and one vehicle discrimination session with 80% or higher responding between test sessions. Substitution tests were conducted with MDPV (0-0.5mg/kg), α-PVP (0-0.5mg/kg), (S)-α-PVP (0-1mg/kg), and (R)-α-PVP (0-10mg/kg) in subjects that continued to meet the aforementioned discrimination criteria. Each dose of each test drug was counterbalanced among the subjects in both training groups.

Data Analysis

Discrimination accuracy with MDPV and substitution with the test compounds was determined by calculating the percentage of responses on the MDPV-lever during substitution tests. Response rate during substitution tests was expressed as responses per second and was calculated by dividing the total number of responses emitted by the number of seconds required to complete the test. Group means were calculated separately for males and females and plotted in dose response curves for visual comparison. A nonlinear regression analysis was performed to estimate the median effective dose (ED_{50}) for each compound. Additionally, for each drug assessed, a two-way repeated-measures (RM) analysis of variance (ANOVA) was conducted to determine main effects of sex and dose and any sex by does interaction on the percentage of drug

lever responses and on response rate. Substitution tests that yielded fewer than 10 responses were considered incomplete and were excluded from statistical and graphic analyses. Animals that failed to complete all test doses of a particular test compound were excluded from statistical analyses, though data from all completed tests were included in the graphic analysis.

RESULTS

This study evaluated stimulus substitution with α-PVP and its enantiomers in male and female rats trained to discriminate MDPV from saline. Stimulus control was established in all eight male and all eight female rats. Male rats met the initial discrimination criteria of 80% correct responses for a minimum of eight out of 10 consecutive sessions within an average of 29 (±3.45) training sessions (Range: 12-43). Seven of the eight female rats met the initial discrimination criteria within an average of 30.4 (±3.6) training sessions (Range: 16-41). Although training was suspended for 13 weeks due to unforeseen circumstances, all 16 subjects met these criteria again when training resumed. Including the initial sessions, prior to the training hiatus, the average total number of sessions to meet the aforementioned criteria was 39.1 (±3.9) for males (Range: 25-53) and 48.6 (±3.3) for females (Range: 31-57). An unpaired t-test on the number of sessions to meet these criteria was not significantly significant between males and females.

Dose response curves for MDPV are displayed in figure 1. MDPV produced a dose-dependent increase in drug-lever responses and full substitution only at the training dose in both males and females. The ED_{50} for MDPV was approximately 0.15mg/kg (95% confidence interval (CI) [0.07 to 0.31 mg/kg]) for males and 0.12mg/kg (95% CI [0.06 to 0.24 mg/kg]) for females. The graphic display of substitution test results includes all completed tests. However, animals that did not complete the substitution tests with all MDPV doses were excluded from the repeated measures two-way ANOVA. Six males and eight females were included in the RM ANOVA of percent MDPV responses. This analysis revealed a statistically significant main effect of dose ($F(3, 36) = 15.7$, $P<0.01$) on percent MDPV-lever responses. No statistically significant differences were observed between sexes ($F(1, 12) = 0.038$, $P=0.85$) nor was there a

statistically significant sex by dose interaction (F (3, 36) = 0.38, P=0.77). Statistical analysis of response rate during MDPV substitution tests included all eight males and all eight females. The main effect of dose (F (3, 42) = 1.65, P=0.19) and sex by dose interaction (F (3, 42) = 0.75, P=0.53) were not statistically significant, though a statistically significant effect of sex was observed on response rate (F (1, 14) = 6.29, P=0.03).

