Methamphetamine and Novel “Legal High” Methamphetamine Mimetics:

Abuse liability, Toxicity, and Potential Pharmacobehavioral Treatments

by

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ABSTRACT

Globally, addiction to stimulants such as methamphetamine (METH) remains a significant public health problem. Despite decades of research, no approved anti-relapse medications for METH or any illicit stimulant exist, and current treatment approaches suffer from high relapse rates. Recently, synthetic cathinones have also emerged as popular abused stimulants, leading to numerous incidences of toxicity and death. However, contrary to traditional illicit stimulants, very little is known about their addiction potential. Given the high relapse rates and lack of approved medications for METH addiction, chapters 2 and 3 of this dissertation assessed three different glutamate receptor ligands as potential anti-relapse medications following METH intravenous self-administration (IVSA) in rats. In chapters 4 through 7, using both IVSA and intracranial self-stimulation (ICSS) procedures, experiments assessed abuse liability of the popular synthetic cathinones 3,4-Methylenedioxypyrovalerone (MDPV) , methylone, α-pyrrolidinovalerophenone (α-PVP) and 4-methylethylcathinone (4-MEC). Results from these seminal studies suggest that these drugs possess similar abuse potential to traditional illicit stimulants such as METH, cocaine, and 3,4-methylenedioxymethamphetamine (MDMA). Finally, studies outlined in chapter 8 assessed the potential neurotoxic or adverse cognitive effects of METH and MDPV following IVSA procedures for the purpose of identifying potential novel pharmacotherapeutic targets. However, results of these final studies did not reveal neurotoxic or adverse cognitive effects when using similar IVSA procedural parameters that were sufficient for establishing addiction potential, suggesting that these parameters do not allow for sufficient drug intake to produce similar neurotoxicity or cognitive

deficits reported in humans. Thus, these models may be inadequate for fully modeling the adverse neural and psychological consequences of stimulant addiction. Together, these studies support the notion for continued research into the abuse liability and toxicity of METH and synthetic cathinones and suggest that refinements to traditional IVSA models are needed for both more effective assessment of potential cognitive and neural deficits induced by these drugs and screening of potentially clinically efficacious pharmacotherapeutics.

DEDICATION

To my wife Liz, for her unconditional love, support, and seemingly inexhaustible patience in putting up with me through these last 13 years, especially during the stressful graduate school years. And to my parents, Rick and Judy, who always encouraged me to do whatever it was that made me happy, even after that meant moving across the country, not having a real job, and not giving them grandchildren as soon as they had hoped.

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TABLE OF CONTENTS

Page

LIST OF TABLES xiv

LIST OF FIGURES xv

CHAPTER

1 GENERAL INTRODUCTION 1

The Costs of Drug Addiction 1

Methamphetamine 2

Synthetic Cathinones 3

2 ATTENUATION OF REINSTATEMENT OF METHAMPHETAMINE-, SUCROSE-, AND FOOD-SEEKING BEHAVIOR IN RATS BY FENOBAM, A METABOTROPIC GLUTAMATE RECEPTOR 5 NEGATIVE ALLOSTERIC MODULATOR 9

Method 12

Subjects 12

Surgical Procedures 13

Methamphetamine, Sucrose, and Food Self-Administration 14

Extinction Procedures 15

Reinstatement Procedures 15

Locomotor Procedures 16

Drugs 16

Statistical Analyses 17

Results 18

CHAPTER Page

Discussion 20

3 ARE AMPA RECEPTOR POSITIVE ALLOSTERIC MODULATORS POTENTIAL PHARMACOTHERAPEUTICS FOR ADDICTION? 25

"Bottom-up": Subcortical Neuroplasticity Drives the Development of Habitual Drug-seeking Behavior 25

"Top-down": Repeated Drug-induced Insults to Prefrontal Dortices Impairs Executive Functioning 27

Glutamatergic Mechanisms in Memory Formation: A Brief Overview 28

Extinction / Exposure Strategies: Rescuing Behavioral Regulation 31

Facilitating ILC-NAcs Glutamate Signaling.....34

AMPA PAMs and Addiction: Preclinical Studies 35

Discussion 40

Conclusions 41

4 POTENT REWARDING AND REINFORCING EFFECTS OF THE SYNTHETIC CATHINONE 3,4-METHYLENEDIOXYPYROVALERONE (MDPV). 42

Method.....44

Subjects 44

Drugs and Assessment of Purity.....45

Experiment 1: Intravenous Self-administration (IVSA) Procedures.....46

Surgical Procedures 46

Apparatus 46

Experimental Design: IVSA Procedures.....47

CHAPTER Page

Experiment 2: ICSS Procedures 48

Surgical Procedures 48

Apparatus 49

Experimental Design: ICSS Procedures.....49

Statistical Analyses 51

Results 53

Experiment 1: Self-Administration of MDPV in 2 hr/day Sessions 53

Progressive Ratio Responding.....54

Self-Administration of MDPV during ShA vs. LgA 54

Experiment 2: Effects of MDPV on Thresholds for ICSS 56

Discussion 56

5 THE REINFORCING AND REWARDING EFFECTS OF METHYLONE, A SYNTHETIC CATHINONE COMMONLY FOUND IN “BATH SALTS.” 62

Method.....66

Subjects 66

Drugs 66

Experiment 1: IVSA Procedures 66

Surgical Procedures 66

Apparatus 67

Experimental Design: IVSA Procedures 68

Experiment 2: ICSS Procedures 69

CHAPTER Page

Surgical Procedures 69

Apparatus 70

Experimental Design: ICSS Procedures.....70

Statistical Analysis.....70

Results 72

Lever Discrimination During ShA 72

Infusions During ShA 73

Progressive Ratio Tests.....74

Assessment of Escalated Intake During LgA.....74

Assessment of ICSS Thresholds.....75

Evidence of Toxicity.....75

Discussion 75

6 EFFECTS OF α-PYRROLIDINOVALEROPHENONE (α-PVP) AND 4-METHYL-N-ETHYLCATHINONE (4-MEC), TWO SYNTHETIC CATHINONES COMMONLY FOUND IN SECOND-GENERATION "BATH SALTS", ON ICSS THRESHOLDS IN RATS. 84

Method.....88

Subjects 88

Drugs 89

ICSS Surgical Procedures.....89

ICSS Apparatus.....90

CHAPTER Page

ICSS Testing Procedures 90

Statistical Analyses 93

Results 94

Discussion 95

7 SENSITIZATION TO THE LOCOMOTOR STIMULANT EFFECTS OF 3,4-METHYLENEDIOXYPYROVALERONE (MDPV) AND CROSS-SENSITIZATION TO METHAMPHETAMINE IN RATS 102

Method.....104

Subjects 104

Drugs 104

Locomotor Testing Procedures.....105

Experiments 1a and 1b − MDPV-induced Locomotor Sensitization with 24 hour Inter-test Intervals.....105

Experiment 2 - MDPV-induced Locomotor Sensitization with 48 hour Inter-treatment Intervals.....106

Experiment 3 - Cross-sensitization of METH-induced Locomotor Sensitization to MDPV 106

Experiment 4 - Cross-sensitization of MDPV-induced Locomotor Sensitization to METH 107

Statistical Analyses 107

Results 108

Acclimation and Repeated Treatment Sessions 108

CHAPTER Page

Experiment 1a 108

Experiment 1b 109

Experiment 2 109

Experiment 3 110

Experiment 4 111

Sensitization Tests.....111

Experiments 1a and 1b.....111

Experiment 2 112

Experiment 3 112

Experiment 4 112

Discussion 113

8 ASSESSMENT OF NEUROTOXIC AND COGNITIVE EFFECTS FOLLOWING CHRONIC INTRAVENOUS MDPV AND METH SELF-ADMINISTRATION 119

Method.....123

Subjects 123

Sucrose, drugs and Assessment of Purity.....124

Surgical Procedures 125

Experiment 1: IVSA Apparatus.....126

Experiment 1: Self-administration Procedures 127

Tissue Processing.....128

CHAPTER Page

Astrogliosis Assessment: Glial Fibrillary Acid Protein (GFAP) Immunohistochemical Staining Procedures 128

Neurodegeneration Assessment: Fluoro-jade C Staining Procedures 129

Image Analysis 130

Experiment 2: Cognitive Task Training 130

Experiment 2: DMTP Apparatus 131

Experiment 2: DMTP Training 131

Experiment 2: DMTP Probe Tests 132

Experiment 2: S+/S- Training and Reversal Apparatus 133

Experiment 2: S+/S- Training 133

Experiment 2: S+/S- Reversal Test 134

Experiment 2: Self-administration Apparatus 134

Data and Statistical Analysis 136

Results 139

Experiment 1: Self-administration 139

Experiment 1: GFAP Total Counts and Percent Total Area 140

Experiment 1: Fluoro-jade C Staining 140

Experiment 2: Self-administration 141

Experiment 2: DMTP Probe Tests 142

Experiment 2: S+/S- Reversal Tests 143

Bodyweights 144

Discussion 144

CHAPTER Page

9 GENERAL DISCUSSION 160

REFERENCES..... 168

APPENDIX

A SECURED PERMISSION TO INCLUDE PUBLISHED RESEARCH 265

B CITATIONS OF PUBLISHED ARTICLES ADAPTED AND/OR USED IN THEIR ENTIRITY IN THIS DISSERTATION 267

C CIRRICULUM VITAE 270

LIST OF TABLES

Table Page

1. Inactive Lever Presses Per 2 hr session During Extinction and Reinstatement Procedures 202

2. Slopes, ED50 Values, and Maximum ICSS Threshold Reductions. 203

LIST OF FIGURES

Figure Page

1. Effects of Fenobam on the Reinstatement of METH-Seeking By Acute METH Administration or METH-Associated Cues 204
2. Effects of Fenobam on the Reinstatement of a Sucrose- and Food-Seeking Induced By Associated Cues 206
3. Effects of Vehicle or Fenobam (10 mg/kg) on Locomotor Behavior.....208
4. Effects of AMPA PAMs on Extinction.....210
5. Effects of AMPA PAMs on Reinstatement. 213
6. MDPV IVSA in ShA 215
7. MDPV IVSA During Progressive Ratio Responding.....217
8. MDPV IVSA During LgA.....219
9. MDPV ICSS.....221
10. Methylone IVSA During ShA. 223
11. Infusions of Methylone Across ShA. 225
12. Infusions During PR and LgA Methylone IVSA.....227
13. Methylone ICSS 229
14. Chemical Structures of Psychostimulants 231
15. α -PVP, 4-MEC, and METH ICSS.....233
16. 24 hr MDPV Sensitization.....235
17. 48 hr MDPV Sensitization.....237
18. 48 hr METH-MDPV Cross-sensitization.....239
19. 48 hr MDPV-METH Cross-sensitization.....241

Figure Page

20. Self-administration of MDPV, METH, and Sucrose for Experiment 1 243

21. Reinforcers Obtained During LgA IVSA Procedures in Experiment 1 245

22. GFAP Staining for CA1 Region. 247

23. GFAP Staining for CA3 Region. 249

24. GFAP Staining for mPFC. 251

25. Results of Fluoro-Jade C Neurodegeneration Staining.....253

26. Self-administration of MDPV, METH, and Sucrose for Experiment 2 255

27. Results from DMTP Probe Tests.....257

28. Total Forfeited and Completed DMTP Probe Trials 259

29. Performance on S+/S- Reversal Tests 261

30. Body Weights. 263

CHAPTER 1

GENERAL INTRODUCTION

The Costs of Drug Addiction

Drug addiction is a chronically relapsing disorder principally characterized by the uncontrollable drive to obtain and failure to limit the use of drugs despite adverse, often severe, consequences. In addition, there is a concomitant loss of interest in engagement in other activities such as work, school, dependent care, and social gatherings, to name a few (Kalivas, Volkow, & Seamans, 2005; Koob, Sanna, & Bloom, 1998). Addiction casts a wide net of consequences, adversely affecting not just the health and well-being of individual users, but devastating families, straining healthcare resources, significantly damaging environments, and imparting a significant economic and medical burden to society as a whole (Ericson, 2001). Globally, the use of addictive drugs is responsible for nearly 10% of the total disease burden (Harwood & Bouchery, 2004). When factoring in the influence of treating drug-related health problems, crime, loss of productivity due to disability and withdrawal from the workforce, and premature death, the economic burden of drug addiction to the US is estimated to exceed half a trillion dollars annually (Substance Abuse and Mental Health Services Administration, 2011). In 2011, 21.6 million persons in the U.S. aged 12 or older met criteria for substance dependence or abuse. However, only 2.3 million received treatment, with the other 19.3 million declining help largely because of the costs and inconvenience of treatment (Substance Abuse and Mental Health Services Administration, 2011). Currently, the prevailing treatment approaches consist of traditional cognitive-behavioral therapies, self-help and social support programs, adjunctive treatment with approved medications (when

applicable), and/or some combination thereof. However, only a small number of approved addiction pharmacotherapeutics exist, and only for nicotine, opioid, and alcohol addiction (Sofuoglu, DeVito, Waters, & Carroll, 2013). For addiction to psychostimulants such as cocaine, methamphetamine, and newer “legal high” designer stimulants, there are currently no approved medications (Sofuoglu, 2010). To make matters worse, even with the best treatment interventions, most individuals (up to 90% for some drugs) will relapse within 12 months of discontinuing drug use (Brandon, Vidrine, & Litvin, 2007; Hendershot, Witkiewitz, George, & Marlatt, 2011). Given these severe consequences to users, families and society, the large number of individuals declining much needed treatment, and high rates of relapse even with treatment, there is a significant demand for more effective treatment strategies designed to limit abuse and decrease the probability of relapse. Thus, there is a tremendous need for research aimed at finding new pharmacotherapeutic targets, developing more effective anti-relapse medications, employing more effective behavioral treatment strategies, or some combination of these approaches.

Methamphetamine

Methamphetamine (METH) is a highly addictive psychostimulant with potent effects on the central nervous system (Shrem & Halkitis, 2008) that can lead to severe adverse neurological and physical effects (Darke, Kaye, McKetin, & Duflou, 2008; Scott et al., 2007). According to the most recent World Drug Report published by the United Nations Office on Drugs and Crime, the use of amphetamine-type stimulants (ATS), which includes amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and METH, has reached epidemic levels globally and continues to increase in most regions of

the world (United Nations, 2013). METH accounts for both the majority (71%) of all ATS use, and is primarily responsible for the continued global increase in use, since general amphetamine use has held steady and the global use of MDMA (i.e. “ecstasy”) is in decline (United Nations, 2013). Furthermore, global increases are primarily driven by crystalline METH (i.e. crystal METH). In the United States, this increased use of METH has translated into an increase in individuals seeking treatment for primary METH addiction (Maxwell & Brecht, 2011). However, even with treatment, approximately 70-90% of METH users will relapse within three years (McKetin et al., 2012). In addition to the adverse physical and psychological effects on individual users, METH use also imparts a significant economic burden to societies. When factoring in the costs of treatments, excessive health care utilization costs, lost productivity, crime, child endangerment, and consequences of METH manufacture, the annual cost to the U.S. is estimated to be around 23.5 billion dollars (Dobkin & Nicosia, 2009; Nicosia, 2009). Given the severe consequences of METH use to both society and individual users, the continued increase in global use, the high rates of relapse even with treatment, and lack of any approved anti-relapse medications, there is a tremendous need for basic research focused on developing more effective pharmacotherapeutics for the treatment of METH (and other psychostimulant) addiction as well as delineating the neurotoxic and adverse cognitive effects of meth use in order to find new therapeutic targets.

Synthetic Cathinones

In recent years, there has been an unprecedented rise in the availability of new psychoactive substances (NPS) on international drug markets. NPS is a catch-all term that describes new substances of abuse that are unregulated yet mimic the effects of other

controlled substances (United Nations, 2013). In 2013, 251 NPS were identified on international drug markets, exceeding the number (234) of substances under international control for the first time in history (United Nations, 2013). While NPS encompass a variety of drug classes, novel designer stimulants known as synthetic cathinones (comprising ~23% of all NPS) have emerged as arguably the most problematic in terms of adverse effects to users as evidenced by a now large and growing body of case reports highlighting bizarre and violent behaviors, toxicity, and death (Prosser & Nelson, 2012; Spiller, Ryan, Weston, & Jansen, 2011).

The use of synthetic cathinones has escalated dramatically in the western world since first appearing in Europe in the mid-2000's, and subsequently in the United States in 2009 (Rosenbaum, Carreiro, & Babu, 2012; Spiller et al., 2011); Concomitant with this escalated use has been an unprecedented increase in the variety of these substances now available in drug markets (Rosenbaum et al., 2012). Experts predict that many more designer stimulants (i.e. multiple generations) are likely to emerge as replacements once popular (i.e. older generation) analogues are banned by regulatory agencies, a pattern that has already been reported (Baumann, Partilla, & Lehner, 2013; Brandt, Sumnall, Measham, & Cole, 2010; D. E. A. United States Department of Justice, 2013b; Baumann et al., 2013; Brandt et al., 2010; D. E. A. United States Department of Justice, 2013b). Of the many synthetic cathinones reported in drug markets, methylenedioxypyrovalerone (MDPV), mephedrone, and methylone, initially comprised the majority (98%) of all synthetic cathinones encountered by law enforcement agencies (D. E. A. United States Department of Justice, 2011c). Use of these drugs has led to numerous published reports of bizarre and violent behavior, multi-organ toxicity, and death (Rosenbaum et al., 2012;

Spiller et al., 2011), forcing regulatory agencies such as the United States Drug Enforcement Administration (DEA) to institute emergency bans citing serious hazards to public health and safety (D. E. A. United States Department of Justice, 2011b). Despite the increasing popularity of these drugs, very little scientific data exists regarding their abuse liability and toxicity from long-term use, and even scarcer information exists for clinicians regarding effective treatments for synthetic cathinone abuse. Given these facts, the scientific study of designer stimulant abuse/addiction liability is of the utmost importance for (a) providing targets for pharmaceutical development (b) evidence-based information to healthcare experts charged with treating abusers of these drugs, (c) guiding government agencies responsible for regulating these substances, and (d) informing the public about the potential risks of abuse of and dependence on synthetic cathinones.

Given the significant negative impacts that METH and synthetic cathinone use have on both individual users and society, incomplete characterization of their neurotoxic and adverse cognitive effects, and the general lack of available treatments, the studies in this dissertation have collectively focused on: (1) the assessment of novel glutamatergic agents as potential anti-relapse medications for METH addiction; (2) assessing the abuse liability of newer synthetic cathinones, and (3) determining the potential neurotoxic and adverse cognitive effects following chronic, voluntary intravenous self-administration of these psychostimulants.

In chapter 2, we present experimental data revealing that the metabotropic glutamate receptor 5 (mGluR5) negative allosteric modulator (NAM) fenobam decreases relapse-like behavior to both METH-prime and METH-associated cue-prime

reinstatement procedures. Prior to starting these experiment, research had shown that similar mGlur5 NAMs were effective at decreasing METH-seeking across various animal models of addiction. In contrast to the other mGluR5 NAMs, fenobam had several advantageous, the most important of which was a favorable safety profile in humans. However, as shown in our results, fenobam displayed non-specific effects on both sucrose- and food-seeking behavior during cue-primed reinstatement procedures, prompting us to discontinue studies assessing fenobam as a potential pharmacotherapeutic treatment.

In chapter 3, we review the published literature detailing efficacy of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor positive allosteric modulators (PAMs) as extinction / cue-exposure therapy adjunctive pharmacotherapeutics, a class of drugs which had shown positive effects across a wide range of neuropsychiatric disorders. Next, we present data showing that the two novel AMPA PAMs CX1837 and CX1739 both facilitate extinction following METH self-administration procedures, but ultimately failed to decrease relapse-like behavior in METH cue-primed reinstatement procedures. As in experiment 2, as a result of disappointing effects in reinstatement procedures, subsequent extinction studies using AMPA PAMs were discontinued in our laboratory.

In chapters 4 and 5, we present the first published studies detailing reinforcing and rewarding effects of MDPV and methylone, two of the most popular synthetic cathinones to emerge as “legal high” psychostimulants on international drug markets. Specifically, in chapter 4, potent MDPV reinforcing and rewarding effects in rats are shown as robust intravenous self-administration (IVSA) and decreases in intracranial

self-stimulation thresholds, suggesting a high addiction potential similar to illicit stimulants such as METH. In chapter 5, using almost identical procedures, we also reveal reinforcing and rewarding effects of methylone. However, in contrast to MDPV, lack of robust escalation in long access (LgA) IVSA and lack of significant ICSS threshold decreases suggest that methylone does not possess the compulsive abuse potential of MDPV or other illicit psychostimulants (METH or cocaine) with known high abuse liability.

In chapter 6, we shown that α-pyrrolidinovalerophenone (α-PVP) and 4-methyl-n-ethylcathinone (4-MEC), two synthetic cathinones that largely replaced MDPV and methylone on international markets following government bans, also possess potent rewarding effects as revealed by significant threshold decreases in ICSS procedures nearly identical to those used in chapters 4 and 5. Results from these experiments suggest that newer second-generation synthetic cathinone analogues likely have similar addiction potential as their first generation counterparts.

In chapter 7, we conducted several MDPV behavioral sensitization and cross-sensitization experiments (MDPV + METH) motivated by evidence suggesting that (1) MDPV abuse is still prevalent despite governmental bans, (2) that MDPV users are typically previous users of illicit amphetamines, and (3) that previous amphetamine use appears to increase the potential adverse effects of MDPV suggesting similar neurochemical effects. Results from these experiments revealed that behavioral responsivity, as shown by increased locomotor behavior, is increased in rats following repeated exposure to MDPV when compared to rats repeatedly treated with saline vehicle. Interestingly, repeated exposure to METH did not increase behavioral

responsivity to a subsequent low-dose MDPV challenge, suggesting that sensitization between MDPV and METH is not entirely bi-directional. Together, results from chapter 7 corroborate human studies suggesting that exposure to both MDPV and METH may increase the potential for addiction or adverse neuropsychopathology following abuse of these drugs.

In chapter 8, as a result of our robust MDPV IVSA results in chapter 4 and cross-sensitization results in chapter 7, we conducted additional MDPV IVSA experiments using similar temporal parameters (2 hr short access (ShA) + 6 hr long access (LgA)) in chapter 4 to assess whether voluntary administration would lead to neurotoxic or adverse cognitive effects seen in other studies with illicit psychostimulants such as METH and cocaine. While these IVSA experimental procedures in chapter 4 revealed potent reinforcing effects suggestive of high addiction potential in humans, they did no produce evidence of neurotoxicity or adverse cognitive effects. When the IVSA experiments from chapter 8 and those published by others are interpreted together, psychostimulant IVSA-induced neurotoxic and adverse cognitive effects appear only in IVSA procedures employing both longer periods of voluntary exposure and protracted withdrawal. Taken together, results from chapter 4 and 8 together suggest that while traditional IVSA procedures are sufficient for establishing abuse liability, they do not appear sufficient for producing a significant level of exposure to produce adverse effects. Thus, as the IVSA procedures employed in this dissertation do not appear to fully capitulate adverse effects reported in human abusers, it is recommended that future research employ IVSA experiments with sufficiently long exposure periods combined with protracted withdrawal.

CHAPTER 2

ATTENUATION OF REINSTATEMENT OF METHAMPHETAMINE-, SUCROSE-, AND FOOD-SEEKING BEHAVIOR IN RATS BY FENOBAM, A METABOTROPIC GLUTAMATE RECEPTOR 5 NEGATIVE ALLOSTERIC MODULATOR

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Methamphetamine (METH) is a highly addictive psychostimulant with potent effects on the central nervous system (Shrem and Halkitis 2008). METH use is highly correlated with various medical and neuropsychiatric disorders and has numerous adverse neurological and other health effects (Darke et al. 2008; Rusyniak 2011; Scott et al. 2007). In many regions of the U.S., METH use has recently reached epidemic levels, and the most recent epidemiological data suggest that METH use is again on the rise despite decreasing trends in use in the mid- to late-2000's (Anglin et al. 2000; Maxwell and Brecht 2011; Maxwell and Rutkowski 2008).

METH's reinforcing effects are generally attributed to its actions as a potent releaser of monoamines (Cruickshank and Dyer 2009; Kish 2008; Sulzer 2011). These effects are caused by a displacement of monoamines from vesicular stores by METH acting as a substrate for vesicular monoamine transporters (VMAT). This results in increased cytoplasmic monoamine levels and a subsequent reversal of plasma membrane monoamine transporter direction. Attempts at developing pharmacological treatments for METH addiction have historically focused on compounds which modulate the actions of METH on VMAT, plasma membrane monoaminergic transporters, or GABAergic functioning within mesolimbic brain circuits (Ciccarone 2011; Karila et al. 2010; Vocci

and Appel 2007). However, as of yet there are currently no medications approved by the U.S. Food and Drug Administration for METH addiction.

Various studies have shown that METH increases extracellular levels of glutamate in forebrain regions such as the striatum, hippocampus, and prefrontal cortex (Mark et al. 2007; Rocher and Gardier 2001; Shoblock et al. 2003; Stephans and Yamamoto 1995). However, in other regions of the brain such as the nucleus accumbens and ventral midbrain, it has been shown that METH can increase, have no effect, or even decrease extracellular levels of glutamate (Ito et al. 2006; Shoblock et al. 2003; Zhang et al. 2001). Thus, the effects of METH on extracellular glutamate appear to be complex, and likely dependent on brain region, dose, and frequency of administration. While most research on METH–induced changes in extracellular glutamate has focused on its role in excitotoxicity, more recent research has revealed a primary role for glutamatergic neurotransmission in mediating the rewarding and reinforcing effects of METH (Gass and Olive 2008). Thus, glutamatergic transmission may be a novel therapeutic target for the treatment of addiction to METH (Kalivas and Volkow 2011; Olive 2009; Olive et al. 2012).

Receptors for glutamate are broadly classified as ionotropic (iGluR) or metabotropic (mGluR) receptors. There are 8 mGluR receptor subtypes (mGluR1 – mGluR8) which are further subclassified into three distinct families (Group I, II, or III) based upon their pharmacology and signaling transduction mechanisms (Conn and Pin 1997; Pin and Duvoisin 1995). In a seminal study by Chiamulera and colleagues, it was shown that mice lacking the mGluR5 gene did not acquire cocaine self-administration and were unresponsive to its locomotor stimulant effects (Chiamulera et al. 2001). Since

this study, numerous investigators have shown that mGluR5 antagonists reduce intravenous drug self-administration and reinstatement of drug-seeking in animal studies, as reviewed elsewhere (Duncan and Lawrence 2012; Kenny and Markou 2004; Olive 2009). We have previously demonstrated that the selective mGluR5 NAM 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine (MTEP) attenuates intravenous METH self-administration while exerting no effects on food self-administration (Gass et al. 2009; Osborne and Olive 2008). In addition, MTEP also attenuated reinstatement of METH-seeking behavior induced by METH-associated cues or acute METH exposure, but did not alter cue-induced reinstatement of food-seeking. Taken together, these studies indicate that mGluR5 receptors play a key role in METH reinforcement and METH-seeking behaviors, and justify further investigation into mGluR5 antagonists as potential anti-addiction therapeutics.

Fenobam was first developed in the 1970's as a non-benzodiazepine anxiolytic for human use despite unknown pharmacological mechanisms of action (Itil et al. 1978; Pecknold et al. 1980; Pecknold et al. 1982). In 2005, it was revealed that fenobam is a selective mGluR5 NAM (Porter et al. 2005), renewing interest in fenobam as a potential therapeutic for the treatment of various dysfunction of the nervous system. Fenobam possesses antidepressant, analgesic, and anxiolytic effects in experimental animals (Jacob et al. 2009; Montana et al. 2009), symptoms that often accompany withdrawal from chronic METH use (Scott et al. 2007). Fenobam and several other mGluR5 NAMs have recently been tested in clinical trials for a number of medical disorders including Fragile X syndrome and L-dopa induced dyskinesias (Berry-Kravis et al. 2009; Hagerman et al. 2008; Jaeschke et al. 2008). While these clinical trials showed that fenobam was

generally well tolerated with only moderate side effects, unfortunately, clinical testing of fenobam was recently discontinued due to somewhat limited efficacy and large variability in plasma levels of the drug following oral administration. Nevertheless, since there is a great need to develop medications for the treatment of METH addiction, particularly with compounds that demonstrate safety and tolerability in human subjects, we sought to determine the effects of fenobam on the reinstatement of METH-seeking behavior. To examine the potential generalization of effects on the reinstatement of seeking of natural reinforcers, we assessed the effects of fenobam on the reinstatement of sucrose- and food-seeking behavior.

Method

Subjects

Fifty-four male Sprague-Dawley rats (Harlan Laboratories, Livermore, CA), weighing approximately 250-275 g, were individually housed upon arrival. Animals were maintained on a 12 hr light-dark cycle (lights off at 0700 hr) in a temperature and humidity controlled rodent colony. All experimentation was conducted during the dark phase of the light-dark cycle, with the exception of a 16 hr overnight operant training session for METH and sucrose self-administration groups which commenced near the end of the dark phase (at approximately 1600 hr) and continued through the light phase into the following morning (ending at approximately 0800 hr). Rats undergoing METH and sucrose self-administration procedures were given ad libitum access to food and water during all phases of the experiment except during drug self-administration and for 12 hr prior to the initial operant training session. Rats undergoing food self-

administration procedures were maintained at approximately 85% of their free-feeding bodyweight and received food in their home cage for one hour each day approximately two hours after operant testing. Rats undergoing locomotor assessment procedures received ad libitum access to food and water during all experimental phases. Two rats were eliminated from the study due to catheter patency failure. All experimental procedures were conducted with the approval of an Institutional Animal Care and Use Committee at Arizona State University and in accordance with the Principles of Laboratory Animal Care and the 8th Edition of the Guide for the Care and the Use of Laboratory Animals (National Research Council, 2011).

Surgical Procedures

Prior to arrival, rats undergoing METH self-administration procedures were prepared with intravenous catheters into the jugular vein by Harlan Laboratories Surgical Services. Upon arrival, rats were allowed one day of acclimation before vascular port implantation. Rats were anesthetized and implanted with vascular access ports (Model 313000BM15, Plastics One, Roanoke, VA, USA) as described previously (Gass et al. 2009). Following surgical procedures, rats were given 5 days of post-operative care during which they received daily intravenous infusions of 70 U/ml heparin (0.2 ml volume) to maintain catheter patency and 100 mg/ml cefazolin (0.1 ml volume) to protect against infection. Rats also received daily subcutaneous injections of 2.5 mg/ml of meloxicam (0.15 ml volume) to relieve surgery-related discomfort. During post-operative care, observation of weight loss on any day resulted in a 5 ml subcutaneous injection of saline to combat dehydration. The surgery site was also treated with topical lidocaine and triple antibiotic ointments to facilitate healing of the wound. Rats undergoing locomotor,

sucrose reinstatement, or food reinstatement procedures did not undergo catheter implantation procedures.

Methamphetamine, Sucrose, and Food Self-Administration

Self-administration, extinction, and reinstatement tests were conducted in operant self-administration chambers (ENV-008, Med Associates, St. Albans, VT, USA) as described previously (Gass et al. 2009). To initiate operant responding for METH and sucrose self-administration, rats were underwent overnight sucrose pellet training according to a fixed-ratio 1 (FR1) schedule of reinforcement as described elsewhere (Gass et al. 2009). Approximately 24 hr following the initial overnight training session, 2 hr daily self-administration sessions were initiated, whereby presses on the active lever resulted in delivery of METH (0.05 mg/kg/infusion, delivered in a volume of 0.06 ml over a 2 sec period) on a FR1 schedule of reinforcement for METH trained animals. Each active lever press was accompanied by a concurrent illumination of a stimulus light located above the active lever, and presentation of an auditory stimulus (~65 dB, 2900 Hz) for 2 sec. Animals trained to self-administer sucrose underwent the same procedures, except each active lever press resulted in delivery of a single 45-mg sucrose pellet (TestDiet, Richmond, IN, USA) according to a FR1 schedule of reinforcement. For food self-administration procedures, rats did not undergo a16 hr overnight training session and began 2 hr self-administration through spontaneous acquisition procedures and active lever presses resulted in delivery of a single 45-mg food pellet (Bio-Serv, Frenchtown, NJ) according to a FR1 schedule of reinforcement. Self-administration sessions were conducted 7 consecutive days per week. For METH self-administration procedures, each session was preceded by intravenous infusion of 0.1 ml of 70 U/ml heparin, and followed

by infusion of 0.1 ml of 70 U/ml of heparin and 0.1 ml of 100 mg/ml cefazolin. Stabilization of self-administration was considered to have been reached when the average number of active lever presses during each 2 hr session differed by less than 15% for 2 consecutive days, after a minimum of 8 days of self-administration. Self-administration (SA) data reported represent the average number of active lever presses during the final two self-administration sessions prior to extinction training.

Extinction Procedures

Extinction sessions were 2 hr in length and commenced following stabilization of self-administration. During extinction training, responding on the previously active lever no longer produced any programmed consequences, as described previously (Gass et al. 2009). Extinction sessions were conducted each day until the number of active lever presses per session was less than 25% of the average number of active lever presses during the final two days of self-administration responding, and when this level of pressing was observed for 2 days.

Reinstatement Procedures

Reinstatement test sessions were 2 hr in length and commenced on the day immediately following the last extinction session. For all groups of rats (METH-prime, METH-cue, sucrose-cue, and food-cue), fenobam or vehicle was injected i.p. 20 min prior to the reinstatement sessions. For the METH-prime group, a single METH injection (0.5 mg/kg i.p.) was given 30 minutes prior to reinstatement testing and ten minutes prior to fenobam administration. Following each reinstatement test session, animals were placed into additional 2 hr extinction sessions starting on the following day. These additional extinction sessions were carried out until extinction criteria were again met at

which point another reinstatement test was conducted on the following day. Each group of rats were subjected to either 3 or 4 reinstatement tests, and each rat received 3 or 4 of the different treatments (vehicle or 3 doses of fenobam) in a randomized counterbalanced design. After the final reinstatement test, the animals were euthanized by anesthesia with isoflurane followed by decapitation.

Locomotor Procedures

Locomotor activity was measured as rotational behavior and recorded by Rotorat version 1.2 software (Med Associates). Rats were placed into stainless steel bowls (40.6 cm diameter x 25.4 cm high; model ENV-500, Med Associates) surrounded by a clear Plexiglas wall to prevent rats from escaping the apparatus. Rats were connected to spring tether secured to the top of the apparatus by a sensitive rotational sensor which recorded activity. A zip-tie collar was placed around the neck of the rat and connected to the spring tether via a stainless steel alligator clip. Measurements taken were full (360° turns) and quarter (90°) turns, in both clockwise and counter-clockwise directions.

Prior to locomotor assessment rats were placed into 90 min daily sessions for two days for acclimation to the locomotor apparatus. Following acclimation procedures, 6 rats were randomly assigned to receive either fenobam (10 mg/kg, i.p.) or vehicle for the first locomotor session. The next day, rats that previously received fenobam were administered, and vice versa. Locomotor sessions were 90 min in length, and fenobam or vehicle injections were given 20 minutes prior to placing rats in the locomotor apparatus.

Drugs

Fenobam (1-(3-chlorophenyl)-3-(3-methyl-5-oxo-4H-imidazol-2-yl)urea) was custom synthesized by Chemir Analytical Services (Maryland Heights, MO, USA).

Fenobam was suspended in a vehicle consisting of 0.3% v/v Tween 80 via sonication. For reinstatement procedures, fenobam was injected by the intraperitoneal (i.p.) route at doses of 5, 10, or 15 mg/kg in an injection volume of 1 ml/kg. These doses were chosen based on previous reports that they do not produce significant signs of sedation or anhedonia (Cleva et al. 2012; Porter et al. 2005). Fenobam (10 mg/kg) was also injected via the i.p. route prior to locomotor assessment. (+)Methamphetamine hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in a sterile saline for intravenous infusion. For METH-primed reinstatement procedures, METH was prepared at a concentration of 0.5 mg/ml in saline and administered i.p. in a volume of 1 ml/kg.

Statistical Analyses

For all groups (METH-prime, METH-cue, sucrose-cue, and food-cue), verification that extinction training produced significant decreases in the number of active lever presses was perfomed by Student's t-tests. The effects of fenobam on reinstatement behavior was analyzed by a one-way repeated measures ANOVA, with the number of active or inactive lever presses with dose/experimental phase (extinction, vehicle, 5, 10, and 15 mg/kg) serving as the repeated measures. Holm-Sidak post hoc tests were used to determine effects of fenobam dose. Furthermore, one-way repeated measures ANOVAs were also conducted on the number of inactive lever presses as an indicator of possible motoric side effects. For locomotor assessment, a repeated measures ANOVA for full and quarter turns with treatment (fenobam 10 mg/kg vs. vehicle) was conducted. When tests of data normality failed, a Friedman ANOVA on ranks was utilized. Level of statistical significance was set to $p < 0.05$ for all tests.

Results

For all groups, extinction training produced a significant reduction in the number of active lever presses during each session (all p's <0.05). During METH-primed reinstatement (n = 12), a significant main effect of fenobam dose/experimental phase on active lever presses was observed ($F[4,32]=4.341$, $p<0.01$; see Fig. 1a). Post-hoc comparisons revealed a reinstatement following the METH-prime injection as evidenced by a significant increase in the number of active lever presses following vehicle administration vs. the average of the last 2 days of extinction ($p<0.05$). Fenobam at both the 10 and 15 mg/kg doses significantly attenuated reinstatement as compared to following vehicle treatment ($p<0.05$ and $p<0.01$, respectively). Analysis of inactive lever presses did not reveal any significant main effects of dose/experimental phase ($p>0.05$; Table 1).

During cue-induced reinstatement of METH-seeking (n = 10), a significant main effect of fenobam dose/experimental phase was observed for active lever presses ($F[4,36]=6.44$, $p<0.001$; see Fig. 1b). Post-hoc tests revealed a significant increase in active lever presses following vehicle administration as compared to extinction responding, indicating that METH-associated cues induced a reinstatement of METH-seeking behavior. Furthermore, fenobam doses of 10 and 15 mg/kg significantly attenuated cue-induced reinstatement ($p<0.01$ and $p<0.001$, respectively). For inactive lever pressing, a repeated measures one-way ANOVA failed on tests of normality, and a Friedman's repeated measures ANOVA on ranks revealed a significant main effect of fenobam dose/experimental phase on inactive lever presses ($\chi^2=26.064$, $p<0.001$). However, Dunn's method of multiple comparisons did not reveal any significant

differences in the number of inactive lever presses during extinction and any of the reinstatement tests ($p>0.05$; Table 1).

During cue-induced reinstatement of sucrose-seeking (n=12), a significant main effect of fenobam dose/experimental phase was observed for active lever presses ($F[4,59]=6.653$, $p=0.001$; see Fig. 2a). Post-hoc tests revealed a significant increase in the number of active lever presses during reinstatement tests following vehicle treatment as compared to extinction values ($p=0.005$), demonstrating reinstatement of sucrose-seeking in response to sucrose-associated cues. Post-hoc tests revealed significant decreases in active lever presses following the 5, 10 and 15 mg/kg doses of fenobam as compared with vehicle ($p<0.01$). A significant main effect of fenobam dose/experimental phase was also found on inactive lever presses ($F[4,59]=4.369$, $p=0.005$). However, post-hoc tests revealed no significant differences in the number of inactive lever presses between extinction and following vehicle treatment, or between vehicle and all doses of fenobam tested ($p>0.05$; Table 1).

During cue-induced reinstatement of food-seeking (n=12), a significant main effect of fenobam dose/experimental phase was observed for active lever presses ($F[4,59]=8.589$, $p=0.001$; see Fig. 2b). Post-hoc tests revealed a significant increase in the number of active lever presses during reinstatement tests following vehicle treatment as compared to extinction values ($p=0.001$), demonstrating reinstatement of food-seeking in response to food-associated cues. Post-hoc tests revealed significant decreases in active lever presses following the 5, 10 and 15 mg/kg doses of fenobam as compared with vehicle ($p<0.001$). For inactive lever presses, no significant effects were observed ($p>0.05$; Table 1).

For locomotor behavior, repeated measures ANOVA did not reveal a significant main effect fenobam dose for full or quarter turns (p's >0.05, Fig. 3).

Discussion

The current study demonstrated that fenobam, an mGluR5 NAM that has been tested in human subjects for treatment of other medical conditions, effectively reduced METH-seeking behavior elicited by either METH-paired cues or a METH priming injection. Specifically, fenobam significantly attenuated reinstatement of METH-seeking behavior elicited by both METH-associated cues and by a METH priming injection at doses of 10 and 15 mg/kg. However, an attenuation of cue-induced reinstatement of sucrose- and food-seeking behavior was also observed, and at each dose tested (5, 10, and 15 mg/kg). These results indicate that fenobam, like previously tested mGluR5 NAMs such as 3-((2-methyl-4-thiazolyl)ethynyl)pyridine (MTEP), attenuates METH-seeking in the reinstatement paradigm (Gass et al. 2009). However, the reduction in cue-induced reinstatement of both sucrose- and food-seeking indicates that fenobam also affects seeking of natural reinforcers.

