Published in final edited form as:

Psychopharmacology (Berl). 2019 March ; 236(3): 903–914. doi:10.1007/s00213-018-5011-8.

Analysis of neurotransmitter levels in addiction-related brain regions during synthetic cathinone self-administration in male Sprague-Dawley rats

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Abstract

Rationale: Synthetic cathinones are used as stimulants of abuse. Different stimulants may induce distinct rates of disease progression, yielding neurochemical changes that may vary across brain regions or neurotransmitter systems.

Objectives: This research sought to behaviorally and chemically differentiate stages of synthetic cathinone abuse through rodent self-administration and measurement of the neurotransmitter profile in multiple brain regions.

Methods: Male rats were trained to self-administer α -PVP, mephedrone (4MMC), or saline. Half of each drug group stopped self-administering after autoshaping; the other half self-administered for another 21 days. Brain tissue from amygdala, hippocampus, hypothalamus, PFC, striatum, and thalamus was profiled with electrochemical detection to assess neurotransmitter levels.

Results: During autoshaping, the majority of infusions were delivered noncontingently. In the self-administration phase, rats responded more for α -PVP and 4MMC than for saline, demonstrating that both synthetic cathinones were reinforcing. Longer durations of exposure elevated 5-HIAA in hypothalamus, PFC, and hippocampus, indicating that learning may produce changes in addiction-related brain regions. Both synthetic cathinones decreased norepinephrine in hippocampus, while α -PVP decreased glutamate in hippocampus and PFC, and 4MMC decreased glutamate in thalamus. Furthermore, α -PVP increased dopaminergic metabolites in striatum, whereas 4MMC decreased serotonin in amygdala, hippocampus, and PFC. Interestingly, neither synthetic cathinone affected dopamine levels despite their functional effects on the dopaminergic system.

Conclusions: In summary, the neurotransmitter changes observed here suggest that synthetic cathinone use likely produces sequential neurochemical changes during the transition from use to abuse. Consequently, treatment need may differ depending on the progression of synthetic cathinone abuse.

Keywords

α -PVP; mephedrone; neurotransmitter; self-administration

1.0 Introduction

Products containing synthetic cathinones have recently flooded the recreational drug market (Baumann et al. 2013; Baumann et al. 2014). These products, often sold under the guise of “bath salts,” contain psychoactive cathinone derivatives and are purchased as “legal” alternatives to illicit drugs (Baumann 2014; Karila et al. 2015). Synthetic cathinone abuse can result in tachycardia, seizures, rhabdomyolysis, hallucinations, delusions, and suicide (German et al. 2014; Prosser and Nelson 2012; Wood et al. 2011). Despite a permanent ban on the most common active compounds found in synthetic cathinone products, including 4-methylmethcathinone (4MMC), 3,4-methylenedioxypyrovalerone (MDPV), and 3,4-methylenedioxymethcathinone (methyldone), these products remain available as manufacturers supply new, unregulated compounds (Fratantonio et al. 2015; Katz et al. 2014; Saha et al. 2015). Synthetic cathinones continue to be widely available throughout the U.S., and availability is increasing in some U.S. markets (U.S. Department of Justice 2017). α -pyrrolidinopentiophenone (α -PVP) was one of the top five most commonly seized synthetic cathinones across the U.S. in 2016 (NDEWS 2016a; NDEWS 2016b; NDEWS 2017), and α -PVP has been implicated in multiple deaths and widespread excited delirium (NDEWS 2015).

Synthetic cathinones produce typical stimulant-like effects. 4MMC, a ring-substituted cathinone, releases dopamine (DA), norepinephrine (NE), and serotonin (5-HT) (Baumann et al. 2012; Cameron et al. 2013; Simmler et al. 2013), but is less potent in DA release than methamphetamine (Baumann et al. 2012). In contrast, α -PVP contains a pyrrolidine ring, which produces DA and NE transporter blockade (Glennon and Young 2016; Marusich et al. 2014). Despite a different mechanism of action, α -PVP and 4MMC produce typical stimulant effects including hyperactivity and stereotyped behavior (Marusich et al. 2014; Marusich et al. 2012; Marusich et al. 2016). Importantly, α -PVP and 4MMC are readily self-administered by rats, indicating a propensity for addiction (Aarde et al. 2015; Creehan et al. 2015; Nguyen et al. 2017a; Nguyen et al. 2017b; Vandewater et al. 2015).