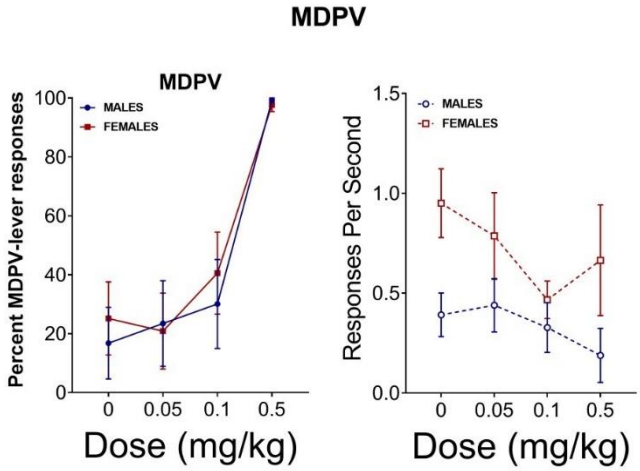

Figure 1. MDPV Response Curves

As the study progressed, two female rats exhibited response disruption during MDPV training sessions and their training dose was subsequently reduced. These animals were excluded from group analysis of subsequent substitution tests. Dose response curves for α-PVP are displayed in figure 2. These graphs show α-PVP produced a dose-dependent increase in MDPV-lever responding, with complete substitution for MDPV at the highest dose of 0.5mg/kg. The dose response curve of α-PVP is similar to that of MDPV with comparable ED_{50}. The ED_{50} was approximately 0.09 mg/kg (95% CI [0.04 to 0.20 mg/kg]) for male rats and approximately 0.13 mg/kg (95% CI [0.06 to 0.32]) for female rats. The statistical analysis for percentage MDPV-lever responses and response rate included eight males and six

females. No statistical significance was found in percentage of MDPV-lever responses between the sexes (F (1, 12) = 0.335, P=0.57) or in dose by sex interaction (F (3, 36) = 0.4217, P=0.74), though there was statistically significance effect of α-PVP dose (F (3, 36) = 7.6, P<0.01). Response rate displayed no significant differences between doses (F (3, 36) = 0.09, P=0.97) or sex by dose interaction (F (3, 36) = 0.10, P=0.96). Response rate following α-PVP treatment was significantly different between the sexes (F (1, 12) = 11.42, P=0.01).

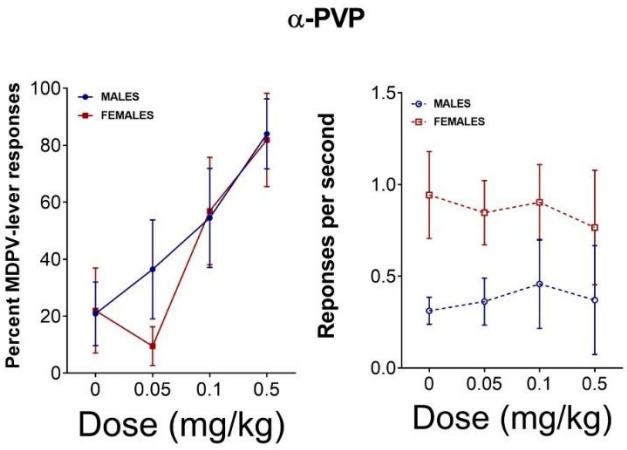

Figure 2. α -PVP Response Curves

The dose response curve for (R)-α-PVP is displayed in figure 3. The graph includes test results from eight males and six females. Although a dose-dependent increase in drug-lever responses was evident in both males and females, full substitution was observed only in females at the highest dose tested (10mg/kg). The ED_{50} of (R)-α-PVP was approximately 2.69 mg/kg (95% CI [1.01 to 6.74]) for females. The RM ANOVA of percent MDPV-lever responses following tests with (R)-α-PVP includes eight male rats and six female rats. (R)-α-PVP produced no statistically significant differences in MDPV-lever responses between the sexes (F (1, 12) = 2.11, P=0.17) or in the sex by dose interaction (F (5, 60) = 0.79, P=0.56). A

statistically significant dose effect was observed with (R)-α-PVP in percent MDPV-lever responses ($F(5, 60) = 7.1$, $P<0.01$). Response rates following (R)-α-PVP treatment were not significantly different between the sexes ($F(1, 12) = 0.001$, $P=0.97$) or among doses ($F(5, 60) = 2.30$, $P=0.06$), though a statistically significant sex by dose interaction was observed ($F(5, 60) = 4.71$, $P<0.01$)