The demonstration that fenobam attenuates METH-seeking under reinstatement conditions extends previous research showing that either genetic or pharmacological blockade of mGluR5 receptors leads to reductions in drug reward, reinforcement, and relapse-like behavior (Duncan and Lawrence 2012; Olive 2009). While the exact mechanisms by which fenobam and other mGluR5 NAMs reduce drug-seeking or relapse-like behavior are not completely understood, a likely mechanism is by decreasing glutamatergic transmission in the nucleus accumbens (NAcc) and/or ventral tegmental

area (VTA). Various studies have revealed that glutamatergic transmision in the NAcc mediates reinstatement of drug-seeking for numerous addictive drugs, including amphetamines (Cornish and Kalivas 2000; Di Ciano et al. 2001; Gass and Olive 2008; Knackstedt and Kalivas 2009; Lalumiere and Kalivas 2008). Furthermore, while drug-, cue-, and stress-primed reinstatement of drug-seeking initially engage distinct neural circuits, these circuits converge onto the regions of the prefrontal cortex which in turn send glutamatergic projections to the NAcc. This prefrontal-NAcc connection has been hypothesized as the final common pathway mediating reinstatement of drug-seeking (Kalivas and McFarland 2003; Kalivas et al. 2005).

With regards to mGluR5 receptors, these receptor proteins are widely distributed in many regions of the brain, with the NAcc and VTA showing moderate to high levels of mGluR5 receptor expression (Mitrano and Smith 2007; Romano et al. 1995; Shigemoto et al. 1993). Bilateral microinfusions of the mGluR5 NAM 2-methyl-6-(phenylethynyl)pyridine (MPEP) or MTEP into the NAcc attenuates the reinstatement of cocaine-seeking elicited by drug priming and drug-associated cues (Backstrom and Hyytia 2007; Kumaresan et al. 2009) as well as cue-elicited alcohol-seeking behavior (Sinclair et al. 2012). Thus, it is likely that systemic administration of fenobam exerts its effects on the reinstatement of METH-seeking by modulating the glutamatergic transmission within these regions. Further studies are needed to confirm this, as well as the role of these regions in mediating fenobam-induced suppression of cue-induced reinstatement of sucrose- and food-seeking behavior.

Another possible mechanism through which fenobam may attenuate METH-, sucrose-, and food-seeking is via effects on brain reward function. Fenobam, along with

the prototypic mGluR5 NAMs MPEP and MTEP, have been shown to decrease brain reward functioning as measured by intracranial self-stimulation (ICSS) (Cleva et al. 2012; Harrison et al. 2002; Kenny et al. 2005; Kenny et al. 2003). Specifically, doses of MPEP (1-9 mg/kg) which significantly decrease drug self-administration, also elevates ICSS thresholds. However, others have found that MPEP does not alter ICSS thresholds, nor does MPEP decrease amphetamine-induced potentiation of brain stimulation reward (Gormley and Rompre 2011). A 3 mg/kg dose of the more selective mGluR5 NAM MTEP has been shown to decrease cue- and drug-primed reinstatement of METH-seeking (Gass et al. 2009) and also elevate ICSS thresholds (Cleva et al. 2012). However, only a high dose of fenobam (30 mg/kg, twice the highest dose tested in the current study) significantly elevated ICSS thresholds, whereas a 10 mg/kg dose (which attenuated cue-induced METH-seeking in the current study) did not significantly increase ICSS thresholds (Cleva et al. 2012). Thus, the inhibitory effects of fenobam to reduce METH-, sucrose-, and food-seeking are not likely explained by an anhedonic state produced by these compounds. In addition, the lack of effects of fenobam on inactive lever presses during reinstatement or locomotor activity, as demonstrated in the present study, suggest that motor impairing effects of fenobam did not likely contribute to its observed effects on reinstatement. However, the effects of fenobam on ICSS thresholds (Cleva et al. 2012) and locomotor activity have only been examined thus far in drug-naïve animals, and fenobam may have differential effects on brain reward function in animals with a history of drug self-administration. This possibility warrants further investigation.

While the 10 and 15 mg/kg doses of fenobam significantly attenuated the reinstatement, all doses of fenobam tested attenuated sucrose- and food-seeking. Although mice lacking mGluR5 receptors do not show an attenuation of food self-administration (Chiamulera et al. 2001), recently it has been shown that mGluR5-deficient mice do show an attenuation of food-seeking under reinstatement conditions relative to wild-type controls (Eiler et al. 2011). This finding is not without precedent, as mGluR5 receptors have been implicated in playing a central role in regulating appetite (Bradbury et al. 2005) and pharmacological blockade of mGluR5 receptors has been shown to decrease responding for food (Paterson and Markou 2005). Although we have previously shown that the selective mGluR5 NAM MTEP does not affect cue-induced reinstatement of food-seeking (Gass et al. 2009), non-specific effects of fenobam may account for these observations. For example, when compared to MPEP and MTEP, fenobam has been observed to exert more non-specific behavioral disruptions in animal models of anxiety, possibly due to yet to be identified active metabolites of fenobam (Porter et al. 2005). In addition, Jacob and colleagues (Jacob et al. 2009) revealed fenobam-induced learning impairments in both the Morris water maze and contextual fear learning paradigms at a dose as low at 10 mg/kg. Furthermore, it was previously shown that in humans, high doses of fenobam exerted some psychostimulant and psychotomimetic effects in a subset of individuals (Pecknold et al. 1982). Other side effects of fenobam that have been reported in humans include dizziness, nausea and sedation (Berry-Kravis et al. 2009). It is therefore possible that such effects may have led to the observed reductions in sucrose-, food-, and METH-seeking behavior produced by fenobam in the current study.

In summary, we observed that fenobam attenuates the reinstatement of METH-seeking behavior induced by acute METH exposure as well as METH-associated cues. These findings have important implications for the potential use of fenobam or fenobam-related compounds as novel treatments for METH addiction, since studies examining the effects of pharmacological agents that are safe and relatively well-tolerated in humans on METH-seeking behavior in preclinical studies are generally lacking. However, we also observed that fenobam also suppressed cue-induced reinstatement of sucrose- and food-seeking. Therefore, fenobam may induce a suppression of general appetitive behaviors, and thus optimization of fenobam analogues (Jaeschke et al. 2007) may be warranted for further development of mGluR5 NAMs as treatments for METH addiction.

CHAPTER 3

ARE AMPA RECEPTOR POSITIVE ALLOSTERIC MODULATORS POTENTIAL PHARMACOTHERAPEUTICS FOR ADDICTION?

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"Bottom-up": Subcortical neuroplasticity drives the development of habitual drug-seeking behavior

Addiction begins with controlled, episodic use motivated primarily by the positive reinforcing and rewarding effects of the drug (Berridge & Robinson, 1998). These hedonic effects, like those of natural reinforcers (i.e., food, water, sex, etc.), are predominantly mediated by increased dopamine (DA) transmission from neurons in the ventral tegmental area (VTA) of the midbrain to the ventral striatum (nucleus accumbens, NAc) (Berridge & Robinson, 1998). This VTA → NAc pathway is generally considered to be the final common "reward" pathway for all reinforcers, both drug and otherwise (Feltenstein & See, 2008). DA transmission is also increased in other regions such as the amygdaloid complex (Amyg), ventral pallidum, hippocampus and prefrontal cortex (PFC) (Feltenstein & See, 2008), which are believed to play more distinct roles in executive function, the formation of associations between drugs and external and interoceptive cues, and modulation of goal-directed behaviors (Hyman & Malenka, 2001). With repeated exposure to rewarding or reinforcing stimuli, DA transmission in these circuits leads to cellular alterations that regulate how the organism behaves in the presence of motivationally relevant environmental stimuli, and mediate the establishment of adaptive responses necessary for acquiring future rewards or reinforcers (Graybiel, 2008; Spanagel & Weiss, 1999). When a natural reward or reinforcer is consumed, DA

transmission in transiently activated and progressively diminishes with repeated exposure. Over time, neutral stimuli become conditioned reinforcers which themselves increase DA transmission, predicting the event and motivating the organism to engage in appropriate behavioral responses (Berridge & Robinson, 1998; Kalivas, 2007). Thus, mesolimbic DA transmission both (1) initially signals the occurrence of motivationally relevant events and (2) later predicts the event from associated cues in order to engage in efficient goal-directed behaviors. With abused drugs, unlike natural reinforcers, increased DA transmission is robust, long-lasting, and *pathologically* reinforcing (Feltenstein & See, 2008). With repeated drug use, associations between the drug and previously neutral environmental stimuli (cues) become *exceedingly* salient conditioned reinforcers (associative "overlearning") which can lead to craving and drive subsequent drug-seeking. Furthermore, with repeatedly reinforced drug-seeking events, this behavior becomes automatic, prepotent, and compulsive (instrumental "overlearning") (Gass & Olive, 2008). Thus, drugs of abuse "hijack" the subcortical systems that subserve normal motivational learning, and the combination of these "overlearning" processes produce lasting neuroadaptations in DA transmission that progressively lead to an escalated cycle of maladaptive (habitual) drug use (Cleva & Gass, 2010).

Historically, researchers have thought that these neuroplastic changes mediated the transition from episodic to compulsive drug use and addiction (Kalivas & O'Brien, 2008; Kalivas, 2002). However, research has shown that these subcortical neuroplastic changes alone are not fully capable in mediating the progression to compulsive drug use. Numerous lines of evidence in the last two decades, from both human neuroimaging and preclinical animal studies, have revealed that repeated drug use also disrupts prefrontal

cortical functioning, resulting in a loss of executive functioning and "top-down" inhibitory control that, under normal circumstances, overrides habitual responding when exposed to adverse consequences (Goldstein & Volkow, 2011; Jentsch & Taylor, 1999; Kalivas et al., 2005; Kalivas & Volkow, 2005). Thus, drug addiction develops from a combination of subcortical alterations that drive automatic, habitual responding with a lack of top-down inhibitory control that regulates behavior in response to negative consequences. Given that most attempts to develop pharmacotherapeutics for addiction have predominantly targeted only the subcortical reward systems (attempting to reduce craving or block rewarding and reinforcing effects of the drug), it is not surprisingly that the vast majority of compounds tested have failed to adequately reduce relapse rates, and only a few approved anti-relapse medications exist (and only for nicotine, alcohol, and opioids).

"Top-down": Repeated drug-induced insults to prefrontal cortices impairs executive functioning

The PFC is responsible for many higher-order cognitive processes, often collectively referred to as executive functions, such as decision-making, response inhibition, planning, working memory, and attention (Goldstein & Volkow, 2011; Miller & Cohen, 2001; Sofuoglu et al., 2013). As mentioned previously, in addition to increased DA signaling in the mesolimbic reward pathway, drugs of abuse also increase DA transmission in the PFC (Koob & Volkow, 2010). Evidence suggests that, while acute drug effects can increase PFC activity and improve cognitive functioning, repeated drug exposure leads to compensatory changes that subsequently both biases attention toward drug-related stimuli and impairs multiple domains of executive functioning (for a

comprehensive review of drug-induced impairments in executive function domains, see (Field & Cox, 2008; Goldstein & Volkow, 2011; Sofuoglu et al., 2013)). In drug addiction, impaired functioning in these domains, combined with attention biased towards drug-related stimuli, culminates in the inability of the PFC to effectively exert "top-down" inhibitory control over habitual drug-seeking behavior (Kalivas & O'Brien, 2008; Kalivas, 2008). While "bottom-up" DA transmission is responsible for innervating prefrontal regions, reciprocal "top-down" signaling from the PFC is mediated by the excitatory neurotransmitter glutamate (Gass & Olive, 2008; Kalivas, LaLumiere, Knackstedt, & Shen, 2009; Tzschentke & Schmidt, 2003). In recent years, addiction research has begun to reveal that changes in glutamatergic signaling within corticostriatal and corticolimbic circuits where DA terminals are embedded are essential in mediating drug reward, reinforcement, and the transition to addiction (Gass & Olive, 2009; LaLumiere, Smith, & Kalivas, 2012; Peters, Kalivas, & Quirk, 2009; Tzschentke & Schmidt, 2003), revealing new potential targets for addiction pharmacotherapeutics (Cleva, Gass, Widholm, & Olive, 2010; Kalivas & Volkow, 2011; Olive, Cleva, Kalivas, & Malcolm, 2012).

Glutamatergic mechanisms in memory formation: a brief overview

Glutamate is the main excitatory neurotransmitter in the mammalian brain and responsible for approximately 70% of the chemical transmission in the central nervous system (Gass & Olive, 2008). Glutamate binds to two major classes of receptors; ionotropic glutamate receptors (N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors) which mediate fast excitatory transmission, and metabotropic glutamate receptors (mGluR1-8) which

mediate slow modulatory transmission through G-protein mediated signaling pathways (Niciu, Kelmendi, & Sanacora, 2012). At the cellular level, learning produces changes in excitatory glutamatergic transmission such as long-lasting increases in synaptic strength and postsynaptic current amplitudes, increasing the efficacy of communication between nerve cells. These changes, known as long-term potentiation (LTP), are generally accepted to be the cellular basis for memory formation and storage (Lisman, Yasuda, & Raghavachari, 2012). While there is an overwhelming amount of evidence suggesting that each of the glutamate receptor types play a unique role in LTP and learning and memory (Nicoll & Roche, 2013), for the purposes of this review, focus will be placed on ionotropic AMPA receptors (for a more comprehensive review on glutamate mechanisms and LTP, see (Lamprecht & LeDoux, 2004; Niciu et al., 2012)). Both early and late phases of LTP require AMPA receptors. First, signaling through AMPA receptors is necessary to slightly depolarize the membrane to approximately -50 mV, at which point the Mg^{2+} block is released from NMDA receptors, allowing Ca^{2+} ions to enter the cell. While Ca^{2+} triggers multiple downstream effects including gene transcription and translation mechanisms that results changes in the levels of numerous synaptic proteins, it also causes an immediate non-genomic increase in AMPA receptor trafficking and insertion of AMPA receptors into the plasma membrane, increasing the size and strength of postsynaptic responses (P. Chang, Verbich, & McKinney, 2012). The long-lasting increase in postsynaptic AMPA receptors is thought to be necessary for the lasting LTP and memory formation. Thus, ligands that increase signaling through AMPA receptors facilitate LTP, learning and memory (Lynch & Gall, 2006).

As mentioned above, while dopaminergic signaling appears necessary for initiating and reinforcing early drug use (positive reinforcement), glutamatergic mechanisms within mesocorticolimbic circuits have also emerged as primary mediators of the transition to compulsive drug use (Gass & Olive, 2008; Kalivas et al., 2009). Specifically, lasting neuroadaptations in corticostriatal and corticolimbic glutamatergic transmission are thought to be largely responsible for the behavioral hallmarks of addiction including (1) the impaired ability to regulate the drive to obtain and use drugs, even in the face of adverse consequences, and (2) a propensity to relapse even after long periods of abstinence (Kalivas & O'Brien, 2008; Kalivas & Volkow, 2005; Kalivas, 2002). In the normal brain, when motivational relevant stimuli are encountered, corticolimbic glutamatergic circuits, comprised of the PFC, amygdala, NAc core and shell (NAcc and NAcs), interact and send relevant environmental information through the NAc to mesostriatal (sensorimotor) circuits involving the dorsal striatum, which in turn communicates with other basal ganglia regions such as the globus pallidus and substantia nigra. Together, these circuits process environmental stimuli in order to establish efficient, goal-directed behaviors. Following repeated drug reinforcement, the influence of corticolimbic glutamatergic projections from the PFC and amygdala into the NAc progressively diminishes, whereas sensorimotor glutamatergic transmission to the dorsal striatum becomes predominant, allowing responses to become automatic (i.e., habitual) and allowing corticolimbic circuits to process other relevant stimuli (Jog, 1999; Kalivas, 2008). However, if reinforcer contingencies change and responses fail to yield expected outcomes, engaged corticolimbic circuits function to both inhibit the prepotent response and signal the motor cortex to generate new adaptive responses (Berridge & Robinson,

1998; Kalivas, 2002). In other words, following repeated drug reinforcement, the influence of corticolimbic glutamate projections from the PFC to the NAc on behavior progressively diminishes, whereas sensorimotor glutamatergic transmission to the dorsal striatum becomes predominant, resulting in more automatic and habitual behaviors. Thus, compulsive drug use develops from a combination of pathologically strengthened "habit" circuitry combined with impaired corticolimbic circuits, rendering drug addicts with impaired behavior regulation who are unable to inhibit drug-seeking behavior in the face of adverse consequences (Everitt & Robbins, 2005; Kalivas & Volkow, 2005; Kalivas, 2002). Given the pivotal role of excitatory transmission in these circuits, treatments aimed at rescuing or increasing behavioral regulation and/or impairing drug-related "habit" memories may be promising avenues for novel addiction treatments.

Extinction / exposure strategies: Rescuing behavioral regulation

One therapeutic approach that has shown some success (although moderate at best) in decreasing relapse by enhancing behavioral regulation is cue exposure therapy (Conklin & Tiffany, 2002; Havermans & Jansen, 2003; Marlatt, 1990). In this approach, clinicians attempt to extinguish (i.e., "break") the associations between drug craving, use, and drug-related stimuli (such as drug paraphernalia) by repeatedly exposing drug users to the drug-related stimuli in the absence of drug availability. In preclinical rodent models of addiction, this process is studied using the extinction-reinstatement paradigm (Epstein, Preston, Stewart, & Shaham, 2006). Here, a rodent or nonhuman primate is allowed to intravenously self-administer (IVSA) a drug, with drug infusions simultaneously paired with discrete cues such as a light and/or tone. Following stabilization of drug-taking, animals undergo either extinction training (ET), where they are placed in the drug-taking

context but the drugs is no longer available (and thus drug-cue associations cannot be further strengthened), or no ET where they remain in their home cage (forced abstinence) (Epstein et al., 2006). Subsequently, animals are tested for reinstatement ("relapse") of drug-seeking by the presentation of drug-associated discrete cues, a small dose of the drug, or a stressful stimulus (Epstein et al., 2006). Historically, a common misconception about exposure and extinction therapies has been that the resulting decrease in responding occurs due to a process of "forgetting". However, evidence suggests that extinction is instead a form of new learning that is highly context dependent (Bouton, 2004). This is evidenced by the fact that, despite a loss of responding during extinction procedures, responding will often re-appear spontaneously with time (spontaneous recovery), when the organism is placed back in the original drug-taking context(s) (renewal), or exposed to discrete cues not present during the extinction procedures (cue-induced reinstatement) (Bouton, 2002; Crombag, Bossert, Koya, & Shaham, 2008; Rescorla, 2004). These phenomena suggest that exposure and extinction strategies do not erase the original drug-seeking memory engram(s), but instead decrease drug-seeking by strengthening "top-down" inhibitory control circuits (Cleva et al., 2010). However, the inconsistent success rates of exposure and extinction therapies in humans is likely attributable to due to either (1) a lack of proper use of extinction procedures due to misunderstandings about the underlying processes of extinction (i.e., new learning vs. forgetting), (2) context-specificity issues (i.e., lack of extinction training in the actual drug-taking context(s)), (3) lack of adequate exposure session time, (4) lack of utilization of highly salient drug cues, or other uncontrolled variables (Conklin & Tiffany, 2002; Taylor, Olausson, Quinn, & Torregrossa, 2009).

Despite mixed results at the clinical level, preclinical studies show that extinction training (ET) decreases reinstated drug-seeking when compared to forced abstinence procedures where animals simply remain in their home cage for a matched amount of time (Fuchs, Branham, & See, 2006; Sutton et al., 2003). Furthermore, reinstatement following forced abstinence is primarily mediated by dorsal striatal "habit" circuitry (Di Ciano, Robbins, & Everitt, 2008), whereas ET engages prefrontal glutamate projections to the NAcs, implying that top-down behavioral regulation circuits are also recruited (Knackstedt et al., 2010; LaLumiere & Kalivas, 2008; LaLumiere et al., 2012; Peters, LaLumiere, & Kalivas, 2008). Furthermore, similar to human imaging studies that have shown that cue-induced drug craving is correlated with anterior cingulate activation (Childress & Mozley, 1999), the homologous prelimbic cortex in rats (Ongür & Price, 2000), which sends glutamatergic projections to the NAcc, is responsible for initiating cue-induced drug-seeking (Kalivas & McFarland, 2003; Kalivas & O'Brien, 2008; LaLumiere & Kalivas, 2008; McFarland & Kalivas, 2001; McFarland, Lapish, & Kalivas, 2003). In contrast, ET enhances glutamatergic transmission from the infralimbic cortex (ILC) to the NAcs, which is a critical locus for the storage and consolidation of extinction learning and subsequent inhibition of cue-induced drug-seeking (Knackstedt et al., 2010; LaLumiere, Niehoff, & Kalivas, 2010; LaLumiere et al., 2012; Peters et al., 2008). Thus, these two parallel PFC-NAc projections are functionally dichotomous, and compete for control of signaling in the NAc to motor circuits that ultimately guide behavior (Kalivas & O'Brien, 2008; Kalivas, 2009). In addition, ET leads to persistent changes in various plasticity-related proteins in the NAc. Specifically, ET upregulates the expression of the GluR1 and GluR2/3 subunits of the AMPA receptor in the NAcs, indicative of the

emergence of an LTP-like “up” state in these specific pathways. Corroborating these effects, viral overexpression of these same subunits also decreases reinstatement of cocaine-seeking (Ghasemzadeh, Vasudevan, Mueller, Seubert, & Mantsch, 2009; Sutton et al., 2003). Conversely, viral overexpression of “pore-dead” GluR1 subunits in the NAcc potentiates reinstated cocaine-seeking (Bachtell et al., 2008; Sutton et al., 2003). Thus, either potentiation or increasing the number of AMPA receptors in the NAcs, antagonism or decreasing the number of AMPA receptors in the NAcc, or both, would theoretically tip the balance of glutamatergic signaling to the NAc back towards favoring of ILC-NAcs mediated inhibitory control.

Facilitating ILC-NAcs glutamate signaling

The fact that ET recruits “top-down” glutamatergic signaling that mediates and is responsible for the consolidation of extinction behavior has led to an increase in research focusing on these pathways as pharmacotherapeutic targets. Recent studies have shown that various glutamate receptor agonists or PAMs enhance the consolidation of both extinguished drug-seeking and increase markers associated with synaptic plasticity in the ILC-NAcs pathway (Knackstedt et al., 2010; LaLumiere et al., 2010, 2012), suggesting that pharmacological compounds that enhance activity or plasticity in this pathway have the potential to be novel therapeutic adjuncts to cue exposure therapies (Cleva et al., 2010). One promising class of glutamate ligands, called AMPA PAMs (Lynch, 2002, 2006), are small molecules that, while displaying a wide range of structural differences, all enhance glutamatergic signaling through positive modulation of AMPA receptors (Arai & Kessler, 2007). The first AMPA PAMs were developed approximately two decades ago, and in the time since have been shown to improve learning, memory and/or

cognition, in both humans and animal subjects, and in a variety of experimental designs, indicating their potential as broad spectrum pharmacotherapeutics (Black, 2005; Lynch & Gall, 2006; Lynch, Palmer, & Gall, 2011; Lynch, 2006; Marenco & Weinberger, 2006; Swanson, 2009). AMPA PAMs work in an activity-dependent manner by maintaining the open-channel state of AMPA receptors after binding of an endogenous ligand (glutamate) (Jin et al., 2005). AMPA PAMs decrease either the rate of desensitization or deactivation of the receptor, thereby increasing cation influx into the postsynaptic cell (ONeill & Bleakman, 2004). However, unlike orthosteric (competitive) glutamate receptor agonists which can produce severe unwanted side effects such as excitoxicity, AMPA PAMs only enhance endogenous activity and are less prone to adverse side effects (Christopoulos, 2002) (but see below). For example, evidence shows that AMPA PAMs can facilitate learning and memory at doses that do not cause excitotoxic damage, a common occurrence with orthosteric agonists (Mattson, 2003; Mehta, Prabhakar, Kumar, Deshmukh, & Sharma, 2013; Olney, 1994; Staubli, Rogers, & Lynch, 1994). However, it has recently been reported that AMPA PAMs may be more excitotoxic at effective doses than previously thought (Shaffer et al., 2013). Nonetheless, most published studies have reported that AMPA PAMs generally have a safe profile at effective doses (Lynch & Gall, 2006; Lynch, 2006).

AMPA PAMs and Addiction: Preclinical Studies

In recent years, a handful of animal studies have assessed the potential use of AMPA PAMs for the treatment of addiction. In the first study of this kind (LaLumiere et al., 2010), rats were allowed to intravenously self-administer (IVSA) cocaine for two weeks in daily 2-hr sessions using standard operant lever pressing procedures. Following

self-administration, rats were first placed into brief (15 min sessions) extinction sessions for five days after which intracranial ILC injections of the AMPA positive modulator 2-[2,6-difluoro-4-({2-[(phenylsulfonyl)amino]ethyl}thio)phenoxy]acetamide (PEPA, 30 ng/side) or vehicle were administered immediately after the extinction session. PEPA is a GluR3/4 preferring AMPA PAM that primarily exerts its effects through attenuation of AMPA receptor desensitization (Sekiguchi, Nishikawa, Aoki, & Wada, 2002). Next, seven additional 2-hr extinction sessions were conducted, after which no post-session PEPA infusions were given, in order to assess for retention of extinction learning. The results showed that ILC injections of PEPA facilitated extinction learning (i.e., decreased presses on the lever that previously resulted in cocaine delivery) during the final two 15-min extinction sessions. Furthermore, PEPA-facilitated extinction also continued through the seven 2-hr extinction sessions, as overall responding was significantly decreased for all remaining ET sessions.

In a follow-up study by the same research group (LaLumiere et al., 2012), rats underwent cocaine self-administration procedures for 2 weeks. Following cocaine IVSA, rats were again placed into ET for at least 10 sessions and remained in extinction until responding decreased to a predetermined criteria (>25 lever presses in 2 consecutive sessions). Following extinction procedures, PEPA microinjections into the ILC (0.075 nmol/hemisphere) were administered immediately prior to testing for cue-induced reinstatement. Two reinstatement tests were given, with rats receiving either PEPA or vehicle in a randomized, counter-balanced design. The results demonstrated that PEPA significantly decreased cue-induced reinstatement of cocaine-seeking compared to vehicle. Importantly, this decrease was not due to alterations in general locomotor

activity. In a subsequent experiment in this study, it was also shown that PEPA-mediated decreases in responding were reversed by microinjections of an AMPA receptor antagonist into the NAcs. Together, these studies demonstrate that ILC glutamate transmission to the NAcs mediates the expression and consolidation of extinction behavior in the reinstatement paradigm.

While these studies demonstrate that facilitating glutamatergic transmission in the ILC→NAcs pathway with AMPA positive modulators is promising, there are no published reports demonstrating that *systemic* administration of AMPA PAMs, which is more translationally relevant for the development of newer treatments for addiction, produces similar promising results. Some AMPA PAMs have been reported to possess the ability to induce the expression and secretion of brain-derived neurotrophic factor (BDNF) (Lynch & Gall, 2006), a neurotrophin that among other things facilitates the induction and maintenance of LTP (Bramham & Messaoudi, 2005). Thus, AMPA PAMs can be further characterized into BDNF-inducing (BDNF AMPA PAM) or non-BDNF-inducing (non-BDNF AMPA PAM) subtypes(Arai & Kessler, 2007; Clarkson et al., 2011). For example, previous work has revealed increased motor recovery following experimental stroke in rats following administration of the BDNF AMPA PAM CX1837 as compared to the non-BDNF AMPA PAM CX1739 (Clarkson et al., 2011), suggesting that BDNF-inducing AMPA PAMs may have superior therapeutic potential.

Recently, in collaboration with Cortex Pharmaceuticals (Glen Rock, NJ), our laboratory has collected novel data on the effects of BDNF vs. non-BDNF AMPA PAMs on the extinction and reinstatement of methamphetamine-seeking behavior. Following two weeks of methamphetamine self-administration in rats, we systemically administered

either the BDNF AMPA PAM CX1837 (0.1 and 1 mg/kg i.p., Fig. 4a) or non-BDNFAMPA PAM CX1739 (0.1, 1, and 10 mg/kg i.p., Fig. 4b) prior to ET sessions. Doses of these compounds were based upon recommendations from Cortex Pharmaceuticals and earlier reports of efficacious effects at similar doses (Clarkson et al., 2011) that do not alter generalized locomotor behavior (Silverman, Oliver, Karras, Gastrell, & Crawley, 2013). Results revealed that systemic treatment with either CX1837 or CX1739 significantly facilitated extinction learning (reduction in active lever presses) on the first day of extinction tests (see Fig. 4a,b). However, statistical analyses did not reveal any significant differences during any of the remaining extinction sessions or any main effects of drug type (CX1837 vs. CX1739). Furthermore, the reduction in responding seen during ET sessions unfortunately did not lead to significant reductions in cue-induced reinstatement of METH-seeking as seen previously following intra-ILC central injections of the AMPA PAM PEPA (Fig. 5)

The observed lack of attenuated reinstatement by these AMPA PAMs is disappointing, especially in light of the aforementioned positive results observed with intra-ILC administration of PEPA following cocaine self-administration and ET. Reasons for the lack of apparent efficacy of either CX1739 or CX1839 in attenuating reinstatement may be attributable to the different drug reinforcers used (methamphetamine vs. cocaine), and it is possible that self-administration of these two psychostimulants produces differential effects on AMPA receptor and/or BDNF expression that may reduce the pharmacological effects of AMPA PAMs (Bowers, Chen, & Bonci, 2010; Ghitza et al., 2010). Alternatively, some studies have shown opposing prelimbic vs. ILC influences on the extinction and reinstatement of drug-seeking

behavior (Willcocks & McNally, 2013), and it possible that potentiation of AMPA and/or BDNF signaling in both of these regions simultaneously following systemic AMPA PAM administration negates any effects of either of these compounds when acting in either region alone, as would be achieved by intracerebral administration. Thirdly, it is possible that CX1739 and/or CX1837 act on AMPA receptors containing subunit configurations that are different than those affected by PEPA (GluR3/4).

Nonetheless, these results do indicate that further research is needed to ascertain the potential therapeutic value of AMPA PAMs in the treatment of drug addiction. Specifically, future studies should examine factors such as drug reinforcer, BDNF vs. non-BDNF AMPA PAM utilized, and selectivity of these compounds for specific AMPA subunit composition and their neuroanatomical localization. Furthermore, it has recently been shown that a novel extinction paradigm, known as memory-retrieval extinction, leads to a reduction in cocaine, heroin, and alcohol reinstatement when compared to standard extinction training (Xue et al., 2012; Zayra Millan, Milligan-Saville, & McNally, 2013). Furthermore, reductions in reinstatement are correlated with an upregulation of protein kinase M zeta (PKM ζ, an atypical member of the protein kinase C family that is thought to be necessary and sufficient for long-term memories and LTP (Sacktor, 2010), although this has recently been challenged (Lee et al., 2013; Volk, Bachman, Johnson, Yu, & Huganir, 2013). PKM ζ, once synthesized, remains persistently active and maintains memories through an increase and maintenance of AMPA receptors in the post-synaptic membrane (Sacktor, 2010). Thus, the attenuated reinstatement observed following memory-retrieval extinction procedures are likely mediated through upregulated AMPA receptor signaling, and further potentiation with

AMPA PAMs may theoretically confer added benefits. This hypothesis, however, remains to be tested.

Discussion

Collectively, the results from the studies outlined above suggest that AMPA PAMs *may* have potential as pharmacological adjuncts to traditional cue-exposure therapies. However, the data thus far are rather limited, and this suggestion needs to remain hypothetical at this point until additional data are collected. Further studies with additional BDNF and non-BDNF AMPA PAMs, utilizing different drug reinforcers, and potentially additional extinction paradigms such as extinction-retrieval, are needed to provide firmer evidence of a therapeutic value of AMPA receptors in the treatment of addiction, such as novel pharmacological adjuncts to cue exposure therapy. Nonetheless, given that the ILC→NAcs glutamate pathway has been shown to mediate both the expression and consolidation of learned extinction of drug-seeking behavior, and AMPA PAMs exert their effects in an activity-dependent manner, a likely mechanism of the observed effects of AMPA PAM administration is potentiated glutamate transmission in this pathway. This hypothesis, while currently unconfirmed, suggests a facilitation of "top-down" inhibitory control over drug-seeking behavior. It is therefore possible that other AMPA PAM mechanisms may or may not contribute to the potential efficacy of these compounds in the context of drug addiction. While it has been suggested in previous work that positive effects AMPA PAMs may be mediated, in part, by alterations in neurotrophin (BDNF) signaling (Clarkson et al., 2011; Lauterborn et al., 2009; Lynch & Gall, 2006; Lynch et al., 2011; Lynch, 1998, 2006), our results did not reveal

significant differences between the BDNF AMPA PAM CX1837 and the non-BDNF AMPA PAM CX1739, and thus does not suggest a significant role of BDNF signaling in the observed facilitated extinction effects. These results should be interpreted with caution however, and additional testing with other BDNF-inducing compounds is needed before definitive conclusions can be made.

Conclusions

Extinction-based cue-exposure therapies have shown limited success decreasing relapse in humans. However, evidence from preclinical studies suggests that extinction training, combined with AMPA PAMs treatment, under some circumstances, facilitates and consolidates extinction learning. Furthermore, under some circumstances AMPA PAM treatment also leads to attenuated cue-induced reinstatement of drug-seeking. These promising preclinical findings point to the need for future research aimed at assessing whether adjunct treatment with AMPA PAMs could potentially improve the success rate of cue-exposure therapies in humans.

CHAPTER 4

POTENT REWARDING AND REINFORCING EFFECTS OF THE SYNTHETIC CATHINONE 3,4-METHYLENEDIOXYPYROVALERONE (MDPV).

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In recent years, there has been a dramatic increase in the use of desiger drugs known as synthetic cathinones in both Europe and the United States (Spiller et al., 2011; Drug Enforcement Administration, 2011a). Use of synthetic cathinones has emerged rapidly, fueled largely by online marketing and widespread availability over the internet and in smoke shops and convenience stores (Kavanagh et al. 2010; Vardakou et al., 2011). These synthetic drugs are derivatives of cathinone, a naturally occurring beta-ketone amphetamine analogue found in khat (*Catha edulus*), a plant that is abused for its stimulant-like effects (Magdum, 2011) . Typically, synthetic cathinones are sold as "bath salts", "plant food", and other misleading terms and marketed as "legal highs" and alternatives to traditionally abused stimulants such cocaine and amphetamines (Drug Enforcement Administration, 2011a). While many potential synthetic cathinones exist and are predicted to emerge as abused substances in the future, the analogues most frequently used at present include mephedrone (4-methylmethcathinone, 4-MMC), 3,4-methylenedioxypyrovalerone (MDPV), and methylone (3,4-methylenedioxymethcathinone, MDMC) (Drug Enforcement Administration, 2011a). As of October 2011, mephedrone, MDPV, and methylone have been temporarily classified in the United States as Schedule I controlled substances (Drug Enforcement Administration, 2011b).

Despite the widespread increase in use of these compounds, very little scientific

data exist regarding their reinforcing effects and abuse potential. Of the three most common synthetic cathinones, most scientific investigations have focused on mephedrone, and recently it has been shown that rats will readily self-administer mephedrone at a dose of 0.24 mg per 10 μl infusion (Hadlock et al., 2011). While mephedrone has been the subject of most popular press coverage and recent scientific investigations, MDPV use is also common and has been marketed as a replacement mephedrone in places where it has previously been banned (Durham, 2011; Coppola & Mondola, 2012) . MDPV is a methylenedioxy analogue of pyrovalerone (Yohannan and Bolenko, 2010), a drug with stimulant-like properties (Holliday et al., 1964) that was once prescribed to treat chronic fatigue and lethargy (Goldberg et al., 1973) before being shown to possess abuse potential in drug addicts (Deniker et al., 1975). Although the precise mechanism(s) of MDPV is currently unknown, it is possible it acts as a monoamine uptake inhibitor as pyrovalerone has been shown to inhibit dopamine and norepinephrine transporters (DAT and NET, respectively), and to a lesser extent serotonin transporters (SERT)(Lancelot et al., 1992)(Meltzer et al., 2006)(Kelly, 2011)(Coppola & Mondola, 2012). MDPV increases extracellular levels of DA in the striatum of mice after oral administration (Fuwa et al., 2009). Behaviorally, MDPV leads to dose-dependent increases in locomotor activity in mice to a greater extent than methamphetamine when using identical doses (Marusich et al., 2011). Together, these data provide early evidence that MDPV possesses stimulant-like properties and corroborates users reports describing subjective effects similar to those of methylphenidate, cocaine, and amphetamines (Psychonaut WebMapping Research Group 2009a,b).

To our knowledge, there have been no published studies directly exploring the

reinforcing and rewarding effects of MDPV. The present study addressed this issue by examining the ability of MDPV to support intravenous self-administration (IVSA) and to lower thresholds for intracranial self-stimulation (ICSS). In Experiment 1, the reinforcing effects MDPV during IVSA were assessed at three doses (0.05, 0.1, and 0.2 mg/kg per infusion) during three phases of experimentation: (1) 2 hr daily access sessions, (2) a progressive ratio (PR) schedule of reinforcement, and (3) short (2 hr daily, ShA) vs. long (6 hr daily, LgA) sessions. A separate group of animals underwent the same procedures but self-administered methamphetamine (0.05 mg/kg/infusion) as a positive control. In Experiment 2, MDPV (0.1, 0.5, 1, and 2 mg/kg, i.p.) was administered acutely to determine effects on thresholds for ICSS, a well-established measure of brain reward function (Kornetsky & Bain, 1992).

Method

Subjects

All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee at Arizona State University, and according to the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health (NIH). Forty-one male Sprague-Dawley rats (Harlan Laboratories, Livermore, CA), weighing approximately 250 g, were individually housed upon arrival. Forty-eight rats were implanted with jugular vein catheters and vascular access ports and underwent IVSA procedures for Experiment 1. Five non-catheterized rats underwent ICSS procedures for Experiment 2. Rats were housed according to NIH standards on a 12 hr light-dark cycle and given *ad libitum* access to food and water during all experimental

procedures except during behavioral testing. All experimental sessions took place during the dark phase, with the exception of a 16 hr overnight lever-press training sessions and PR tests which began at 4:00 p.m. and ended the following morning at approximately 8:00 a.m. Throughout the course of experiments, 12 of the 48 rats in Experiment 1 were removed due to catheter patency failure and one of the 5 rats in Experiment 2 was removed due to health-related issues.

Drugs and Assessment of Purity

MDPV was obtained through an internet website www.researchchemz.com (Laboratory Supply USA, San Diego, CA). Ten mg samples of MDPV were analyzed by LC-MS for purity at Research Triangle Institute (Durham, NC). Samples were analyzed using a Waters Synapt HDMS quadrupole time of flight (Q-TOF) mass spectrometer interfaced to a Waters Acquity UPLC system. Data were acquired using a capillary voltage of 3 kV, source temperature of 150 ˚C, desolvation temperature of 500 ˚C, sampling cone at 30 V, and extraction cone at 4 V. The mass spectrometer was externally calibrated from 50 - 700 Da using a sodium formate solution, and mass shifts during acquisition were corrected for using leucine enkephalin as a lockmass. Liquid chromatography was performed using a BEH C_{18} column (2.1 x 50 mm, 1.7 µm particles) held at 40˚C. Sample identity was confirmed based on exact mass, retention time, and fragmentation match to a certified reference standard from Cerilliant (Round Rock, TX). MDPV samples were determined to have an apparent purity of >95%. For all behavioral studies, MDPV and methamphetamine (Sigma-Aldrich, St. Louis, MO) were dissolved in sterile saline. For Experiment 2, MDPV was administered i.p. in a volume of 1 ml/kg.

Experiment 1: Intravenous Self-administration (IVSA)Procedure

Surgical Procedures

Prior to arrival, rats were implanted with intravenous catheters into the jugular vein at Harlan Laboratories. On the day following arrival, rats were anesthetized with isoflurane (2% v/v) vaporized oxygen at a flow rate of 2 L/min. A 2.5 cm longitudinal incision was made between the scapulae for implantation of a threaded vascular access port (Plastics One, Roanoke, VA, USA). Threaded vascular access ports were attached to be mesh collar sutured underneath the surrounding tissue within the incision. Access ports were sealed with a piece of Tygon tubing closed at one end and a protective cap. All rats were given allowed to recover from surgery for 5 days prior to the initiation of behavioral testing, and during this time animals received daily intravenous infusions of 70 U/ml heparin (0.2 ml volume) to maintain catheter patency and 100 mg/ml cefazolin (0.1 ml volume) to protect against infection. Meloxicam (2.5 mg/ml s.c.) was administered for the first 3 days following surgical procedures to provide additional relief post-surgical discomfort. In addition, rats were given ten 45 mg sucrose pellets in their homecage four days prior to IVSA procedures to eliminate neophobia to sucrose pellets that could delay acquisition of self-administration during 16 hr overnight training sessions.

Apparatus

Drug self-administration sessions were conducted in operant self-administration chambers (ENV-008, Med Associates, St. Albans, VT, USA). All self-administration chambers were located inside sound-attenuating cubicles equipped with a house light and exhaust fan designed to mask external noise and odors, and were interfaced to a PC

computer. Chambers were equipped with two stainless steel response levers located on one wall with a 4.2 × 5 cm food pellet receptacle placed between levers. Each response lever was located approximately 7 cm above a stainless steel grid floor, and positioned above each lever was a 2.5 cm diameter white stimulus light. Located near the top of the self-administration chambers was a Sonalert speaker that provided an auditory stimulus during drug delivery. Outside each chamber was a syringe pump that was interfaced to the computer and delivered the drug solution via a single-channel liquid swivel mounted atop the chamber via polyethylene tubing.