Metabolomic techniques are ideal for studying stimulant-induced neuroplasticity and biomarker discovery (Ghanbari and Sumner 2018; Patkar et al. 2009; Zaitsev et al. 2014). Changes in neurotransmitter levels and their metabolites can be simultaneously measured using a liquid chromatography electrochemical array as a broad spectrum, non-targeted metabolomic platform (Kaddurah-Daouk et al. 2008; Reinhoud et al. 2013). This electrochemical method allows for much faster screening of neurotransmitters compared to commonly used HPLC methods in which a single neurotransmitter is measured at a time. In order to understand the molecular pathways by which stimulant use leads to addiction, several studies have examined stimulant effects on neurotransmitter levels in various brain regions. Experimenter-administered cocaine and methamphetamine produced changes in neurotransmitters and their metabolites in hippocampus, prefrontal cortex (PFC), and striatum, which were identified using metabolomic analyses (Bu et al. 2013; Kaplan et al. 2013; Li et al. 2012). Repeated experimenter administered 4MMC altered 5-HT, DA, and their metabolites in striatum and hippocampus (Motbey et al. 2012). Furthermore, cocaine self-administration increased GABA and glutamate in PFC and nucleus accumbens,

respectively, but fewer neurotransmitter changes were found after 3 weeks of withdrawal (Zhang et al. 2016). Thus, stimulant-induced changes in neurotransmitter levels across various brain regions appears to be dependent on the stage of drug abuse, and can be studied using metabolomic analyses, giving insight into global brain changes.

Stimulant-induced neurochemical changes may not occur simultaneously across affected brain regions or affected neurotransmitter systems (Koob and Volkow 2010). Consequently, treatment need may differ depending on the progression of stimulant abuse, defined by the amount of drug consumed over time, and genetic vulnerability (Koob and Volkow 2010). Different stimulants may also induce distinct rates of disease progression, which may explain divergent use patterns. The synthetic cathinone-induced psychoses in humans appears to be something unique, or at least accelerated compared to other drugs of abuse since such bizarre behavior can be caused by only one use of synthetic cathinones (Derungs et al. 2011; Striebel and Pierre 2011; Warrick et al. 2012). Therefore, the unique psychosis observed with synthetic cathinones (German et al. 2014; Prosser and Nelson 2012; Wood et al. 2011) may arise from altered regional dysfunction and disease progression compared to classical stimulants.

The purpose of this study was to probe the potential chronology of synthetic cathinone abuse. The underlying neurotransmitter profiles were measured in addiction-related brain regions to assess how neuronal signaling differed between two synthetic cathinones with different mechanisms of action, and how signaling changed as a function of duration of drug exposure. Rats self-administered synthetic cathinones or vehicle for 7 or 28 days in an attempt to model the binge/intoxication or preoccupation/anticipation stages of drug abuse, respectively (Koob and Volkow 2010). Then neurotransmitter levels and their metabolites were examined in several brain regions.

2.0 Methods

2.1 Subjects

Adult male Sprague-Dawley rats (Envigo, Frederick, MD, USA) (total n=48), aged approximately 65–70 days at the start of the experiment, were housed individually in polycarbonate cages with hardwood bedding. Rats were housed in temperature-controlled conditions (20–24°C) with a 12 h standard light-dark cycle (lights on at 0700). Rats had free access to water in the home cage, and were lightly food restricted (e.g. 20 g daily). Experiments were approved by the Institutional Animal Care and Use Committee at Mipro Biotech. All research was conducted as humanely as possible, and followed the principles of laboratory animal care (National Research Council 2011).

2.2 Drugs

α -PVP and 4MMC were synthesized in house using standard synthetic procedures. They were formulated as recrystallized salt and were > 97% pure. The purity was assessed by carbon, hydrogen, nitrogen (CHN) combustion analysis, and proton nuclear magnetic resonance spectroscopy. Compounds were dissolved in sterile saline USP (Butler Schein,

Dublin, OH). Gentamicin USP and heparin USP, used for maintaining catheter patency, were purchased from Butler Schein.

2.3 Apparatus

Experimental sessions were conducted in operant conditioning chambers for rats (MED Associates, St. Albans, VT) housed inside sound-attenuating chambers (MED Associates). Each operant conditioning chamber contained two retractable levers, with a stimulus light above each lever, and a house light. One lever was designated as the active lever and the other lever was designated as inactive.

The side of the operant conditioning chamber associated with the active lever was counterbalanced across subjects. Fans provided ventilation for each chamber and speakers provided white noise. Infusion pumps (Med Associates) were located outside the chamber. Experimental events were arranged and recorded by MED-PC software (Med-Associates) on a computer in the experimental room.

A Thermo Scientific CoulArray Multi-Channel ECD Array system (model 5600A; Thermo Scientific, Waltham, MA) was used to analyze neurotransmitter levels. The array detector contained a series of 16 coulometric electrochemical cells that provided quantitation of several neurotransmitters and metabolites simultaneously. An Agilent 1100 HPLC System (Santa Clara, CA) and an Applied Biosystems API 4000 Triple Quadrupole liquid chromatography mass spectrometer (LC-MS) with Turbo Ion Spray source (Foster City, CA) were used for quantitation of glutamate.

2.4 Surgical Procedures

Rats were surgically implanted with chronic indwelling jugular catheters under general anesthesia as previously described (Marusich et al. 2010; Marusich et al. 2011). The external end of the catheter was secured by a quick connect harness. Rats were given a minimum of 7 days to recover from surgery before beginning the experiment. Catheters were flushed daily with saline prior to the session, and with post-flush solution (0.01% gentamicin, 0.03% heparin, 99.6% sterile saline USP) after the session to maintain patency. All catheters were checked for patency prior to the start of the experiment.