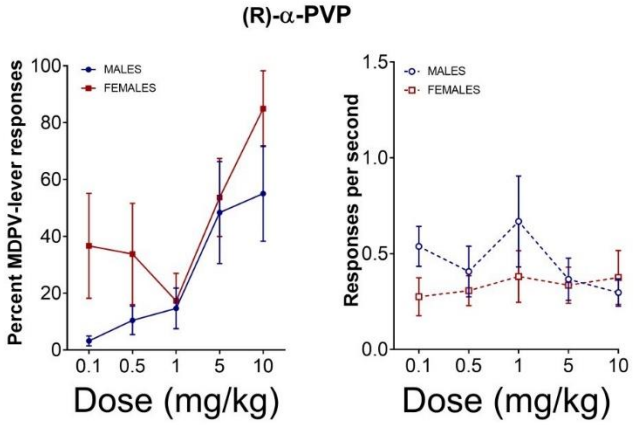

Figure 3. (R)-α -PVP Response Curves

The (S)-α-PVP dose response curve is displayed in figure 4. The graph includes test results from eight males and six females. Full substitution for MDPV was observed following 0.5 and 1mg/kg. The ED_{50} was approximately 0.05mg/kg (95% CI [0.02 to 0.11]) for the males and approximately 0.1 mg/kg (95% CI [0.05 to 0.18]) for the females. The results from two males were excluded from the RM ANOVA following tests with (S)-α-PVP because they failed to complete response requirement. Additionally, 1mg/kg (S)-α-PVP was excluded from the two-way RM ANOVA tests because only three male rats completed the response requirement following this dose; the other five males failed to respond after treatment with this dose. Therefore, only six males and six females were included in the two-way RM ANOVA for MDPV-lever responses with (S)-α-PVP substitution tests. This analysis, showed no statistically

significant effect of sex (F (1, 10) = 0.80, P=0.39) or sex by dose interaction (F (3, 30) = 0.90, P=0.45), although a statistically significant effect of dose was observed (F (3, 30) = 12.26, P<0.01). Statistical analysis of the response rate included all eight males and six females. This analysis showed no statistically significant effect of dose (F (4, 48) = 1.23, P=0.31), sex (F (1, 12) = 0.18, P=0.68) or dose by sex interaction (F (4, 48) = 1.52, P=0.21).

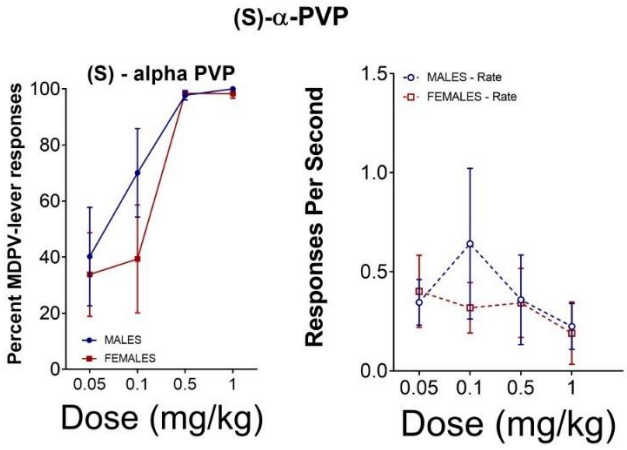

Figure 4. (S)-α -PVP Response Curves

DISCUSSION

The present study examined the discriminative stimulus effects of α-PVP and its isomers, (S)-α-PVP and (R)-α-PVP, in male and female rats trained to discriminate MDPV from saline. The number of training sessions required to establish stimulus control with MDPV were comparable between males and females in this study and consistent with previous studies conducted with male rats (Risca et al., 2019) and female rats (Thomas et al. unpublished findings) under similar conditions in the same laboratory setting.

The primary objectives of this study were to assess substitution of α-PVP and its isomers for MDPV and determine if these effects were sex-dependent. As predicted, α-PVP and its isomers substituted for MDPV. All three substances produced dose-dependent increases in MDPV-lever responses and full substitution at the highest dose tested in most rats, with the exception of (R)-α-PVP in males. Dose response curves for MDPV, α-PVP, and (S)-α-PVP were nearly identical in males and females, indicating that these effects are not sex-dependent. Interestingly, sex differences were observed in response rate following treatment with MDPV and α-PVP. Sex differences in response rate were not observed with either α-PVP isomer. The reason for this discrepancy is not clear. Whereas tests with the isomers were conducted later in the study, it is possible these differences are an artifact of timing. Therefore, additional tests to re-determine the dose effect curve with MDPV are ongoing.