Experimental Design: IVSA Procedures

Following recovery from surgical procedures, self-administration sessions commenced. During all self-administration sessions, except during progressive ratio training, each press on the active lever delivered the reinforcer on an FR1 schedule of reinforcement. Reinforcer delivery was accompanied by concurrent illumination of a stimulus light and presentation of an auditory stimulus for two seconds followed by a 20-sec timeout period during which additional lever presses were recorded but produced no programmed responses. Inactive lever presses were recorded but produced no programmed consequences. Self-administration procedures were initiated with a 16 hr overnight training session whereby active lever presses delivered a 45 mg sucrose pellet (TestDiet, Richmond, IN). Approximately 24 hr following sucrose training, rats were separated into one of four groups based upon MDPV dose (0.05, 0.1, or 0.2 mg/kg/infusion) or as a positive control, methamphetamine (0.05 mg/kg/infusion). Each drug infusion was delivered in a volume of 0.06 ml. Next, daily 2 hr self-administration sessions were commenced with intravenous MDPV or methamphetamine as the

reinforcer. MDPV or methamphetamine was delivered to the vascular access port by polyethylene tubing housed in a stainless steel spring tether that was attached to the liquid swivel. Self-administration sessions were conducted 7 consecutive days per week, and each session was preceded and followed by an intravenous infusion of 0.1 ml of 70 U/ml heparin plus 100 mg/ml cefazolin to maintain catheter patency. Daily 2 hr self-administration sessions were conducted for a minimum of 10 days and until stability criterion was reached (<15% deviation in active lever pressing for each dose group for two consecutive days). All groups met stability on day 10.

Following ten days of 2 hr IVSA sessions, a 16 hr overnight progressive ratio (PR) schedule was conducted to assess the reinforcing efficacy of MDPV. During PR tests, the number of lever presses required to obtain a single infusion of MDPV was determined by the following the equation: responses per reinforcer delivery = $5 \times e^{(\text{injection number} - 0.2)} - 5$ (i.e., 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, etc.) (Richardson and Roberts, 1996). Breakpoints were considered to be met when rats did not emit any lever presses for 2 hours. Following PR testing, each dose group of rats (0.05, 0.1, or 0.2 mg/kg/infusion of MDPV) was divided into two subgroups, such that half of the rats in each dose group continued with 2 hr daily self-administration sessions for ten days (short access, ShA), while the other half began 6 hr daily sessions (long access, LgA) for ten days. All aspects of the self-administration procedures were identical except for session length (2 vs. 6 hr per day). For rats self-administering methamphetamine, all rats were assigned to the LgA group to demonstrate escalation of drug intake.

Experiment 2: ICSS Procedures

Surgical Procedures

Rats were anesthetized with isoflurane (2% v/v) vaporized oxygen at a flow rate of 2 L/min and placed into a stereotaxic frame. A stainless-steel bipolar electrode (PlasticsOne, Roanoke, VA, USA, 2 mm diameter, insulated except at the ventral tip) was implanted into the medial forebrain bundle (AP -0.05 mm; ML ± 1.7 mm, DV -8.3 mm from dura). Four skull screws and dental cement were used to permanently secure electrodes to the skull. To counterbalance for any hemispheric differences, half the animals received electrodes in the left hemisphere and the other in the right hemisphere. Following surgery, rats were given 7 days to recover before beginning ICSS procedures during which they received daily injections of 2.5 mg/ml meloxicam (0.15 ml volume) to minimize post-surgical discomfort.

Apparatus

All ICSS testing was conducted in operant chambers (ENV-007CT, Med Associates). Chambers were housed inside sound-attenuating cubicles equipped with an exhaust fan to mask external noise and odors. Chambers contained a house light on the back wall and a front wall mounted nose-poke aperture with LED stimulus lights located inside the access hole (ENV-114M, Med Associates). The nose-poke aperture was 2.5 cm in diameter, located 5 cm above the stainless steel grid floor, and contained an infrared detector placed 0.64 cm from the front edge of the panel for recording responses. Located outside chambers was a dual programmable ICSS stimulator (PHM-150B/2, Med Associates) that was interfaced to a computer to deliver electrical current to the electrode. Chambers were interfaced to a PC computer using Med-PC IV software that controlled all stimulation parameters, test functions, and data collection (Med Associates).

Experimental Design: ICSS Procedures

The procedure for measuring reward thresholds was a modified version of the discrete trials current-threshold method (Kornetsky et al., 1979; Markou and Koob, 1992). During all ICSS phases, stimulation availability was signaled by illumination of the nose poke aperture by the LED stimulus light complex. Rats initiated training on a FR1 schedule of reinforcement where nose pokes resulted in the delivery of a 200 µsec square-wave cathodal pulses at 100 Hz at a current of 120 µA. After acquisition criteria were met (> 600 responses in 30 min for 2 sessions), rats began discrete-trials training procedures. Each discrete trial began with a free stimulation of 120 µA, followed by a 7.5 second period during which the first response (trial response) yielded an identical stimulation. Following the trial response, LED lights turned off and subsequent responses (inter-trial interval (ITI) responses) were recorded, but yielded no stimulation. Progression through discrete trials training required rats to meet criterion (>60% of total response were trial responses) at four ITI lengths (2, 5, 10, and 15 sec). Upon completing training, rats then began discrete-trials current-threshold determination procedures. Each current threshold determination session began with 120 µA of current and progressed through 4 cycles of ascending and descending current intensities. At a given current intensity, trial blocks began with a free stimulation, followed by 7.5 seconds during which the animal could emit a nose-poke response to receive an identical stimulation. Following a single trial response, LED stimulus lights turned off initiating an ITI period between 7.5 and 15 seconds (mean of 10 second) that separated trials. Responses during the ITI interval further lengthened the ITI by 12.5 seconds. When animals emitted appropriate responses on ≥3 of 5 trials, electrical stimulation decreased by 5 µA for the next 5-trial block. Block intensities continued to descend until rats responded ≤2 out of 5

trials during a given trial block, at which point the current intensities reversed into ascending mode, with increases in current intensities of5 μA each for the subsequent block. Thus, the procedure determined the minimum amount of current (threshold) for which the rat was willing to respond. Thresholds were calculated by averaging the midpoint of current intensities between positive (responses on ≥3 of 5 trials) or negative (responses on ≤2 of 5) trial blocks. Rats received a minimum of 10 days of baseline threshold assessment and were required to meet stable baseline criteria prior to administration of MDPV, defined as when the average of thresholds for the last 4 days minus the first 4 days of an 8-day window was less than 10% of the average of the full 8 days. Rats continued to receive baseline testing throughout the course of the experiment 4 days per week. Rats received vehicle injections 20 min prior to placement in ICSS procedures. MDPV doses were assigned randomly and injections given 20 minutes prior to threshold determination procedures. All rats, with the exception of one that was removed halfway through MDPV testing due to loss of cranial implant, underwent 2 determinations of each dose of MDPV, and 5 determinations of vehicle.

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics version 19 (Armonk, New York, USA). All data points represent mean ± SEM. A significance criterion of $p<0.05$ was used for all analyses. For the first 10 IVSA sessions, the ability of MDPV to maintain responding was first analyzed separately for each dose of MDPV by a mixed analysis of variance (ANOVA) with lever (active vs. inactive) and session as factors. Post-hoc one-way ANOVAs were also conducted to determine the number of sessions required to obtain lever discrimination. The total number of MDPV infusions

obtained per session was analyzed by a mixed ANOVA with MDPV dose and session as factors. Holm-Sidak post-hoc tests determined overall dose effects, and one-way ANOVAs followed by Holm-Sidak post-hoc tests further determined dose effects during each session. Analysis of the total number of infusions obtained during PR sessions at different doses of MDPV were analyzed by a one-way between subjects ANOVA followed by Holm-Sidak post-hoc tests. The 0.05 mg/kg dose of methamphetamine and MDPV was analyzed separately by an independent samples t-test. For ShA vs LgA IVSA sessions, the effects of session length (ShA vs. LgA) on total infusions obtained was analyzed by mixed ANOVA for each dose of MDPV or methamphetamine. Post-hoc one-way ANOVAs further explored differences in the number of infusions obtained across MDPV doses for each session. To determine escalation of drug intake, mixed ANOVAs for each dose of MDPV or methamphetamine were conducted with infusions obtained in ShA vs. LgA (first 2 hr only) and session as factors. Post-hoc tests compared each session separately. For the 0.1 and 0.2 mg/kg dose groups, repeated measures ANOVAs were conducted separately for ShA, LgA, and LgA (first 2 hr) to determine if drug intake escalated across time (session 1 – 10) as determined by significant increases over the first session of the ShA vs. LgA phase. For Experiment 2, raw ICSS current intensity thresholds (in μA) for all baseline sessions conducted after drug-administration tests began were first compared to vehicle sessions with a t-test to assess for potential injection effects. Next, ICSS current intensity thresholds were obtained following all doses, including vehicle, and converted to scores reflecting the percent change from thresholds obtained following vehicle administration for each rat. Threshold measures following vehicle treatment were calculated by averaging ICSS thresholds obtained across the 5

vehicle test days. Percentage change scores were analyzed by one-way repeated measures ANOVA.

Results

Experiment 1: Self-Administration of MDPV in 2 hr/day Sessions

For the 0.05 mg/kg dose group, a significant main effect of lever ($F[1,13]=8.67$, $p<0.01$) and session ($F[9,117]=2.64$, $p<0.01$) was observed, but a lever x session interaction was not found. Presses on the active lever were significantly greater than those on the inactive lever for sessions 4 through 10 ($p<0.01$), indicating that rats successfully learned to discriminate between active and inactive levers after 4 experimental sessions (Fig. 6a).

For the 0.1 mg/kg dose group, a significant main effect of lever ($F[1,13]=6.06$, $p<0.05$) was observed, but significant effects of session or a lever x session interaction were not observed. Presses on the active lever were significantly greater than those on the inactive lever for sessions 4 through 10 ($p<0.01$), indicating that rats successfully learned discriminate between the active and inactive levers after 4 experimental sessions (Fig. 6b).

For the 0.2 mg/kg dose group, a significant effect of lever ($F[1,16]=14.06$, $p<0.01$), session ($F[9,144]=3.872$, $p<0.001$), and a lever x session interaction ($F[9,144]=2.731$, $p<0.01$) were observed. Presses on the active lever were significantly greater than those on the inactive lever for all sessions ($p<0.01$), indicating that rats successfully discriminated between the active and inactive levers (Fig. 6c). Similar lever discrimination was observed in rats self-administering methamphetamine (data not shown).

When analyzing overall drug intake (number of drug infusions), significant main effects of MDPV dose ($F[2,24]=6.96$, $p<0.01$), session ($F[9,216]=3.791$, $p<0.01$), and a dose x session interaction ($F[18, 216]=2.15$, $p<0.01$) were observed. The overall number of infusion obtained per 2 hr session across all 10 sessions was significantly greater in the 0.05 mg/kg dose group as compared to 0.2 mg/kg dose groups ($p<0.05$) and approached significance compared to the 0.1 mg/kg dose group ($p=0.07$). Post-hoc comparisons revealed significant differences in the number of infusions obtained in the 0.05 vs. 0.1 mg/kg dose groups, and in the 0.05 vs. 0.2 mg/kg dose group for sessions 6 through 10 ($p<0.05$, Fig. 6d)

Progressive Ratio Responding

Under a PR schedule of reinforcement, a significant effect of MDPV dose ($F[2,24]=7.472$, $p<0.01$) was observed for the total number of infusions obtained prior to cessation of responding (i.e., breakpoints) (Fig. 7). Post-hoc tests revealed that the number of infusions obtained in the 0.2 mg/kg dose group were significantly greater than those in the 0.05 ($p<0.001$) and the 0.1 mg/kg ($p<0.05$) dose groups. Thus, there appeared to be positive relationship between MDPV dose and breakpoints for MDPV reinforcement. Rats self-administering methamphetamine exhibited breakpoints that were similar to those in rats self-administering the 0.05 mg/kg dose of MDPV and a t-test revealed no significant difference, $p >0.05$.

Self-Administration of MDPV during ShA vs. LgA

For the 0.05 mg/kg dose group, no significant effects of session or session length were observed (Fig. 8a). For the 0.1 mg/kg dose group, a significant effect of session length ($F[1,7]=18.644$, $p<0.01$) was observed, but no effect of session or a session length

x session interaction were found. The number of infusions obtained was significantly greater in LgA vs. ShA groups for all experimental sessions ($p<0.05$). Additionally, a significant effect of session was observed for LgA rats ($F[9,27]=2.285$, $p<0.05$), but not for ShA, such that the number of infusions obtained during sessions 8, 9 and10 were significantly greater than those observed during session 1 ($p <0.05$, Fig. 8b).

For the 0.2 mg/kg dose group (Fig. 8c), a significant effect of session length ($F[1,7]=50.209$, $p<0.001$) was observed, but no effect of session nor a session length x session interaction was observed. The number of infusions obtained was significantly greater in LgA vs. ShA groups for all experimental sessions ($p<0.01$). A significant effect of session for LgA rats was observed ($F[9,27]=2.288$, $p<0.05$), and post-hoc tests revealed that the number of infusions obtained was significantly higher during sessions 4 through 10 as compared to session 1 ($p<0.05$). Taken together, these results revealed that rats self-administering the 0.1 or 0.2 mg/kg/infusion dose of MDPV under LgA conditions displayed escalated drug intake across experimental sessions.

Additional analyses were conducted to determine if escalation of intake also occurred during the first 2 hr of 6 hr LgA sessions. No significant increases in the number of infusions during the first 2 hr of LgA sessions were evident in rats self-administering the 0.1 mg/kg dose of MDPV. However, in rats self-administering the 0.2 mg/kg dose, a significant effect of session ($F[9,36]=3.924$, $p <0.005$) was observed. Post-hoc tests revealed significant differences in the number of infusions obtained during the first 2 hr of LgA during sessions 3 through 10 as compared with session 1 ($p<0.001$). Thus, only rats self-administering the 0.2 mg/kg dose of MDPV displayed escalated drug intake during the first 2 hr of LgA.

Rats self-administering methamphetamine under LgA conditions (Fig. 8d), a significant effect of session was observed for the number of infusions obtained during the entire 6 session ($F[9,72]=7.413$, $p < 0.001$) as well as during the first 2 hrs of the LgA sessions ($F[9,72]=6.359$, $p < 0.001$) Post-hoc tests revealed significant differences in the number of infusions obtained during the entire LgA during sessions 5 through 10 as compared with session 1 ($p<0.001$), as well as significant differences in number of infusions obtained in the first 2 hr of LgA during sessions 6 through 10 as compared with session 1 ($p<0.001$).

Experiment 2: Effects of MDPV on Thresholds for ICSS

An independent samples t-test revealed no significant differences between baseline and vehicle scores ($t[58] = -1.39$, $p > 0.05$). A significant effect of MDPV dose ($F_{4,35}=11.549$, $p<0.001$) on thresholds for ICSS was observed (Fig. 9). When compared to vehicle, ICSS thresholds following MDPV administration were significantly lower at all doses tested ($p<0.05$).

Discussion

To our knowledge, this is the first systematic verification of the reinforcing effects of MDPV in rats. The current study revealed that during daily 2 hr IVSA sessions, all doses of MDPV tested maintained active lever responding across experimental sessions, and rats successfully discriminated between active and inactive levers by the 4th day of self-administration. Furthermore, significant dose effects on MDPV intake were observed as measured by the total number of infusions obtained during experimental sessions. Following stable responding on IVSA procedures, a PR test revealed a positive

relationship between MDPV dose and reinforcing efficacy, as measured by breakpoints for MDPV self-administration. Breakpoints for methamphetamine reinforcement at a dose of 0.05 mg/kg/infusion were similar to those obtained for the same dose of MDPV. Under extended access conditions (6 hr/day), an escalation of MDPV intake at the 0.1 and 0.2 mg/kg doses was observed for the entire extended access session, and this also occurred during the first 2 hr of LgA sessions for the 0.2 mg/kg dose, but not for other doses. Extended access to methamphetamine also produced escalation of drug intake. Finally, a reduction in ICSS thresholds across all doses of MDPV following acute administration was observed, indicating an increase in brain reward function.

The IVSA method was chosen for the present study given the high degree correspondence between drugs that can have addictive potential in humans and drugs that function as reinforcers in IVSA procedures in animals (Collins et al., 1983). In order to establish that a drug functions as a reinforcer in IVSA procedures, a number of criteria need to be met, including higher responding on the active vs. inactive lever, and responding must show orderly and differential effects across a range of drug doses (Meisch, 1987). The first criterion was verified across the first 10 days of IVSA procedures during which all MDPV doses maintained active lever pressing while inactive lever pressing progressively declined. These results suggest that responding occurred due to the reinforcing effects of MDPV and not as the result of any indirect locomotor or general response-enhancing effects of MDPV. The second criterion was also met when results revealed an orderly inverse dose-effect on total drug intake (i.e., number of infusions obtained) such that animals received the fewest infusions for the 0.2 mg/kg dose, followed sequentially by the 0.1 and 0.05 mg/kg doses. This inverse pattern

between dose and drug intake replicates findings of abused stimulants under continuous schedules of reinforcement, and likely represents the upper end of the typical inverse U-shaped pattern typically seen across wider dose ranges (Panlilio, 2011). In addition, the results of the present study are strikingly similar to self-administration patterns for methamphetamine under nearly identical experimental conditions and doses (present study and (Gass et al., 2009). This finding provides evidence of similar potencies between MDPV and methamphetamine. Together, these findings indicate that MDPV likely possess a potential for abuse similar to that of methamphetamine and other stimulants.

The progressive ratio schedule of reinforcement has been used extensively to evaluate the reinforcing efficacy of drugs of abuse, as it is an index of the motivation to obtain infusions of the drug in the face of increasing behavioral demand. PR schedules have consistently shown a positive relationship between dose and reinforcer efficacy, and this relationship has been consistently observed with other abused stimulants such cocaine (Roberts et al., 1989), d-amphetamine, and methamphetamine (Richardson and Roberts, 1996). The results from the present study also revealed this positive relationship between MDPV dose breakpoints for MDPV reinforcement. As with responding on the FR1 schedule above, under the same PR schedule with identical doses (0.1 and 0.2 mg/kg/infusion), breakpoints for MDPV self-administration were similar to those we and others have previously observed for methamphetamine (Gass et al., 2009; Richardson and Roberts, 1996) as well as D-amphetamine (Richardson and Roberts, 1996). In addition, breakpoints for MDPV self-administration under PR conditions at a dose of 0.05 mg/kg/infusion were similar to those observed in rats self-administering the same dose of

methamphetamine, further demonstrating methamphetamine-like potency and reinforcing efficacy of MDPV.

While demonstrating that a drug functions as a reinforcer is an important first step in determining abuse liability, such observations do not unequivocally indicate the potential for addiction potential in humans (Ahmed, 2011). One of the defining characteristics of drug addiction is an escalation in drug use, often due to tolerance to the reinforcing effects of the drug (American Psychiatric Association, 2004). As a result, a common procedure for modeling human patterns in animals has been termed the "escalation model" (Ahmed and Koob, 1998). In this procedure, animals are given extended access to the drug (typically 6 – 12 hr/day access sessions) vs. traditionally employed shorter access (1 – 2 hr/day). As a result of extended access to the drug, animals display an escalation in drug intake that parallels intake patterns characteristic of compulsive drug-seeking and addiction in humans (Ahmed, 2011). The current study revealed that, during extended access to MDPV, rats responding for the two highest doses of MDPV displayed a significant escalation in overall drug intake across the final 10 experimental sessions. Furthermore, this escalation was also seen during the first 2 hrs of LgA sessions for the high dose of 0.2 mg/kg. These findings are similar to those reported for other addictive stimulants including cocaine (Ahmed and Koob, 1998), D-amphetamine (Gipson and Bardo, 2009) and methamphetamine (Kitamura et al., 2006), and the present study also demonstrated escalation of methamphetamine intake at a dose of 0.05 mg/kg/infusion . Unlike these studies, however, our results revealed escalation of drug intake at higher rather than lower doses. These data suggest that MDPV may possess some unique reinforcing properties that are not reflective of other prototypical stimulants

such as methamphetamine. While additional comparative studies are needed to further corroborate these findings, the current results further strengthen the possibility that MDPV possesses the potential for compulsive use in humans.

Olds and Milner first discovered that rats would show a place preference for and perform an operant task to receive ICSS (Olds and Milner, 1954), and numerous studies have revealed that both ICSS and drug reinforcers likely active the same brain reward circuitry (Wise, 1996). Drug-induced lowering of ICSS thresholds is generally accepted to be due to the facilitation of brain reward functioning, providing a direct measure of the hedonic and rewarding properties of drugs of abuse (Panlilio, 2011), and nearly all abused stimulants including cocaine (Esposito et al., 1978), amphetamine (Horovitz et al., 1972), and methamphetamine (Sarkar and Kornetsky, 1995) lower ICSS thresholds. The current results reveal that, when using the discrete-trials current threshold procedure, MDPV lowers ICSS thresholds across a wide range of doses as compared to vehicle. Thus, these findings both parallel previous findings with other addictive stimulants and provide further evidence that MDPV possesses similar rewarding properties.

In summary, the current study demonstrates that the synthetic cathinone MDPV possesses potent reinforcing properties and suggests a high degree of abuse potential in humans. The results revealed that MDPV dose-dependently functions as a reinforcer on a continuous reinforcement schedule. A positive relationship between MDPV dose and reinforcer efficacy was demonstrated in during progressive ratio testing, and breakpoints for MDPV reinforcement at the lowest dose tested were similar to those for the same dose of methamphetamine. Extended access to MDPV produced escalated intake over time for the two higher doses, indicative of a compulsive pattern of intake characteristic of

addiction in humans. Finally, the ability of MDPV to lower thresholds for ICSS provides further evidence of hedonic and rewarding effects of MDPV. Taken together, these results suggest that that MDPV possesses a strong potential for compulsive use and addiction in humans. These findings have important implications for future research on synthetic cathinone addiction, as well the development of appropriate drug policies and legislative measures regarding its status as a controlled substance.

CHAPTER 5

THE REINFORCING AND REWARDING EFFECTS OF METHYLONE, A SYNTHETIC CATHINONE COMMONLY FOUND IN "BATH SALTS."

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Methylone (3,4-methylenedioxymethcathinone (MDMC), 2-methylamino-1-(3,4-methylenedioxyphenyl)propan-1-one, or *bk*-MDMA) is a member of the designer drug class known as synthetic cathinones. These emerging drugs of abuse are derivatives of cathinone, a beta-ketone amphetamine with known abuse potential (Patel, 2009) and the primary active alkaloid of the *Catha edulis* (Khat) plant (Magdum, 2011). In recent years, synthetic cathinones have become increasingly popular as "legal highs" due to online marketing, media coverage, and availability in convenience stores, head shops, and the internet (D. E. A. United States Department of Justice, 2011c). While most commonly sold as "bath salts", these drugs have been falsely sold as many different commercial products such as "plant food", "room odorizer", and "iPod cleaner", and typically contain labels stating "not for human consumption" as a means of evading regulatory controls (D. E. A. United States Department of Justice, 2011a). Desired effects of these drugs include euphoria, appetite suppression, and increases in energy, focus, libido, and empathy (Prosser & Nelson, 2012). However, an increasing number of calls to national poison control centers (American Association of Poison Control Centers, 2012) and numerous reports of toxicity (Prosser & Nelson, 2012), adverse psychological and behavioral effects (Spiller et al., 2011), and death (8-10) have been reported. While many synthetic cathinone analogues have been discovered in drug seizures, the three most common are methylenedioxypyrovalerone (MDPV), methylone, and mephedrone (D. E. A. United

States Department of Justice, 2011c). In October 2011, these three substances were temporarily classified as Schedule I substances in the United States (D. E. A. United States Department of Justice, 2011b). Interestingly, only mephedrone and MDPV were *permanently* classified as Schedule I substances with the Synthetic Drug Abuse Prevention Act in July 2012 (One Hundred Twelfth Congress of the United States of America, 2012) while the *temporary* schedule I status of methylone was extended until April, 2013 (D. E. A. United States Department of Justice, 2012). Despite these new regulatory controls, U.S. Poison Control Centers continue to receive calls regarding "bath salts" (American Association of Poison Control Centers, 2012), likely from continued abuse of mephedrone, MDPV, and methylone along with unscheduled structurally similar analogues.

While the rise in abuse of synthetic cathinones is now well documented, little information exists about the relative abuse liability of these compounds and whether consumption patterns are primarily *episodic* (i.e., recreational) or *compulsive* (i.e., characteristic of addiction). Given recent permanent scheduling for only mephedrone and MDPV, it is not surprising that most investigations of abuse potential have focused on these two synthetic cathinones, and relatively little attention has been given to methylone. We have recently shown that rats will dose-dependently maintain intravenous MDPV self-administration in short access (ShA, 2 hr/day) intravenous self-administration (IVSA) sessions. We also demonstrated significant dose effects for reinforcer efficacy (i.e., breakpoints) between each of the doses tested (0.05, 0.1, and 0.2 mg/kg/infusion) on a progressive ratio schedule of reinforcement. Finally, under long access (LgA, 6 hr/day) conditions, rats in the two highest dose groups also displayed escalated MDPV intake

suggesting the potential for compulsive use in humans. The reinforcing effects of MDPV were complemented with significant dose-dependent reductions in ICSS thresholds, indicative of hedonic and rewarding properties (Watterson et al., 2014). With regards to mephedrone, studies by other investigators have shown that rats will self-administer mephedrone intravenously under ShA conditions (Hadlock et al., 2011), and in mice mephedrone elicits conditioned place preference (CPP) and increases in locomotor activity (Kehr et al., 2011), leads to locomotor sensitization (Lisek et al., 2012), and lowers ICSS thresholds (J. Robinson, Agoglia, Fish, Krouse, & Malanga, 2012).

The existing behavioral and neurochemical data suggest that methylone may possess the potential for compulsive use. Behavioral studies have shown that methylone elicits CPP at doses of 2.5 mg/kg or higher in mice (Miyazawa, Kojima, & Nakaji, 2011) and substitutes for 3,4-methylenedioxymethamphetamine (MDMA) in a drug-discrimination paradigm (Dal Cason, Young, & Glennon, 1997). Furthermore, methylone possesses psychomotor stimulant effects in mice, but to a lesser extent compared to methamphetamine (Dal Cason et al., 1997; Marusich, Grant, Blough, & Wiley, 2012).

As mentioned above, synthetic cathinones are similar in chemical structure to amphetamines. Methylone is the benzylic ketone analog of MDMA and, not surprisingly, has been shown to have similar neurochemical effects on monoamine transporters. Uptake inhibition studies have reported that methylone blocks the reuptake of norepinephrine, dopamine, and serotonin plasma membrane transporters (NET, DAT, and SERT, respectively) with a profile similar to that of methamphetamine and MDMA, but with greater potency than methamphetamine for SERT, and three-fold less potency for SERT compared to MDMA (22,23). Methylone has also been shown to be a less potent at

inhibiting the vesicular monoamine transporter 2 (VMAT2) compared to methamphetamine, MDMA (Cozzi et al., 1999), and mephedrone (López-Arnau, Martinez-Clemente, Pubill, Escubedo, & Camarasa, 2012). However, while uptake assays suggest that methylone functions as a transporter blocker, these assays are unable to discern between drugs that are transporter blockers versus those that are monoamine releasing agents, as tissue accumulation of radiolabeled transmitters is decreased by both drug types (Baumann et al., 2012). However, additional studies have clarified these discrepancies and reveal that methylone is a non-selective monoamine releaser with properties similar to MDMA (Baumann et al., 2012; Nagai, Nonaka, & Satoh Hisashi Kamimura, 2007). Also, in comparison to MDMA, methylone produces qualitatively similar, but less potent, increases in extracellular monoamine levels in the nucleus accumbens (Baumann et al., 2012).

These neurochemical effects, along with the few behavioral studies outlined above, suggest that methylone may possess the potential for compulsive use. However, to our knowledge, there are no published reports showing that laboratory animals will acquire intravenous self-administration of methylone or if extended access to methylone (i.e., 6 hr/day) leads to escalated drug intake, a consumption pattern predictive of compulsive use in humans (Ahmed, 2012). The present study examined whether methylone would support IVSA at doses of 0.05, 0.1, 0.2, or 0.5 (mg/kg/infusion) under short (ShA, 2 hr/day) and long (LgA, 6 hr/day) access conditions. A separate group of animals was tested for effects of methylone (0.1-10 mg/kg i.p.) on current intensity thresholds for ICSS.

Method

Subjects

All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee at Arizona State University and according to the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health. Male Sprague-Dawley rats (n=48), weighing approximately 250 g upon arrival, were implanted with jugular vein catheters at Harlan Laboratories (Livermore, CA, USA) and used for intravenous self-administration procedures. An additional 4 non-catheterized male Sprague-Dawley rats, weighing approximately 250 g (Harlan Laboratories) were used for ICSS procedures. Upon arrival, all rats were individually housed on a 12-hour light-dark cycle and provided *ad libitum* access to food and water during all procedures, except during surgical and behavioral testing procedures. All experimental procedures were conducted during the dark phase with the exception of 16 hr overnight progressive ratio tests which began at approximately 4:00 PM and ended the following morning at approximately 8:00 AM.

Drugs

Methylone was synthesized by the Department of Discovery and Analytical Sciences at Research Triangle Institute (RTI) International (Research Triangle Park, NC, USA). Methylone was dissolved in sterile physiological saline for intravenous self-administration and intraperitoneal administration.

Experiment 1: IVSA Procedures

Surgical Procedures

Following one day of acclimation to housing conditions, rats were anesthetized with isoflurane (2% v/v) vaporized oxygen at a flow rate of 2 l/min. A 2.5-cm longitudinal incision was made between the scapulae for implantation of a threaded vascular access port (Plastics One, Roanoke, VA, USA). A mesh collar attached to the port was sutured underneath the surrounding tissue within the incision. Access ports were sealed with a piece of Tygon tubing and protective cap. Rats received one week of post-operative care including daily infusions of 0.4 ml Timentin (66.6 mg/ml, in 70 U/ml heparinized saline) to protect against infection and ensure catheter patency. Meloxicam (2.5 mg/kg, s.c.) was administered for the first 3 days following surgery procedures to provide relief from post-surgical discomfort. Rats also received approximately 8–10 pieces of a sweetened cereal in their home cage each day during the recovery period to minimize post-surgical weight loss.

Apparatus

Operant drug self-administration sessions were conducted in modular self-administration chambers (ENV-008, Med Associates, St. Albans, VT, USA). All self-administration chambers were located inside sound-attenuating cubicles containing a house light and exhaust fan designed to mask external noise and odors, and were interfaced to a personal computer. Chambers contained two stainless steel response levers located on one wall with a 4.2 x 5 cm food pellet receptacle placed between the levers. Response levers were located approximately 7 cm above the grid floor and positioned above each lever was a 2.5-cm diameter white stimulus light. Located near the top of the chambers was a Sonalert speaker that provided an auditory stimulus during drug delivery. Syringe pumps were located outside each chamber, interfaced to a PC

computer, and delivered methylone solution via a single-channel liquid swivel mounted atop the chambers via polyethylene tubing.

Experimental Design: IVSA Procedures

Following recovery from surgical procedures, rats began experimental sessions and were allowed to spontaneously acquire intravenous self-administration in 2-hour daily (ShA) sessions for 21 days. IVSA procedures were conducted 7 days a week as described elsewhere (13,27). Briefly, active lever presses delivered the drug reinforcer on an FR1 schedule of reinforcement. Methylone was delivered to the vascular access port by polyethylene tubing housed in a stainless steel spring tether that was attached to a liquid swivel. Reinforcers were accompanied by activation of a stimulus light and tone complex for 2 sec, followed by a 20-sec timeout period during which additional lever presses were recorded but produced no consequences. Inactive lever presses were also recorded, but produced no programmed responses at any time during the experiment. Rats were randomly assigned to one of four groups based upon methylone dose (0.05, 0.1, 0.2, or 0.5 mg/kg per infusion). Each drug infusion was delivered in a volume of 0.06 ml. Both before and after each IVSA session, access ports were flushed with 0.2 ml Timentin (66.6 mg/ml, in 70 U/ml heparinized saline) to protect against infection and ensure catheter patency.

Following 21 days of ShA IVSA, rats were in a 16 hr overnight progressive ratio (PR) sessions. For PR sessions, methylone was delivered on a schedule determined by the following equation: responses per reinforcer delivery $=5 \times e^{(\text{injection number} - 0.2)} - 5$ (i.e. 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, etc.) (N. R. Richardson & Roberts, 1996). Breakpoints were considered to be obtained when rats did not emit any active lever presses for 2 hr.

Following PR tests, all rats were placed into 6 hr LgA sessions on a FR1 schedule for 10 additional days to assess escalation of drug intake. Finally, following 10 days of LgA sessions, rats were placed into an additional PR session to assess any changes in reinforcer efficacy following extended access. For both PR and LgA sessions, all aspects of IVSA sessions were identical except for session length and number of lever presses required for an infusion.

Based on earlier MDMA self-administration studies (Schenk et al., 2007), methylone was considered to function as a reinforcer for individual rats when responding on the active lever exceeded 10 lever presses per session. Figure 11b shows the percentage of rats for each dose group that responding greater than 10 times on the active lever for each experimental session.

Experiment 2: ICSS Procedures

Surgical Procedures

Rats were anesthetized with isoflurane (2% v/v) vaporized oxygen at a flow rate of 2 l/min and placed into a stereotaxic frame. A twisted stainless steel bipolar electrode (PlasticsOne, Roanoke, VA, USA; 2 mm diameter, insulated except at the ventral tip) was implanted into the medial forebrain bundle (anterior-posterior -0.05 mm; medial-lateral, 1.7 mm, dorsal-ventral, -8.3 mm from dura and bregma). Four skull screws and dental cement were used to permanently secure electrodes to the skull. To counterbalance for any hemispheric differences, half of the animals received electrodes in the left hemisphere and the other in the right hemisphere. Following surgery, the rats were given 7 days of recovery prior to commencement of ICSS procedures, during which they

received daily injections of 2.5 mg/ml meloxicam (0.15 ml volume) to minimize post-surgical discomfort.

Apparatus

All ICSS testing was conducted in modular chambers (ENV-007CT; Med Associates). Chambers were housed inside sound-attenuating cubicles equipped with an exhaust fan to mask external noise and odors. Chambers contained front wall mounted nose-poke aperture with light-emitting diode (LED) stimulus lights located inside the access hole (ENV-114M; Med Associates). The nosepoke aperture was 2.5 cm in diameter, located 5 cm above the stainless steel grid floor, and contained an infrared detector placed 0.64 cm from the front edge of the panel for recording responses. Located outside the chamber was a dual programmable ICSS stimulator (PHM-150B/2; Med Associates) that was interfaced to a PC which delivered electrical current to the electrode. MED-PC IV software was used to control all stimulation parameters, test functions, and data collection.

Experimental design: ICSS procedures

The procedures for determination of ICSS thresholds was a modified version of the discrete trials current-threshold method (Kornetsky et al., 1979; Markou & Koob, 1992). For a detailed review of the procedures used here, please see methods in (Watterson et al., 2014). Briefly, following acquisition procedures, reward threshold training commenced and rats were tested for a minimum of 10 days until stable baseline levels of reward thresholds were achieved (defined as when the average of thresholds for the last 4 days minus the first 4 days of an 8-day window was less than 10% of the average of the full 8 days). Rats continued to undergo baseline (i.e., no drug

administration) testing throughout the course of experiments every three days. Rats received vehicle injections 20 min prior to ICSS threshold determination procedures every 3 days. Methylone doses (0.1, 0.5, 1, 3, 5, and 10 mg/kg, i.p.) were assigned randomly and injections given 20 min prior to threshold determination procedures every three days. All rats received 4 to 5 threshold determinations at each dose of methylone and at least 10 threshold determinations following administration of saline vehicle.

Statistical Analysis

All statistical analyses were conducted using SPSS version 20 (Armonk, NY, USA). All data points represent the mean ± standard error of the mean (SEM). A significance criterion of $p<0.05$ was used for all analyses. For Experiment 1 during the initial 21 days of self-administration procedures, individual methylone doses were analyzed by a mixed analysis of variance (ANOVA) with lever (active versus inactive) as between measures factors and session number (1–21) as the repeated measures factor. Post-hoc one-way ANOVAs were conducted to determine when successful lever discrimination occurred. The total number of infusions obtained during experimental sessions was also analyzed with a mixed ANOVA with methylone dose (0.05, 0.1, 0.2, and 0.5 mg/kg) as between measures factors and session number (1-21) as the repeated measures factor. Holm-Sidak post-hoc tests further analyzed dose effects for each session. The total number of infusions obtained during PR tests was analyzed in a mixed ANOVA with dose as the between measures and PR tests (before and after LgA) as the repeated measures with Holm-Sidak post-hoc tests. For LgA sessions, the total number of infusions obtained during experimental sessions was analyzed with a mixed ANOVA with methylone dose and session number (1-10) as the repeated measures factor.

Furthermore, one-way repeated measures ANOVAs were conducted for each dose separately to analyze for escalation of drug intake across experimental sessions. Data from rats removed from the study due to overdose or loss of catheter patency were removed from statistical analyses. For Experiment 2, raw ICSS current intensity thresholds (in μA) obtained following all doses and vehicle were converted to scores reflecting the percent change from mean baseline scores obtained following stabilization for each rat. Threshold measures following baseline days were calculated by averaging ICSS thresholds obtained across all baseline days following initial stabilization. Percentage change scores were analyzed by one-way repeated measures ANOVA.

Results

Lever Discrimination During ShA

Throughout the course of the study, 3 of 48 rats were removed from experimental procedures due to catheter patency failure. Also, 2 additional rats in the 0.5 mg/kg/infusion group died on LgA days 7 and 10, respectively, presumably due to overdose. For the 0.05 mg/kg dose group (as shown in Fig. 10a), significant main effects of lever ($F[1,21]=8.54$, $p<0.01$) and session number ($F[20,420]=2.07$, $p<0.01$) were observed, as well as a significant lever X session number interaction ($F[20,420]=2.73$, $p<0.001$). Presses on the active lever were significantly greater than on the inactive lever for sessions 3–21 ($p<0.05$) indicating that rats in the 0.05 mg/kg dose group successfully discriminated between the active and inactive levers after 3 experimental sessions.

For the 0.1 mg/kg dose group (as shown in Fig. 10b), significant main effects of lever ($F[1,20]=8.59$, $p<0.01$) and session number ($F[20,400]=2.15$, $p<0.01$) were

observed, as well as a significant lever X session number interaction ($F[20,400]=2.48$, $p<0.001$). Presses on the active lever were significantly greater than on the inactive lever for sessions 6–21 ($p<0.05$) indicating that rats in the 0.1 mg/kg dose group successfully discriminated between the active and inactive levers after 6 experimental sessions.

For the 0.2 mg/kg dose group (as shown in Fig. 10c), significant main effects of lever ($F[1,20]=6.07$, $p<0.05$) and session number ($F[20,400]=3.03$, $p<0.001$) were observed, as well as a significant lever X session number interaction ($F[20,400]=4.49$, $p<0.001$). Presses on the active lever were significantly greater than on the inactive lever for sessions 8–21 ($p<0.05$) indicating that rats in the 0.2 mg/kg dose group successfully discriminated between the active and inactive levers after 8 experimental sessions.

For the 0.5 mg/kg dose group (as shown in Fig. 10d), significant main effects of lever ($F[1,22]=30.42$, $p<0.05$) and session number ($F[20,440]=6.25$, $p<0.001$) were observed, as well as a significant lever X session number interaction ($F[20,440]=7.14$, $p<0.001$). Presses on the active lever were significantly greater than on the inactive lever for sessions 2–21 ($p<0.05$) indicating that rats in the 0.1 mg/kg dose group successfully discriminated between the active and inactive levers after 2 experimental sessions.

Infusions During ShA

For overall methylone intake (as determined from the total number of drug infusions obtained, Fig. 11a) significant main effects of methylone dose ($F[3,41]=6.477$, $p<0.001$) and session ($F[20,820]=25.67$, $p<0.001$) were observed, along with a significant dose X sessions interaction ($F[60,820]=4.579$, $p<0.001$). The overall number of methylone infusions obtained per 2 hr session across all 21 sessions was significantly greater in the 0.5 mg/kg group versus the 0.05 mg/kg group ($p<0.01$) and the 0.1 mg/kg

group ($p<0.01$). Post-hoc tests revealed a greater number of infusions obtained at the 0.5 mg/kg dose versus the 0.05 and 0.1 mg/kg dose group for days 11–21 ($p<0.05$).

Progressive Ratio Tests

Analysis of breakpoints (Fig. 12a) during PR sessions revealed significant main effects of methylone dose ($F[3,37]=9.209$, $p<0.001$) and PR session ($F[1,37]=34.691$, $p<0.001$). However, a significant dose X PR session interaction was not observed ($F[3,37]=1.166$, $p>0.05$). For all doses tested, breakpoints decreased significantly from the first PR test (5.93 ± 0.52, mean ± SEM) to the PR test following LgA (3.34 ± 0.33, mean ± SEM). Post-hoc tests revealed that the total number of infusions obtained in the 0.5 mg/kg dose group was significantly greater than that of the 0.05 mg/kg ($p<0.001$), 0.1 mg/kg ($p<0.001$), and 0.2 mg/kg doses ($p<0.01$). While there were no significant differences observed among the three lower doses, there did appear to be a positive relationship between methylone dose and breakpoints.