2.5 Drug Self-Administration

Rats were randomly assigned to one of three drug groups: α -PVP (0.1 mg/kg/infusion), 4MMC (0.5 mg/kg/infusion), or saline. The dose of α -PVP was chosen because it has led to more consistent acquisition of self-administration, and shorter latency to stable responding than lower doses (Gannon et al. 2018; Nguyen et al. 2017a), the dose of 4MMC was chosen because it is the most commonly used dose for acquisition of 4MMC self-administration (Creehan et al. 2015; Motbey et al. 2013; Nguyen et al. 2017b; Vandewater et al. 2015), and these doses were at the peak of the dose-effect curve in most past studies (Aarde et al. 2015; Gannon et al. 2017; Nguyen et al. 2017b). Furthermore, 3.0 mg/kg α -PVP and 10 mg/kg 4MMC produce similar levels of locomotor activity at a 0.5 log unit dose differential (Marusich et al. 2014; Marusich et al. 2012). Thus, the present study used 0.1 mg/kg/infusion α -PVP and 0.5 mg/kg/infusion 4MMC, a 0.7 log unit differential, in attempt to

yield similar levels of self-administration, which would indicate that the doses were functionally equivalent. Within each drug group, rats were randomly assigned to one of two duration groups: autoshaping only (AO), or autoshaping followed by an additional 21 days of self-administration (self-administration; SA) (n=8/group).

All rats were first trained to self-administer through an autoshaping procedure for 7 days (Carroll and Lac 1993; Marusich et al. 2010) to ensure that rats in the AO groups received a consistent, limited amount of drug exposure, regardless of how quickly they acquired self-administration. This limited drug exposure may be sufficient to produce neurochemical changes indicative of the binge/intoxication stage of drug abuse (Koob and Volkow 2010). During autoshaping sessions, active lever extension was paired with an infusion (0.1 ml) based on a random time 60 s schedule. Following 15 s of lever extension, or immediately after a lever press, an infusion was delivered over 5.9 s. Infusions were followed by a 20-s timeout signaled by illumination of both stimulus lights. Throughout training and self-administration sessions, the inactive lever was extended, and presses on this lever were recorded, but had no programmed consequence. Autoshaping sessions delivered 15 infusions within the first 30 min of the session. Rats then remained in the operant conditioning chamber for 15 min with only the inactive lever present and no drug infusions available, which provided additional exposure to the lack of programmed consequences associated with the inactive lever. Following autoshaping, self-administration stopped for rats in AO groups, whereas SA groups continued to self-administer on a fixed ratio 1 schedule of reinforcement (FR1) for an additional 21 days during daily 60 min sessions.

2.6 Brain Sample Collection

Rats were euthanized by rapid decapitation one day after their last self-administration session to avoid direct drug effects. The hypothalamus was removed from the ventral side of the brain and divided into halves. Then the brain was cut down the mid line and each cortical half was opened, and the hippocampus was removed (Spijker 2011). Next, each half was cut into three coronal slices using mid line anatomical markers moving rostral to caudal. The first cut was made at the beginning of the corpus callosum, the second at the fornix, and the third cut at the end of the corpus callosum. PFC was taken from the first section. Striatum was removed from the second slice. Finally, thalamus and amygdala were removed from the third section (Chiu et al. 2007; Honkanen 1999). All tissue samples were placed in aluminum foil, inserted into a 2 ml cryovial, flash frozen in liquid nitrogen, and stored at -80°C .

2.7 Neurotransmitter Quantitation

The tissue buffer consisted of 0.05 M Na_2HPO_4 , 0.03 M citric acid, and 2 mM ascorbic acid with pH 3. The first internal standard solution contained 200 ng/ml 3,4-dihydroxybenzylamine (DHBA) in tissue buffer. The second internal standard solution contained 10 $\mu\text{g}/\text{ml}$ L-glutamic $-2,3,3,4,4\text{-d}_5$ acid ($\text{d}_5\text{-GLU}$) in water. Tissue samples were weighed prior to analysis. Internal standard solution was then added to each sample at a ratio of 20 $\mu\text{l}/\text{mg}$ of tissue. Five 2.8-mm stainless steel grinding balls were added to each sample for homogenation. Samples were homogenated by two 30-s cycles on the genogrinder, and then centrifuged. Aliquots were removed and processed as described below.

2.7.1 Electrochemical methods.—Ultra-high pressure liquid chromatography (UPLC) coupled with electrochemical detection (ECD) was used to simultaneously measure DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, 5-hydroxy-3-acetic acid (5-HIAA), and NE. A 10- μ l aliquot was injected onto a Luna Omega 2.1 $\times$ 150 mm column (Phenomenex, Torrance, CA) coupled to a LPG-3400RS pump, WPS-3000TBRS autosampler, and a CoulArray electrochemical detector. The column was heated to 30°C. The mobile phase consisted of 50 mM sodium phosphate, 47 mM citric acid, 0.14 mM EDTA, 0.64 mM octanesulfonic acid, and 5% methanol, with a flow rate of 0.4 ml/min. The detector was set to sequentially deliver potentials of –150 mV, 150 mV, 400 mV, and 600 mV.