Only a few published studies were found that assessed sex differences in the behavioral effects of MDPV (King et al., 2015; Hambuchen et al., 2018; Risca et al., 2020). Although these studies utilized different behavioral assessments from the present study, the results are worth noting for comparison. King et al. (2015) evaluated male and female SD rats in an assessment conditioned taste aversion and conditioned place preference with MDPV. Although

no sex differences were observed in CPP induced by MDPV, females exhibited a weaker taste avoidance than males (King et al., 2015). Consistent with those findings, Risca et al. (2020) demonstrated minimal differences between male and female rats in MDPV-induced CPP, though females displayed greater increases in activity following treatment with 1 or 3.2 mg/kg MDPV. In an investigation of the pharmacokinetic profile of intravenous MDPV and its (R)- and (S)-enantiomers, Hambuchen et al. (2018) also reported a statistically significant sex differences in the locomotor effects following i.p. injections of MDPV in Sprague-Dawley rats, though they found greater increases in horizontal activity following 3 mg/kg MDPV in males compared to females. Additionally, they reported a higher magnitude and variance in bioavailability in males compared to females. Considered together, these findings along with the current results indicate MDPV produces differential effects in males and females in some behavioral measures and not others.

Two published studies were found that assessed α-PVP in female rats. Jayadi-Paydar et al., (2018) utilized female rats with activity wheel access to assess α-PVP self-administration and binge-like behavior, and reported a binge-like initial acquisition and a decrease of value of the wheel as a reinforcer. Nelson et al. (2019b) compared male and female Sprague-Dawley rats in a study of α-PVP-induced taste avoidance, place preference, and locomotor activity. They demonstrated that males exhibited a higher preference at lower α-PVP doses, while females only exhibited CPP following treatments with 6mg/kg α-PVP. They also reported females were slightly more sensitive to the locomotor effects of α-PVP and displayed weaker taste avoidance (Nelson et al., 2019). These findings suggest that males may be more susceptible to the rewarding and aversive effects of α-PVP.

Nelson et al. (2019a) compared the α-PVP enantiomers in a conditioned taste avoidance study with male rats. Both (S)-α-PVP and (RS)-α-PVP induced conditioned taste avoidance whereas (R)-α-PVP failed to do so. Schindler et al. (2020) also conducted a study evaluating α-PVP and its enantiomers in male rats, where they evaluated self-administration, in vivo microdialysis, and telemetric assessment of cardiovascular parameters. They found that all three substances block dopamine and norepinephrine transporters, but not serotonin transporters. They also found that (S)- α-PVP increases dopamine in the nucleus accumbens as well as locomotor activity. Increased elevation of blood pressure and heart rate were observed with α-PVP and (S)- α-PVP with low doses, whereas these effects were observed with a higher dose of (R)- α-PVP (6mg/kg). These findings demonstrate (S) α-PVP is approximately 30 times more potent than (R) α-PVP. This difference in potency is consistent with the current finding that (S) α-PVP produced substitution for MDPV at lower doses than (R)- α-PVP.

Previous findings indicate the involvement of D_1 dopamine in MDPV's interoceptive stimulus effects (Risca & Baker, 2019). In consideration of the present study results that α-PVP substitutes fully for MDPV, it is likely that dopaminergic mechanisms also mediate the discriminative stimulus effects of α-PVP. As noted above, Schindler et al. (2020) reported that α-PVP and its isomers block dopamine and norepinephrine reuptake. To characterize the specific involvement of dopaminergic and/or noradrenergic mechanisms contributing to α-PVP discrimination, future investigations are required to assess the effects of selective dopamine receptor antagonists in animals trained to discriminate α-PVP.

In conclusion, the current findings add to the psychopharmacology literature devoted to characterizing the behavioral effects of novel drugs of abuse. Investigations into the neurochemical mechanisms of novel drugs are crucial to medical advances in

treating substance use disorders associated with synthetic cathinones. A variety of illicit “bath salts” are frequently combined with other recreational drugs, and users may not always be aware of the specific chemicals they consume. This contributes to the public health problem associated with frequent abuse of these substances, specifically relevant to emergency departments and law enforcement. Although hospital emergency department protocols may be in place for treating individuals presenting with signs and symptoms of overdose with commonly abused drugs, the psychopharmacology of a wide variety of new synthetic cathinones is still not well understood. Further investigation into the neurochemical mechanisms underlying the behavioral effects of popular synthetic cathinones can serve to inform medical management of individuals seeking treatment for substance abuse or presenting in emergency departments with adverse effects from their use.

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APPENDIX

WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

Date: March 27, 2019

To: Lisa Baker, Principal Investigator

From: Kathryn Eckler, Vice Chair

Re: IACUC Protocol Number 19-02-01

Your protocol titled "Drug Discrimination Studies of Psychoactive Drugs in Rats" has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: **March 26, 2020**

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