Assessment of Escalated Intake During LgA

Analysis of the total number of infusions obtained during LgA sessions (Fig. 12b) revealed a significant main effect of dose ($F[3,38]=7.035$, $p<0.001$). However, there was no significant main effect of session ($p>0.05$), and a dose X session interaction only revealed a trend towards significance ($F[27,342]=1.466$, $p=0.06$). Pairwise comparisons revealed significant overall differences between the 0.05 mg/kg vs. the 0.2 and 0.5 mg/kg doses ($p<0.05$ and 0.001, respectively). No other pairwise comparisons between doses were significant. Analysis of escalation for each dose independently did not reveal escalated intake across experimental sessions for the 0.1 mg/kg and 0.2 mg/kg doses ($p>0.05$). Significance was obtained for both the 0.5 mg/kg ($F[9,81]=2.315$, $p<0.05$) and

0.05 mg/kg doses ($F[9,81]=4.829$, $p<0.05$). However this occurred as a result of reduced numbers of infusions across the 10 LgA sessions. For the 0.5 mg/kg dose group, pairwise comparisons did not reveal significant differences between individual sessions. For the 0.05 mg/kg group, pairwise comparisons revealed that the significance occurred only between day 1 and day 2 ($p<0.01$). No other pairwise comparisons were significant.

Assessment of ICSS Thresholds

Repeated measures ANOVA did not reveal a significant effect of methylone dose on ICSS thresholds (Fig. 13), however a trend was observed ($F[7,163]=1.783$, $p=0.09$).

Evidence of Toxicity

In addition to the IVSA and ICSS results outlined above, it is also important to mention that during LgA sessions, a number of adverse effects of methylone self-administration were observed. The most common adverse effects were porphyrin staining and foaming at the mouth that typically began during sessions 3-4 of LgA conditions. These effects were observed in nearly all animals in the 0.5 mg/kg and roughly half of the 0.2 mg/kg group during the course of LgA procedures and, once manifested, typically continued until completion of the experiment. Additionally, two rats in the 0.5 mg/kg group self-administered methylone to the point of seizure (after 114 and 138 total infusions each), and despite being immediately removed from the self-administration chamber, died within 20 mins of removal.

Discussion

The present study revealed that methylone serves as a reinforcer as rats dose-dependently acquired IVSA of methylone through spontaneous acquisition procedures.

In Experiment 1, during 21 days of 2-hr daily access sessions, orderly dose-dependent differences in overall drug intake were observed across groups and rats successfully discriminated between active and inactive levers by days 3, 6, 8, and 2 for the 0.05, 0.1, 0.2, and 0.5 mg/kg per infusion groups, respectively. These findings indicate that responding occurred due to the reinforcing effects of methylone and not from any non-specific response-enhancing effects of methylone. This study further revealed a positive dose-dependent relationship between methylone dose and reinforcer efficacy as measured by breakpoints obtained during PR sessions both prior to and following LgA. Furthermore, while methylone intake was greater in LgA when compared to asymptotic responding during ShA sessions, none of the dose groups displayed escalated drug intake across experimental sessions. Finally, Experiment 2 revealed that methylone did not significantly decrease ICSS thresholds, suggesting a lack of effect on brain reward function.

To our knowledge, this study is the first to systematically verify that methylone serves as a reinforcer in the IVSA paradigm in drug-naïve animals. To date, discussions about reinforcing effects and abuse liability of synthetic cathinones have largely come from its comparison to amphetamine-type stimulants such as methamphetamine and MDMA. Most often, methylone has been compared to MDMA due to its similar chemical structure, similar in vitro binding and in vivo neurochemical data, and the early reports that methylone produced subjective effects similar to MDMA, but lacked the "unique magic" produced by MDMA (Bossong, Van Dijk, & Niesink, 2005). Given this precedent, a comparison of the current results to previous work with MDMA serves as a logical starting point. However, it is important to state from the outset that comparisons

of the present results with earlier findings must be interpreted with caution, as each of these studies employed somewhat different experimental procedures. Initial MDMA IVSA experiments by multiple investigators that revealed that MDMA functions as a reinforcer in rats. However, inconsistent and low response rates indicated that MDMA was a weak-to-moderate reinforcer since only a subset of animals acquired self-administration (Bossong et al., 2005; Cole & Sumnall, 2003). Additional IVSA studies with rhesus monkeys and baboons also revealed similar weak-to-moderate reinforcing properties (Beardsley, Balster, & Harris, 1986; Fantegrossi et al., 2004). Later studies by Schenk and colleagues found that in a subset of rats, MDMA could support higher rates of self-administration than those observed in earlier reports (Schenk et al., 2007; Schenk, Colussi-Mas, Do, & Bird, 2012; Schenk, Gittings, Johnstone, & Daniela, 2003), corroborating reports in humans that compulsive use is possible in certain individuals (Jansen, 1999). When compared to the collective results from these studies of MDMA self-administration, methylone appears to support more robust self-administration responding than does MDMA, albeit with a rightward shift in the inverted dose-effect curve. All methylone doses tested in the present study supported IVSA, and lever-discrimination occurred for all rats on days 3, 6, 8, and 2 for the 0.05, 0.1, 0.2, and 0.5 mg/kg/infusion groups, respectively. Furthermore, as revealed in Fig. 11b, while only about 40-60% of rats pressed the active lever more than 10 times a session for the 0.05 mg/kg group between sessions 15-21, the 0.1, 0.2, and 0.5 mg/kg/infusion groups demonstrated group acquisition percentages that were approximately 60, 80, and 100%, respectively. Thus, at the higher doses, a larger percentage of rats acquired methylone IVSA in 2 hr daily sessions than rats self-administering MDMA in 6 hr daily access

sessions (i.e., 60% for both the 0.25 mg/kg/infusion and 1 mg/kg/infusion MDMA dose groups) using nearly identical acquisition criterion (>10 lever presses per session) (Schenk et al., 2007). More recent work by Schenk and colleagues revealed that across 25 days of 2 hr IVSA sessions MDMA (1.0 mg/kg/infusion), only 49% (63 of 128 rats) acquired a total of ≥ 90 infusions across experimental sessions (Schenk et al., 2012). The present study revealed that only 5 rats failed to accumulate ≥ 90 infusions by the end of session 21, with 3 of those rats being in the 0.05 mg/kg group, 1 in the 0.1 mg/kg group, 1 in the 0.2 mg/kg group, and 0 in the 0.5 mg/kg group (data not shown). Together, these results are also consistent with previous studies showing that lower doses of methylone (≥2.5 mg/kg/i.p.) vs. MDMA (≥9 mg/kg/i.p.) elicit conditioned place preference in mice (Miyazawa et al., 2011; Robledo, Balerio, Berrendero, & Maldonado, 2004; Salzmann, Marie-Claire, Le Guen, Roques, & Noble, 2003; Tzschentke, 2007)(19,41-43).

In addition, it is also important to mention differences in lever pressing behavior for methylone observed in the present study as compared to our previously findings with MDPV (Watterson et al., 2014), as these are the first two published studies to establish initial dose-effect curves for IVSA of these two synthetic cathinones. Specifically, the highest methylone dose tested in the present study (0.5 mg/kg/infusion) lead to a maximum number of lever press of approximately 100 after roughly twenty 2 hr IVSA sessions. In our previous MDPV study, the lowest dose tested (0.05 mg/kg/infusion) lead to approximately 200 active lever presses after only seven 2 hr IVSA sessions. Thus, while future studies must establish full IVSA dose-effect curves before direct comparisons can be made between methylone and MDPV, our initial results suggest that MDPV is a much more potent reinforcer than methylone.

While our 2 hr IVSA acquisition data suggest stronger reinforcing properties of methylone compared to MDMA, our PR data appear similar to those obtained from previous studies on PR responding for MDMA. For example, the total number of infusions obtained for MDMA doses of 0.25 and 1.0 mg/kg/infusion were approximately 4.5 and 12.5, respectively (Schenk et al., 2007), whereas our present methylone results with (0.2 and 0.5 mg/kg/infusion) revealed similar breakpoints with approximately 5.5 and 9.5 infusions, respectively. When compared to previous results with MDPV and prototypical stimulants d-amphetamine and methamphetamine, and using an identical PR procedures and doses (0.05, 0.1, and 0.2, mg/kg/infusion), methylone breakpoints in the present study were comparatively much lower (Gass et al., 2008; N. R. Richardson & Roberts, 1996; Watterson et al., 2014). Specifically, methylone breakpoints for these doses were approximately 4, 5, and 5.5, respectively, whereas our MDPV breakpoints were approximately 8, 10, and 15, respectively. Thus, the reinforcer efficacy of methylone appears to be significantly lower than that of MDPV. In addition to the initial progressive ratio tests following ShA procedures, the PR tests following extended access revealed similar dose effects, but compared to initial PR tests, the overall reinforcing efficacy was lower during the second test. This apparent decease in motivation to seek methylone following LgA is in contrast to previous work with cocaine (Paterson & Markou, 2003) and methamphetamine (Sunmee Wee, Wang, Woolverton, Pulvirenti, & Koob, 2007) which have been shown to elicit greater PR responding following extended access. To our knowledge, PR data following LgA has not been reported for MDMA. These results suggest that methylone likely possesses a reinforcer efficacy that more

closely resembles MDMA and is similarly weaker compared to other prototypic stimulants (Ahmed, 2012)..

In addition to the results obtained during ShA and PR procedures, LgA sessions did not lead to escalated drug intake across experimental sessions. Our previous findings of responding for MDPV reinforcement under extended access conditions revealed escalated intake across LgA sessions (Watterson et al., 2014), similar to previous findings with cocaine and methamphetamine (Ahmed & Koob, 1998; Kitamura et al., 2006). While others have found evidence of escalated MDMA intake (Schenk, 2009), this phenomenon only occurred with extended testing, and to our knowledge, there are no reports of escalated MDMA intake across experimental LgA following prior asymptotic responding on ShA. While none of the doses of methylone tested here led to escalated intake, it is possible that escalation of intake might occur with higher doses of methylone (i.e., 1.0 mg/kg/infusion), as we have shown that only higher doses of the MDPV produce escalation of intake (Watterson et al., 2014). Furthermore, while these data suggest that the potential for *compulsive* use of methylone in humans appears less likely than prototypic stimulants, replication of these results with additional animal experiments, as well as human studies, are ultimately needed before conclusions about abuse liability can be made.

Despite our non-significant ICSS results, a trend towards dose-dependent threshold decreases suggest that methylone may possesses hedonic properties, corroborating reports of euphoric subjective effects in humans (Spiller et al., 2011; Warrick et al., 2012). Furthermore, while it could be argued that higher doses and/or greater experimental power (observed power in the present study was 0.326) may have

yielded significance, the decrease in reward thresholds produced by methylone here appear similar, but slightly weaker in magnitude, to those previously reported for MDMA (Hubner, Bird, & Rassnick, 1988; Lin, Jackson, Atrens, Christie, & McGregor, 1997), and are much less robust than decreases reported for cocaine, d-amphetamine, methamphetamine, and MDPV (Vlachou & Markou, 2011; Watterson et al., 2014). In the present study, the highest dose of methylone (10 mg/kg) produced threshold reductions that were smaller (13%) than the lowest dose (0.1 mg/kg) of MDPV (16%) previously tested under identical ICSS procedures (Watterson et al., 2014). Thus, while our self-administration data suggests that methylone functions as a stronger reinforcer than MDMA, our ICSS data suggest similar or weaker rewarding properties compared to MDMA. This effect is somewhat surprising in light of the more robust self-administration and stronger rewarding effects of methylone, as revealed in CPP studies mentioned above, as compared to MDMA. Thus, replication of these findings is needed before definitive conclusions can be reached.

In addition to the aforementioned measures of abuse liability, rats in the two higher dose groups showed signs of toxicity including porphyrin staining, foaming at the mouth, and death. For the two rats in the 0.5 mg/kg/infusion group which self-administered methylone to the point seizure and death, the total infusions obtained during this session were 114 and 138. Interestingly, both of these subjects had previously obtained a higher number of infusions in earlier LgA sessions, the highest being 214 and 198, respectively. Thus, these fatalities appear to be the result of repeated methylone self-administration and not necessarily the acute effects of a single high dose. These observations reveal the need for further studies regarding the toxic effects of methylone

that may provide additional information about various reports of toxicity and death associated with methylone use in humans (Cawrse et al., 2012; Pearson et al., 2012; Warrick et al., 2012).

Overall, these results fit with previous research showing that the ratio of dopamine-serotonin release induced by psychostimulants is positively correlated with self-administration patterns, ICSS threshold-lowering ability, and addiction liability (Bauer, Banks, Blough, & Negus, 2013a; Rothman & Baumann, 2003; Schenk et al., 2007; S Wee et al., 2005). The *in vitro* release data from Baumann (2011) revealed a DAT/SERT transporter mediated release for methylone (1.82) to be similar to MDMA (0.97), along with qualitatively similar microdialysis release data. In contrast, methamphetamine and d-amphetamine DAT/SERT ratios are 152.0 and 219.5, respectively (Baumann et al., 2012; S Wee et al., 2005). While our results generally conform to this hypothesis, the results here also demonstrate the importance of behavioral experiments in assessing pharmacological nuances not explicitly revealed in neurochemical assays. Prior to the current study, methylone was primarily compared to MDMA and predicted to exert similar effects. However, our data revealed more robust self-administration during 2 hr sessions than previously shown for MDMA, predictive of a greater addiction liability and suggestive of other possible neurochemical differences between methylone and MDMA not accounted for in previous monoamine assays. Furthermore, given the lack of escalation in LgA, relatively weak PR responding, and weak variable ICSS results, our study demonstrates the importance of testing beyond basic self-administration. The lack of escalation during LgA and weak variable ICSS

effects may be reflective of lower dopamine-serotonin ratios and more indicative of lower compulsive use liability (episodic vs. compulsive use).

Finally, it is also important to mention some limitations of the current study. The primary limitation of the current study is a relatively small number of subjects in both the IVSA experiments (n=48) and ICSS experiments (n=4). One of the main conclusions made from the current study is that methylone possesses a relatively low abuse liability given the lack of escalation in LgA. While it does not appear likely that any dose group would display significant escalated intake, it is possible that with additional subjects, escalated intake might have been observed in a subset of animals. Further studies are needed to evaluate this possibility. In addition, ICSS experiments were performed with only 4 rats, and it is possible that additional subjects would have yielded statistical significance, as only a trend towards significant (p=0.09) was observed. Another limitation of the current study is that is was conducted with drug-naïve animals. While demographic information regarding methylone users is scarce, it is possible that individuals with previous experience with illicit stimulants may be more sensitized to the reinforcing properties methylone. These possibilities warrant further investigation. Finally, it is important to reiterate that while our results suggest more potent reinforcing properties of methylone as compared to MDMA, and weaker reinforcing properties compared to MDPV and other prototypic stimulants, our study is the first to demonstrate methylone IVSA. Thus, replication of our initial IVSA results, as well as additional studies directly comparing methylone to MDMA and other psychostimulants, is needed before definitive conclusions can be made.

In general, the IVSA results from the present study reveal that MDMA functions as a moderate reinforcer that appears stronger than MDMA given the more rapid rate and greater percentage of rats acquiring self-administration compared to previous MDMA self-administration studies. However, the weak PR responding and lack of escalated methylone intake in LgA indicate that methylone is weaker than prototypic stimulants. These results are complimented by our ICSS results which reveal a trend towards lowering ICSS thresholds similar to previous studies on MDMA. These results provide initial evidence which suggests that methylone possesses an abuse liability similar to or slightly greater than MDMA, but significantly lower other prototypic stimulants. In humans, MDMA is generally considered to have a low *addiction* liability as consumption patterns are generally intermittent rather than compulsive (De La Garza, Fabrizio, & Gupta, 2007). This is not without exception, however, as MDMA dependence has been reported in some individuals (Jansen, 1999). Extrapolating from our results, one would predict that methylone dependence may be possible in a subset of individuals, but that consumption patterns would also generally stay intermittent and typically not advance to compulsive use. However, this conjecture requires validation from additional human experimental and epidemiological research, and definitive conclusions about human consumption patterns cannot be made at this time. Nonetheless, our findings provide an initial behavioral characterization of the reinforcing and rewarding effects of methylone and have important implications for future synthetic cathinone research, treatment specialists, and the development of appropriate regulatory policies.

CHAPTER 6

EFFECTS OF α-PYRROLIDINOVALEROPHENONE (α-PVP) AND 4-METHYL-N-ETHYLCATHINONE (4-MEC), TWO SYNTHETIC CATHINONES COMMONLY FOUND IN SECOND-GENERATION "BATH SALTS", ON ICSS THRESHOLDS IN RATS.

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For the first time in history, the number of unregulated novel psychoactive substances on international drug markets now exceeds those under international control (United Nations, 2013). One of the most problematic classes of novel psychoactive substances to emerge are synthetic cathinones, comprising approximately 18% of all unregulated substances in international markets (United Nations, 2013). Synthetic cathinones first appeared in Europe in the mid 2000's and in the United States around 2009, and their use led to numerous reports of abuse, bizarre behavior, toxicity, and death (Prosser & Nelson, 2012; Rosenbaum et al., 2012). The rise in popularity of synthetic cathinones is linked to their ease of procurement over the internet, and in gas stations, smoke shops, and novelty stores (Spiller et al., 2011). Synthetic cathinones have been falsely marketed as numerous products, the most recognizable being "bath salts", and are typically labeled "not for human consumption" or "for research purposes only" in order to evade drug laws (N. D. I. C. United States Department of Justice, 2011). While many synthetic cathinone derivatives exist, 3,4-methylenedioxypyrovalerone (MDPV), mephedrone, or methylone initially comprised approximately 98% of all synthetic cathinones encountered in U.S. drugs seizures (D. E. A. United States Department of

Justice, 2011a). Citing imminent threats to public health and safety, the U.S. DEA used their emergency scheduling authority to temporarily classify these three drugs (now often referred to as first-generation bath salts) as Schedule I substances in October of 2011 (D. E. A. United States Department of Justice, 2011b). As of 2013, these first-generation synthetic cathinones are now permanently classified as Schedule I substances in the U.S. (One Hundred Twelfth Congress of the United States of America, 2012; D. E. A. United States Department of Justice, 2013a).

On January 22, 2013, the U.S. DEA published a request for information specifically regarding 8 additional synthetic cathinones, two of the most prominent being 4-methyl-N-ethcathinone (4-MEC) and alpha-pyrrolidinopentiophenone (α-PVP) (DEA, 2013). Their similarity in chemical structure suggests that α-PVP and 4-MEC likely emerged as replacements for MDPV and mephedrone, respectively (see Fig. 14).

While literature regarding the neurochemistry, toxicology, and abuse liability of first generation synthetic cathinones has emerged in recent years (Spiller et al., 2011; Coppola and Mondola, 2012; Baumann et al., 2013; Simmler et al., 2013; Watterson et al., 2013; Watterson and Olive, 2014), relatively little information exists regarding second-generation analogues such as α-PVP and 4-MEC (D. E. A. United States Department of Justice, 2013b). With regards to abuse liability, the potential for compulsive use (i.e. addiction) of stimulant drugs generally increases as dopamine to serotonin transporter (DAT/SERT) reuptake (IC_{50} values) and/or release (EC_{50} values) ratios increase (i.e. synaptic levels of DA are greater than 5-hydroxytryptamine, 5-HT). On the other hand, higher SERT/DAT ratios are generally associated with more entactogenic effects and episodic abuse patterns (Bauer et al., 2013a; Rothman &

Baumann, 2003, 2006). Studies have revealed that α-PVP is a potent dopamine and norepinephrine transporter (DAT and NET, respectively) inhibitor, has relatively little affinity for the serotonin transporter (DAT/SERT IC_{50} >781) (Marusich et al., 2014; Meltzer et al., 2006), increases extracellular dopamine (DA) release in the striatum (Kaizaki, Tanaka, & Numazawa, 2014), and has locomotor enhancing properties similar to MDPV (DAT/SERT $IC_{50} \approx$ 806-816) and methamphetamine (DAT/SERT $IC_{50} \approx$ 10 – 25; DAT/SERT $EC_{50} \approx$ 152) (Baumann, Partilla, Lehner, et al., 2013; Kaizaki et al., 2014; Marusich et al., 2014; Rothman & Baumann, 2003). On the other hand, *in vitro* assays have shown that 4-MEC inhibits the reuptake of DAT, NET and SERT with approximately equal affinity, but is also acts as 5-HT releaser with a similar DAT/SERT ratio (DAT/SERT $IC_{50} \approx$ 1.85) (Iversen et al., 2013; Simmler, Rickli, Hoener, & Liechti, 2014) to methylone (DAT/SERT $IC_{50} \approx$ 2; DAT/SERT $EC_{50} \approx$ 1.82; Baumann et al., 2012a) and 3,4-methylenedioxymethamphetamine (MDMA; DAT/SERT $EC_{50} \approx$ 0.97; Baumann et al., 2012a). To our knowledge, the effects of 4-MEC on locomotor activity have not been reported. It also is important to note here that DAT/SERT ratios differ slightly between laboratories and/or as a result of cell types used (e.g. rat brain synaptosomes, HEK 293 cells expressing human transporters, etc.). Thus, despite their somewhat unique in vitro profiles, these newer synthetic cathinones appear to exert effects on monoaminergic signaling, and suggest that α-PVP will have stimulant effects and high compulsive abuse potential similar to METH and the first generation synthetic cathinone MDPV (Aarde, Huang, Creehan, Dickerson, & Taffe, 2013; Watterson et al., 2014). In contrast, 4-MEC is predicted to have entactogenic effects and relatively lower

compulsive abuse potential (i.e., episodic use) similar to MDMA and the first generation synthetic cathinone methylone (Watterson et al., 2012).

However, there are currently no published behavioral studies that have directly assessed the potential abuse liability of α-PVP and 4-MEC. Thus, the current study sought to determine the effects of α-PVP and 4-MEC, along with methamphetamine for comparison, on thresholds for intracranial self-stimulation (ICSS) using a discrete trials current threshold determination procedure (Markou and Koob, 1992). The discrete trials current threshold ICSS task is a commonly employed to assess abuse liability, with reductions in ICSS thresholds representing facilitation of brain reward functioning, and increases in ICSS threshold representing anhedonic/depression-like effects and inhibition of brain reward function (Markou & Koob, 1992; Vlachou & Markou, 2011).

Method

Subjects

All procedures were conducted with the approval of the Institutional Animal Care and Use Committee at Arizona State University in accordance with the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health (NIH). Male Sprague-Dawley rats (n=5 for α-PVP, n=5 for 4-MEC, and n=4 for METH) were obtained from Harlan Laboratories (Livermore, CA, USA), and weighed approximately 250 g on arrival. Rats were individually housed according to NIH standards on a reversed 12-hour light–dark cycle (lights off at 6:00 AM) and given *ad libitum* access to food and water during all experimental procedures, except during behavioral testing. All behavioral testing occurred during the dark phase between 8:00 AM – 6:00 PM.

Drugs

α-PVP and 4-MEC were both obtained through internet websites (NicePriceResearchChems.biz and www.researchchemz.com, respectively). 10 mg samples of both drugs were analyzed by liquid chromatography–mass spectrometry for purity and chemical composition at the Research Triangle Institute (Durham, NC, USA). Samples were dissolved in methanol and analyzed using a Waters Synapt HDMS (Milford, MA, USA) quadrupole time-of-flight mass spectrometer interfaced to a Waters Acquity UPLC system. Data were acquired using a capillary voltage of 3 kV, source temperature of 120°C, desolvation temperature of 450°C, sampling cone at 30 V and extraction cone at 3 V. The mass spectrometer was externally calibrated from 50 to 700 Da using sodium formate solution, and mass shifts during acquisition were corrected using leucine enkephalin as a lockmass. Liquid chromatography was performed using a BEH C18 column (2.1 X 50 mm, 1.7 µm particles) held at 30°C. Sample identity was confirmed based on exact mass, retention time and fragmentation match to a certified reference standard from Cerilliant (Round Rock, TX, USA). Both samples were determined to have an apparent purity of >95%. For all behavioral studies, α-PVP, 4-MEC, and methamphetamine hydrochloride (METH, Sigma Aldrich, St. Louis, MO) were dissolved in 0.9% sterile saline and administered via the intraperitoneal (i.p.) route in a volume of 1 ml/kg.

ICSS Surgical Procedures

Rats were anesthetized with isoflurane (2% v/v) vaporized oxygen and unilaterally implanted (right and left hemispheres counterbalanced across rats) with a stainless steel bipolar electrode (PlasticsOne, Roanoke, VA, USA; 2 mm diameter,

insulated except at the ventral tip) into the medial forebrain bundle (anterior-posterior -0.05 mm; medial-lateral ±1.7 mm, dorsal-ventral -8.3 mm from dura) and secured to the skull with skull screws and dental cement. Rats were given 7 days to recover from surgery before commencement of ICSS procedures, during which they received daily injections of 2.5 mg/ml meloxicam (0.15 ml volume) to minimize post-surgical discomfort.

ICSS Apparatus

All ICSS testing was conducted in operant conditioning chambers (ENV-007CT; Med Associates) housed in sound-attenuating cubicles equipped with an exhaust fan to mask external noise. Chambers were equipped with a house light and a nose-poke aperture containing a light-emitting diode (LED) stimulus light (ENV-114M; Med Associates). The nose-poke aperture was 2.5 cm in diameter, located 5 cm above a stainless steel grid floor, and contained an infrared detector placed 0.64 cm from the front edge of the panel for recording responses. Located outside the chambers was a dual programmable ICSS stimulator (PHM-150B/2; Med Associates) interfaced to a computer to deliver electrical current to the electrode. Chambers were interfaced to a PC using Med-PC IV software that controlled all stimulation parameters, test functions, and data collection.

ICSS testing procedures

The discrete-trials current threshold procedure ICSS used in the present study were identical to those described in previous publications from our laboratory (Watterson et al., 2012, 2014; Watterson, Watterson, et al., 2013), also see Markou and Koob, 1992; Vlachou and Markou, 2011). During all ICSS testing procedures, stimulation availability

was signaled by illumination of the LED stimulus light located within the nose-poke aperture. Training began by allowing rats to spontaneously acquire nose-poke responses on an FR1 schedule of reinforcement, which delivered a 200-ms square-wave cathodal pulse of 120 µA at 100 Hz. Rats were required to exert a minimum of 600 nose-pokes in a 30 min session for two sessions in order to progress to discrete trials training. During discrete trials training, each trial began with a free stimulation of 120 µA, followed by a 7.5-s period during which the LED light remained on until the rat emitted a response that would yield an identical stimulation. Following the initial trial, the LED light was turned off and an inter-trial interval (ITI) was initiated, during which responses were recorded but yielded no stimulation. Progression through discrete trial training required rats to meet criterion (> 60% of total (trial + ITI) responses were correct trial responses) at four ITI lengths (2, 5, 10 and 15 s). Once rats completed discrete trials training, discrete trial current threshold determination procedures began. All discrete trials current threshold sessions began with a stimulus intensity of 120 µA and progressed through four cycles of ascending and descending blocks of trials. At a given current intensity, 5-trial blocks began with a free stimulation, followed by a 7.5-s interval during which rats could emit a nose-poke response to receive an identical stimulation. Following a single trial response, the LED stimulus light was turned off, initiating an ITI period between 7.5 and 15 s (mean 10 s) that separated trials. Responses during the ITI further lengthened the ITI by 12.5 s. When rats emitted an appropriate response on ≥3 out of 5 trials, electrical stimulation was decreased by 5 µA for the next five-trial block. Stimulation intensities continued to descend until the rats responded ≤2 out of 5 trials during a given trial block, at which point the current intensities reversed into ascending mode, with 5 µA increases

in current intensity for the subsequent blocks. Therefore, the discrete trial current threshold procedure determined the lowest amount of current intensity (threshold) for which rat was willing to emit responses. For each session, raw threshold scores were calculated by averaging the midpoint of current intensities between positive (responses on ≥3 out of 5 trials) or negative (responses on ≤2 out of 5) trial blocks.

Prior to all drug and vehicle testing, rats received a minimum of 10 days of baseline threshold assessment and were required to meet stable baseline criteria. These criteria were determined by threshold means for the most recent 8 sessions, as well as sub-means for the first and last 4 of these sessions. The difference in sub-means was divided by the overall mean, and threshold stability is considered to be met if the resulting percentage was less than 5 (Sidman, 1960). Baseline testing continued throughout experimentation to monitor stability, and drug testing was stopped if animals no longer displayed stability across baseline scores. In all cases, loss of stability either occurred as a result of loosening or complete detachment of the electrode implant from the skull.

Drugs were administered 20 min prior to placement into ICSS procedures. Drug doses were given in a randomized block design such that rats received each dose once before beginning another block of testing. At the beginning of experiment, all subjects were to receive 5 determinations of each dose; however, loss of electrode implant or baseline stability meant some subjects received less. Rats that were administered 4-MEC received 2 – 5 determinations at each dose and all rats that were administered α-PVP received 1-4 determinations at each dose. A 100 mg/kg dose for 4-MEC and 5 mg/kg dose for α-PVP were also administered, but only once and only for a subset of rats (N=4

for α-PVP; N = 3 for 4-MEC) due to apparent aversive effects as indicated by robust ICSS threshold elevations. Because only a subset of rats received these higher doses, ICSS threshold determinations were not included in the statistical analysis. Rats receiving METH received 2 – 3 determinations at each dose with the exception of the 3 mg/kg dose which was only assessed once in rats also because of ICSS threshold increases. However, because all rats received a 3 mg/kg determination, ICSS thresholds at this dose for METH were included in statistical analyses.

Statistical Analyses

All statistical analyses were conducted using SigmaPlot (Systat Software, Inc. San Jose, CA, USA). A significance criterion of $P < 0.05$ was used for all analyses. For each rat, raw ICSS current intensity thresholds (in μA) for all vehicle and drug sessions conducted once drug administration began were converted to scores reflecting the percent change from the mean of baseline thresholds obtained after reaching stabilization. For each dose, including vehicle, scores reflect the average percent change from the baseline score which immediately preceded its determination for each individual animal. In addition to dose means, corresponding 95% confidence intervals (Fig. 2) were calculated and significance (between individual doses and vehicle) occurred when the 95% confidence intervals between individual doses and vehicle did not overlap (Cardinal and Aitken, 2006).

In order to perform dose-effect comparisons across the different drugs tested, for each animal doses were log transformed and a linear slope (line of best fit) was calculated on group means for the descending portion of dose-effect curves starting with the lowest dose tested and ending with the dose producing the largest observed mean maximal

reduction in ICSS thresholds. Linear slopes were then used to calculate ED50 values for each drug and animal. Maximal reductions for each animal, regardless of dose, were also calculated. Next, slopes for the log transformed drug doses on the linear portion of the descending slopes (ED50 values in mg/kg) and maximal ICSS threshold decreases for METH, α-PVP, and 4-MEC were compared by a one-way between subjects ANOVA with bonferroni post-doc tests. Slope and ED50 values from previously published data (Watterson et al.,2012; Watterson et al. 2014) were also calculated (see Table 2), but were not compared statistically due to the possibility of cohort effects.

Results

For 4-MEC (Fig. 15A), significant reductions in ICSS thresholds versus vehicle was seen in the 10 (M = -11.86) and 30 (M = -15.09) mg/kg dose groups. For a-PVP (Fig. 15B), significant reductions versus vehicle were seen in the 0.3 (M = -13.79) and 1 (M = -19.03) mg/kg doses. For METH (Fig. 15C), significant reductions versus vehicle were seen in the 0.3 (M = -13.40) and 1 (M = -19.82) mg/kg doses.

For α-PVP, 4-MEC, and METH, higher doses (5, 100, and 3 mg/kg, respectively) produced elevations in ICSS threshold values, with only METH producing significant elevations (mean ± 95% CI; α-PVP, 19.83 ± 38.64; 4-MEC, 28.00 ± 31.72; METH, 84.39 ± 69.48%).

The slope, ED50, and maximal effect values are shown in Table 2. There were no significant differences in slopes of the linear portions of the dose-response curves between the three drugs ($F[2,11] = 1.63$, $p > 0.05$). For ED50 values, a significant effect of drug was observed ($F[2,11] = 46.05$, $p < 0.001$, and post-hoc analyses revealed

significant differences between METH and 4-MEC, METH and α-PVP, and METH and 4-MEC (all p's < 0.001). For comparison purposes, slope and ED50 values for MDPV and methylone were also calculated from previously published data(Watterson et al.,2012; Watterson et al. 2014), but not compared statistically. Finally, there were no significant differences observed in maximal reductions in ICSS thresholds ($F[2,11] = 1.64$, $p > 0.05$).

Discussion

To our knowledge, there are currently no published studies directly assessing the potential abuse liability of the second generation synthetic cathinones α-PVP or 4-MEC. The present study revealed that, similar to methamphetamine in the present study and to MDPV and methylone in previous studies (Bonano, Glennon, De Felice, Banks, & Negus, 2014; Watterson et al., 2012, 2014), both α-PVP and 4-MEC dose-dependently decreased ICSS thresholds in a discrete trials current threshold procedure. ICSS threshold reductions are commonly accepted as indicative of facilitated brain reward function and the interoceptive rewarding effects of drugs of abuse, and thus provide evidence of abuse potential in humans (Vlachou & Markou, 2011). At the highest doses tested, α-PVP (5 mg/kg), 4-MEC (100 mg/kg), and METH (3 mg/kg) no longer decreased, but instead produced increased ICSS thresholds (although these increases were not significant for α-PVP or 4-MEC). Increases in ICSS thresholds have been postulated to indicate decreases in brain reward function, aversive effects, and/or depression-like anhedonia (Vlachou & Markou, 2011). These higher doses were only assessed once in the current study and only in a subset of animals receiving 4-MEC and

α-PVP due to the appearance of apparent aversive effects, and thus were not included in statistical analyses. However, these observations suggest that high doses of all three of these psychostimulants may result in the emergence of deficits in brain reward function and/or aversion and anhedonia.

For α-PVP, the observed decreases in ICSS thresholds were very similar those reported here for METH as well as those previously reported for MDPV (Watterson et al., 2014). The most robust threshold decrease observed for α-PVP was ~19% at the 1 mg/kg dose was similar to the 1 mg/kg dose of METH presented here (~20%) and the 0.5 mg/kg dose of MDPV (~18%) (Watterson et al., 2012). Both METH and α-PVP resulted in significant ICSS threshold reductions at the 0.3 and 1 mg/kg doses. However, at the 3 mg/kg dose, METH led to an increase in ICSS thresholds (84.39 ± 69.48, mean ± 95% CI), an effect not seen in until a dose of 5 mg/kg α-PVP was administered (19.83 ± 38.64; mean ± 95% CI). Although these effect were not previously observed with MDPV doses tested up to 2 mg/kg (Watterson et al., 2014), it is likely that higher doses of MDPV would also produce elevations in ICSS thresholds effects similar to those observed in the current study with high doses of α-PVP and METH. In addition, slope of the descending portion of the dose-effect curves for α-PVP was most similar to those observed after METH (-46.70 vs -50.23), along with the maximal ICSS threshold reductions (25.76% vs 21.11%) and ED50 values (0.35 mg/kg vs 0.20 mg/kg, see Table 2). Thus, α-PVP and METH are approximately equipotent in reducing ICSS thresholds; however, when compared to our previously published data on MDPV, maximal ICSS threshold reductions and slopes for both α-PVP and METH were approximately half those produced by MDPV (maximal reduction = 42.03%, slope = -96.07) under identical

experimental procedures (Watterson et al., 2014). The ED50 dose for MDPV was the same α-PVP at 0.35 mg/kg. Again, however, this dose was determined by maximal ICSS reductions which were approximately twice as robust for MDPV (42.03%) as α-PVP (25.76%). Thus, the ability of α-PVP to reduce ICSS thresholds is most similar to that of METH but approximately half that of MDPV.

For 4-MEC, the changes in ICSS thresholds observed in the present study closely resemble those previously observed for methylone (Watterson et al., 2012), albeit with a rightward shift in the dose response curve. The most robust decrease produced by 4-MEC was at the 30 mg/kg dose (~15%), similar to the 0.3 mg/kg doses of α-PVP (~14%) and (METH (~13%) in the present study and lowest dose MDPV (0.1 mg/kg, ~17%) tested previously (Watterson et al., 2014), and most robust, but non-significant, methylone dose (10 mg/kg, ~13%) previously reported (Watterson et al., 2012). This maximal ICSS threshold decrease is also similar to that produced by MDMA (Hubner et al., 1988), but significantly less than maximal decreases produced by MDPV and METH (Watterson et al., 2014). At the 100 mg/kg dose (only assessed in a subset of animals, see Methods), 4-MEC increased ICSS thresholds (28.00% ± 31.73; mean ± 95% CI) indicative of a biphasic dose-response pattern that is typical of other illicit stimulants (Vlachou and Markou, 2011). In addition, neither the slope of the descending portion of the dose-effect curve for 4-MEC (-21.12) nor the maximal ICSS threshold decrease (17.40%) were different from α-PVP or METH. However, the ED50 value for 4-MEC was significantly different from α-PVP and METH, indicating that 4-MEC is less potent than these drugs. When compared to our previously published ICSS data for methylone (Watterson et al., 2012), 4-MEC lead to similar maximal ICSS threshold reductions (-

17.40% vs -21.50%), but much higher ED50 values (6.41 vs. 1.00) and a slightly steeper slopes (-21.12 vs. -17.59). However, our previously published study on methylone did not assess doses higher than 10 mg/kg; thus, it is possible that methylone may have led to greater maximal ICSS threshold reductions at higher doses. While the results from the present study suggest that 4-MEC appears to be less potent than methylone, yet more effective in reducing ICSS thresholds, further experimentation is needed before definitive conclusions can be made.

The present study is, to our knowledge, the first to directly assess the potential abuse liability of second generation synthetic cathinones. These results reveal that, like the first generation synthetic cathinones now classified as Schedule I substances for their high abuse potential, replacement synthetic cathinones possess similar rewarding effects as measured in ICSS procedures, and thus likely possess similar degrees of abuse liability in humans. Furthermore, as with first generation synthetic cathinones, these newer replacement cathinones appear to produce rewarding effects similar to the illicit stimulants methamphetamine and MDMA (Hubner et al., 1988; Vlachou & Markou, 2011). When considering the ICSS data from the present study, along with data previously published for MDPV (Watterson et al., 2014) and methylone (Watterson et al., 2012) under identical ICSS experimental conditions, the rank order potency of these drugs is MDPV > METH ≈ α-PVP > methylone ≈ 4-MEC.

Synthetic cathinones, like prototypical psychostimulants, primarily exert their effects through substrate releasing or plasma membrane transporter blocking effects at monoaminergic terminals (Iversen et al., 2013; Lehner & Baumann, 2013; Marusich et al., 2014; Meltzer et al., 2006; Simmler et al., 2014). As mentioned previously, numerous

studies suggest that there is a large degree of correspondence between ICSS threshold reductions, propensity for drug self-administration, and the balance between effects on DA vs. 5-HT transmission in the mesolimbic reward pathway (Bauer et al., 2013a; Bauer, Banks, Blough, & Negus, 2013b; Rothman & Baumann, 2003, 2006; S Wee et al., 2005). Specifically, higher DA/5-HT transporter affinity/release ratios correlate with increased self-administration propensity, more robust decreases in ICSS thresholds, and greater abuse liability as indicated by a progressively increased risk for compulsive use. Alternatively, lower DA/5-HT ratios correlate with reduced propensity for self-administration, less robust decreases in ICSS thresholds, and a lower potential for compulsive drug intake. When considering all of the ICSS data from the present study along with previously published data on first generation synthetic cathinones (Watterson et al., 2012, 2014) and prototypical psychostimulants (Hubner et al., 1988; Vlachou & Markou, 2011), these data generally support a strong positive relationship between differential effects on DA/5-HT signaling and rewarding effects, as revealed by maximal ICSS thresholds and steepness of slope of the descending portion of dose response curve. However, individual comparisons of the *in vitro* DAT/SERT affinities of all of these compounds is not appropriate, since some act primarily as presynaptic plasma membrane transporter blockers (MDPV and α-PVP), others are transporter substrates and monoamine releasers (METH, methylone, and MDMA), or a combination of both (4-MEC). Thus, direct comparison of DAT/SERT data (i.e., IC_{50} values) derived from inhibition of transporter function as assessed in competitive binding assays and data from monoamine release assays (i.e., EC_{50} values) is problematic. However, when considering the aforementioned rank order potency (MDPV > α-PVP > 4-MEC) based on ICSS

threshold decreases and slopes of dose response curves within the class of monoamine transporter blockers, this rank order of potency is in close agreement with DAT/SERT ratios derived from IC_{50} values obtained in previous in vitro studies (MDPV (806-816) > α-PVP (806) > 4-MEC (1.85) (Baumann et al., 2012b; Baumann et al., 2013; Marusich et al., 2014; Simmler et al., 2014). As mentioned previously, 4-MEC is also a weak 5-HT releaser (Simmler et al., 2014) which likely further decreases its abuse liability. When considering maximal ICSS threshold decreases and slopes of the dose response curve for monoamine releasers, their rank-order potency is METH > methylone, which corresponds with their DAT/SERT ratios derived from EC_{50} values obtain in vitro (METH (152) > methylone (1.82 – 2))(Baumann et al., 2012; Baumann et al., 2013).

Taken together, we predict that α-PVP possesses a potential for compulsive abuse (i.e. addiction) that is roughly similar to that of METH and MDPV, but much greater than that of 4-MEC, methylone, and MDMA. Accordingly, we also predict that 4-MEC will have a relatively lower potential for compulsive use than that of MDPV and METH, would be most similar to methylone and MDMA (i.e. episodic use), and may exert primarily entactogenic effects.