2.7.2 LC-MS methods.—Glutamic acid (GLU) concentration was analyzed separately with liquid chromatography mass spectrometry (LC-MS). A 10- μ l aliquot of supernatant was transferred to a 700 μ l deep, 96 well plate, and diluted with 490 μ l of mobile phase A. The plate was mixed at 1000 RPM for 4 min. Then 100 μ l was transferred to a new 700 μ l deep, 96 well plate and diluted with an additional 400 μ l of mobile phase A (total dilution of 250 fold). A 50 μ l aliquot of 10 μ g/ml d₅-GLU was added. The plate was sealed and mixed at 1000 RPM for 4 min prior to analysis.

Then a 10- μ l aliquot was injected onto an XBridge HILIC 4.6 $\times$ 150 mm column (Water, Ireland) coupled to the HPLC and LC-MS systems. Mobile phase A consisted of 5 mM ammonium acetate and 0.1% formic acid (aqueous). Mobile phase B consisted of acetonitrile. Mobile phase A was held at 50% for 1 min, then increased linearly to 90% for 1.5 min, and held for 2.5 min before returning to initial conditions. The flow rate was 0.5 ml/min.

2.8 Data Analysis

One rat in the α -PVP SA group failed the catheter patency test before the study began, and was, therefore, dropped from the study. Additionally, one rat in the 4MMC SA group and one in the saline SA group were exposed to an experimenter error that increased infusion volume. All self-administration and neurotransmitter data for these rats were excluded from graphs and analyses. Statistical analyses were conducted using NCSS (Number Cruncher Statistical Systems, Kaysville, Utah, USA). For all analyses, α -PVP and 4MMC were analyzed separately, and compared to saline.

For autoshaping self-administration data, separate mixed factors ANOVAs (drug $\times$ lever $\times$ exposure duration) were used to compare active and inactive lever presses for drug and saline groups, with drug and duration as between-subjects factors, and lever as a within-subjects factor. The additional 21 days of self-administration for SA groups were analyzed with separate mixed factors ANOVAs (drug $\times$ lever $\times$ session). These analyses compared active and inactive lever presses for drug groups to the saline group, with drug as a between-subjects factor, and lever and session number as within-subjects factors.

Neurotransmitter and metabolite data were analyzed with between-factors duration (AO vs SA) $\times$ drug group (cathinone vs saline) ANOVAs. Each brain region and neurotransmitter

(or metabolite) combination was analyzed separately. All tests were considered significant at $p < 0.05$, and were followed with Tukey's post hoc tests as appropriate.

3.0 Results

3.1 Self-administration

Self-administration data from the autoshaping phase are shown in Figure 1. Lever presses for rats in the AO and SA groups did not significantly differ during autoshaping, nor were there interactions between duration and drug group, or duration and lever during autoshaping ($p > 0.05$). Therefore, data were collapsed across AO and SA groups within drug for graphical representation (Figure 1). Saline rats lever pressed more than α -PVP or 4MMC rats during autoshaping [α -PVP: $F(1, 26) = 6.79$, $p < 0.05$; 4MMC: $F(1, 26) = 10.24$, $p < 0.01$]. Across drug groups, rats responded more on the active than inactive lever, [α -PVP: $F(1, 26) = 12.47$, $p < 0.01$; 4MMC: $F(1, 26) = 10.87$, $p < 0.01$]. Saline rats lever pressed more on the active than inactive lever, and responded more on the active lever than α -PVP or 4MMC rats (drug $\times$ lever interaction) [α -PVP: $F(1, 26) = 5.20$, $p < 0.05$; 4MMC: $F(1, 26) = 6.50$, $p < 0.05$]. These results indicate that during autoshaping, saline rats self-administered a small portion of the infusions they received, whereas most α -PVP and 4MMC rats did not self-administer any of the infusions they received. Therefore, the majority of infusions for all groups were delivered noncontingently during autoshaping.

Self-administration data from the FR1 phase for SA groups are shown in Figure 2. α -PVP rats responded more than saline rats (Figure 2 top panel), and rats responded more on the active than inactive lever [main effect of drug: $F(1, 12) = 9.30$, $p < 0.05$; main effect of lever: $F(1, 12) = 133.87$, $p < 0.001$]. Both α -PVP and saline active responses were greater than the respective inactive responses within drug group, and α -PVP active responses were greater than saline active responses, [drug $\times$ lever interaction: $F(1, 12) = 47.40$, $p < 0.001$]. Active responses increased from session 1 for sessions 5–21, and active responses were greater than inactive responses during sessions 3–21 [main effect of session: $F(20, 240) = 1.77$, $p < 0.05$; lever $\times$ session interaction: $F(20, 240) = 4.77$, $p < 0.001$]. There was no significant drug $\times$ session interaction. Therefore, rats acquired self-administration of α -PVP, and self-administered significantly more α -PVP than saline.

During the 21 days of self-administration, rats self-administered more 4MMC than saline, responded more on the active than inactive lever, and made more 4MMC active responses than both 4MMC inactive responses and saline active responses [main effect of drug: $F(1, 12) = 71.44$, $p < 0.001$; main effect of lever: $F(1, 12) = 140.20$, $p < 0.001$; drug $\times$ lever interaction: $F(1, 12) = 92.12$, $p < 0.001$]. Responding for 4MMC increased from session 1 for sessions 3–21, and responding for 4MMC was greater than responding for saline for sessions 3–21 [main effect of session: $F(20, 240) = 5.83$, $p < 0.001$; drug $\times$ session interaction: $F(20, 240) = 3.96$, $p < 0.001$]. Rats responded more on the active than inactive lever during sessions 2–21, and active lever presses were higher than session 1 for sessions 3–21 [lever $\times$ session interaction: $F(20, 240) = 7.91$, $p < 0.001$]. These data indicate that rats acquired self-administration of 4MMC during the first few sessions, and then number of 4MMC infusions earned remained fairly stable. Interestingly, rats in α -PVP and 4MMC groups showed elevated responding on session 15, which persisted through session 16 for α -PVP; however,

these elevations were not statistically significant. These sessions occurred on the same day, and the cause of this increase is unknown.

3.2 Neurotransmitters

Neurotransmitter and metabolite concentrations for all brain regions and all rat groups are shown in Figure 3. There were several differences between drug groups, between durations (AO vs SA), and interactions between drug and duration.

3.2.1 Effects of duration.—All statistically significant effects of exposure duration are shown in Table 1. Hippocampus showed elevated 5-HIAA following greater durations of α -PVP and 4MMC exposure, and elevated NE following greater durations of α -PVP exposure (Table 1 and Figure 3). Hypothalamus showed the most increases in neurotransmitters as a function of duration of exposure with increases in 5-HIAA and DOPAC for both drugs, and an increase in DA for 4MMC. In PFC, greater duration of exposure to either synthetic cathinone led to decreased GLU, and longer exposure to α -PVP increased 5-HT and 5-HIAA. Thalamus also showed differential drug effects as a result of duration. Longer 4MMC exposure decreased NE, while increased α -PVP exposure elevated 5-HIAA. In striatum, α -PVP groups showed increased 5-HT and 5-HIAA as a function of duration. Finally, differential GLU effects across brain regions were observed for α -PVP, with longer duration of exposure increasing GLU in amygdala, but decreasing GLU in PFC. Duration did not alter HVA for either drug (data not shown).

3.2.2 Effects of drug.—All statistically significant effects of drug on neurotransmitters, and interactions between duration and drug are shown in Table 2. Some drug effects only occurred for one duration; these interactions are denoted by the duration names (AO or SA) in the corresponding cells in the table. Despite α -PVP and 4MMC being indirect-acting DA receptor agonists, there were surprisingly no drug effects on DA in any brain region for either drug; however, α -PVP increased DOPAC and HVA in striatum, indicating metabolism of DA in this brain region (Table 2 and Figure 3). 4MMC produced several serotonergic effects. 4MMC decreased 5-HT in amygdala, hippocampus, and PFC, although the decreases in hippocampus and PFC only occurred for rats with extended durations of exposure (SA). 4MMC also decreased 5-HIAA in hippocampus. In contrast, α -PVP had no effect on 5-HT or 5-HIAA. There was a wide range of drug effects on GLU, that varied by drug, brain region, and duration. Interestingly, α -PVP produced differential glutamatergic effects across brain regions and duration. Following brief α -PVP exposure (AO), GLU decreased in hippocampus, but when α -PVP was self-administered longer (SA), GLU increased in amygdala and decreased in PFC. 4MMC decreased GLU in thalamus, but only after a longer duration of exposure (SA). α -PVP and 4MMC both decreased NE in hippocampus, and α -PVP also decreased NE in thalamus (AO).

4.0 Discussion

Rats were exposed to α -PVP, 4MMC, and saline during autoshaping. SA rats acquired self-administration of α -PVP and 4MMC during their additional 21 days of self-administration. These results are consistent with past studies in which male rats acquired self-administration

of these synthetic cathinones using the same doses and an FR1 schedule of reinforcement (Aarde et al. 2015; Nguyen et al. 2017a; Nguyen et al. 2017b; Vandewater et al. 2015). Synthetic cathinone administration, and duration of exposure produced several effects on neurotransmitters. In summary, both drugs decreased NE in localized brain regions, and both produced alterations in GLU levels, which varied by direction of effect and brain region. α -PVP increased dopaminergic metabolites in striatum, while 4MMC decreased 5-HT in amygdala, hippocampus, and PFC.