Finally, it is also important to mention that both first and second generation synthetic cathinones are often sold as mixtures. Specifically, synthetic cathinones products have been shown to often contain more than one cathinone, as well as other adulterants including illicit amphetamines, piperazines, cutting/binding agents, caffeine, and topical anesthetics (Brandt et al., 2010; German, Fleckenstein, & Hanson, 2014). Thus, while abuse liability assessment of these individual drugs is now emerging, assessment of the effects and abuse potential of combinations of these drugs will be more

difficult, yet should be a central focus of future research. Together, the results of the present study suggest that second generation synthetic cathinones likely possess a similar potential for abuse as their first generation predecessors as well as the illicit amphetamines they are designed to mimic. Furthermore, these findings have important implications for future research on synthetic cathinone abuse, dependence, and legislative efforts to classify these drugs according to the proper controlled substance schedule

CHAPTER 7

SENSITIZATION TO THE LOCOMOTOR STIMULANT EFFECTS OF 3,4-METHYLENEDIOXYPYROVALERONE (MDPV) AND CROSS-SENSITIZATION TO METHAMPHETAMINE IN RATS

Synthetic cathinones, often falsely marketed as “bath salts” or “legal high” alternatives to illicit psychostimulants such as methamphetamine (METH), cocaine, or 3,4-methylenedioxymethampehtamine (MDMA), are a class of designer stimulants that have become increasingly popular drugs of abuse in recent years. In the United States, 3,4-methylenedioxypyrovalerone (MDPV), mephedrone, and methylone initially emerged as the most prominent “bath salts” constituents, comprising 98% of all synthetic cathinones obtained in drug seizures prior to their permanent classification as Schedule I substances (One Hundred and Twelfth Congress of the United States of America, 2012). Of these three, MDPV was the most commonly abused in the U.S. (Bonano et al., 2014; Uralets, Rana, Morgan, & Ross, 2014) and identified in numerous case reports of synthetic cathinone related toxicity, bizarre behaviors, and death (Spiller et al., 2011; Murray et al., 2012; Penders and Gestring, 2011; Ross et al., 2012; Penders et al., 2013; Wright et al., 2013; Wyman et al., 2013).

Despite being permanently classified as a Schedule I substance in 2012, MDPV continues to be abused (NMS Labs, 2014) and recent reports of MDPV addiction have emerged (Nouredine Sadeg et al., 2014). While MDPV-related toxicity is now well established, the scientific assessment of abuse liability is still in its infancy (Watterson et al., 2013: Gregg and Rawls, 2014). Preclinical animal studies have revealed that MDPV has potent reinforcing (Aarde et al., 2013; Watterson et al., 2014) and rewarding effects

(De Felice et al., 2013; Bonano et al., 2014; Watterson et al., 2014), fully substitutes for cocaine and methamphetamine in drug discrimination tests (Fantegrossi et al., 2013; Gatch, Taylor, & Forster, 2013), and elevates locomotor activity in a manner indicative of psychostimulants (Baumann et al., 2012; Fantegrossi et al., 2013; Aarde et al., 2013; Marusich et al., 2012, 2014) . In humans, concurrent use of MDPV and other illicit stimulants is prevalent and evidence suggests that prior stimulant use enhances severity of adverse sympathomimetic effects during acute MDPV use (Spiller et al., 2011). However, despite mounting preclinical literature suggesting an abuse potential of MDPV, and human literature suggesting enhanced vulnerability to MDPV toxicity with prior amphetamine use, there are currently no published reports detailing whether MDPV use alters behavioral sensitivity and responsiveness, and thus potentially abuse vulnerability, for traditional illicit psychostimulants (e.g. METH). Conversely, prior research has not yet established whether use of traditional psychostimulants such as METH enhance behavioral sensitivity and responsiveness and thus abuse potential of MDPV. One method for assessing lasting changes in behavioral sensitivity and responsiveness is via locomotor sensitization, in which repeated exposure to a drug leads to a progressive and enduring enhancement of locomotor behavior elicited by a subsequent drug challenge (Vanderschuren & Kalivas, 2000).

With regards to synthetic cathinones, it has been previously demonstrated that prior exposure to mephedrone produces sensitization in rats when given a subsequent mephedrone or cocaine challenge (Lisek et al., 2012; Gregg et al., 2013a, 2013b; Shortall et al., 2013). Furthermore, repeated oral administration of cathinone, the parent compound of synthetic cathinones and the primary psychoactive alkaloid found in *Catha*

edulis, also leads to locomotor sensitization in rats (Banjaw et al., 2005; Banjaw and Schmidt, 2005, 2006). However, to our knowledge, the phenomenon of locomotor sensitization has not yet been established for MDPV. Therefore, the purpose of the present study was to assess the ability of repeated MDPV administration to produce locomotor sensitization. We also sought to determine if cross-sensitization between MDPV and METH could be observed.

Method

Subjects

Male Sprague-Dawley rats (Harlan Laboratories, Livermore, CA), weighing approximately 250-275 g upon arrival, were housed in a humidity- and temperature-controlled colony, maintained on a 12:12 reversed light/dark cycle and were provided *ad libitum* access to food and water except during locomotor testing procedures. All experimentation was conducted during the dark phase (7 AM – 7 PM). All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee at Arizona State University and in accordance with the principles of the National Research Council's *Guide for the Care and Use of Laboratory Animals (2011)*.

Drugs

MDPV was purchased from Laboratory Supply USA (San Diego, CA, USA). A 10-mg sample of MDPV was analyzed by liquid chromatography–mass spectrometry for purity at the Research Triangle Institute (Durham, NC, USA) and determined to have an apparent purity of >95%, as previously reported (Watterson et al., 2014). For all experiments, MDPV and methamphetamine (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in sterile saline and administered intraperitoneally (i.p.) in a volume of 1 ml/kg.

Locomotor Testing Procedures

Locomotor activity was assessed using a Rotorat system apparatus (Med Associates, Mt. St Albans, VT). This apparatus measures rotational ambulation and locomotor behavior quantified by quarter turns (90° rotations) in a bowl-shaped arena as previously described in our laboratory (Kufahl et al., 2013; Watterson et al., 2013). For all experiments, drug or vehicle injections were administered i.p. immediately prior to being placed into the arena for 90 minutes. Prior to all drug administration procedures, all rats received two days of acclimation to the testing apparatus. On the first acclimation day, rats were placed into and allowed to freely explore the arena for 90 min during which no locomotor activity was recorded. On the second day, rats were first fitted with a plastic neck collar. Next, rats received a saline injection immediately prior to being placed into the arena and allowed to freely explore the arena for 90 minutes. Locomotor activity was recorded by a rotating actuator mounted at the top of the arena that was connected to the rat via a stainless steel spring tether and a metal clamp affixed to the plastic collar. For all subsequent sessions, locomotor activity was assessed with identical 90 min procedures. Saline was used as vehicle for all experiments.

Experiments 1a and 1b – MDPV-induced Locomotor Sensitization with 24 hr Inter-test Intervals

Initial doses of MDPV of 1 and 5 mg/kg were chosen based upon previously published findings by our laboratory and others of lowered thresholds for intracranial self-stimulation at similar doses (Bonano et al., 2013; Watterson et al., 2014; De Felice et al., 2014) as well as acute locomotor stimulant properties of MDPV (Aarde et al., 2013; Fantegrossi et al., 2013; Gatch et al., 2013; Marusich et al., 2014, 2012). In Experiment

1a, rats were injected with either MDPV 5 mg/kg (N = 8) or vehicle (N=8) and placed into locomotor chambers for 90-min sessions for 5 consecutive sessions (24 hours apart: Sessions 1-5 = Days 1 - 5). Experiment 1b followed the same timeline, but rats were injected with either MDPV 1 mg/kg (N=8) or vehicle (N=8) for 5 consecutive days (24 hrs apart: Sessions 1-5 = Days 1 - 5) prior to placement into locomotor chambers. Following a 5 day incubation period, rats with a 5 mg/kg or vehicle dosing regimen history received a 1 mg/kg challenge prior to the final locomotor test session. For rats receiving either 1 mg/kg or vehicle, a challenge dose of 0.5 mg/kg was administered prior to the final locomotor test session.

Experiment 2 - MDPV-induced Locomotor Sensitization with 48 hour Inter-treatment Intervals

For experiment 2, rats received either MDPV 1 mg/kg (N = 10) or vehicle (N=8) immediately prior to 90 min sessions for five sessions (48 hrs apart: Sessions 1-5 = Days 1 - 9). Following a 5-day incubation period, all rats regardless of dosing history received a vehicle injection immediately prior to a 90-min test session to assess for any residual non-specific locomotor effects (e.g. context related locomotion). The next day, all rats received a MDPV 0.5 mg/kg challenge dose prior to the final locomotor test session.

Experiment 3 - Cross-sensitization of METH-induced Locomotor Sensitization to MDPV

For Experiment 3, rats were treated with either METH 1 mg/kg (N = 16) or vehicle (N=16) immediately prior to 90-min sessions for five sessions (48 hrs apart: Sessions 1-5 = Days 1 - 9). The 48 hour inter-test interval was chosen to be consistent with Experiment 2. Following a 5-day incubation period, all rats regardless of dosing history were received saline immediately prior to a test session to assess for any residual

non-specific locomotor effects. The next day, all rats received a MDPV 0.5 mg/kg challenge dose prior to the final locomotor test session.

Experiment 4 - Cross-sensitization of MDPV-induced Locomotor Sensitization to METH

For Experiment 4, rats were administered either MDPV 5 mg/kg (N=6), MDPV 1 mg/kg (N=10) or vehicle (N=8), immediately prior to 90-min sessions for five sessions (48 hrs apart: Sessions 1-5 = Days 1 – 9). The 48 hour inter-test interval was chosen to be consistent with Experiments 2 and 3 in which sensitization to the locomotor stimulant effects of MDPV were observed (see Results). Following a 5-day incubation period, all rats regardless of dosing history received saline injection immediately prior to a test session to assess for any residual non-specific locomotor effects. The next day, all rats received a METH 0.5 mg/kg challenge dose prior to the final locomotor test session.

Statistical Analyses

Data analysis was conducted using Prism 5 software (GraphPad, La Jolla, CA). For all experiments, the dependent measure was total number of quarter turns (sum of clockwise and counter-clockwise quarter turns) occurring during each of the five 90 min sessions, as well as during saline and challenge test sessions. For Experiments 1 - 3, locomotor activity across the five repeated treatment sessions were analyzed with 2 × 5 mixed-model ANOVAs, with *dose* (vehicle, drug) as the between-subjects factor and *session* (1-5) as the within-subjects factor. One-way repeated measures ANOVAs were always conducted for vehicle and drug groups individually with *post hoc* pairwise comparisons to compare session effects. For Experiment 4, locomotor activity across the five repeated treatment sessions was analyzed with a 3 × 5 mixed-model ANOVA, with *dose* (vehicle, MDPV 1 mg/kg, MDPV 5 mg/kg) as the between-subjects factor and

session (1-5) as the within-subjects factor. For both saline and drug challenge tests, locomotor activity measures were analyzed with independent samples t-tests (Experiments 1 – 3) and Dunnett's test (Experiment 4). More specifically, for Experiment 1a, the drug challenge (MDPV 1 mg/kg) sensitization test between rats with MDPV (5 mg/kg) vs vehicle dosing history was analyzed with an independent samples t-test. For Experiment 1b, the drug challenge (MDPV 0.5 mg/kg) sensitization test between rats with MDPV (1 mg/kg) vs vehicle dosing history was analyzed with independent samples t-test. For Experiment 2, both the saline and drug challenge (MDPV 0.5 mg/kg) sensitization tests between rats with MDPV (1 mg/kg) vs vehicle dosing histories were analyzed with an independent samples t-test. For Experiment 3, the saline and drug challenge (MDPV 0.5 mg/kg) sensitization tests between rats with METH (1 mg/kg) vs vehicle dosing histories were analyzed with an independent samples t-test. For experiment 4, saline and drug challenge sensitization (METH 0.5 mg/kg) tests were analyzed with a Dunnett's multiple-comparison test for the 1 and 5 mg/kg MDPV groups using vehicle as the common control (C. Dunnett, 1955; Holson, Freshwater, Maurissen, Moser, & Phang, 2008). Numerical results are displayed as mean ± SEM, where appropriate.

Results

Acclimation and Repeated Treatment Sessions

Experiment 1a

For experiment 1a (Fig. 16a) statistical analyses revealed a significant difference in baseline locomotor activity such that rats subsequently assigned to the saline treatment

group (874.88 ± 148.33 turns) displayed more quarter turns than rats subsequently assigned to the MDPV 1 mg/kg group (468.76 ± 91.56 turns) ($t[7]=2.60$, $p<0.05$). For the five treatment sessions (sessions 1-5; days 1-5), there was a significant main effect of *session* ($F[4,56] = 4.30$, $p <0.05$), *dose* (F]1,14) = 88.05, $p<0.001$), and a *dose* × *session* interaction ($F[4,56]=7.49$, $p<0.05$). Post-hoc analyses revealed that total quarter turns were significantly greater during sessions 1 and 2 than session 5 ($p < 0.05$). No other session differences were observed. No significant differences were found across sessions in rats receiving saline. *Post hoc* tests also revealed significantly increased locomotor activity in rats receiving MDPV 1 mg/kg than rats receiving saline across all treatment sessions ($p<0.05$).

Experiment 1b

For experiment 1b (Fig. 16b), statistical analyses did not reveal a significant difference in locomotor activity for the initial acclimation session such that rats subsequently assigned to the saline treatment group (763.89 ± 169.21 turns) displayed similar numbers of quarter turns than rats subsequently assigned to the MDPV 5 mg/kg group (640.63 ± 85.13 turns, $p>0.05$). For the next five days of testing (sessions 1-5; days 1-5), there was neither a significant main effect of *session* nor a significant *dose* × *session* interaction. However, there was a significant main effect of *dose* ($F[1,15] = 21.12$, $p < 0.001$), with rats receiving MDPV 5 mg/kg displaying more quarter turns (2117.175 ± 799.68) than rats receiving saline (642.98 ± 104.23).

Experiment 2

For experiment 2 (Fig. 17a), statistical analyses did not reveal a significant difference in locomotor activity for the initial acclimation session such that rats

subsequently assigned to the saline treatment group (524.00 ± 86.75 turns) or MDPV 1 mg/kg group (510.50 ± 75.24). For the five treatment sessions (sessions 1-5; days 1-9), a 2-way mixed ANOVA revealed a significant main effect of *session* ($F[4,64] = 4.12$, $p < 0.01$), *dose* ($F[1,16] = 87.825$, $p < 0.001$), and a significant *dose* × *session* interaction ($F[4,64] = 2.75$, $p < 0.05$). *Post-hoc* analyses revealed a significant differences in locomotor activity across treatment sessions in the MDPV 1 mg/kg group ($F[4,36] = 4.35$, $p < 0.01$), with locomotor activity in session 3 significant greater than all other sessions (p-values < 0.05). Locomotor activity in session 1 was significantly lower than that in sessions 2 and 3, but not 4 or 5. No significant differences were found across sessions in rats receiving saline. Furthermore, locomotor activity in rats receiving MDPV 1 mg/kg was significantly higher than rats receiving saline across all treatment sessions (p-values < 0.05).

Experiment 3

For experiment 3 (Fig. 18a), here were no significant differences in baseline locomotor activity between rats subsequently assigned to the saline treatment group (463.00 ± 72.30 turns) or METH 1 mg/kg groups (442.75 ± 49.70 turns). For the five treatment sessions (sessions 1-5; days 1-9), 2-way mixed ANOVA revealed a significant main effect of *session* ($F[4,120]=3.42$, $p< 0.05$), *dose* ($F[1,30]=28.246$, $p<0.001$, and a significant *dose* × *session* interaction ($F[4,120]=3.31$, $p<0.05$. *Post-hoc* analyses revealed a significant difference across treatment sessions in the METH 1 mg/kg group ($F[4,60]=4.14$, $p<0.01$), with sessions 1, 2 and 3 being significantly lower than session 5, and session 2 being significantly lower than session 4. No significant differences in locomotor activity were found across sessions in rats receiving saline. Rats treated with

METH 1 mg/kg showed significantly greater activity than rats receiving saline across all treatment conditions (p-values <0.05).

Experiment 4

For experiment 4 (Fig. 19a), there were no significant differences in baseline locomotor activity between rats subsequently assigned to the saline treatment group (497.88± 96.47 turns), MDPV 1 mg/kg group (513.90± 91.62 turns) or MDPV 5 mg/kg group (529.30 ±90.46 turns). For the five treatment sessions (sessions 1-5; days 1-9), 2-way mixed ANOVA did not reveal a significant main effect of *session* but did reveal a significant main effect of *dose* ($F[1,21]=5.07$, $p<0.05$) and a significant *dose* × *session* interaction ($F[8,84]=2.614$, $p<.05$). Pairwise comparison revealed significant differences between the saline and MDPV 1 mg/kg groups as well as between the saline and MDPV 5 mg/kg groups (p-values < 0.05). One-way ANOVA revealed significant differences between sessions 1 and 3 (days 1 and 5, respectively; p-values < 0.05). For both sessions (1 and 3), the locomotor activity in the saline treatment group was significantly different from the MDPV 5 mg/kg group (p-values < 0.05).

Sensitization Tests

Experiment 1a and 1b

The results of sensitization tests following drug challenge are shown in Figure 16. In Experiment 1a (Fig. 16a), no significant differences in locomotor activity following the MDPV 0.5 mg/kg challenge dose were observed between rats with a history of MDPV 1 mg/kg or saline (see Fig. 16a). In Experiment 1b (Fig. 16b), rats with a history of saline treatment showed increased locomotor activity following administration of

MDPV (1 mg/kg) as compared to rats with a history of MDPV (5 mg/kg) treatment ($t[7]=2.63$, $p<0.05$, see Fig. 16b).

Experiment 2

In Experiment 2 (Fig. 17b), rats with a history of repeated treatment with 1 mg/kg MDPV demonstrated an elevated locomotor response to the 0.5 mg/kg challenge of MDPV as compared to rats with a history of saline treatment ($t[7]= 3.04$, $p < 0.05$). In contrast, no significant differences between treatment groups were observed in the locomotor response to saline challenge ($p>0.05$; see Fig. 17b).

Experiment 3

In Experiment 3 (Fig. 18b), there were no significant differences in the locomotor response to the 0.5 mg/kg MDPV challenge between rats with a history of repeated METH vs. saline treatment ($p>0.05$). Furthermore, there were no significant differences in locomotor response to the saline challenge between rats with a history of METH vs. saline treatment ($p>0.05$; see Fig. 3B).

Experiment 4

In Experiment 4 (Fig. 19b), Dunnett's test revealed that rats with a history of repeated treatment of 1 mg/kg MDPV exhibited increased locomotor activity in response to a 0.5 mg/kg METH challenge as compared to saline treated animals ($p < 0.05$). However, rats with a history of treatment with 5 mg/kg MDPV did not exhibit increased locomotor activity in response to the 0.5 mg/kg MDPV challenge ($p=0.13$). There were also no significant differences in locomotor responses following saline challenge between rats with a history of METH 1 mg/kg vs. saline treatment or MDPV 5 mg/kg vs. saline (see Fig. 4B).

Discussion

The present study revealed that acute systemic administration of MDPV leads to increased locomotor behavior when compared to saline vehicle controls, and that repeated intermittent (48 hr interval) acute administration also leads to an enhancement of locomotor activity when subjects were subsequently tested with a challenge dose of either MDPV or METH, indicating the development of locomotor sensitization and cross-sensitization to METH, respectively. However, when MDPV treatments were separated by 24 hrs, sensitization to the locomotor stimulant effects of an MDPV challenge was not observed. Furthermore, rats receiving intermittent (48 hr interval) administration of METH did not display cross-sensitization to a subsequent MDPV challenge. In all experiments in which sensitization or cross-sensitization occurred, enhanced locomotor activity was not observed following saline challenge, suggesting that augmented locomotion during drug challenge tests were drug-specific and not driven by contextual conditioning factors such as re-exposure to the testing environment. To our knowledge, this is the first report of locomotor sensitization to MDPV, as well as cross-sensitization to METH, following repeated MDPV administration.

The augmented locomotor response seen following repeated exposure to psychostimulants is a robust and common phenomenon observed in laboratory animals (T. Robinson & Berridge, 2000, 2001; Steketee & Kalivas, 2011). The *expression* of behavioral sensitization is thought to reflect lasting neural adaptations that develop with repeated drug exposure (i.e. the *incubation* of sensitization) (Vanderschuren & Kalivas, 2000). Furthermore, evidence suggests that these neuroadaptations may, at least in part, contribute to the transition to compulsive drug use (Cornish & Kalivas, 2001; Robinson

& Berridge, 2000), as repeated drug exposure not only potentiates behavioral responsivity and sensitivity to the drug, but also the development of drug self-administration and reward (Vezina, Lorrain, Arnold, Austin, & Suto, 2002; Vezina, 2004; Zernig et al., 2007). Drug-induced increases in locomotor activity are mediated by increases in extracellular dopamine in limbic and motor circuits. Similarly, the progressive increase in locomotor activity characteristic of sensitization is also paralleled by augmented dopamine neurotransmission in limbic and motor regions such as the nucleus accumbens (Kalivas & Stewart, 1991; Vezina et al., 2002; Vezina, 2004).

The primary mechanism of action of MDPV is similar to cocaine in that it is a potent inhibitor of presynaptic plasma membrane dopamine and norepinephrine transporters (DAT and NET, respectively), with little effects on presynaptic plasma membrane serotonin transporters (SERT) (Baumann et al., 2013; Baumann et al., 2013; Eshleman et al., 2013; Simmler et al., 2013). Compared to cocaine, however, the potency of MDPV at inhibiting DAT and NET are 50 and 10 times greater, respectively (Baumann, Partilla, & Lehner, 2013; Baumann, Partilla, Lehner, et al., 2013). Furthermore, like cocaine, MDPV induces outward (hyperpolarizing) electrical currents in human DAT cells expressed in *Xenopus laevis* oocytes. Alternatively, inward DAT currents are produced by other synthetic cathinones such and mephedrone and methylone, as well as METH (Cameron et al., 2013, 2013). Unlike MPDV, these psychostimulants primarily exert their neurochemical effects as monoamine substrate releasers with varying levels of preference across DAT, NET and SERT as well as vesicular monoamine transporters (Baumann et al., 2013, 2012a). Systemic administration of MDPV elevates extracellular dopamine levels in the nucleus accumbens with at least 10

times greater potency than cocaine and with much longer lasting effects (Baumann et al., 2013; Baumann et al., 2013; Marusich et al., 2014). In locomotor assays, MDPV is a powerful locomotor stimulant (Aarde et al., 2013; Fantegrossi et al., 2013; Gatch et al., 2013; Marusich et al., 2012, 2014) and it is likely that these locomotor effects are mediated by D1 receptors in the nucleus accumbens as has been shown for other psychostimulants (Lobo & Nestler, 2011; Smith, Lobo, Spencer, & Kalivas, 2013). Thus, consistent with previous studies on cocaine and amphetamines, the ability of MDPV to induce locomotor sensitization is likely mediated by its ability to augment extracellular dopamine levels in limbic and motor regions via potent DAT inhibition.

Cross-sensitization has been shown to occur with both cocaine and amphetamines as well as other drug classes (Akimoto et al., 1990; Kalivas and Stewart, 1991; Fitzgerald et al., 1996). Although the primary pharmacological mechanisms of action differ across drug classes, the ability to increase mesolimbic dopaminergic transmission is common amongst drugs of abuse and is thought to mediate their locomotor stimulant properties (Robinson et al., 1988; Kalivas and Duffy, 1990; Fitzgerald et al., 1996). As such, cross-sensitization is thought to occur when two drugs share overlapping mechanisms of action, albeit often differing from their primary mechanism of action (Steketee & Kalivas, 2011). Thus, while MDPV increases extracellular dopamine through DAT inhibition, and METH increases extracellular dopamine through monoamine substrate releasing effects, the net common effect of increased dopamine transmission on post-synaptic dopamine receptors is a likely possibility. Specifically, persistent increases in DA transmission by both drugs could increase postsynaptic responsiveness to dopamine through either increases in DA receptor density or sensitivity (Kalivas & Stewart, 1991). Furthermore,

repeated exposure to both cocaine and amphetamines also enhance glutamate signaling in corticolimbic circuits (Kalivas et al., 2009), which likely also plays a role in the locomotor sensitizing effects of MDPV and its cross-sensitization to METH (Steketee & Kalivas, 2011). However, glutamatergic effects of MDPV exposure have yet to be explored, but should be a central focus on future research on this synthetic cathinone.

The lack of cross-sensitization to MDPV in animals with a history of METH exposure is puzzling given that a cross-sensitizing effect of METH was observed in rats with a history of MDPV exposure. Many experimental variables may have contributed to these negative observations, including drug dose, number of exposures, dosing schedule, route of administration, and species/strain effects (Phillips et al., 2011). Thus, the possibility remains that bidirectional cross-sensitization between MDPV and METH may occur under certain experimental conditions. Given the mechanistic similarity between cocaine and MDPV, we predicted full cross-sensitization for both drugs. It is possible, however, that the lack of bidirectional cross-sensitization may be related to known effects of METH on serotonergic transmission, which is known to modulate rewarding and reinforcing effects of various psychostimulants (Rothman & Baumann, 2006). Clearly, further studies would be needed to dissect the precise monoaminergic mechanisms underlying cross-sensitization, or lack thereof, between MDPV and METH.

In experiments where sensitization to a drug challenge occurred, we observed no significant increases were observed when subjects were given a saline challenge test the previous day. The lack of a sensitization response in animals when tested with saline is important as evidence suggests that the convergence of drug exposure and associated environmental stimuli (context) together contribute to sensitized responding (Vezina et

al., 1989; Vezina and Leyton, 2009). Because the expression of sensitization is often context-dependent, future research should assess whether repeated exposure to MDPV or METH in a separate distinct environment (e.g. home cage) would also produce sensitized responding when tested in an alternative environment.

The lack of locomotor sensitization to MDPV observed in Experiments 1a and 1b, where the inter-treatment interval was 24 hrs, is not without precedent. Previous work has revealed that intermittent drug administration with longer inter-treatment intervals (e.g. 24 or 48 hrs vs. 3, 6, or 12 hrs) produces greater locomotor sensitization for methamphetamine, cocaine, and morphine (Kuribara, 1996). In comparison with cocaine, MDPV-induced elevations in extracellular dopamine are much longer in duration, likely reflecting either a longer half-life and/or centrally active metabolites such as 3,4-dihydroxypyrovalerone (3,4-catechol-PV) and 4-hydroxy-3-methoxypyrovalerone (4-OH-3-MeO-PV) (Anizan et al., 2014). Research on the pharmacokinetic and/or pharmacodynamics of MDPV and its bioactive metabolites is generally lacking and should be a focus of future research studies.

Repeated intermittent exposure to psychostimulant drugs not only increases locomotor behavior during subsequent drug exposure, but can also enhance drug self-administration behavior, reward, and the development of psychostimulant-related psychosis. The findings presented here suggest that repeated use of MDPV can increase the sensitivity and behavioral responsivity to the drug, which may lead to increased vulnerability to addiction to MDPV and/or METH. Increased responsivity to MDPV may also explain he increased propensity to develop psychostimulant-induced psychosis or toxicity with subsequent use (Spiller et al., 2011; Prosser and Nelson, 2012). While the

present study only evaluated cross-sensitization of the locomotor stimulant effects of MDPV to that of METH, cross-sensitization across other drug classes has also been reported, and future research should focus on the drug-drug combinations for MDPV as well as other synthetic cathinones.

CHAPTER 8

ASSESSMENT OF NEUROTOXIC AND COGNITIVE EFFECTS FOLLOWING CHRONIC INTRAVENOUS MDPV AND METH SELF-ADMINISTRATION

Acute MDPV use has led to numerous reports of toxicity and death (Coppola & Mondola, 2012; Kesha et al., 2013; Mas-Morey et al., 2013; Mugele, Nañagas, & Tormoehlen, 2012; Murray et al., 2012; Penders & Gestring, 2011; Prosser & Nelson, 2012; Ross et al., 2012; Spiller et al., 2011; Stoica & Felthous, 2013; Wright et al., 2013; Wyman et al., 2013). In fact, the majority of the published reports on MDPV exposure and use are case reports detailing the various adverse effects of acute exposure and overdose. More recently, however, evidence has emerged suggesting that the dangers of MDPV extend far beyond the adverse effects that can accompany acute overdose and/or toxicity. Specifically, studies have revealed that MDPV is highly reinforcing (Aarde et al., 2013; Watterson et al., 2014) and rewarding in animals (Bonano et al., 2014; De Felice et al., 2013; Watterson et al., 2014) and has methamphetamine and cocaine-like subjective effects (Fantegrossi et al., 2013; Ross et al., 2012; Ross, Watson, & Goldberger, 2011). Furthermore, human users often report a persistent desire to continue using MDPV despite these adverse effects, resulting in binge-like patterns of consumption that can last for days (Fass, Fass, & Garcia, 2012; Johnson, Johnson, & Portier, 2013; Maxwell, 2013; Penders, Gestring, & Vilensky, 2012; Penders & Gestring, 2011; Ross et al., 2012; Slomski, 2012). While relatively scarce in comparison to case reports reporting intoxication, excited delirium, serotonin syndrome, hallucinatory psychosis, multi-organ toxicity, and death, reports of MDPV addiction have recently begun to appear in the literature (Sadeg et al., 2013; Winder, Stern, & Hosanagar, 2012).

To make matters worse, only protocols for supportive care following overdose have been established. Treatment strategies for MDPV addiction are not scientifically established, and basic research aimed at discovering pharmacotherapeutic targets for MDPV addiction is also scarce (Glennon, 2014; Jordan & Harrison, 2013). Thus, the emergence of reports detailing MDPV addiction and the lack of any established treatments highlight the importance of basic preclinical research focusing on assessing the consequences of chronic, long-term use and finding potential neurological targets for the development of effective cognitive, behavioral, and/or pharmacotherapeutic treatments (Glennon, 2014).

As previously mentioned, MDPV is a potent DAT and NET reuptake inhibitor, with weak effects at SERT, that most closely resembles the neurochemical properties of cocaine (Baumann, Partilla, & Lehner, 2013; Baumann, Partilla, Lehner, et al., 2013). However, when compared with cocaine, MDPV is at least 50 times more potent in inhibiting DAT, 10 times more potent in inhibiting NET, and is at least 10-times more potent and longer lasting at increasing extracellular dopamine levels in the nucleus accumbens. For example, elevations in extracellular DA levels are significantly elevated above baseline levels 60 minutes after administration of MDPV, whereas cocaine-induced elevations return to baseline approximately 40 minutes after administration (Baumann, Partilla, & Lehner, 2013; Baumann, Partilla, Lehner, et al., 2013). Behavioral effects of MDPV, however, most closely resemble METH, with similar or greater reinforcing, rewarding, and locomotor stimulant effects (Aarde et al., 2013; Marusich et al., 2012;Watterson, et al., 2013; Watterson et al., 2014). Together, these effects suggest that long-term repeated exposure to MDPV may cause similar neurological toxic effects and cognitive deficits that are often reported after chronic heavy use of cocaine and/or

METH (Simon et al., 2001). However, to date, there are currently no studies assessing possible of neurotoxicity following long-term chronic MDPV use.

Human imaging studies have revealed that repeated use of METH or cocaine is correlated with substantial and enduring macroscale changes in regional volumes of and functional activity in various brain regions (Aron & Paulus, 2007; Ersche, Williams, Robbins, & Bullmore, 2013). In addition to neurotoxic effects secondary to cerebrovascular infarcts, seizures, and stroke, commonly reported structural abnormalities in psychostimulant users include changes in striatal volumes (Ersche, Jones, Williams, Robbins, & Bullmore, 2013; Ersche, Williams, et al., 2013; Franklin et al., 2002; Hanlon, Dufault, Wesley, & Porrino, 2011), decreased gray matter volumes in frontal as well as cingulate, limbic and paralimbic cortices, and shrinkage of the hippocampus. These changes are correlated with deficits in various domains of cognitive functioning include impaired inhibitory control, abnormal preservation, decreased attentional control, impairments in learning and memory, among other deficits (Baicy & London, 2007; Wood, Sage, Shuman, & Anagnostaras, 2014). However, as with all studies using human subjects, the possibility exists that these brain abnormalities or cognitive deficits reflect pre-existing differences or polysubstance abuse, instead of the toxic effects of a particular drug (Aron & Paulus, 2007; Ersche, Williams, et al., 2013; Majewska, 1996).

While it is possible that such pre-existing differences precede psychostimulant abuse, animal studies have demonstrated a causal relationship between psychostimulant use and toxicity at the cellular level that likely mediate this macroscale alterations. The most commonly reported cellular neurotoxic effects are alterations in monoaminergic

signaling (mostly DA and 5-HT) as a result reduced presynaptic monoamine plasma membrane and vesicular transporters, formation of reactive oxygen and nitrogen species, DA quinones, and inflammatory cytokines (Berman, O'Neill, Fears, Bartzokis, & London, 2008; Fleckenstein, Volz, Riddle, Gibb, & Hanson, 2007; German et al., 2014; Halpin, Collins, & Yamamoto, 2013; Krasnova & Cadet, 2009; Larsen, Fon, Hastings, Edwards, & Sulzer, 2002; Majewska, 1996; Marshall & O'Dell, 2012; Panenka et al., 2012; Schwendt, Rocha, & See, 2009; Zhu, Xu, & Angulo, 2006). Others have also reported that psychostimulant use can lead to neurodegeneration through autophagic and apoptotic mechanisms (Cadet, Jayanthi, & Deng, 2003; Cunha-Oliveira et al., 2006; Davidson, Gow, Lee, & Ellinwood, 2001; Krasnova & Cadet, 2009; Nassogne, Louahed, Evrard, & Courtoy, 1997; Zhu et al., 2006). As a result of these neurodegenerative effects, astroglial proliferation (i.e. reactive astrogliosis) can also occur, reflecting both neuroinflammation early after insult or neuronal scarring long after insult (Krasnova & Cadet, 2009; Simões et al., 2008). Together, the loss of both terminals and cell bodies presumably underlie, at least partially, volumetric reductions and gray matter loss in the forebrain, striatum, and hippocampus reported following repeated psychostimulant use.

Given the facts that MDPV is highly reinforcing in rodents and there is evidence of compulsive use in humans, as well as an absence of studies examining neurotoxic and adverse cognitive effects of chronic long-term MDPV use, the overall purpose of the following experiments was to assess potential toxic effects, specifically neurodegeneration and astrogliosis, as well as cognitive deficits (decreased working memory and set shifting ability) following intravenous MDPV self-administration.

Furthermore, for comparison purposes separate groups of animals self-administered either METH or, as a non-drug control, sucrose.

Method

Subjects

All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee at Arizona State University and were according to the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health (NIH). For all experimental procedures, rats were housed according to NIH standards on a 12-hour light–dark cycle and behavioral testing sessions took place during the dark phase.

For experiment one, twenty-four male Sprague Dawley rats (Harlan Laboratories, Livermore, CA, USA), weighing approximately 250 g, were individually housed upon arrival. Rats were given *ad libitum* access to food and water during all experimental procedures, except during behavioral testing. Sixteen rats were implanted with jugular vein catheters and vascular access ports (see below for surgical methods) and underwent IVSA procedures. Eight additional rats placed into sucrose self-administration procedures did not receive surgery.

For experiment 2, twenty-four male Sprague Dawley rats (Harlan Laboratories, Livermore, CA, USA), weighing approximately 250 g, were individually housed upon arrival. After 2 acclimation days, all rats were placed into delayed-match-to-position and S+/S- reversal learning task training (see below for experiment 2 methods) before beginning IVSA procedures. Throughout all behavioral procedures in experiment 2, rats

were given 1 hour access to food following cognitive testing procedure (approximately 11 AM each day) prior to subsequent placement into self-administration procedures to maintain 85% of their free-feeding bodyweight throughout experimental testing. Rats were given *ad libitum* to water during all experimental procedures, except during behavioral testing. Sixteen rats were implanted with jugular vein catheters and vascular access ports and underwent IVSA procedures. Eight additional rats placed into sucrose self-administration procedures did not receive surgery. Four rats receiving catheterization lost patency prior to beginning IVSA experiments and were switched to oral self-administration.

Sucrose, drugs and Assessment of Purity

MDPV was obtained through an Internet website (http://www.researchchemz.com) (Laboratory Supply USA, San Diego, CA, USA). A 10-mg sample of MDPV was analyzed by liquid chromatography–mass spectrometry for purity at the Research Triangle Institute (Durham, NC, USA). Samples were analyzed using a Waters Synapt HDMS (Milford, MA, USA) quadrupole time-of-flight mass spectrometer interfaced to a Waters Acquity UPLC system. Data were acquired using a capillary voltage of 3 kV, source temperature of 150°C, desolvation temperature of 500°C, sampling cone at 30V and extraction cone at 4V. The mass spectrometer was externally calibrated from 50 to 700 Da using sodium formate solution, and mass shifts during acquisition were corrected using leucine enkephalin as a lockmass. Liquid chromatography was performed using a BEH C_{18} column (2.1 ¥ 50 mm, 1.7 µm particles) held at 40°C. Sample identity was confirmed based on exact mass, retention time and fragmentation match to a certified reference standard from Cerilliant (Round Rock, TX,

USA). MDPV samples were determined to have an apparent purity of >95%. For all behavioral studies, MDPV and methamphetamine (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in sterile saline. Rats placed into sucrose self-administration procedures received one 45 mg sucrose pellet (Test- Diet, Richmond, IN, USA) as a reinforcer. During cognitive testing procedures (see below under experiment 2), chocolate and banana flavored 45 mg sucrose pellets (Bio-Serv, NJ, USA) served as reinforcers for DMTP and S+/S- procedures, respectively. However, it should be noted that for DMTP procedures after LgA session 6, chocolate pellets became unavailable due to manufacturing issues with the vendor, at which point standard 45 mg sucrose pellets were used.

Surgical Procedures

For IVSA surgical procedures (experiments 1 and 2), rats were anesthetized with isoflurane (2% v/v, Butler Schein Animal Health, Dublin, OH, USA) vaporized in oxygen at a flow rate of 2 l/min. Rats received pre-incision injections of buprenorphine (0.05 mg/kg, s.c., Reckitt Benckiser, Richmond, VA, USA) and meloxicam (1 mg/kg, s.c., Boehringer Ingelheim, St. Joseph, MO, USA). Surgical sites were shaved and cleaned with 1% iodine. A ~2 cm incision was made in order to isolate the right or left jugular vein. A sterile silastic catheter filled with 100 U/ml heparin was inserted 2.5cm into the vein. The catheter was secured to the surrounding tissue with sutures, and the opposite end of the catheter was tunneled subcutaneously to the dorsum where it exited the skin between the scapulae. The catheter was secured to the surrounding tissue by sutures and a mesh collar attached to a threaded vascular access port (Plastics One, Roanoke, VA, USA). Access ports were sealed with a piece of Tygon tubing (Cole-

Parmer, Vernon Hills, IL, USA) closed at one end and a protective cap. Rats received one week of post-operative care including daily infusions of 0.4 ml Timentin (66.6 mg/ml, in 70 U/ ml heparinized saline) to protect against infection and ensure catheter patency. Meloxicam (2.5 mg/ml, s.c.) was administered for the first 3 days following surgical procedures to provide additional relief of post-surgical discomfort. In addition, the rats were given ten 45 mg sucrose pellets in their home cage 4 days prior to IVSA procedures to eliminate neophobia to sucrose pellets that could delay acquisition of self-administration during the two 2 hr training sessions.

Experiment 1: IVSA Apparatus

Drug and sucrose self-administration sessions were conducted in operant self-administration chambers (ENV-008; Med Associates, St. Albans, VT, USA). All self-administration chambers were located inside the sound-attenuating cubicles equipped with a house light and exhaust fan designed to mask external noise and odors, and were interfaced to a personal computer (PC). Chambers were equipped with two stainless steel response levers located on one wall with a 4.2 x 5 cm food pellet receptacle placed between levers. Each response lever was located approximately 7 cm above a stainless steel grid floor, and positioned above each lever was a 2.5-cm diameter white stimulus light. Located near the top of the self- administration chambers was a Sonalert speaker that provided an auditory stimulus during drug delivery. For drug self-administration, outside each chamber was a syringe pump that was interfaced to the computer and delivered the drug solution via a single-channel liquid swivel mounted atop the chamber via polyethylene tubing. Sucrose pellets were delivered through the food pellet receptacle between the two levers.

Experiment 1: Self-administration Procedures

Following recovery from the surgical procedures, self-administration sessions commenced with two 2-hour sucrose self-administration sessions to initiate lever pressing. During all self-administration sessions, each press on the active lever delivered the reinforcer on a fixed-ratio 1 (FR1) schedule of reinforcement. Reinforcer delivery was accompanied by concurrent illumination of a stimulus light and presentation of an auditory stimulus for 2 seconds, followed by a 20-second timeout period, during which additional lever presses were recorded but produced no programmed responses. Inactive lever presses were recorded but produced no programmed responses. Rats were separated into one of three reinforce groups with 8 subjects initial per group; however, one rat from each of the MDPV and METH IVSA groups was removed from the study for catheter patency failure. The three results groups were: an MDPV group receiving 0.05 mg/kg per infusion (N=7), a METH group receiving 0.05 mg/kg per infusion (N=7), or a sucrose group (N=8) receiving a single 45 mg sucrose pellet per reinforcer delivery. Each drug infusion was delivered in a volume of 0.06 ml. MDPV and methamphetamine reinforcers were delivered to the vascular access port by polyethylene tubing housed in a stainless steel spring tether that was attached to the liquid swivel. Sucrose pellets were delivered to the pellet receptacle located between the active and inactive levers. Self-administration sessions were conducted 7 consecutive days per week, and each session was preceded and followed by an intravenous infusion of 0.1 ml Timentin (66.6 mg/ml, in 70 U/ ml heparinized saline) to maintain catheter patency. Daily 2-hr self-administration sessions were conducted for 16 days. Next, all rats began extended access sessions during which

the session length was extended to six hours. All other aspects of the self-administration procedures remained the same.

Tissue Processing

24 hours following the last 6-hour self-administration session, rats were deeply anesthetized with sodium pentobarbital, 150 mg/kg i.p. and perfused transcardially with 100 ml of phosphate-buffered saline (PBS, pH=7.4) followed by 200 ml 4% w/v paraformaldehyde in PBS (pH=7.4). Next, the skull was processed by removal of the skin, ears, fascia, eyes, and cartilaginous nose tips from the skull. The skull was post-fixed in 4% w/v paraformaldehyde at 4°C for 12 hours. Next, the skull is removed and immersed in PBS at 4°C. PBS was replaced every 24 hours for the first five days at which point the skull was agitated for 2-3 minutes. Next, brains were removed from the skull, cryoprotected with 30% sucrose for 2 days at 4°C, and cut into coronal sections (40 μm thickness) on a cryostat (Leica CM9000). Coronal adjacent serial sections (in groups of 6) were taken through the regions of interest and placed sequentially into cell culture dish wells containing 0.1 M phosphate buffer (i.e., 1 well per intended stain). Serial sections for orbital staining began at bregma +3.20 mm. Serial sections for striatal (caudate, putamen, nucleus accumbens core and shell) sections began at bregma +1.60 mm and serial sections for the hippocampus began at bregma -3.14 mm.

Astrogliosis Assessment: Glial Fibrillary Acid Protein (GFAP) Immunohistochemical Staining Procedures

For immunohistochemical staining, sections were incubated in 10% methanol to quench endogenous peroxidase activity, washed in PBS, preblocked for 1 hr in PBS containing 0.1% Tween 20, 1 M glycine, and 5% v/v donkey serum, followed by

overnight incubation with GFAP primary (1:5000) antisera (product code AB7260, Abcam, MA, USA) at 4°C. On the next day, sections are washed, incubated with biotinylated secondary antisera (1:500; Jackson ImmunoResearch), following by chromogenic detection using a HRP-based Vectastain Elite ABC kit and nickel-enhanced diaminobenzidine (DAB) as a substrate (Vector Laboratories) to generate gray/black color. Sections were again washed, mounted onto microscope slides, and coverslipped with VectaMount Permanent mounting media. Photomicrographs were taken under brightfield microscopy at 10x magnification (Leica Microsystems; Bannockburn, IL, USA) using a digital camera interfaced to a PC computer.

Neurodegeneration Assessment: Fluoro-jade C Staining Procedures

For assessment of neurodegeneration, sections were stained with FluoroJade C (product code AG325, Merck Millipore, MA, USA) using procedures previously described elsewhere (Gass & Olive, 2009; Schmued, Stowers, Scallet, & Xu, 2005). Tissue sections were first mounted on 1% pig skin gelatin from distilled water. Slides were then air dried on a slide warmer for 60 minutes at 50 °C. Next, slides were immersed in a solution of 1% sodium hydroxide in 80% histological grade ethanol for 5 minutes. Slides were then rinsed in 70% ethanol for 2 minutes, then distilled water for 2 minutes, followed by incubation in 0.06% potassium permanganate solution for 10 minutes. Next, slides were immersed in a 0.0001% FluoroJade C solution of 0.1% acetic acid. Slides were then rinsed three times in distilled water for 1 minute per rinse before air-drying for 10 minutes on the slide warmer at 50 °C. Slides were then clear with xylenes for 1 minute and, before being allowed to dry, coverslipped with DePex (Sigma-Aldrich, St. Louis, MO, USA) mounting media.

In order to verify staining efficacy, one male Sprague-Dawley rat was used as a positive control and given a single 1 µg/µl intracerebroventricular (ICV) administration of kainic acid (KA) and transcardially perfused 24 hours later. Previous research has shown that method of KA treatment produces robust neurodegeneration in hippocampal tissue (Nadler, Perry, & Cotman, 1978; Sperk, 1994). During staining of each region of interest (ROI), hippocampal tissue from the KA positive control subject was included to verify staining continuity across assays. 40 µm sections were then obtained using identical tissue processing procedures described above. Photomicrographs were taken under epifluorescence microscopy at 488 nm excitation using a Leica DMLB microscope equipped with a digital camera that was interfaced to a PC computer.

Image Analysis

For GFAP staining, Image analysis was performed using ImageJ (National Institutes of Health, Bethesda, MD, USA) by two independent experiments. Subsequent inter-rater reliability was greater than 95%. Following contrast adjustment, both cell counts and percent total area were calculated for the ROIs (CA1 and CA3 of the hippocampus; medial PFC) in stained sections (striatal GFAP quantification was not possible due to non-specific interference from striosome staining). For neurodegeneration staining, no evidence of positive FluoroJade C staining was apparent when assessed by two independent experiments blind to groups of animals for ROIs including hippocampus, medial PFC, and striatum.

Experiment 2: Cognitive Task Training

After two acclimation days, rats begin both S+/S- reversal learning and delayed-match-to-position (DMTP) training (see methods below). During initial training, 50 trials

were conducted daily for both tasks until all rats met performance criterion (85% correct responses for 2 consecutive days). Following acquisition of performance criterion for both tasks, rats were placed into DMTP probe tasks or the first reversal for the S+/S- task in order to assess baseline cognitive performance before placement into one of three self-administration groups (see below until experiment 2 IVSA methods). Throughout all DMTP and S+/S- reversal procedures, rats were given food for 1 hour a day 30 minutes following behavioral procedures to maintain 85% of their free feeding bodyweight.

Experiment 2: DMTP Apparatus

DMTP tasks were conducted in operant chambers (ENV-008; Med Associates, St. Albans, VT, USA). All operant chambers were located inside the sound-attenuating cubicles equipped with a house light and exhaust fan designed to mask external noise and odors, and were interfaced to a personal computer (PC). Chambers were equipped with two stainless steel retractable response levers located on one wall with a 4.2 x 5 cm sucrose pellet receptacle placed between levers. Each response lever was located approximately 7 cm above a stainless steel grid floor. Chocolate sucrose pellets were delivered through the food pellet receptacle between the two levers. Prior to beginning daily DMTP testing, four drops of orange scented oil extract were placed into the bedding located in pans located beneath the grid floor to further aid in testing environment discrimination between DMTP from S+/S- tasks.

Experiment 2: DMTP Training

For DMTP, rats were placed into the chamber with the house light off and both levers retracted, and after an initial 10 sec delay, the house light turned on and the session began with the first trial. For each trial, one of the two retractable levers (i.e. the sample

lever) would be presented for 10 sec. Presses on the sample lever yielded a chocolate flavored sucrose pellet on an FR1 schedule and initiated a 1 sec waiting period where both levers were retracted. Failure to press on the sample lever was termed a forfeited trial and initiated a 30 sec inter-trial-interval during which both lever were retracted and the house light turned off (blackout ITI). After the 1 sec waiting period, both levers were ejected. Presses on the previous sample lever yielded a chocolate pellet reinforcer on an FR1 schedule and initiated a 10 sec inter-trial-interval where the house light remained on. Presses on the non-sample lever initiated a 30 sec blackout ITI period and no reinforcer was given. Failure to press on either lever during the choice phase after 10 sec was recorded as an incorrect response and initiated a 30 sec blackout ITI. Sessions ended after 50 completed trials or 100 forfeited trials, whichever occurred first.

Experiment 2: DMTP Probe Tests

After meeting criterion on DMTP training tasks, a DMTP probe tasks was initiated to assess baseline cognitive performance in all rats. During probe tasks, the initial 10 trials were identical to training trials described above. Following the initial 10 trials, four the remaining 40 trials, a series of 4 waiting periods (consisting of 5, 10, 30 and 60 sec) were presented following presses on the sample lever. These four waiting period were presented randomly without replacement ever four trials until all waiting periods were introduced in the four trial block (i.e. across 40 trials, 10 four trial block were presented). All other task parameters remained identical to DMTP training sessions. Following probe tasks, all rats were placed back into DMTP maintenance (identical to parameters used during training) training every 48 hours to maintain task performance prior to later probe tasks. The second probe test was conducted following 15 or 16 days

of 2 hour short access (ShA) self-administration procedures (see methods below) and a third reversal test was conducted following 15 or 16 days of 6 hour long access (LgA) self-administration procedures (see methods below).

Experiment 2: S+/S- Training and Reversal Apparatus

S+/S- training sessions were conducted in operant chambers (ENV-008; Med Associates, St. Albans, VT, USA). All operant chambers were located inside the sound-attenuating cubicles equipped with a house light and exhaust fan designed to mask external noise and odors, and were interfaced to a personal computer (PC). Chambers were equipped with two stainless steel non-retractable response levers located on one wall with a 4.2 x 5 cm sucrose pellet receptacle placed between levers. Each response lever was located approximately 7 cm above a stainless steel grid floor, and positioned above each lever was a 2.5-cm diameter white stimulus light. Banana flavored sucrose pellets were delivered through the food pellet receptacle between the two levers. Prior to beginning daily S+/S- testing, four drops of anise scented oil extract were placed into the bedding located in pans located beneath the grid floor to further aid in testing environment discrimination between DMTP from S+/S- tasks.

Experiment 2: S+/S- Training

For S+/S- training, rats were placed into the chamber with the house light off and both levers ejected. Sessions began when the house light turned on and both stimulus lights over the levers turned on. Initially, the right lever was deemed the S+ lever and left lever deemed the S- lever. Responses on the right lever yielded a banana flavored sucrose pellet reinforcer on an FR1 schedule, initiated a 2 sec tone stimulus and initiated a 2 second inter-trial interval during which both stimulus lights turned off and additional

presses were recorded but produced no programmed consequences. Responses on the left lever yielded no reinforcement but initiated a 5 sec ITI during which both stimulus light turned off and presses were recorded but had no programmed consequences. After the 5 sec ITI, both stimulus light above the levers were illuminated signaling the beginning of the next trial. Sessions ended after 50 completed trials or 2 hours, whichever occurred first.

Experiment 2: S+/S- Reversal Test

After meeting criterion on S+/S- training task, a reversal test was initiated to assess baseline set shifting performance in all rats. During the reversal task, all task parameters remained identical to training with the only change being that the right lever now became S- (i.e. trial responses now longer reinforced) and the left lever became S+ (i.e. trial responses now reinforced). Following the initial reversal test, the left lever remained S+ and right lever S- until the next reversal test was conducted during which sessions were conducted every 48 hours, alternating test days with the DMTP maintenance testing. The second reversal test was conducted following 15 or 16 days of 2 hour ShA self-administration procedures (see below) and a third reversal test was conducted following 15 or 16 days of 6 hour LgA self-administration procedures (see below).

Experiment 2: Self-administration Apparatus

Drug and sucrose self-administration sessions were conducted in operant self-administration chambers (ENV-008; Med Associates, St. Albans, VT, USA). All self-administration chambers were located inside the sound-attenuating cubicles equipped with a house light and exhaust fan designed to mask external noise and odors, and were

interfaced to a personal computer (PC). Chambers were equipped with two nosepokes located on one wall with a 4.2 x 5 cm food pellet receptacle placed between levers. Each nosepoke was located approximately 6 cm above a stainless steel grid floor and inside each nosepoke was a 1 cm diameter white stimulus light. Located near the top of the self-administration chambers was a Sonalert speaker that provided an auditory stimulus during drug delivery. For drug self-administration, outside each chamber was a syringe pump that was interfaced to the computer and delivered the drug solution via a single-channel liquid swivel mounted atop the chamber via polyethylene tubing. Sucrose pellets were delivered through the food pellet receptacle between the two nosepokes.

Following the initial DMTP probe test and first reversal test, rats were placed into three separate groups of eight rats such that baseline performance on the DMTP probe was approximately equal across the groups. The three groups were placed into either a METH (0.05 mg/kg/infusion; N = 6) or MDPV (0.05 mg/kg/infusion; N = 7) IVSA group, or an oral sucrose self-administration group (n = 12). Rats placed into the METH and MDPV IVSA groups underwent surgical procedures (described above) and allowed to recovery for five days. Rats in the oral sucrose group received no surgery. Following recovery from surgical procedures, self-administration sessions commenced with no nosepoke training (i.e. spontaneous acquisition). During all IVSA procedures, the active nosepoke was signaled by illumination of the stimulus inside the nosepoke aperture and was randomly altered between the right and left nosepoke after each reinforcer delivery. During all self-administration sessions, each response on the active nosepoke delivered the reinforcer on a fixed-ratio 1 (FR1) schedule of reinforcement. Reinforcer delivery was accompanied by concurrent presentation of an auditory stimulus for 2 seconds,

followed by a 20-second timeout period, during which the stimulus light inside the nosepoke turned off and additional nosepokes were recorded but produced no programmed responses. Responses on the inactive nosepoke were recorded but produced no programmed responses. As mentioned above, rats were separated into one of three reinforcer groups, based upon cognitive performance on the initial DMTP probe test, with initially 8 subjects per group. However, two rats from each of the MDPV and METH IVSA groups lost patency prior to IVSA procedures and were transferred to the oral sucrose self-administration group. The three resulting groups were: an MDPV group receiving 0.05 mg/kg per infusion (N=6), a METH group receiving 0.05 mg/kg per infusion (N=6), or a sucrose group (N=12) receiving a single 45 mg sucrose pellet per reinforcer delivery. Each drug infusion was delivered in a volume of 0.06 ml. MDPV and methamphetamine reinforcers were delivered to the vascular access port by polyethylene tubing housed in a stainless steel spring tether that was attached to the liquid swivel. Sucrose pellets were delivered to the pellet receptacle located between the active and inactive nosepokes. Self-administration sessions were conducted 7 consecutive days per week, and each session was preceded and followed by an intravenous infusion of 0.1 ml Timentin (66.6 mg/ml, in 70 U/ ml heparinized saline) to maintain catheter patency. Daily 2 hour ShA self-administration sessions were conducted for 16 days. Next, all rats began long access sessions during which the session length was extended to six hours for a total of 16 sessions. All other aspects of the self-administration procedures remained the same.

Data and Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics version 21 (Armonk, New York, USA). All data points represent mean ± SEM. A significance criterion of $p<0.05$ was used for all analyses.

For experiment 1, the first 16 ShA IVSA sessions were analyzed with a 2 (lever: active vs. inactive) X 16 (session: 1 – 16) mixed analysis of variance (ANOVA) for MDPV (N = 7), METH (N = 7), and sucrose groups (N=8) separately. Post-hoc t-tests were conducted at each session to assess differences between active and inactive lever presses. For LgA procedures, total number of reinforcers were analyzed with repeated measures ANOVAs with session as repeated measures (sessions 1 – 10) to assess for escalation from the first session of LgA for the MDPV, METH, and sucrose groups separately. Pairwise comparisons were used to assess for significant differences in total reinforcer intake versus LgA session 1. For GFAP, data represents the mean generated by total counts and total percent area from 2 – 4 brain slices from both hemispheres (i.e. mean of 4 – 8 separate determinations for each subject). Final total counts and percent area represent the mean generated by two independent raters showing greater than 95% inter-rater-reliability. GFAP cell counts and total percent area of the region of interest were analyzed with one-way ANOVAs with group (MDPV, METH, or sucrose: N = 7, 7, and 8, respectively) as the between subjects factor. For FluoroJade C neurodegeneration staining, one-way ANOVAs with group (MDPV, METH, or sucrose; N = 7,7, and 8, respectively) as the between subjects factor were planned, but visual inspection of slices revealed no positive cell counts and thus quantitative analyses were not performed (see Fig. 25 for representative images).

For experiment 2, the first 16 ShA IVSA sessions were analyzed with a 2 (nosepoke: active vs. inactive) X 16 (session: 1 – 16) mixed analysis of variance (ANOVA) for MDPV (N = 6), METH (N = 6), and sucrose groups (N = 12) separately. Post-hoc t-tests were conducted at each session to assess differences between active and inactive lever presses. For LgA procedures, total number of reinforcers were analyzed with repeated measures ANOVAs with session as repeated measures (sessions 1 – 16) to assess for escalation from the first session of LgA for the MDPV (N = 6), METH (N = 6), and sucrose groups (N = 12) separately. For DMTP probe tests, percent correct responses at each waiting interval (1, 5, 10, 30, 60, and total) were analyzed with one-way ANOVAs with group (MDPV, METH, or sucrose; N = 6, 6, and 8, respectively) as the between subjects factor. Also for DMTP probe tests, total number of completed and forfeited trials were also analyzed with one-way ANOVAs with group (MDPV, METH, or sucrose) as the between subjects factor and post-hoc Holm-Sidak tests for group comparison. For S+/S- reversal tests, percent correct responses and total number of trials completed were analyzed with one-way ANOVAs with group (MDPV, METH, or sucrose; N = 6, 5, and 11, respectively) serving as the between subjects factor with Holm-Sidak post-hoc tests used for individual group comparison. One subject from both the METH and sucrose group did not reach acquisition criterion prior to the initial reversal test and were not included in any of the S+/S- reversal tests. Finally, bodyweight assessed the morning of each of the cognitive testing time points was analyzed with a one-way ANOVA with group (MDPV, METH, or sucrose) as the between subjects factor and Holm-Sidak post-hoc tests between individual groups.

Results

Experiment 1: Self-administration

For MDPV, across 2-hour (ShA) sessions for 16 days, 2 (active vs inactive levers) X 16 (ShA session) mixed ANOVAs revealed a significant main effect of session ($F[15,195] = 2.52$, $p < 0.01$), lever ($F[1,13] = 18.55$, $p < 0.001$), and session X lever interaction ($F[15,195] = 2.939$, $p < 0.001$). Post-hoc t-tests revealed that MDPV rats successfully discriminated between and active and inactive levers with active lever presses greater than inactive lever presses across all sessions, all p's < 0.05 (Fig. 20a).

For METH, across 2-hour (ShA) sessions for 16 days, 2 (active vs inactive levers) X 16 (ShA session) mixed ANOVAs revealed a significant main effect of session ($F[15,210] = 3.75$, $p < 0.001$), lever ($F[1,14] = 13.15$, $p < 0.01$), and session X lever interaction ($F[15,210] = 2.31$, $p < 0.01$). Post-hoc t-tests revealed that METH rats successfully discriminated between and active and inactive levers with active lever presses greater than inactive lever presses across all sessions, all p's < 0.05 (Fig. 20b).

For sucrose, across 2-hour (ShA) sessions for 16 days, 2 (active vs inactive levers) X 16 (ShA session) mixed ANOVAs revealed a significant main effect of session ([15,210] = 4.80, $p < 0.001$) lever ($F[1,14] = 42.77$, $p < 0.001$), and session X lever interaction ($F[15,210] = 4.95$, $p < 0.001$). Post-hoc t-tests revealed that sucrose rats successfully discriminated between and active and inactive levers with active lever presses greater than inactive lever presses across all sessions, all p's < 0.05 (Fig. 20c).

Following ShA, rats were placed into LgA session for 10 additional days. ANOVAs revealed a significant effect of session in the METH ($F[9,54] = 2.09$, $p< 0.05$) and MDPV ($F [9,54] = 3.05$, $p< 0.005$) groups, but not in sucrose rats ($F[9,63]= 1.37$, p>

0.05) (Fig. 21a). Pairwise comparisons revealed a trend toward escalation of intake as measured by an increase in the number of reinforcers on LgA session 10 vs LgA session 1 for the MDPV group, $p = 0.052$. A trend toward escalation of intake was also found for METH LgA session 10 vs LgA session 1 for the METH group, $p = 0.061$. Sucrose rats did not significantly escalate intake across LgA sessions.

For the first two hours of LgA for MDPV, there was a significant main effect of session ($F[9,54] = 2.86$, $p < 0.005$), but when compared to day 1, pairwise comparisons for LgA session 10 only showed a trend towards escalation, $p = 0.10$. Rats in the METH group did not significantly escalate intake in the first two hours ($F[9,54] = 1.27$, $p > 0.05$). Rats in the sucrose groups showed significant changes in reinforcers obtained across the first two hours of LgA, but did not escalate from day 1 ($F[9,63] = 2.49$, $p < 0.05$) (Fig. 21b).

Experiment 1: GFAP Total Counts and Percent Total Area

For the CA1 area, ANOVA did not reveal significant group different for total positive cell counts ($F[2,19] = 1.13$, $p > 0.05$) (Fig. 22a), or percent total area ($F[2,19] = 1.22$, $p > 0.05$) (Fig. 22b). For the CA3 area, ANOVA did not reveal significant group different for total positive cell counts ($F[2,19] = 0.03$, $p > 0.05$) (Fig. 23a), or percent total area ($F[2,19] = 0.67$, $p > 0.05$) (Fig. 23b). For the medial PFC area, ANOVA did not reveal significant group differences for total positive cell counts ($F[2,19] = 2.12$, $p > 0.05$) (Fig. 24a), or percent total area ($F[2,19] = 2.24$, $p > 0.05$) (Fig. 24b).

Experiment 1: FluoroJade C Staining

No FluoroJade C staining was observed in the dorsal hippocampus, striatum, or medial prefrontal cortex (Fig. 25). Fluorojade C staining was, however, observed in the dorsal hippocampus of the rat treated with KA (Fig. 25).

Experiment 2: Self-administration

For MDPV, across 2-hour (ShA) sessions for 16 days, 2 (active vs inactive nosepokes) X 16 (ShA session) mixed ANOVAs revealed a significant main effect of session ($F[15,150] = 2.52$, $p < 0.01$), nosepokes ($F[1,10] = 8.13$, $p < 0.05$), and session X nosepoke interaction ($F[15,150] = 2.32$, $p < 0.001$). Post-hoc t-tests revealed that MDPV rats successfully discriminated between and active and inactive nosepokes with active responses greater than inactive responses for all sessions after ShA session 4, all p's < 0.05 (Fig. 26a).

For METH, across 2-hour (ShA) sessions for 16 days, 2 (active vs inactive nosepokes) X 16 (ShA session) mixed ANOVAs revealed a significant main effect of session ($F[15,150] = 2.54$, $p < 0.01$), nosepokes ($F[1,10] = 83.13$, $p < 0.01$, and session X nosepoke interaction ($F[15,150] = 2.32$, $p < 0.01$). Post-hoc t-tests revealed that METH rats successfully discriminated between and active and inactive nosepokes with active responses greater than inactive responses across all sessions, all p's < 0.05 (Fig. 26b).

For sucrose, across 2-hour (ShA) sessions for 16 days, 2 (active vs inactive nosepokes) X 16 (ShA session) mixed ANOVAs revealed a significant main effect of session ($F[15,330] = 5.60$, $p < 0.001$) nosepokes ($F[1,22] = 430.66$, $p < 0.001$), and session X nosepoke interaction ($F[15,330] = 6.40$, $p < 0.001$). Post-hoc t-tests revealed that sucrose rats successfully discriminated between and active and inactive nosepokes

with active responses greater than inactive responses across all sessions, all p's < 0.05 (Fig. 26c).

Following ShA, rats were placed into LgA sessions for 16 additional days. ANOVAs revealed a significant effect of session in the sucrose ($F[15,165]= 6.89$, $p < .001$) and MDPV ($F[15,75] = 2.07$, $p < 0.05$) groups, but not for METH ($F[15,75] = 1.25$, $p>.05$) (Fig. 26d). For sucrose reinforcers, compared to LgA session 1, LgA sessions 3, 11, and 13-16 were significantly lower (p's < 0.05). For MDPV infusions, pairwise comparisons did not reveal significant any significant escalation for any LgA session compared to LgA session 1.

Experiment 2: DMTP Probe Tests

For percent correct responses on the Pre-IVSA DMTP probe test (Fig. 27a), ANOVA revealed a significant main effect of waiting time ($F[4,84] = 35.176$, $p < .001$), but not treatment group ($F[2,21] = 0.433$, $p > 0.05$), or a waiting time X treatment group interaction ($F[8,84] = 0.399$, $p > 0.05$). Pairwise comparisons revealed performance steadily decreased as waiting times increased, within significance occurring between all waiting times (p's < 0.05) with the exception of the 30 and 60 sec waiting time. For total forfeited and completed trials (Figs. 28a and b, respectively), ANOVA revealed no significant differences between treatment groups (p's > 0.05).

For percent correct responses on the Post-ShA DMTP probe test (Fig. 27b), ANOVA revealed a significant main effect of waiting time ($F[4,84] = 38.93$, $p < 0.001$), but not treatment group ($F[2,21] = 0.45$, $p > 0.05$), or a waiting time X treatment group interaction ($F[8,84] = 0.74$, $p > 0.05$). Pairwise comparisons revealed the following differences across waiting times: performance steadily decreased as waiting times

increased between the 1 and 5 sec waiting times compared with all waiting times (p's < 0.05), (2) no differences were found between 10, 30 and 60 sec waiting times. For total forfeited and completed trials (Figs. 28a and b, respectively), ANOVA revealed no significant differences between treatment groups (p's > .05).

For percent correct responses on the Post-LgA DMTP probe test (Fig. 27c), ANOVA revealed a significant main effect of waiting time ($F[4,84] = 33.28$, $p < 0.001$), but not treatment group ($F[2,21] = 0.38$, $p > 0.05$), or a waiting time X treatment group interaction ($F[8,84] = 1.23$, $p > 0.05$). Pairwise comparisons revealed the following differences across waiting times: (1) performance steadily decreased from the 1 sec waiting time for all other waiting times, (2) performance during the 5 sec waiting time was significantly better than the 30 and 60 sec waiting time, (3) performance during the 10 sec waiting time was better than the 60 sec waiting time (p's < 0.05). No other pairwise comparisons were significant. For total completed trials (Fig. 28b), ANOVA revealed no significant differences between treatment groups (p's > 0.05). For forfeited trials (Fig. 28a), ANOVA revealed a significant difference in the number of forfeited trials ($F[2,21] = 9.937$, $p < 0.001$), with Holm-Sidak post-hoc tests showing that sucrose rats forfeited significantly more trials than rats in either the MDPV or METH group (p's < 0.05 and 0.01, respectively).

Experiment 2: S+/S- Reversal Tests

For percent correct on the Pre-IVSA S+/S- reversal test (Fig. 29), ANOVA revealed no significant effect of treatment group ($F[2,19] = 1.09$, $p > 0.05$). For percent correct on the Post-ShA S+/S- reversal test (Fig. 29), ANOVA revealed no significant effect of treatment group ($F[2,19] = 0.52$, $p > 0.05$). For percent correct on the Post-LgA

S+/S- reversal test (Fig. 29), ANOVA revealed no significant effect of treatment group ($F[2,19] = 2.97$, $p > 0.05$). Finally, no significant differences in total number of completed trials were found across the three S+/S- reversal tests as all subjects in each group completed all 50 trials (p's > 0.05).

Body weights

For body weights (Fig. 30), ANOVA did not reveal any significant differences between treatment groups for the Pre-IVSA cognitive tests (p's > 0.05). For the Post-ShA test, body weight was significantly different ($F[2,21] = 5.10$, $p < 0.05$), with Holm-Sidak post-hoc tests revealing a greater body weight in the sucrose group compared to MDPV group ($p < 0.05$). For the Post-LgA test, body weights were significantly different ($F[2,21] = 66.492$, $p < 0.001$), with Holm-Sidak post-hoc tests revealing greater body weight in the sucrose group compared to MDPV and METH groups (p's < 0.001).

Discussion

The results of the present study revealed that during both ShA and LgA IVSA, MDPV produces robust IVSA behavior similar to that of METH, replicating previously published findings from both our laboratory (Watterson et al., 2014) and others (Aarde et al., 2013). However, as shown in experiment 1, when compared to oral sucrose controls, self-administratin of neither MDPV nor METH produced evidence of neurotoxicity in the three ROIs assessed (medial PFC, dorsal hippocampus, or striatum). Specifically, no evidence of astrogliosis was revealed by changes in GFAP immunoreactivity between the three groups , and no evidence of neurodegeneration (as evidenced by a lack of FluoroJade C staining) was found in any experimental animals other than a positive

control subject injected with KA. In experiment 2, following both 16 days of ShA or LgA self-administration procedures, no evidence of working memory deficits or abnormal perseveration were revealed from either the DMTP nor S+/S- tasks, respectively, for either drug when compared to sucrose controls. Together, results from this study suggest that IVSA of MDPV and METH under the current procedural parameters does not lead to neurotoxic or adverse cognitive effects similar to those reported in human users of illicit psychostimulants.

GFAP is a cytoskeletal intermediate filament protein expressed exclusively in astrocytes. GFAP is a validated biomarker of toxicity as damage to both neurons and glial cells can elicit astrogliosis that can result from numerous types of toxic insults including drugs, chemicals, organic trauma and disease (O'Callaghan & Sriram, 2005; O'Callaghan, 1991). Astrogliosis can occur both shortly after insult as a neuroimflammatory response or later as glial scarring (Hostenbach, Cambron, D'haeseleer, Kooijman, & De Keyser, 2014). For amphetamine-type stimulants, increased GFAP activity has become a popular measure of toxicity and has extensively been used as a biomarker of CNS damage (O'Callaghan & Miller, 1993, 1994). Numerous studies have now shown robust astrogliosis in rodents following exposure to METH (Achat-Mendes, Anderson, & Itzhak, 2007; Deng, Ladenheim, Tsao, & Cadet, 1999; Kuczenski et al., 2007; Zhu, Xu, & Angulo, 2005; Cappon, Pu, & Vorhees, 2000; Zhu et al., 2005; Friend & Keefe, 2013; Krasnova et al., 2010), cocaine (Blanco-Calvo et al., 2014; Bowers & Kalivas, 2003; Fattore et al., 2002), and the synthetic cathinone mephedrone (Martínez-Clemente et al., 2014). Furthermore, these effects have been reported in numerous brain regions including the cortex, striatum, and hippocampus (for

reviews see (Gonçalves, Baptista, & Silva, 2014; Krasnova & Cadet, 2009). As mentioned previously, MDPV is neurochemically most similar to cocaine as a selective DAT blocker, but produces much longer lasting effects on striatal extracellular DA and similar patterns of IVSA that most closely resembles that of METH (Aarde et al., 2013; Baumann, Partilla, Lehner, et al., 2013; Watterson et al., 2014). As such, we predicted that IVSA of MDPV and METH would also produce astrogliosis in our ROIs (hippocampus, striatum, and medial PFC). Despite the robust self-administration across both ShA and LgA, levels of GFAP immunoreactivity in the the hippocampus and medial PFC were not significantly different for either drug than rats self-admiinstrering sucrose. Striatal GFAP quantification was not possible due to non-specific interference from striosome staining.

While GFAP immunoreactivity in rats self-administering either MDPV or METH was not significantly greater from sucrose rats as we had predicted, this is perhaps not entirely surprising as the majority of studies previously reporting astrogliosis following either cocaine, mephedrone, or METH exposure, have used high-dose experimenter-administered (non-contingent) treatment regimens of either single large bolus or repeated binge-like doses (for reviews see (Krasnova & Cadet, 2009; Gonçalves et al., 2014). Indeed, to our knowledge, only a limitied number of published studies have employed METH self-administration procedures and subsequently assessed GFAP levels (Schwendt et al., 2009; Krasnova et al., 2010; Reichel, Ramsey, Schwendt, McGinty, & See, 2012), and no mephedrone self-administration studies assessing GFAP levels have been published. In the first of these IVSA METH studies (Schwendt et al., 2009), male Long-Evans rats either self-administered METH (0.02 mg/infusion) or received yoked saline

for 7 – 10 1-hr sessions, followed by 12-14 days of 6 hr LgA and then approximately 2 weeks of extinction sessions. Results from this study revealed that despite an escalation in METH intake, no resulting increases in GFAP immunoreactivity (as assessed by immunoblotting) occurred in the prefrontal cortex, dorsal striatum, or nucleus accumbens when compared to saline rats. Furthermore, other common markers of toxicity including reductions in immunostaining for tyrosine hydroxylase, NET, or SERT, or immunostaining for the microglial activation marker Iba-1, were also not significantly different between METH and saline rats in these same regions. Only decreases in DAT immunoreactivity in the PFC and striatum were significant. In a second study (Reichel et al., 2012), rats received either an experimentally administered binge-dose regimen of METH (four 4 mg/kg i.p. injections given every 2 hours) or METH IVSA (0.02 mg/infusion, 7 days for 1 h/day, followed by 14 days for 6 h/day) followed by 8 days of abstinence. In rats receiving the experimenter-administered (non-contingent) binge-dose regimen, GFAP striatal immunoreactivity (as assessed by immunoblotting) was increased relative to saline controls; however, GFAP levels were not assessed in any other regions. Furthermore, TH and DAT were also decreased in striatal tissue. However, in rats undergoing IVSA procedures, no significant differences were found in GFAP, TH, or DAT immunoreactivity, underscoring that important differences occur following experimenter administered vs voluntary self-administration of METH. In the third study (Krasnova et al., 2010), rats were placed into either METH IVSA (0.1 mg/kg/infusion) procedures or yoked saline control procedures for 15 hours a day for 8 consecutive days. After 7 days of abstinence, both striatal and cortical tissue showed robust increases in GFAP levels (as assessed by immunoblotting) vs saline controls; furthermore, GFAP

immunoreactivity was significantly correlated with total METH intake across the 8 days of IVSA in both regions. Increased GFAP immunoreactivity was also accompanied by significant decreases in TH and DAT levels in these same regions.

Thus, in other studies uisng IVSA procedures similar to those in the present study with METH and MDPV, effects on GFAP expression in various brain regions is inconsistent which are likely the result of the different task or dosing parameters employed across these studies. Major disparities between our study and those discussed above include differences in METH dose, overall amount of METH intake, tissue assays employed (IHC in the present study and immunoblotting in the others), and the length of time following IVSA procedures when tissue was harvested (24 hr in the present study vs 7 – 14 days in the other studies). The most likely explanation for the lack of changes in GFAP levels is that the overall amount of METH and MDPV intake was not sufficient to induce astrogliosis. In the current study, across both ShA and LgA, MDPV and METH rats self-administered approximately 140 and 70 mg/kg, respectively. These totals are similar to those reported for two weeks of LgA METH (7-140 mg/kg) in the studies of Schwendt et al. (2009) and Reichel et al. (2012) which also reported no effects on GFAP levels. In contrast, Krasnova et al., (2010) reported total intake levels between 40-160 mg/kg over 8 days (15 hr / day) and a significant elevation in GFAP immunoreactivity in the striatum and cortex, and these increases in GFAP levels were also positively correlated with overall METH intake. Thus, the most likely scenario for the lack of effects of MDPV or METH on GFAP immunoreactivity in the present study and others mentioned above is an insufficient amount of drug taken in a short enough period of time to induce toxicity.

Still another explanation for lack of GFAP immunoreactivity could also be related to the timecourse of changes in GFAP expression. In the current study, rats were sacrificed 24 hours following the last LgA session. In mice, following 1 single injection of METH (30 mg/kg, i.p.), GFAP expression is not significantly increased versus saline controls at 24 hours post-treatment, but is elevated two days later with peak effects occuring at day three (Zhu et al., 2005). In rats, four 10 mg/kg s.c. doses of METH given every two hours have been shown to increase striatal GFAP levels at both 48 hour and 32 day timepoints (Friend & Keefe, 2013) and others have also shown that a single 40 mg/kg i.p. dose of METH increases striatal GFAP at 10 days following administration (Cappon, Pu, & Vorhees, 2000). Finally, in rats receiving an escalation binge-dosing regimen of METH (10, 15, 15, 20, 20, 25, 30 mg/kg, s.c. over 7 consecutive days) and sacrificed 24 hours later, no significant differences in GFAP immunoreactivity were found compared to saline controls in either the striatum or frontal cortex (Simões et al., 2008). Thus, when astrogliosis does occur following psychostimulant exposure, it generally is found in tissue harvested at least 2 days following the last drug treatment. It is therefore possible that differences in GFAP levels may have emerged had rats been sacrificed at a timepoint longer than 24 hours. However, lack of observed changes in GFAP in the studies by Schwendt et al., (2009) and Reichel et al., (2012), in which tissue was collected between 7-14 days after cessation of drug intake, while others showing that it can last over 30 days (Friend & Keefe, 2013), argues against this notion.

Finally, and perhaps most important for the relationship between astrogliosis and stimulant addiction, a recent report has suggested that GFAP immunoreactivity is not significantly altered in human METH addicts (Kitamura et al., 2010). In this study,

striatal tissue from METH users (N = 12) who died of drug overdose was immunohistochemically stained for GFAP. While a positive trend was noted, there was no significant increase in GFAP staining when compared to tissue from non-drug using control subjects (N = 13). Based on these findings, the authors argued that astrogliosis is not likely involved in the transition to the loss of control over drug intake characterizing METH addiction. Thus, a likely scenario is that astrogliosis does not typically occur following repeated voluntary administration of METH and when it does occur in animals, the large bolus doses given are perhaps physiologically irrelevant for addiction.

Both cupric silver or FluoroJade C staining procedures can detect degenerating neurons from a variety of drug, chemical, or organic insults, and stain all degenerating regions of neurons including somata, axons, and terminals (Schmued et al., 2005; Switzer, 2000). Evidence for neuronal degeneration using these techniques has been shown in numerous animal studies followed large non-contingent doses of amphetamines in regions such the striatum, hippocampus and various cortical regions (Ares-Santos, Granado, Espadas, Martinez-Murillo, & Moratalla, 2014; Bowyer & Ali, 2006; Commins, 1987; Eisch, Schmued, & Marshall, 1998; Jensen & Olin, 1993; Kuroda, Ornthanalai, Kato, & Murphy, 2010; Ricaurte, Guillery, & Seiden, 1982; Schmued, 2003; also see reviews by (Cadet et al., 2003; Krasnova & Cadet, 2009). To our knowledge, there have been no published studies showing neuronal degeneration with cupric silver or FluoroJade C staining following self-administration of any psychostimulant, but has been demonstrated to occur in the granule cell layer of the hippocampus of alcohol dependent rats following self-administration (Richardson, Chan, & Crawford, 2009). Cell death has also been demonstrated by immunostaining for activated caspase-3 (AC-3), a marker of

apoptotic activity (AC-3), following ShA and LgA METH IVSA (Mandyam et al., 2008; Recinto et al., 2012). However, a number of psychostimulant self-administration studies have been published in which neuronal degeneration has been inferred by reductions in DAT, TH, or SERT staining (for example see (Kousik, Carvey, & Napier, 2014) as decreases in these proteins often occur comcomittantly with neuronal degeneration (Marshall & O'Dell, 2012). Furthermore, a number of prominent human imaging studies have also inferred neurodegeneration from decreased DAT binding and gray matter volumes (Chang, Alicata, Ernst, & Volkow, 2007). However, a note of caution must be urged in making the inference of neuronal degeneration from reductions in these other markers. For example, some studies have shown that reductions in these aforementioned proteins due to amphetamine exposure can occur at timepoints earlier than the emergence of positive cupric silver or FluoroJade C staining (Ares-Santos et al., 2014; Baumann, Wang, & Rothman, 2007) or other overt signs associated with terminal degeneration such as loss of VMAT immunoreactivity (Moszczynska et al., 2004; Wilson et al., 1996). Thus, while these various markers typically co-occur, it has been argued that reductions in transporters or rate-limiting enzymes such as TH can reflect earlier adaptive processes (e.g. simple downregulation of protein expression) which do not ncessarily reflectactive degenerative processes (Volkow, Chang, Wang, Fowler, Franceschi, et al., 2001; Volkow, Chang, Wang, Fowler, Leonido-Yee, et al., 2001).

In addition to amphetamines, studies using either cupric silver or FluoroJade C staining techniques following cocaine exposure have generally failed to produce evidence of neuronal degeneration in striatum or hippocampus, although patterns of degeneration in the lateral habenula and occasional, but seemingly random, cortical pyramidal cells

were found (Ellison, 1992; Goodman & Sloviter, 1993). In the study by Goodman and Slovier (1993), cocaine injections (40 mg/kg) were given daily for 3 months, producing seizures in many of the animals. Like the present study, a positive control animal injected with KA did produce evidence of hippocampus neuronal degeneration. However, despite 3 months of high level cocaine exposure and seizures, no evidence of cupric silver staining was shown. The reason for numerous accounts of amphetamine related neuronal degeneration and lack of robust effects in cocaine is not entirely clear, but it has been argued that differential effects at DAT (cocaine being a blocker, with amphetamines being monoamine releasers) leads to greater continued DA release by amphetamines which translates into greater toxicity (Ellison, 1992). As MDPV acts primarily as a DAT blocker, and not a monoamine releaser, this is one possible explanation for the lack of effects in FluoroJade C staining in MDPV rats in the present study. Still, lack of FluoroJade C staining with METH in the current study may be related to other factors as evidenced by other METH IVSA studies.