4MMC is the most widely studied synthetic cathinone of abuse, and the few studies that have examined its effects on neurotransmitters and their metabolites show some agreement, but also some conflicting results with the current study. Repeated experimenter administered 4MMC had no effect on hippocampal 5-HT in one study (Shortall et al. 2013), but decreased hippocampal 5-HT in other studies (Hadlock et al. 2011; Motbey et al. 2012), including the present study. Similarly, in accordance with a past studies of repeated experimenter administered 4MMC, the present results showed that 4MMC did not affect DA, DOPAC, or 5-HIAA in striatum or PFC, nor did it affect DA in hippocampus (Hadlock et al. 2011; Shortall et al. 2013). The present study, however, differs from a past study showing 4MMC increased DOPAC in hippocampus, had no effect on 5-HT in PFC, and had no effect on 5-HIAA in hippocampus (Shortall et al. 2013). The conflicting results between the present study and those of past studies may be due to brain sample collection 1 day after the last drug exposure in the present study, but 3–7 days after the last drug exposure in past studies (Hadlock et al. 2011; Motbey et al. 2013; Shortall et al. 2013). 4MMC also produced long lasting elevations in 5-HIAA in hippocampus, and elevated DA, and lowered HVA and DOPAC in striatum 47 days after drug exposure (Motbey et al. 2012).

The single past study on 4MMC self-administration found that 4MMC had no effect on DA, HVA, DOPAC, or 5-HT in striatum, which is consistent with the present study, but showed decreased 5-HIAA in striatum, which is inconsistent with the present study (Motbey et al. 2013). The present 4MMC results are also consistent with results from methamphetamine self-administration, which produced no effect on DA, HVA, DOPAC, 5-HT, and 5-HIAA in striatum (Motbey et al. 2013). To the best of our knowledge, this is the first study to examine neurotransmitter concentrations in vitro for α -PVP.

Interestingly, duration of exposure to synthetic cathinones or vehicle produced several changes in neurotransmitter levels that were not a result of drug effects (Table 1). Longer duration groups (SA) showed elevated 5-HIAA in 5 brain regions for α -PVP groups and in 2 brain regions for 4MMC groups. The longer duration also elevated DOPAC for both drugs, and elevated DA for 4MMC in hypothalamus. Learning may be responsible for the effects of duration, since even saline rats learned that pressing the active lever turned on the cue light. It is also possible that noncontingent (autoshaping) vs contingent drug/saline exposure produced the effects of duration. Previous research shows that the effects of abused drugs on neurotransmitters may differ depending upon whether they are administered by the experimenter or self-administered (Orejana et al. 2009). Contingent cocaine increased basal DA levels in the nucleus accumbens compared to noncontingent cocaine (Lecca et al. 2007). In contrast, self-administered and noncontingent methamphetamine produced similar levels of striatal DA depletion (Brennan et al. 2010; Lacan et al. 2013). These differential

effects of contingency may be due to methodological differences in measurement of DA. Future research should confirm if these neurotransmitter changes are a result of learning, cumulative drug exposure, contingent vs. noncontingent drug/saline exposure, the passage of time, or aging. Since the present study used adult rats, only 3 weeks of time passed between when AO and SA brain samples were collected, and several effects of duration occurred for drug groups and saline groups (see effects on 5-HIAA in hippocampus and hypothalamus in Figure 3), learning may be the most parsimonious explanation for the effects of duration on neurotransmitters.

Despite their similarities in dopaminergic agonist effects, α -PVP and 4MMC produced different changes in neurotransmitter levels. A longer duration of α -PVP exposure decreased GLU in PFC, increased GLU amygdala, and increased NE in thalamus, whereas a longer duration of 4MMC exposure decreased 5-HT in PFC, and decreased GLU in thalamus (Table 2). Furthermore, 4MMC decreased 5-HT and 5-HIAA in multiple brain regions, which is not surprising given that 4MMC is a relatively potent 5-HT releaser (Baumann et al. 2012), whereas α -PVP had no serotonergic effects (Table 2), which is consistent with its lack of functional serotonergic effects (Marusich et al. 2014). In contrast, α -PVP increased dopaminergic metabolites in striatum, whereas 4MMC did not alter DA or DA metabolite levels. Both drugs affected GLU, but did so in different brain regions. Similarly, both drugs decreased NE in hippocampus, but even this effect differed across drugs because it only occurred following a longer duration of exposure (SA) for 4MMC, while the α -PVP-induced decrease in NE was similar across durations (Table 2). The observed differences in neurotransmitter profiles in the brain for α -PVP and 4MMC may be due to their different mechanisms of action, reuptake inhibitor or releaser, respectively, or may be a result of differences in their relative selectivities for monoamine transporters. α -PVP has considerably greater selectivity for the DA transporter vs. 5-HT transporter compared to 4MMC, and α -PVP produces very little 5-HT transporter activity (Baumann et al. 2012; Marusich et al. 2014).