As mentioned above, in METH IVSA studies, neurodegeneration has generally been inferred from the aforementioned markers. In the METH IVSA study by Schwendt et al. (2009) discussed earlier (7-10 1-hr sessions + 12-14 6 hr sessions + 2 weeks of extinction), decreases in DAT levels in the PFC and striatum were revealed, but levels of TH, NET or SERT were not significantly different saline rats in these same regions. As a result, the authors argued that decreased DAT occuring in the absence of these other markers likely signified DAT terminal related neuroadaptation and not overt terminal degeneration (Schwendt et al., 2009). Moreover, in the aforementioned study by Reichel et al., (2012), rats receiving the experimenter-administered (non-contingent) binge-dose

regimen (four 4 mg/kg i.p. injections given every 2 hours), *but not* METH IVSA (7 1 hr sessions +, 14 6 hr sessions + 8 days of abstinence), showed decreases in TH and DAT levles in striatal tissue. Together, these results suggest that IVSA procedures similar to those here do not allow for a sufficient amount of intake to result in neurodegenerative changes. Alternatively, in the aforementioned by Krasnova et al. (2010), METH IVSA (0.1 mg/kg/infusion; 8 15-hr sessions + 7 days of abstinence) did produce significant decreases in TH and DAT levels in striatal and cortical tissue. Finally, in a METH IVSA study (Mandyam, Wee, Eisch, Richardson, & Koob, 2007) in which rats receiving either ShA, intermittent ShA (2 days a week), or LgA to METH (0.05 mg/kg/infusion) for 49 days (42 days of LgA), but no abstinence period, a substantial increase in the number of dying cells (pyknotic cells) was found in the medial PFC for all three IVSA groups when compared to drug-naïve controls receiving no behavioral testing. These data suggest that standard ShA and LgA procedures, using the same dose of METH used in the present study (0.05 mg/kg/infusion) can lead to neurodegenerative effects in the medial PFC without a withdrawal period, but only after 49 days of continuous access. Together, these studies suggest that lack of sufficient exposure most likely explains the null effects of the current study. However, in a recently published study (Kousik et al., 2014), METH IVSA (0.1 mg/kg/infusion) for only 3 hours a day for 14 sessions produced decreases in striatal and accumbal TH levels, but only when rats were sacrified at least 14-56 days later. Subjects sacrificed 24 hours after IVSA procedures showed no decreases in TH staining. Moreover, TH staining was also decreased in the VTA and substantia nigra, but only at 28-56 day timepoints. Furthermore, reduced Fluorogold (a retrograde tracer) staining of these DAergic pathways was also observed, suggesting that METH-induced

terminal field neurodegeneration (striatum / accumbens) caused neurodegenerative effects in VTA/substantia nigra in a retrograde manner. Thus, these authors argue that METH IVSA can induce DAergic-related neurodegenerative effects, even after limited exposure, but only after much longer abstinence periods.

Given these collective findings and the lack of degeneration found in our study, it is most likely that our IVSA procedures failed to produce overt signs of neurodegeneration as a result of (1) insufficient accesstime to acquire a sufficient amount of drug (either METH or MDPV) or (2) lack of protracted withdrawal procedures. Indeed, Krasnova et al. (2012) argue that traditional IVSA procedures similar to those of the present study likely do not mimic the large quantity and binge-like METH-taking seen in humans and recommend that animal models aiming to replicate human METH use employ longer access (i.e. 15 hours instead of standard 2 or 6 hr) procedures. Moreover, Kousik et al. (2014) also recommend sufficiently long withdrawal periods to fully capture METH-induced neurodegeneration. Thus, LgA METH IVSA does appear to cause neurodegenerative effects when the number of sessions is much higher than those in the present study even without withdrawal. However, as discussed with Kousik et al., (2014), lower exposure levels can lead to pathological effects, but only after long abstinence periods. Thus, neurotoxicity only appears to manifest without withdrawal after sufficiently long IVSA access, or without large amounts of exposure only after a sufficiently long withdrawal period..

With regards to cognitive functioning, numerous studies have reported that long-term abuse of psychostimulants such as amphetamines or cocaine in humans can lead to adverse cognitive effects in domains such as information processing speed, motor skills,

attention, decision-making, planning, set shifting, working memory, long-term memory, and others (for reviews see (Nordahl, Salo, & Leamon, 2003; Scott et al., 2007). In the current study, results of cognitive tests revealed no deficits following either METH or MDPV self-administration for either of the two domains of cognitive functioning tested (working memory with the DMTP task and set shifting with the S+/S- reversal learning task). While puzzling, several possibilities likely explain these null results.

Research shows that performance on the DMTP and other working memory tests is dependent on both hippocampal and medial PFC functioning as lesions and/or pharmacological blockade impaired performance (Aggleton, Keith, & Rawlins, 1992; S. Dunnett, Wareham, & Torres, 1990). Set switching, as assessed in various discrimination reversal learning tasks and the Wisconsin Card Sorting Task, is also dependent on both hippocampal and medial PFC functioning, specifically the ILC/PLC regions in rodents) (Izquierdo & Jentsch, 2012). Thus, successful performance on these tasks is dependent upon functioning in the same regions showing no overt signs of neurodegeneration in Experiment 1 under nearly identical IVSA procedures. Given the lack of astrogliosis or neurodegenerative effects from Experiment 1, most likely as a result of insufficient access / intake of either MDPV or METH or lack of a sufficiently long withdrawal period, the most parsimonious explanation for lack of cognitive effects in Experiment 2 is also insufficient access / intake of both drugs. Indeed, of the few IVSA studies published assessing cognitive deficits with similar procedures, adverse effects are typically seen following much longer treatment regimens combined with long withdrawal periods.

For example, in a study by George et al., (2008), rats were placed into either 1 hr ShA or 6 hr LgA cocaine (0.5 mg/kg) IVSA procedures for a minimum of 85 sessions.

Escalated intake was found in the LgA, but not ShA, rats across the 85 IVSA sessions. Three to fifteen days following IVSA procedures, rats were tested in two hippocampal dependent tasks, the spontaneous alternation Y-maze task (day 3 of abstinence) and the delayed-nonmatching-to-sample (DMTS) t-maze task (day 15-17 of abstinence). On the DMTS task, LgA rats performed significantly worse than ShA rats, with the magnitude of cocaine escalation negatively correlating with performance (i.e. as cocaine intake increase, DMTS performance decreased). Furthermore, working memory impairments were also correlated with an overall decrease in medial PFC neurons when tissue was collected 2 months after IVSA, suggesting working memory impairments were related to the neurodegenerative effects of cocaine after a long withdrawal period. On the spontaneous alternation task, no differences were noted between ShA or LgA rats. In a recently published study (Recinto et al., 2012), METH IVSA procedures were paired with the same cognitive tests describe above (George, Mandyam, Wee, & Koob, 2008). Here, rats were placed into either ShA, intermittent ShA (1 hr/day 2 days, Monday and Thursday, a week) or LgA METH (0.05 mg/kg) IVSA procedures for 22 days. As shown previously by this same group, rats in the LgA group displayed significant escalation of METH intake across sessinos (Mandyam et al., 2008, 2007). Three to fifteen days following IVSA procedures, rats were tested in the spontaneous alternation Y-maze task (day 3 of abstinence) and the delayed-nonmatching-to-sample t-maze task (days 15-17 of abstinence). Here, LgA rats were significantly worse than either ShA, intermittent ShA, or drug-naïve controls rats on both tasks. Interestingly, drug-naïve controls were impaired relative to the intermittent ShA group for both tasks, highlighting that voluntary METH consumption under certain conditions can actually improve performance. Indeed,

improved performance across a number of cognitive domains has been shown in humans following acute or intermittent METH exposure (for review see Hart, Marvin, Silver, & Smith, 2012). Finally, LgA also lead to significant increases in the apoptotic marker AC-3 and decreases in neurogenesis in the hippocampal dentate gyrus, whereas the intermittant ShA group has increased neurogenesis, suggesting that neurodegenerative processes likely contributed to impaired working memory performance. In another METH (0.02 mg/infusion) IVSA study, rats underwent 10 days of 1 or 2 hr ShA, followed by 14 days of either 1, 2, or 6 hr LgA. After 10 days of extinction training, rats were tested on a hippocampal dependent (Cohen & Stackman Jr, 2014) novel object recognition test (object exploration task) (Rogers, De Santis, & See, 2008). LgA METH rats displayed impaired recognition memory compared to saline controls. In a follow-up study by the same group under similar IVSA procedures (7 1 hr ShA +14 6 hr LgA sessions vs. yoked saline controls and escalation of METH intake across LgA), novel object recognition memory was again impaired following 7 days of abstinence (Reichel et al., 2012).

Thus, while it is difficult to pinpoint why cognitive deficits were not found in our study, the most likely reasons are insufficient access / intake of either METH or MDPV or assessment of cognitive effects concurrently with IVSA procedures instead of during protracted withdrawal. In each of the studies discussed above, LgA lead to signficant escalation of intake, an effect not seen in Experiment 2 of the present study. Furthermore, in each of the above studies, cognitive effects were assessed during protracted withdrawal at least 3 days following cessation of IVSA procedures. Thus, it is possible that cognitive effects may have emerged had they been assessed perhaps starting 3 days after IVSA

experiments. In our study, rats were assessed only during the *acute* withdrawal phase (each morning prior to IVSA) which may not have been a sufficient to detect declines in cognitive performance. Still another possibility is that sucrose rats were less motivated to perform the cognitive tests on the final LgA DMTP test as they had significantly more forfeited trials and significantly greater body weights. These effects were not seen during the prior two DMTP tests and suggest that, perhaps being less motivated compared to MDPV or METH rats, their performance was artificially decreased and masked potential performance differences. However, lack of differences in percent correct responding across all three DMTP tests for any group argues against this possibility. Finally, another possibility is that, while many studies in human abusers of illicit psychostimulant have reported cognitive impairments, the evidence is often inconsistent. Indeed, a number of recent reviews have shown that most published studies have revealed little to only moderate impairments in neuropsychological and cognitive functioning in psychostimulants abusers (Hart et al., 2012; Scott et al., 2007; Wood et al., 2014).

In conclusion, the overall lack of toxic or adverse cognitive effects in the present study largely suggest that standard IVSA models comprised of ShA (1 – 2 hours) + LgA (6 hour) that do not incorporate either a large number of sessions or protracted withdrawal periods do not lead to toxic or adverse cognitive effects reported in human psychostimulant abusers. While these traditional IVSA procedures appear sufficient to establish reinforcing properties of psychostimulants, and perhaps model to the emergence of loss of control and/or tolerance seen in addiction (Ahmed & Koob, 1998; Kitamura et al., 2006), the overall picture that emerges is that they do not fully capitulate many of the resulting adverse toxic effects reported in human METH or cocaine addicts. Thus, future

IVSA experiments assessing toxic or cognitive deficits should, at the very least, employ longer within-session acquisition periods (such as the 15 hr/day procedures used in Krasnova et al., (2012) or even newer 96 hr access used elsewhere (Cornett & Goeders, 2013), more total LgA sessions (22-85) seen in George et al., (2008), Mandyam et al., (2012), or Recinto et al., (2012)), along with protracted withdrawal (at least 3 days after IVSA). Finally, the lack of neurotoxicity seen in these traditional IVSA models, but overabundance of effects seen following non-contingent large dose treatment regimens, suggests that these latter approaches use doses that are physiologically irrelevant to addiction and may only model potential neurotoxic effects related to accidental overdose.

CHAPTER 9

GENERAL DISCUSSION

The abuse of illicit psychostimulants such as METH, cocaine, MDMA, and newer designer cathinones continues to be a major public health issues around the world. In the latest World Drug Reports, approximately 80 million people used either cocaine or an amphetamine-type stimulant (ATS) at least once in the previous calendar year (UNODC, 2014). In the United States, epidemiological data suggests that despite abuse already existing at epidemic levels, total ATS use, of which METH is the most popular, continues to increase (Maxwell & Brecht, 2011; UNODC, 2014). METH use also continues to grow in parts of the Middle East, Eastern Europe, Oceania, Asia, and Southeast Asia. In recent years, synthetic cathinones also emerged as "legal" stimulants, further compounding problems associated with stimulant abuse. As a result, legislative actions by many countries have banned the use of many of these new drugs. However, while these bans have had the desired effect of decreasing availability certain synthetic cathinones, drug manufacturers have responded by producing new analogues often as dangerous as the drugs they were designed to replace. This has led to a legislative cat and mouse game, where newer generations of synthetic cathinones continue to emerge to elude authorities (Cohen, 2014). Indeed, when synthetic cathinones emerged in 2009, only a handful of synthetic cathinone were available on drug markets with MDPV, mephedrone and methylone comprising 98% of these in the United States (D. E. A. United States Department of Justice, 2011c). As of last count, over 40+ synthetic cathinones have been discovered on international markets (Glennon, 2014), most of which have yet to be assessed in any type of scientific study. Thus, it is clear that global stimulant abuse has

grown and continued research regarding the abuse liability, toxicity, and therapeutic intervention for stimulant addiction is desperately needed. The studies in this dissertation have focused on these goals and collectively have generated of novel findings that have made significant impacts on the studies of stimulant addiction, highlight knowledge gaps where future research is needed, and suggest changes in rodent IVSA models to better model stimulant addiction in the future.

In chapter 2, we assessed the ability of the mGluR5 NAM fenobam to attenuate reinstatement to METH-seeking following ShA IVSA procedures. Fenobam was chosen as it has been shown to be well tolerated in humans (Berry-Kravis et al., 2009). The mGluR5 receptor has become a target of tremendous interest for stimulant addiction as previous work had revealed that reinforcing and locomotor effects of cocaine were absent in mGluR5 knockout mice, and preclinical studies with other mGluR5 NAMs had shown positive effects in attenuating cocaine-seeking (for example see (Kumaresan et al., 2009). Our results showed that fenobam had the effect of decreasing METH-seeking after both METH-prime and cue-prime reinstatement procedures. However, decreased responding for food and sucrose during cue-primed procedures suggested non-specific effects that would limit clinical adoption. Furthermore, other laboratories reported similar effects of mGluR5 NAMs on sucrose-seeking during cocaine studies (Keck et al., 2013). Despite these non-specific effects and discontinued studies with fenobam, attenuated METH reinstatement justifies continued assessment of mGluR5 as a potential therapeutic target. Indeed both newer fenobam analogues (Gichinga et al., 2011) and mGluR5 NAMs are being developed, with a recent study showing that MPEP analogue MFZ 10-7 inhibits cocaine self-administration and cocaine-seeking behavior in reinstatement procedures

with less overall non-specific effects on sucrose intake (Keck et al., 2014). Thus, while interest in the drug fenobam has decreased in recent years, subsequent studies with newer mGluR5 ligands have renewed interest in this target.

In chapter 3, we review the literature regarding the use of AMPA PAMs as potential cue-exposure therapy adjuncts for stimulant addiction. We also present data from two novel AMPA PAMs, CX1739 and CX1837. Our interest in these drugs was motivated by positive reports of the AMPA PAM (4-[2-(phenylsulfonylamino)ethylthio]-2,6-difluorophenoxyacetamide; PEPA) and its ability to facilitate extinction following cocaine IVSA and subsequently decrease cocaine reinstatement (LaLumiere et al., 2010, 2012) as well as facilitated motor recovery from an animal model of stroke with both of these compounds (Clarkson et al., 2011). While both of these drugs proved capable of facilitating extinction following METH IVSA procedures, decreased extinction responding did not translate into attenuated cue-primed reinstatement of METH-seeking. As with fenobam, these disappointing results prompted us to discontinue testing these compounds. Nonetheless, facilitated effects during extinction procedures and the development of newer AMPA PAMs showing promise in numerous other neuropsychiatric disorders (Lynch, 2006) point to the need for further research in this area.

In chapters 4 and 5, we showed for the first time that MDPV and methylone, two of the most popular synthetic cathinones to initially emerge as “legal highs” on international markets, possess similar to greater reinforcing (IVSA) and rewarding (ICSS) effects as compared to METH and MDMA, the two illicit psychostimulants they were designed to mimic, respectively. Since publishing these initial studies, MDPV IVSA

has been replicated by us (chapter 8) and others (Aarde et al., 2013; Fantegrossi et al., 2013). Furthermore, others have shown MDPV facilitation of ICSS (Bonano et al., 2014; De Felice et al., 2013), locomotor stimulant effects (Baumann, Partilla, Lehner, et al., 2013; Fantegrossi et al., 2013; Glennon, 2014; Marusich et al., 2014), and full discrimination for cocaine, methamphetamine and MDMA (Fantegrossi et al., 2013; Gatch et al., 2013; Glennon, 2014). Furthermore, as shown in chapter 7, repeated intermittent treatment with MDPV produces both behavioral sensitization and cross-sensitization with METH, corroborating in vitro data showing common neurological targets (DAT and NET) and human data suggesting that a history of amphetamine abuse increases the sympathomimetic and neurotoxic effects with subsequent MDPV use (Spiller et al., 2011). Thus, based on our initial IVSA and ICSS studies, we predicted that subsequent work would reveal METH-like stimulant effects, high abuse addictive liability in humans, and continued MDPV abuse after legislative bans. Each of these predictions appears to have come true, as subsequent animal studies have revealed METH-like behavioral effects (see above), human case reports of MDPV addiction (Sadeg et al., 2014), and findings that MDPV is still being abused despite its now Schedule I status (NMS Labs, 2014). With regards to methylone, our IVSA experiments revealed greater reinforcing effects compared to MDMA, but also did not produce escalated intake suggesting a lower abuse potential than MDPV or METH. Thus, based upon our IVSA and ICSS results, we predicted subjective effects and episodic abuse patterns in humans most akin to MDMA. Interestingly, subsequent drug discrimination work has shown full substitution for both cocaine and METH (Gatch et al., 2013) and ICSS studies using a different methods revealed greater facilitated ICSS effects that those

shown in our study (Bonano et al., 2014). Furthermore, recent studies have also demonstrated MDMA-like depletion of serotonin and serotonin metabolites in rats following experimenter-administered binge-treatment regimens (den Hollander et al., 2013). Together, these animal studies suggest a methylone addiction potential similar to greater than that of MDMA. To our knowledge, no reports of methylone dependence have been reported in humans, but the most recent published data on use patterns suggests that methylone continues to be used despite its now Schedule I status (NMS Labs, 2014).

In chapter 6, our ICSS results for α-PVP and 4-MEC suggest that these newer second generation synthetic cathinones, which largely emerged as MDPV and methylone alternatives following their classification as Schedule I substances, produce similar rewarding effects and thus likely have a similar degree of addiction potential as METH and MDMA, respectively. Indeed, our data shows that α-PVP, while about half as potent as MDPV in reducing ICSS thresholds, was equipotent with METH. These results demonstrated for the first time rewarding effects for either of these drugs. Additional research has determined that α-PVP is a potent catecholamine transporter blocker similar to MDPV and produces robust locomotor stimulant properties (Marusich et al., 2014). While early data suggests high abuse liability, reinforcing effects in IVSA experiments have yet to be published and no reports of α-PVP addiction in humans has been reported. For 4-MEC, recent work has shown that this drug produces locomotor stimulant effects and fully substitutes for METH in drug discrimination assays(Gatch, Rutledge, & Forster, 2014), suggestive of a relatively high addiction potential and corroborating our ICSS findings. Thus, while our ICSS data predicts similar abuse liability of these drugs as

compared to first generation synthetic cathinones, only a handful of animal studies have been published, and additional studies are needed before definitive predictions regarding addiction potential in humans can be made.

Despite an abundance of data showing neurotoxic or adverse cognitive effects in humans and animals following chronic METH exposure, and the acute toxic effects of MDPV reported in humans, neither IVSA of METH nor MDPV produced evidence of neurotoxicity or cognitive deficits as shown in chapter 8. While these results were surprising, when viewed in light of the published literature, the overall lack of toxic or adverse cognitive effects in the present study largely suggest that standard IVSA models that even incorporate a sufficient LgA (10-16 sessions) procedures that lead to escalation of drug intake, do not lead to toxic or adverse cognitive effects reported in human psychostimulant abusers. Instead, evidence from the literature collectively suggests that escalation of intake alone is not sufficient, but needs to be combined with a longer duration of exposure (i.e. 22-85 sessions) and/or protracted withdrawal periods (i.e. at least 3 days following IVSA procedures) in order to produce toxic or adverse cognitive effects. Thus, while it is possible that voluntary MDPV intake simply does not produce the toxic effects, our lack of effects for METH when others have reported neurotoxic and adverse cognitive effects in rats following IVSA suggest that our IVSA parameters in chapter 8 were not optimal for producing these effects. Interestingly, review papers discussing the relationship between escalation with LgA and resulting toxic or cognitive deficits do not explicitly discuss the importance of duration of drug exposure or inclusion of withdrawal periods, but instead focus entirely on escalation itself (Ahmed, 2010, 2012). Thus, it is recommended that future studies employ more non-traditional IVSA

procedures consisting of either much longer within-session times (e.g. 15 hr or 96 hr binge regimens), more total sessions (22-85) if standard 6-hr LgA is used, and/or protracted withdrawal periods (at least 3 days).

In conclusion, assessment of the abuse liability and toxicity of synthetic cathinones is still in its infancy, and there are still no pharmacological treatments for addiction to any stimulant. With specific regards to synthetic cathinones, several questions remain. 1) Will the predictions of high addiction potential from animal models be corroborated with an epidemic of synthetic cathinone addiction, or will abuse of synthetic cathinones become only a minor class of abuse substances? Thus far, while a recent case report of MDPV addiction has emerged, most reports of compulsive synthetic cathinone use have been anecdotal. 2) What other neurotransmitter systems are involved in the rewarding, reinforcing, or psychological effects of these drugs? There is substantial evidence for contribution of non-monoaminergic transmitters such as acetylcholine, glutamate, and neuropeptides in psychostimulant reward and reinforcement. 3) Do any of these substances have potential as pharmacotherapeutics for disorders such as depression, ADHD, narcolepsy, or perhaps even as agonist-replacement therapies for traditional illicit stimulants such as METH or cocaine? Indeed, the drug buproprion is a FDA approved synthetic cathinone for both depression and smoking cessation, has relatively low abuse liability, and has shown some efficacy in clinical trials for cocaine addiction (Rush & Stoops, 2012). It is likely that among the multitude of potential synthetic cathinones, some of these will possess favorable neurochemical profiles for pharmaceutical development. 4) While numerous case reports have detailed immediate adverse effects and intoxication with synthetic cathinones, what are the long term neurotoxic or adverse

cognitive effects of acute or chronic use of these drugs? 5) Will newer synthetic cathinones continue to flood drug markets as replacements after governmental bans, and if so, how should policy-makers adjust their strategy to limit such effects? While the current legislative strategies have improved some outcomes such as decreasing use of those drugs specifically banned, they have also motivated drug manufacturers to market newer, and sometimes more dangerous, drugs of which we possess even less understanding.

With regards to addiction to more traditional illicit stimulants such as METH or cocaine, there is still a great deal of work needed to develop more effective interventions. While numerous compounds have demonstrated efficacy in animal models, the dismal translation into approved medications suggests that significant improvements are needed for better drug development and screening at the preclinical level. Perhaps with such improvements, more effective treatment interventions will be developed and reduce the impact that these devastating drugs can have on individuals and society.

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Table 1. Inactive lever presses per 2 hr session during extinction and reinstatement procedures

		Reinstatement			
Group	Ext	Vehicle	5 mg/kg	10 mg/kg	15 mg/kg
Drug—METH	3.35±0.71	3.36±0.92	3.67±1.45	1.66±0.73	1.14±0.77
Cue—METH	6.67±0.86	3.60±1.55	1.00±0.49	0.90±0.60	0.30±0.21
Cue—sucrose	5.96±1.03	3.33±1.17	3.33±1.88	1.67±0.91	0.17±0.17
Cue—food	6.92±2.26	6.00±2.02	3.42±1.28	3.00±0.86	4.92±2.28

Data are presented as mean ± SEM. Extinction (Ext) data represent the average of the last 2 days of extinction training prior to the first reinstatement test.

Table 2. Slopes, ED50 values, and maximum ICSS threshold reductions.

Drug	Slope	ED50 mg/kg	Maximal % Reduction
METH	-50.23	0.20: UL = .45, LL = 0.004	-21.11
α-PVP	-46.70	0.35: UL = 0.82, LL = 0.023	-25.76
4-MEC	-21.12	6.41: UL = 9.23, LL = 4.37	-17.40
MDPV	-96.07	0.35: UL = 0.55, LL = 0.17	-42.03
methylone	-17.59	1.00: UL = 1.51, LL = 0.58	-21.50

Slope values represent the means for the descending linear slope of log-transformed doses. ED50 values represent the mean dose leading to 50% maximal response with upper 95% confidence limits (UL) and lower 95% confidence limit (LL). Maximal response values represent the mean maximum intracranial self-stimulation (ICSS) threshold reduction (independent of dose) ± 95% confidence intervals. Results reported for methylenedioxypyrovalerone (MDPV) and methylone were obtained from previous publications (Watterson et al., 2012, 2014).

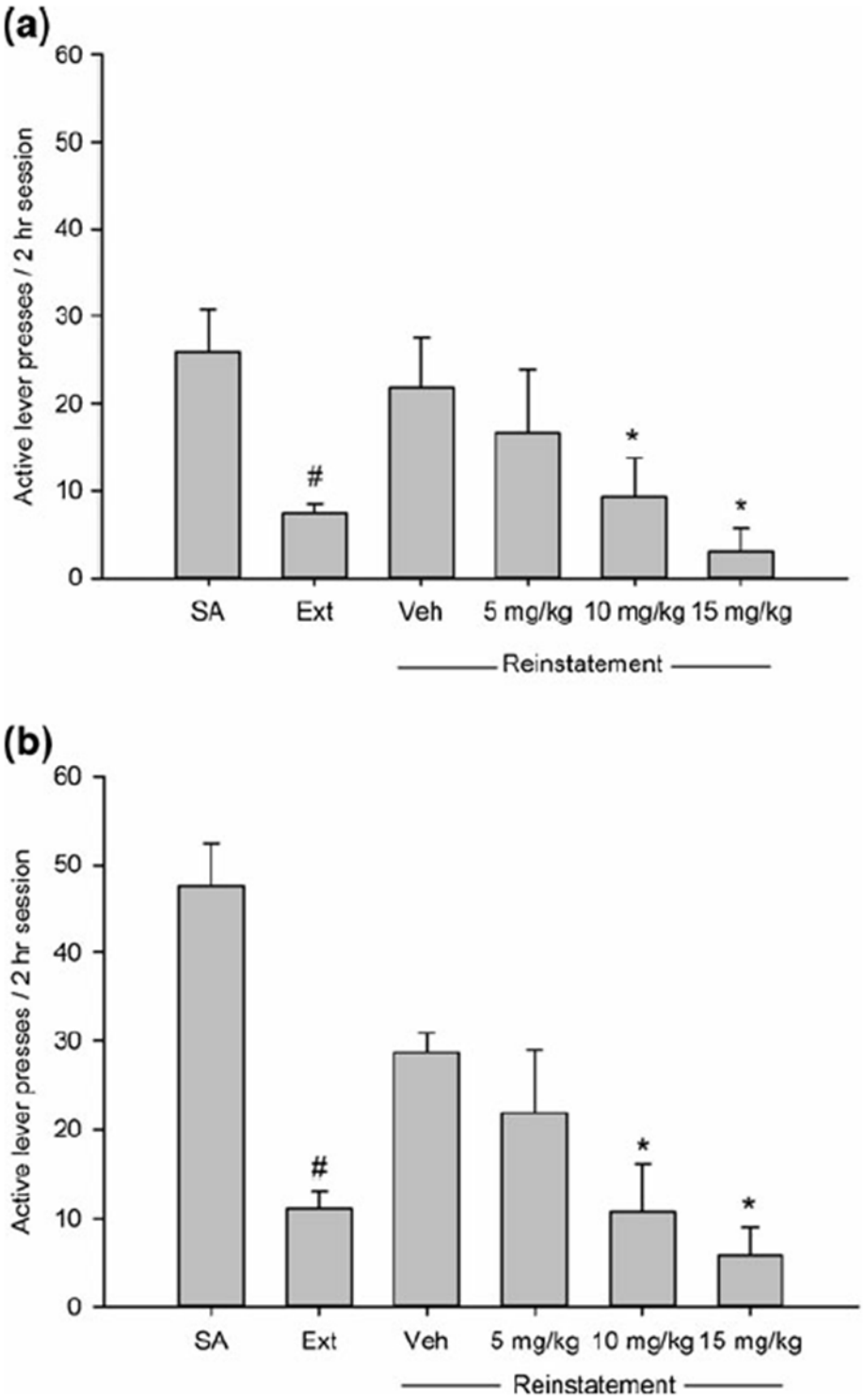

(a)
Active lever presses / 2 hr session
60
50
40
30
20
10
0
#
*
*
SA
Ext
Veh
5 mg/kg
10 mg/kg
15 mg/kg
Reinstatement
(b)
Active lever presses / 2 hr session
60
50
40
30
20
10
0
#
*
*
SA
Ext
Veh
5 mg/kg
10 mg/kg
15 mg/kg
Reinstatement

Figure 1. Effects of fenobam on the reinstatement of METH-seeking by acute METH administration or METH-associated cues. Effects of fenobam on the reinstatement of METH-seeking induced by (a) acute administration of METH (0.5 mg/kg i.p.,) or (b) METH-associated cues. SA values represent the average of the last 2 days of METH self-administration. Extinction (Ext) values represent the average of the last 2 days of extinction training prior to the first reinstatement test. *$p<0.05$ vs. vehicle treatment, #$p<0.05$ vs. SA. Data are presented as mean ± SEM.

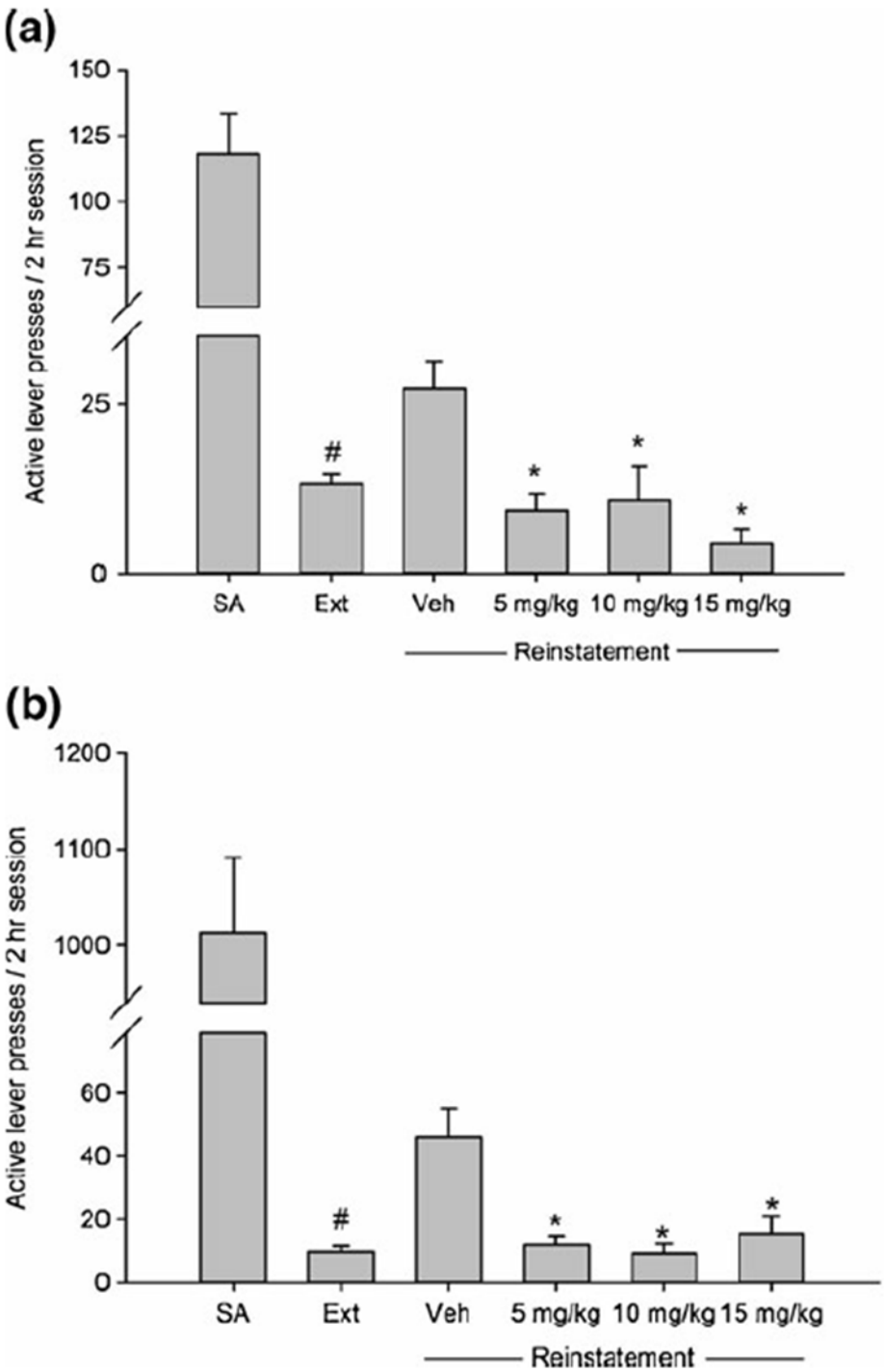

(a)
Active lever presses / 2 hr session
150
125
100
75
25
0
#
*
*
*
SA
Ext
Veh
5 mg/kg
10 mg/kg
15 mg/kg
Reinstatement
(b)
Active lever presses / 2 hr session
1200
1100
1000
60
40
20
0
#
*
*
*
SA
Ext
Veh
5 mg/kg
10 mg/kg
15 mg/kg
Reinstatement

Figure 2. Effects of fenobam on the reinstatement of a sucrose- and food-seeking induced associated by cues. (a) Effects of fenobam on the reinstatement of a sucrose-seeking by sucrose-associated cues and (b) food-seeking induced by food-associated cues. SAvalues represent the average of the last 2 days of sucrose or food self-administration. Extinction (Ext) values represent the average of the last 2 days of extinction training prior to the first reinstatement test. *$p<0.05$ vs. vehicle treatment, #$p<0.05$ vs. SA. Data are presented as mean ± SEM

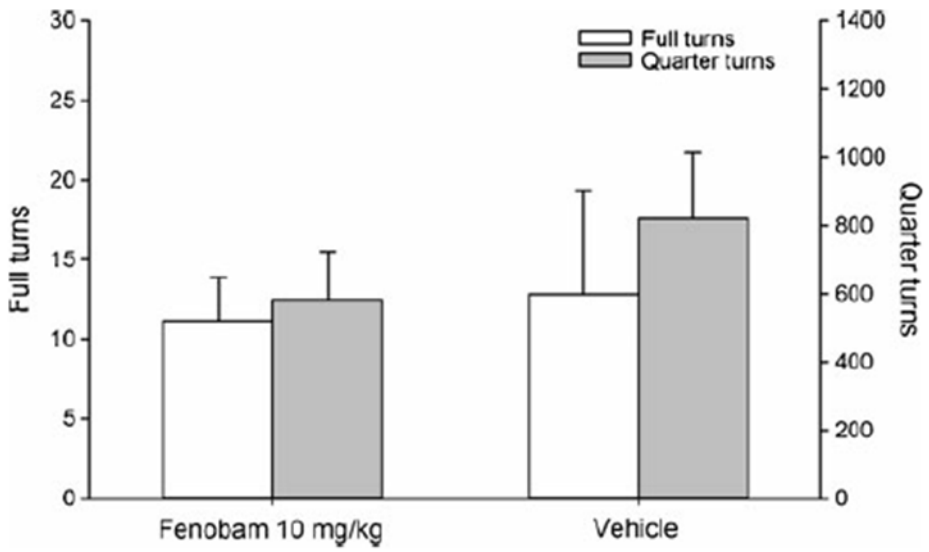

Full turns
Quarter turns
30
25
20
15
10
5
0
1400
1200
1000
800
600
400
200
0
Full turns
Quarter turns
Fenobam 10 mg/kg
Vehicle

Figure 3. Effects of vehicle or fenobam (10 mg/kg) on locomotor behavior. Data are presented as mean ± SEM and represent the average number of full turns (open bars and left y-axis) or quarter turns (shaded bars and right y-axis) during 90 min locomotor test sessions.

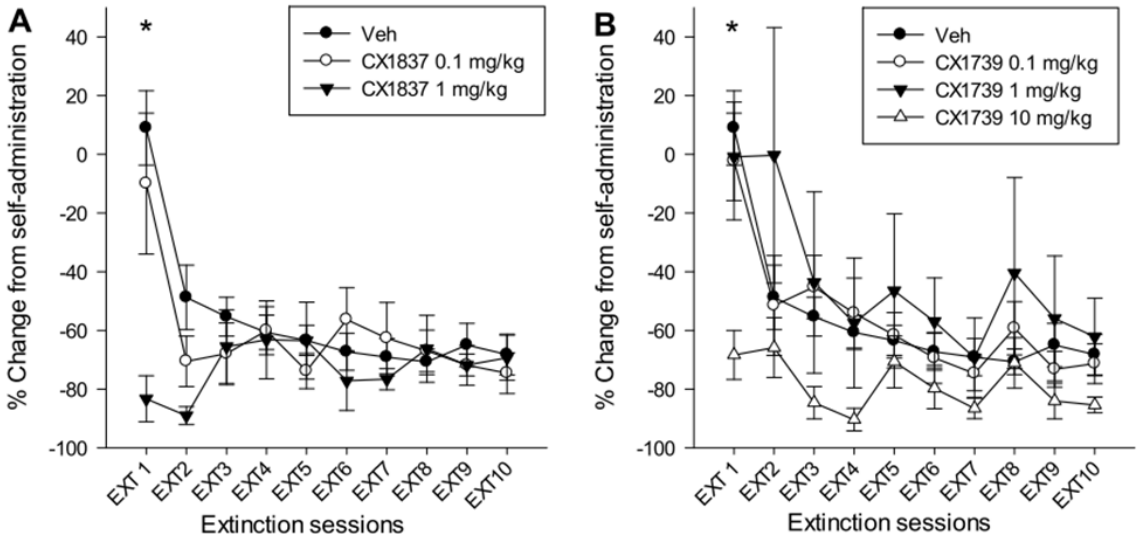

A
*
Veh
CX1837 0.1 mg/kg
CX1837 1 mg/kg
% Change from self-administration
40
20
0
-20
-40
-60
-80
-100
EXT 1
EXT2
EXT3
EXT4
EXT5
EXT6
EXT7
EXT8
EXT9
EXT10
Extinction sessions
B
*
Veh
CX1739 0.1 mg/kg
CX1739 1 mg/kg
CX1739 10 mg/kg
% Change from self-administration
40
20
0
-20
-40
-60
-80
-100
EXT 1
EXT2
EXT3
EXT4
EXT5
EXT6
EXT7
EXT8
EXT9
EXT10
Extinction sessions

Figure 4. Effects of AMPA PAMs on extinction. Male Sprague-Dawley rats were placed into 2 h daily methamphetamine IVSA-administration sessions for 10 days.Presses on an active lever produced methamphetamine infusions (0.05 mg/kg/infusion) on an FR1 schedule of reinforcement with a simultaneous 2 s light-tone stimulus complex. Following stable acquisition of methamphetamine IVSA, rats were placed into daily 2 h extinction sessions for 10 days during which active-lever presses no longer produced drug infusions or presentation of the stimulus complex. Twenty min prior to being placed into each extinction session, rats received intraperitoneal (i.p.) administration of either vehicle (Veh, 30% w/v 2-hydroxypropyl-β-cyclodextrin), CX1837 0.1 mg/kg (N = 6) CX1837 1 mg/kg (N = 12) CX1739 0.1 mg/kg (N = 7), 1 mg/kg (N = 7), or 10 mg/kg (N = 9). Vehicle treated rats (N = 20) were used for comparison for both CX1739 and CX1837. Data points represent the mean percent change (± SEM) from self-administration (mean of the final 2 days of self-administration procedures) for active lever presses. For CX1837, a mixed ANOVA analysis revealed a significant main effect of extinction session ($F[9,306] = 5.78$, $p < 0.001$), a significant extinction session x dose interaction ($F[18, 306] = 2.77$, $p < 0.001$), but no main effect of dose ($F[2,34] = 1.32$, $p > 0.05$). Post-hoc analyses revealed a significant reduction in responding on extinction day one by the 1 mg/kg dose of CX1837 versus vehicle ($F[2,34] = 4.86$, $p < 0.05$). No other measures were significantly different. For CX1739, a significant main effect of extinction session ($F[9,351] = 15.180$, $p < 0.001$), a significant extinction session X dose interaction ($F[27,351] = 1.94$, $p < 0.004$), but not a significant main effect of dose ($F[3,39] = 2.60$ $p > 0.05$). Post-hoc analyses revealed a significant reduction in responding on extinction day one by the 10 mg/kg dose of CX1739 vs. vehicle ($F[3,39] = 5.476$, $p < 0.003$). No

other measures were significantly different. All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee at Arizona State University and according to the Guide for Care and Use of Laboratory Animals as adopted by the National Institutes of Health (NIH).