Perhaps the most surprising result of the present study was the lack of dopaminergic effects. Both synthetic cathinones produce robust functional changes in the DA system, albeit by different mechanisms of action. 4MMC releases DA, while α -PVP produces DA transporter blockade (Baumann et al. 2012; Cameron et al. 2013; Glennon and Young 2016; Marusich et al. 2014; Simmler et al. 2013). Interestingly, the current study showed that neither synthetic cathinone affected DA levels, although α -PVP increased DA metabolites in striatum (Table 2). Greater duration of exposure increased DA and DOPAC in hypothalamus for 4MMC (Table 1), but this effect was not isolated to 4MMC or saline, as can be seen in the similarity of DOPAC levels in hypothalamus within a duration group (Figure 3). One possibility for the lack of dopaminergic effects is that brain samples were collected one day after final drug exposure to avoid interference from direct drug effects during analyses. Therefore, while α -PVP and 4MMC elevate DA in brain tissue (Baumann et al. 2012; Baumann et al. 2017; Kehr et al. 2011; Marusich et al. 2014), the duration of this effect appears transient.

One methodological caveat of the current study is that whole tissue concentrations were measured rather than extracellular neurotransmitter levels, the latter of which can be obtained through in vivo microdialysis (Darvesh et al. 2011), fast scanning cyclic

voltammetry (FSCV) (Garris et al. 1997), or more recently, fast-scanning controlled absorption voltammetry (FSCAV) (Burrell et al. 2015). Whole tissue measurements do not detect changes in the amount of neurotransmitter released, spontaneously or in concert with a behavior, in a spatiotemporal fashion, but those were not goals of the current study, which sought to provide a broad assessment of neurotransmitter and inflammatory markers in order to measure tissue changes in multiple brain regions simultaneously, and potentially uncover tissue-specific toxic effects due to long term drug exposure. Microdialysis, FSCV, and FSCAV cannot assess multiple brain regions simultaneously, or assess other markers such as cytokines. For example, while FSCV provides highly precise spatiotemporal resolution of extracellular neurotransmitter change, and microdialysis coupled with an ECD can quantify total extracellular neurotransmitter levels, both methods are limited by placement of the electrode in a localized region, and measurement of a specific released neurotransmitter (Rodeberg et al. 2017). The microdialysis probe can also change DA activity in brain tissue (Nesbitt et al. 2013). Methods that measure extracellular neurotransmitter levels are well suited for measuring a specific change (neurotransmitter, brain region) in response to a behavior, but are not suitable for measuring total tissue change across the entire brain due to chronic drug exposure.

One goal of the present study was to examine the potential chronology of synthetic cathinone abuse in order to investigate if stimulant-induced neurochemical changes occur simultaneously or in stages (cf. Koob and Le Moal 2005; Koob and Le Moal 2008; Koob and Volkow 2010). Some drug effects in the present study were attributable to the drug alone; however, the majority of drug effects were dependent on duration of exposure (Table 2). Seven days of exposure to α -PVP decreased NE in thalamus, and GLU in hippocampus; effects that were not observed following 21 days of self-administration. In contrast, longer α -PVP self-administration increased HVA in striatum, increased GLU in amygdala, and decreased GLU in PFC. There were no effects of 4MMC on neurotransmitters that were isolated to 7 days of drug exposure. In contrast, extended 4MMC self-administration decreased 5-HT and NE in hippocampus, decreased 5-HT in PFC, and decreased GLU in thalamus. The contrasting effects of neurotransmitters across brain regions, and α -PVP's effects on GLU in particular, illustrate that duration of synthetic cathinone exposure impacts the same neurotransmitter differently across brain regions.

Some neurotransmitter changes in specific brain regions in the present study align with past theories of the trajectory of neurochemical changes. Effects of α -PVP on DOPAC in striatum, observed in both AO and SA groups, align with the binge/intoxication stage of abuse. Additionally, effects of α -PVP on GLU in hippocampus and PFC, observed in the AO and SA groups, respectively, align with the preoccupation/anticipation stage of abuse (Koob and Volkow 2010). While it appears that synthetic cathinones altered neurotransmitter levels, it is also possible that changes in neurotransmitter levels altered the reinforcing effects of synthetic cathinones. Drug reward and reinforcement are associated with changes in DA (Koob and Le Moal 2008), which were generally lacking in the present study. In contrast, drug withdrawal, which could impart negative reinforcement properties on synthetic cathinones and thereby maintain self-administration, is associated with decreased DA and 5-HT, and increased NE in nucleus accumbens, amygdala, and hypothalamus (Koob and Le Moal 2008; Koob and Volkow 2010). Exposure to 4MMC decreased 5-HT in amygdala,

suggesting that rats in 4MMC groups may have been in the withdrawal/negative affect stage of abuse. Thus, noncontingent delivery of α -PVP during autoshaping and α -PVP self-administration produced neurochemical changes indicative of the binge/intoxication and preoccupation/anticipation stages of abuse. In contrast, 4MMC self-administration produced changes indicative of the withdrawal/negative affect stage (Koob and Volkow 2010). While the other drug effects on neurotransmitter and brain region pairings observed in the present study do not completely align with previous theories, the changes seen here nevertheless suggest that synthetic cathinone use and abuse likely produces some sequential neurochemical changes.

5.0 Conclusion

In summary, rats acquired self-administration of α -PVP and 4MMC. While these synthetic cathinones produced regional alterations in neurotransmitter systems, longer durations of exposure also elevated neurotransmitter levels, particularly in hypothalamus, PFC, and hippocampus, suggesting that learning may produce changes in addiction-related brain regions. The mechanism of action of synthetic cathinones also appears to play a role in neurotransmitter profiles in the brain, as evidenced by the differential effects on GLU, NE, and DA metabolites. Interestingly, neither α -PVP nor 4MMC altered DA levels when measured one day after the last drug exposure, indicating that the dopaminergic effects of these synthetic cathinones is short lived. Finally, the changes in neurotransmitter levels observed here suggest that synthetic cathinone use likely produces differential neurochemical changes during the transition from use to abuse. Consequently, treatment need may differ depending on the stage of synthetic cathinone abuse.

Acknowledgements

The authors have no conflicts of interest. The authors thank Daniel Barrus, Ricardo Cortes, Tony Landavazo, Timothy Lefever, Nikita Pulley, Shanequa Taylor, Scott Watson, and Jenny Wiley for technical assistance. Research was generously supported by National Institute of Health grants DA039315 and DA012970. The funding source had no other role other than financial support.

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Figure 1.

Mean responses on the active (filled symbols) and inactive (open symbols) levers as a function of session for rats self-administering α -PVP (top panel), 4MMC (bottom panel), or saline (both panels) during autoshaping. * indicates a significant difference from saline for active lever presses, and \$ indicates a significant difference between active and inactive lever presses within drug group (drug $\times$ lever interactions). $n=15/\text{group}$.

Figure 2.

Mean responses on the active (filled symbols) and inactive (open symbols) levers as a function of session for rats self-administering α -PVP (top panel), 4MMC (bottom panel), or saline (both panels) on an FR1 schedule of reinforcement. * indicates a significant difference from saline for active lever presses, and \$ indicates a significant difference between active and inactive lever presses within drug group (drug $\times$ lever interactions). # indicates a significant difference from session 1 for 4MMC (drug $\times$ session interaction). $n=7/\text{group}$.

Figure 3.

Mean neurotransmitter and metabolite concentrations as a function of brain region. Note different y axis scale for hypothalamus and striatum. * indicates a significant difference from saline for the same duration (main effect or interaction), and \$ indicates a significant difference from AO for the same drug (main effect or interaction). Sal = saline; n=7–8/group.

Table 1.

Significant main effects of duration of synthetic cathinone exposure on neurotransmitter concentrations ($p < 0.05$). Degrees of freedom are shown in parenthesis. Arrows indicate the direction of effects of duration compared to the AO group.

Brain Region	5-HT	5-HIAA	DA	DOPAC	NE	GLU
α-PVP						
Amygdala						$\uparrow F(1,26)=5.40$
Hippocampus		$\uparrow F(1,26)=7.29$			$\uparrow F(1,25)=7.54$	
Hypothalamus		$\uparrow F(1,24)=5.50$		$\uparrow F(1,26)=10.75$		
PFC	$\uparrow F(1,26)=8.03$	$\uparrow F(1,26)=12.87$				$\downarrow F(1,26)=25.51$
Striatum	$\uparrow F(1,26)=5.08$	$\uparrow F(1,26)=4.03$				
Thalamus		$\uparrow F(1,25)=7.88$				
4MMC						
Hippocampus		$\uparrow F(1,26)=5.70$				
Hypothalamus		$\uparrow F(1,26)=5.34$	$\uparrow F(1,26)=5.32$	$\uparrow F(1,26)=10.95$		
PFC						$\downarrow F(1,26)=8.34$
Thalamus					$\downarrow F(1,25)=7.58$	

Table 2.

Significant main effects of drug, and significant duration by drug interactions on neurotransmitter concentrations ($p < 0.05$). Degrees of freedom are shown in parenthesis. Arrows indicate the direction of drug effects compared to the saline group. Cells containing duration names (AO or SA) indicate interactions that were only significant for one duration. Cells without duration names indicate main effects.

Brain Region	5-HT	5-HIAA	DA	DOPAC	HVA	NE	GLU
α-PVP							
Amygdala							↑ SA $F(1,26)=13.73$
Hippocampus						↓ $F(1,25)=6.55$	↓ AO $F(1,26)=6.76$
PFC							↓ $F(1,26)=11.33$
							↓ SA $F(1,26)=5.35$
Striatum				↑ $F(1,26)=9.48$	↑ $F(1,26)=4.69$		
					↑ SA $F(1,26)=4.45$		
Thalamus						↓ AO $F(1,25)=11.17$	
4MMC							
Amygdala	↓ $F(1,26)=5.72$						
Hippocampus	↓ $F(1,26)=10.25$	↓ $F(1,26)=4.59$				↓ $F(1,26)=5.05$	
	↓ SA $F(1,26)=6.24$					↓ SA $F(1,26)=4.31$	
PFC	↓ SA $F(1,26)=20.73$						
Thalamus							↓ $F(1,26)=4.98$
							↓ SA $F(1,26)=4.98$