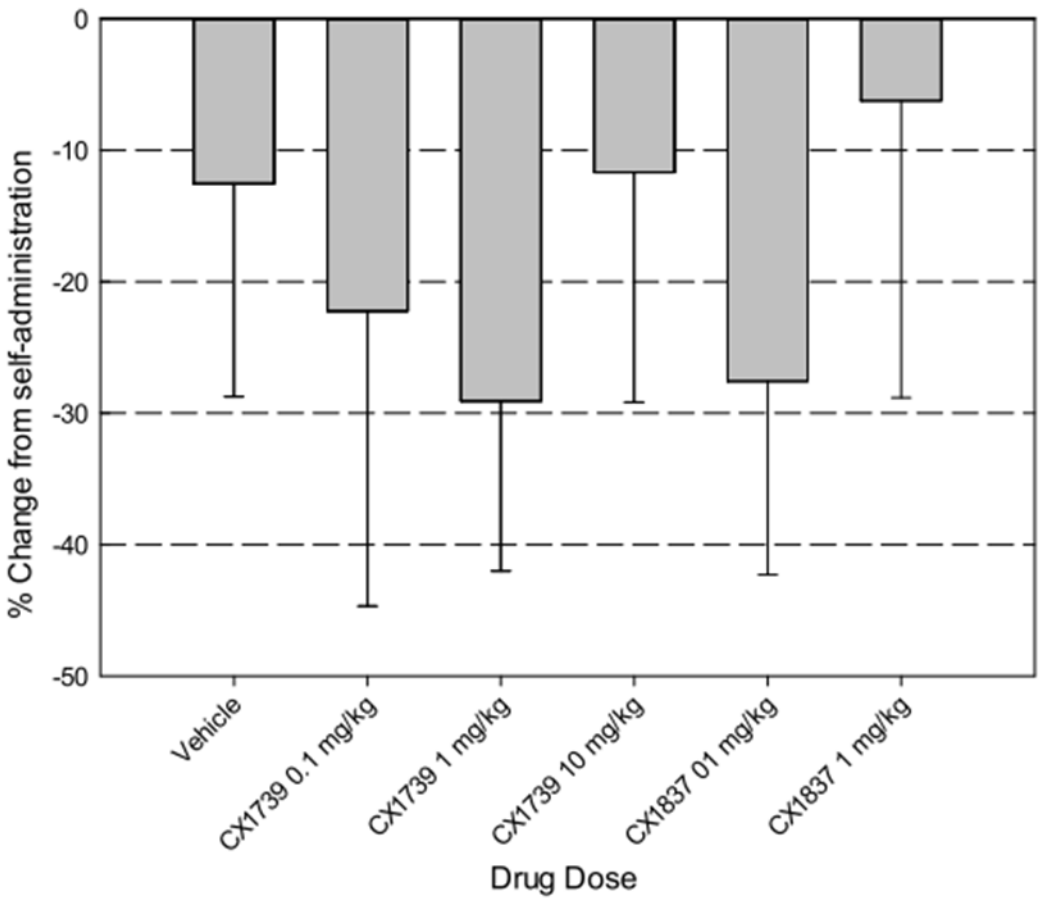

0
-10
-20
-30
-40
-50
% Change from self-administration
Vehicle
CX1739 0.1 mg/kg
CX1739 1 mg/kg
CX1739 10 mg/kg
CX1837 01 mg/kg
CX1837 1 mg/kg
Drug Dose

Figure 5. Effects of AMPA PAMs on reinstatement. Following extinction sessions, rats were placed into cue-primed reinstatement procedures to assess the retention of extinction learning. Data points represent the mean percent change (±SEM) from self-administration (mean of the final 2 days of self-administration procedures) for active lever presses. A one-way ANOVA did not reveal significant differences between vehicle or any CX1837 doses (0.1 or 1 mg/kg, i.p.) ($F[3,39] = 0.161$, $p = 0.922$), nor any significant differences between vehicle and of the doses of CX1739 tested (0.1, 1, or 10 mg/kg, i.p.)($F[2,35] = 0.294$, $p = 0.747$).

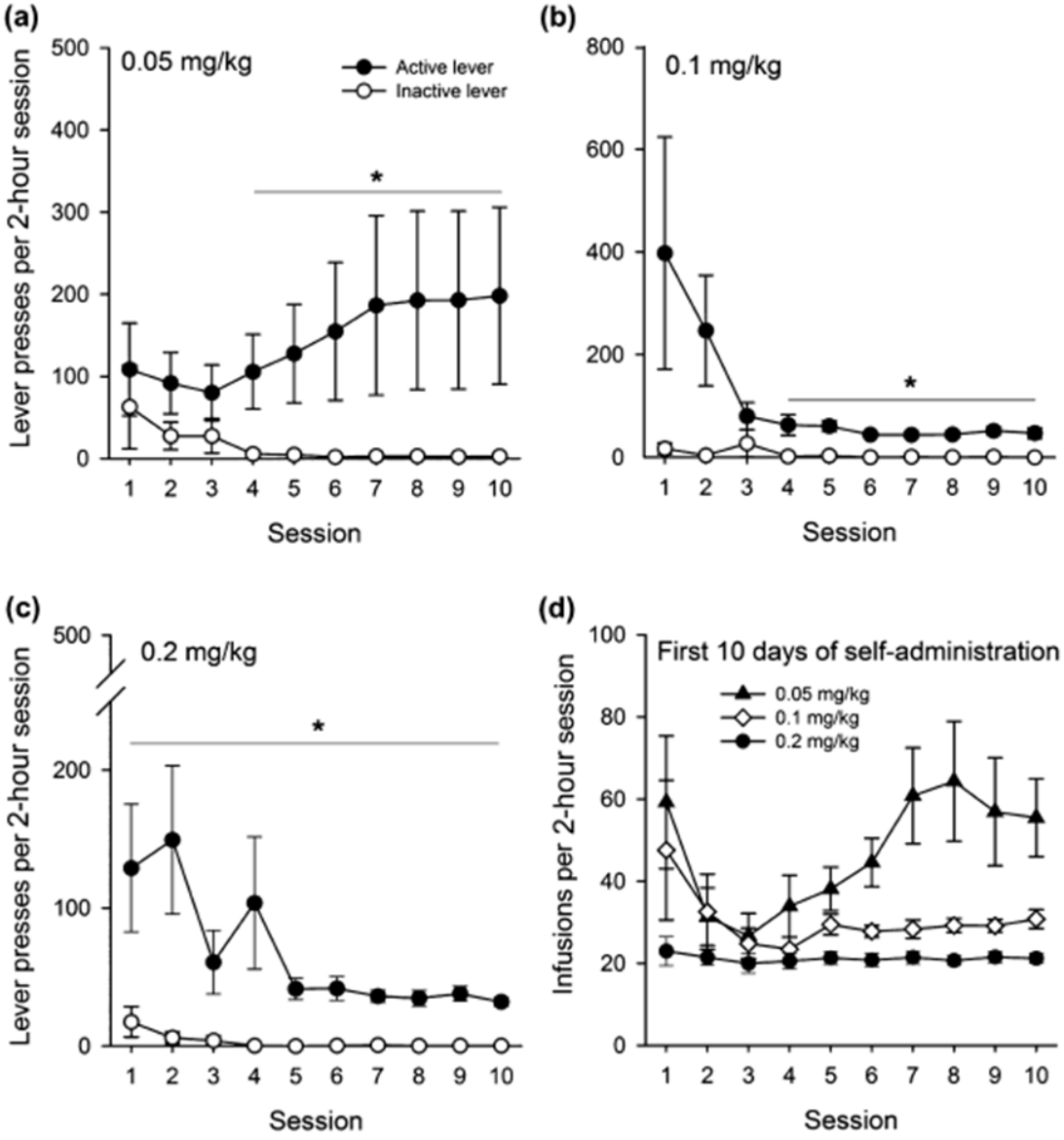

(a)
0.05 mg/kg
Active lever
Inactive lever
Lever presses per 2-hour session
Session
(b)
0.1 mg/kg
Session
(c)
0.2 mg/kg
Lever presses per 2-hour session
Session
(d)
First 10 days of self-administration
0.05 mg/kg
0.1 mg/kg
0.2 mg/kg
Infusions per 2-hour session
Session

Figure 6. MDPV IVSA in ShA. Intravenous self-administration (IVSA) of 3,4-methylenedioxypyrovalerone (MDPV). Data presented are active and inactive lever presses across the first 10 days of IVSA procedures for the (a) 0.05, (b) 0.1 and (c) 0.2 mg/kg per infusion groups (n = 9 for each group). *$P < 0.05$ between active and inactive lever presses. (d) Total number of infusions during 2-hour daily access session across the first 10 days of IVSA and for each dose of MDPV tested.

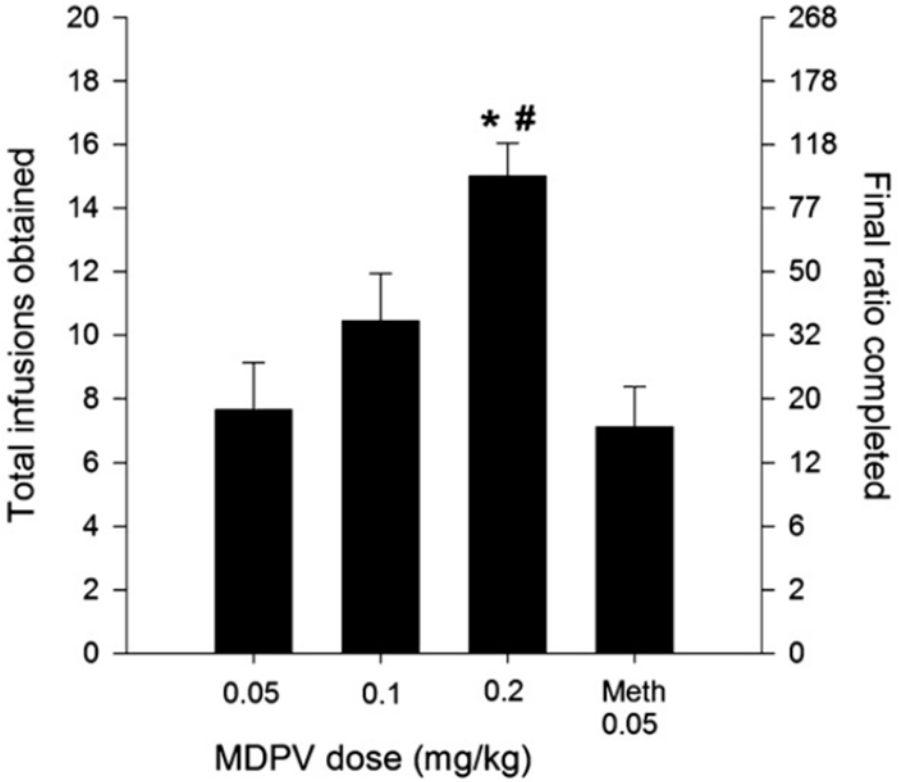

Total infusions obtained
Final ratio completed
20
18
16
14
12
10
8
6
4
2
0
268
178
118
77
50
32
20
12
6
2
0
* #
0.05
0.1
0.2
Meth
0.05
MDPV dose (mg/kg)

Figure 7.MDPV IVSA during progressive ratio responding. Total number of infusions earned during progressive ratio (PR) responding for the 0.05, 0.1 and 0.2 mg/kg per infusion doses of 3,4-methylenedioxypyrovalerone (MDPV) (n = 9 for each group) as well as a separate group of rats self-administering methamphetamine at a dose of 0.05 mg/kg per infusion (n = 9). The total number of infusions earned during the PR session is plotted along the left y-axis. As a reference, the total number of active lever presses completed during the test is plotted along the right y-axis. *P < 0.05 versus the 0.05 mg/kg dose of MDPV. #P < 0.05 versus the 0.1 mg/kg dose of MDPV.

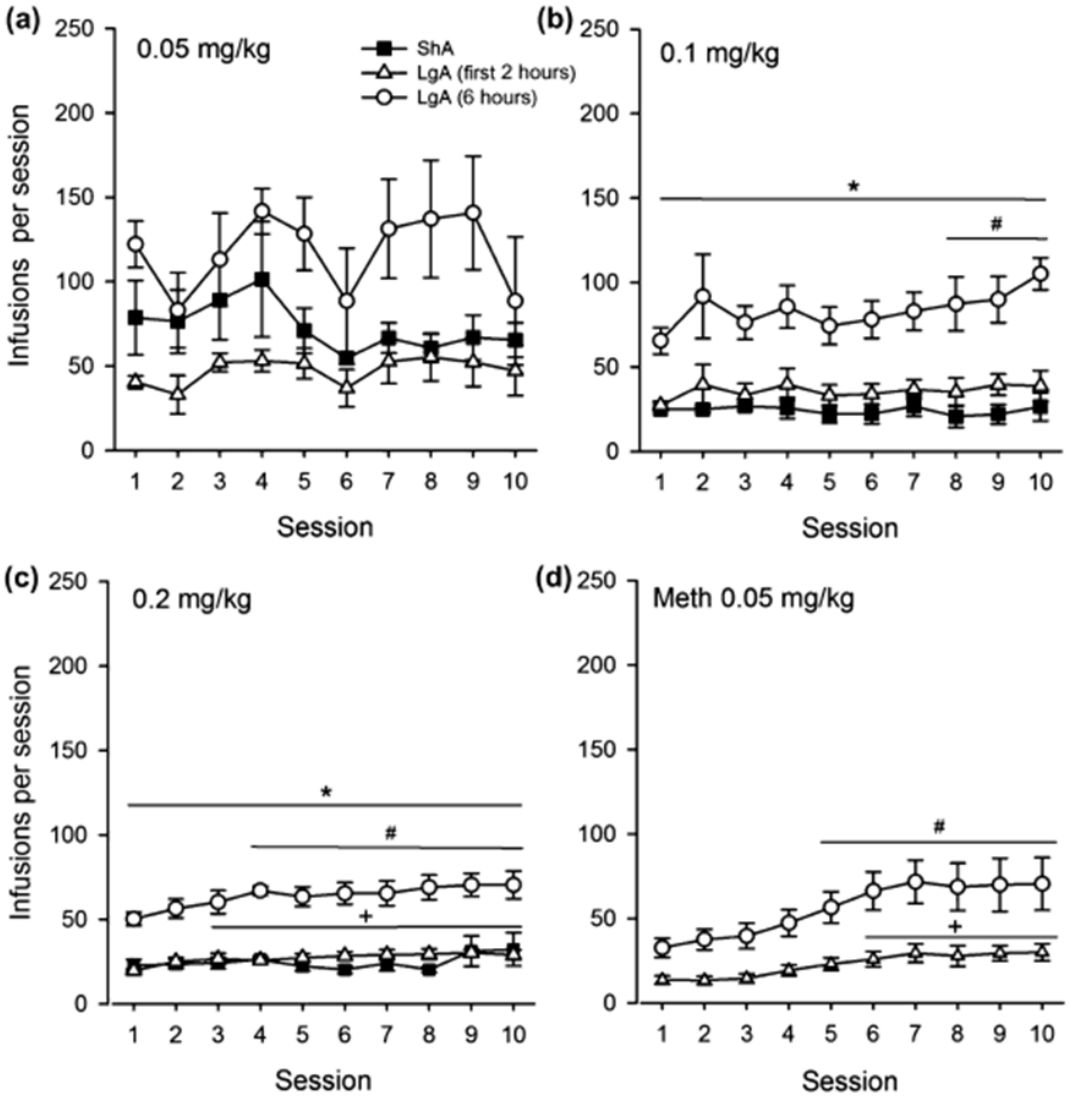

(a)
0.05 mg/kg
ShA
LgA (first 2 hours)
LgA (6 hours)
Infusions per session
Session
(b)
0.1 mg/kg
*
#
Session
(c)
0.2 mg/kg
*
#
+
Infusions per session
Session
(d)
Meth 0.05 mg/kg
#
+
Session

Figure 8. MDPV IVSA during LgA. Total number of infusions obtained during short access (ShA), long access (LgA) and the first 2 hours of LgA sessions across the final 10 days of intravenous self-administration (IVSA) procedures for the (a) 0.05, (b) 0.1 and (c) 0.2 mg/kg per infusion doses of 3,4- methylenedioxypyrovalerone groups (n = 5 for each LgA group), as well as rats self-administering methamphetamine at a dose of 0.05 mg/kg per infusion (d, n = 9). *P < 0.05 for sessions in which the number of total infusions obtained during LgA was significantly greater than total infusions obtained during ShA. #P < 0.05 for total number of infusions obtained during LgA sessions versus day 1 of LgA.+P < 0.05 for total number of infusions obtained during the first 2 hours of LgA sessions versus day 1 of LgA (first 2 hours).

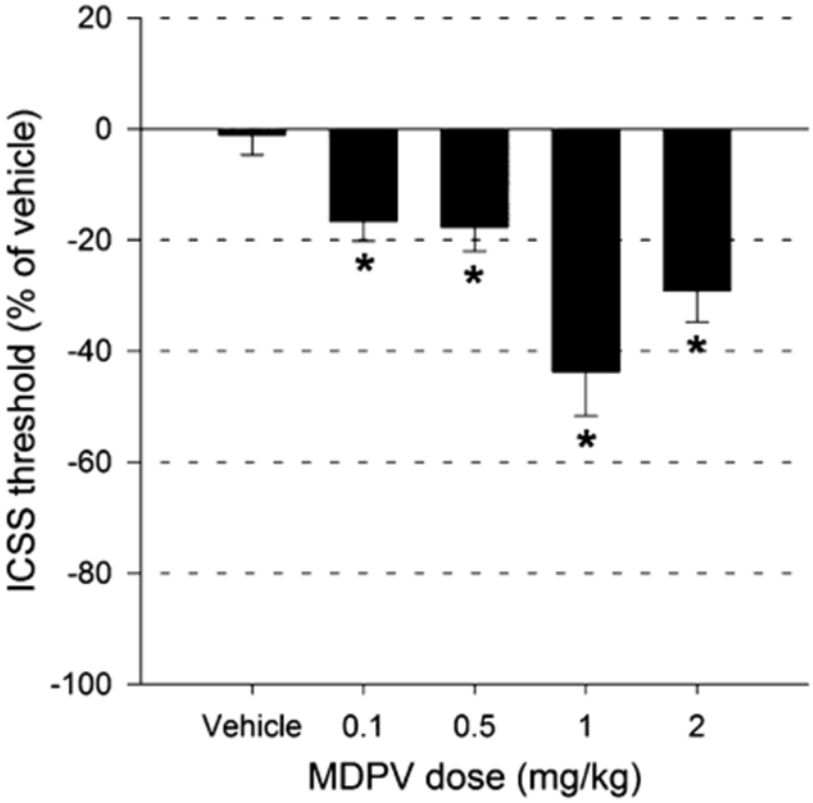

20
0
-20
-40
-60
-80
-100
ICSS threshold (% of vehicle)
*
*
*
*
Vehicle
0.1
0.5
1
2
MDPV dose (mg/kg)

Figure 9. MDPV ICSS. Effects of vehicle and 3,4-methylenedioxypyrovalerone (MDPV) (0.1, 0.5, 1 and 2 mg/kg, i.p.) on thresholds for intracranial self-stimulation (ICSS) (n = 5). *P < 0.05 versus vehicle.

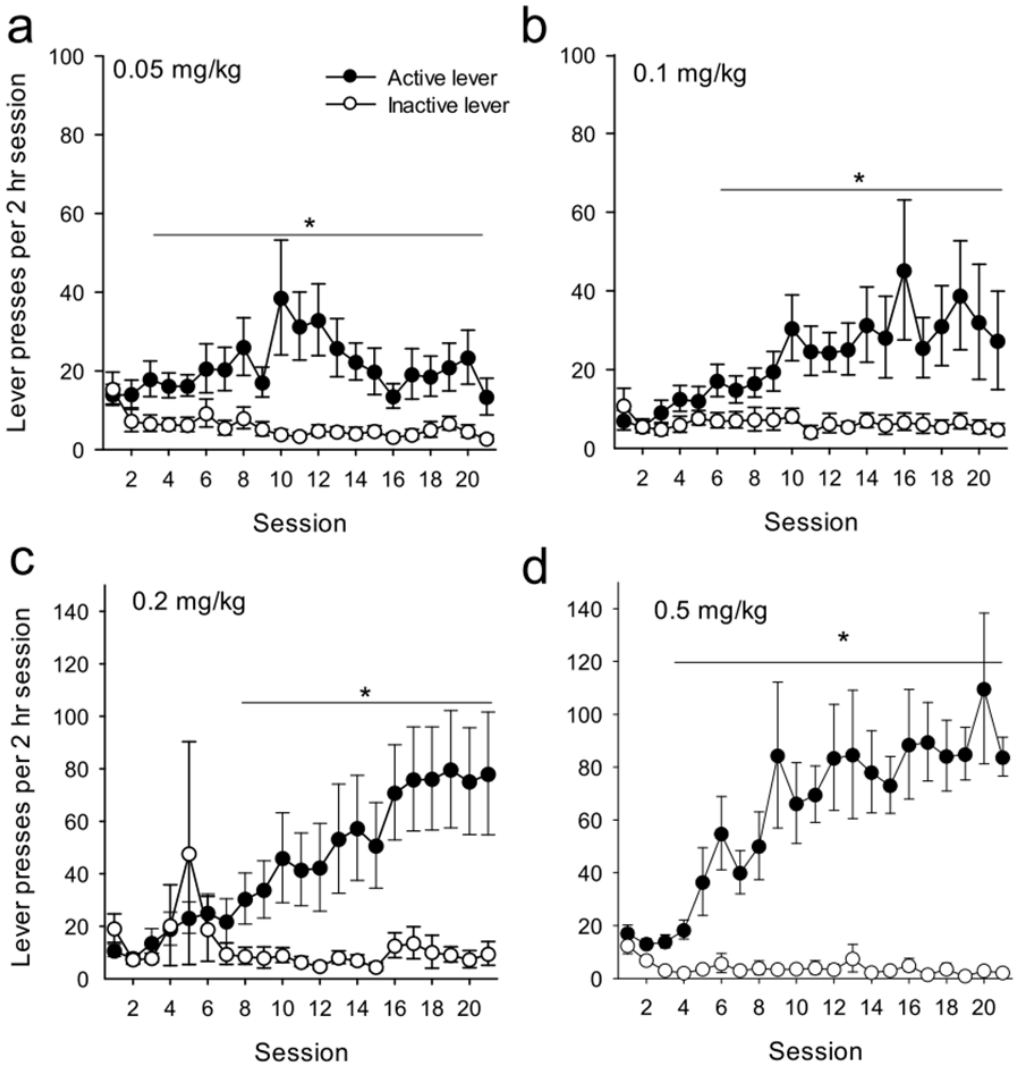

a
0.05 mg/kg
Active lever
Inactive lever
Lever presses per 2 hr session
Session
b
0.1 mg/kg
Session
c
0.2 mg/kg
Lever presses per 2 hr session
Session
d
0.5 mg/kg
Session

Figure 10. Methylone IVSA during ShA. Intravenous self-administration (IVSA) of methylone. Data presented are active and inactive lever presses across the first 21 days of IVSA sessions for the (a) 0.05 (b) 0.1 (c) 0.2 and (d) 0.5 mg/kg/infusion groups (n = 12 for 0.05 and 0.5 mg/kg/infusion groups; n = 11 for the 0.1 and 0.2 mg/kg/infusion groups). *$p<0.05$ between active and inactive lever presses.

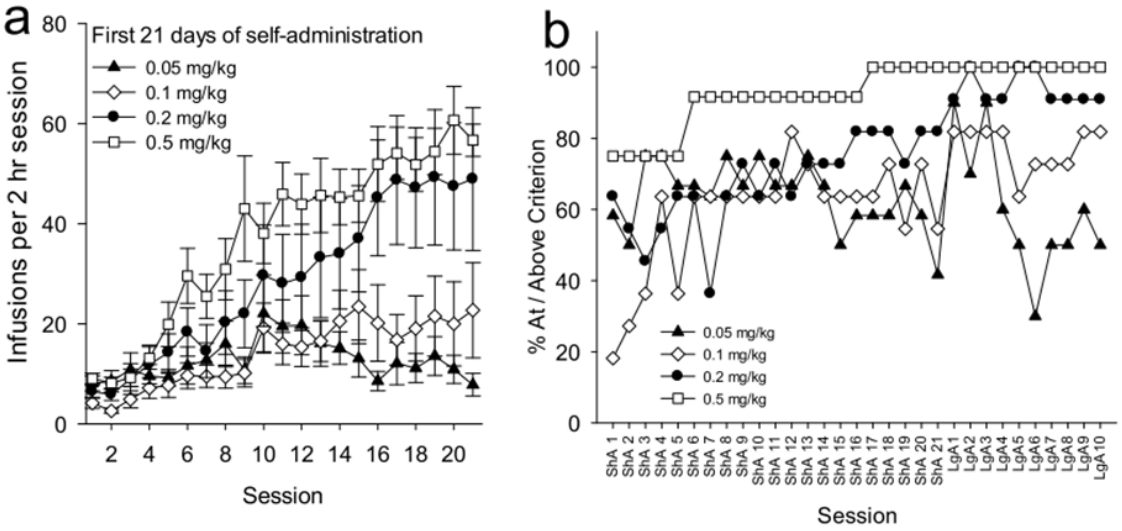

a
First 21 days of self-administration
0.05 mg/kg
0.1 mg/kg
0.2 mg/kg
0.5 mg/kg
Infusions per 2 hr session
Session
b
% At / Above Criterion
0.05 mg/kg
0.1 mg/kg
0.2 mg/kg
0.5 mg/kg
Session

Figure 11. Infusions of methylone across ShA. (a) Total number of infusions obtained during the first 21 days of 2 hr daily access sessions for each dose of methylone tested (n=12 for 0.05 and 0.5 mg/kg/infusion groups; n = 11 for the 0.1 and 0.2 mg/kg/infusion groups). (b) Percent of animals at or above criterion (10 active lever presses per session) for each experimental session.

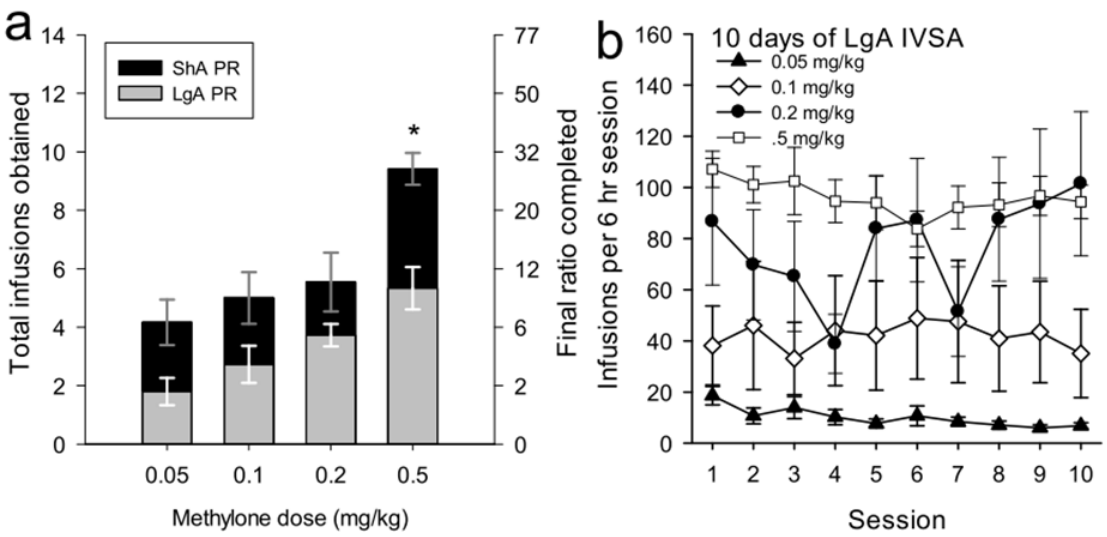

a
ShA PR
LgA PR
Total infusions obtained
Final ratio completed
Methylone dose (mg/kg)
0.05
0.1
0.2
0.5
*
b
10 days of LgA IVSA
0.05 mg/kg
0.1 mg/kg
0.2 mg/kg
.5 mg/kg
Infusions per 6 hr session
Session

Figure 12. Infusions during PR and LgA methylone IVSA. Total number of infusions earned during progressive ratio (PR) tests following 21 days of ShA sessions (ShA PR) and 10 days of LgA sessions (LgA PR) for the 0.05, 0.1, 0.2, and 0.5 mg/kg/infusion groups (n = 12 for 0.05 and 0.5 mg/kg/infusion groups; n = 11 for the 0.1 and 0.2 mg/kg/infusion groups). *$p<0.05$ compared to the 0.05, 0.1, and 0.2 mg/kg/infusion dose groups. (b) Total number of infusions obtained during the 10 days of LgA IVSA sessions for each dose of methylone tested (n=10 for 0.05 and 0.5 mg/kg/infusion groups; n = 11 for the 0.1 and 0.2 mg/kg/infusion groups).

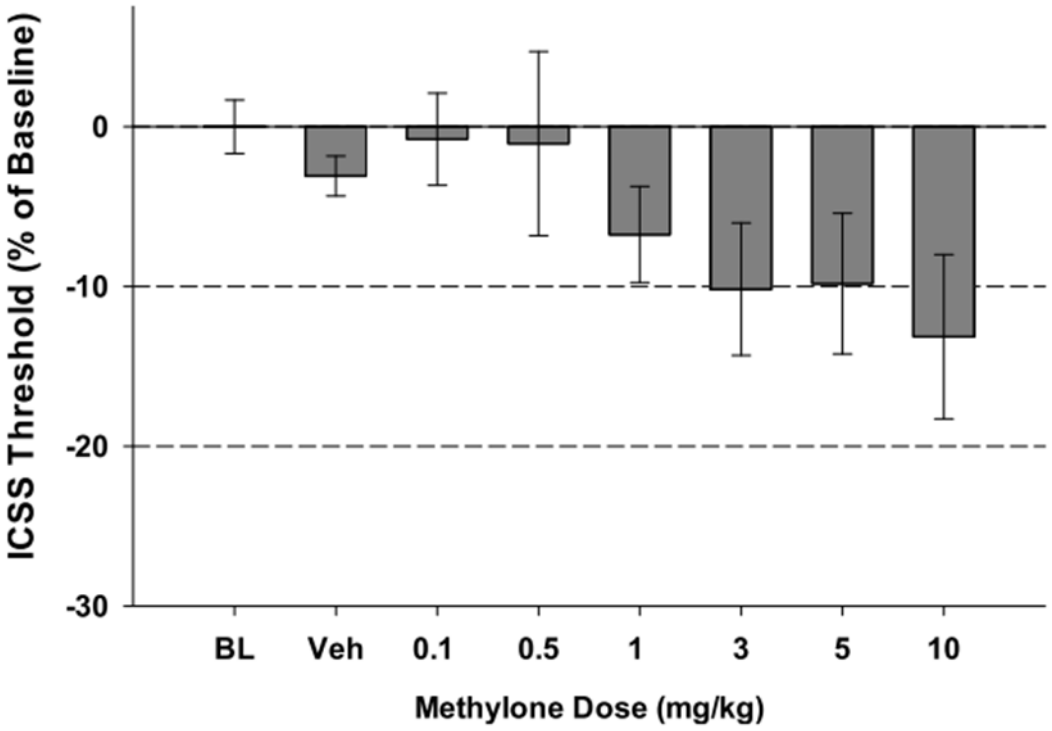

ICSS Threshold (% of Baseline)
0
-10
-20
-30
BL
Veh
0.1
0.5
1
3
5
10
Methylone Dose (mg/kg)

Figure 13.Methylone ICSS. Effects of saline vehicle and methylone (0.1, 0.5, 1, 3, 5, and 10 mg/kg, i.p.) on intracranial self-stimulation (ICSS) current-intensity thresholds (n=4).

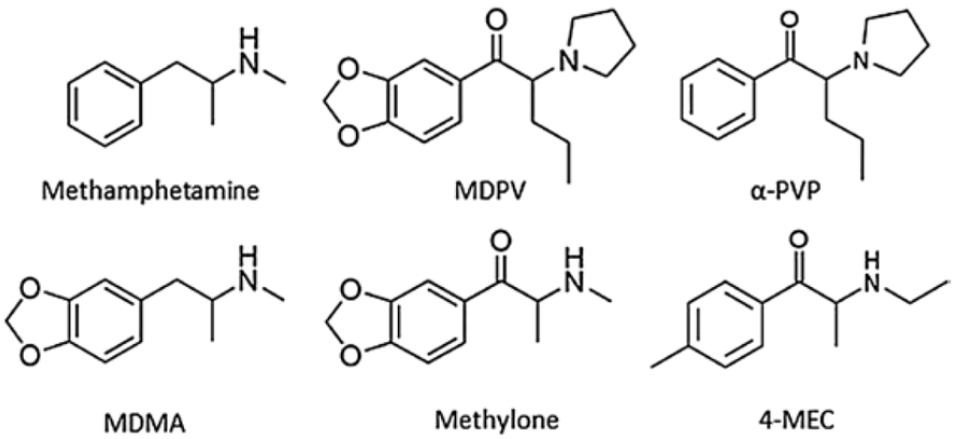

Methamphetamine
MDPV
α-PVP
MDMA
Methylone
4-MEC

Figure 14. Chemical structures of psychostimulants. Chemical structures of the traditional psychostimulants methamphetamine (METH) and methylenedioxymethamphetamine (MDMA) first-generation synthetic cathinones methylenedioxypyrovalerone (MDPV) and methylone, and second-generation synthetic cathinones α-pyrrolidinopentiophenone (α-PVP) and 4-methyl-N-ethcathinone (4-MEC).

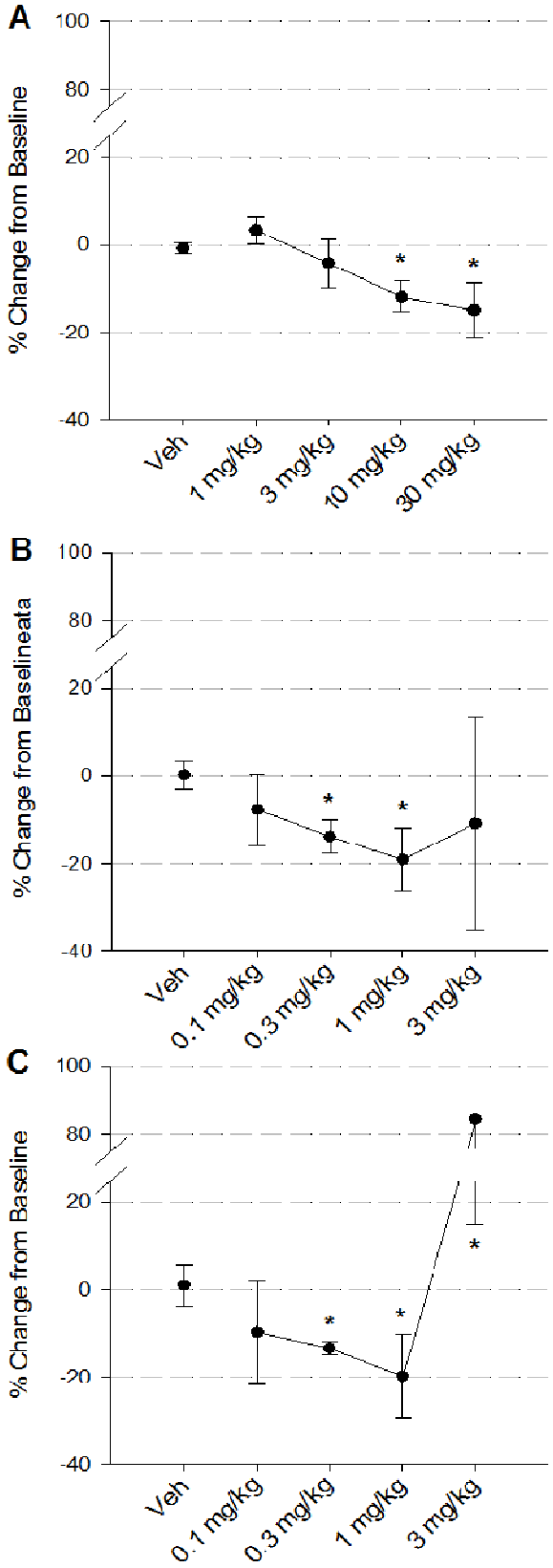

A
100
80
20
0
-20
-40
% Change from Baseline
*
*
Veh
1 mg/kg
3 mg/kg
10 mg/kg
30 mg/kg
B
100
80
20
0
-20
-40
% Change from Baselineata
*
*
Veh
0.1 mg/kg
0.3 mg/kg
1 mg/kg
3 mg/kg
C
100
80
20
0
-20
-40
% Change from Baseline
*
*
*
Veh
0.1 mg/kg
0.3 mg/kg
1 mg/kg
3 mg/kg

Figure 15. α-PVP, 4-MEC, and METH ICSS. Effects of the second-generation synthetic cathinone (A) 4-methyl-N-ethcathinone (4-MEC) (1, 3, 10, 30, mg/kg), (B) α-pyrrolidinopentiophenone (α-PVP) (0.1, 0.3, 1, and 3 mg/kg), and the traditional psychostimulant (C) methamphetamine hydrochloride (METH) (0.1, 0.3, 1, and 3 mg/kg) on intracranial self-stimulation (ICSS) thresholds. Data represent mean ± 95% confidence interval and are expressed as a percent change in ICSS thresholds relative to the previous baseline session. N = 5, 5, and 4 in A, B, and C, respectively. *Symbols represent $P < .05$ vs. saline. In C, the confidence interval upper limit (no shown) for the 3-mg/kg dose = 153.87.

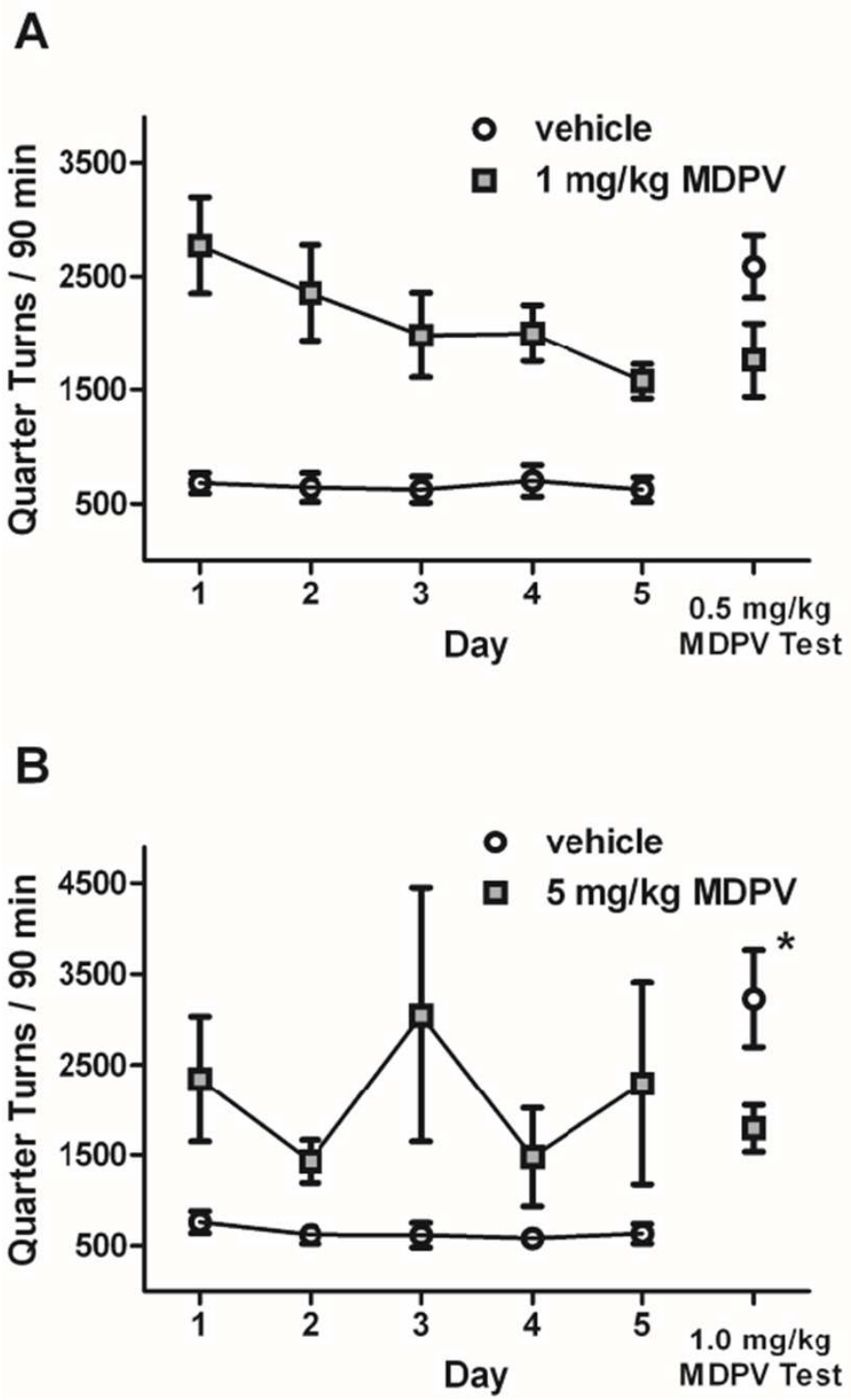

A
vehicle
1 mg/kg MDPV
Quarter Turns / 90 min
3500
2500
1500
500
1
2
3
4
5
Day
0.5 mg/kg
MDPV Test
B
vehicle
5 mg/kg MDPV
*
Quarter Turns / 90 min
4500
3500
2500
1500
500
1
2
3
4
5
Day
1.0 mg/kg
MDPV Test

Figure 16. 24 hr MDPV sensitization. Effects of five repeated MDPV administrations separated by 24 hrs on locomotion and MDPV behavioral sensitization. For experiment 1A (A), 1 mg/kg MDPV (filled squares)(N=8) or saline vehicle (open circles)(N=8) were administered via the intraperitoneal route across five treatment sessions separated by 24 hr intervals. For experiment 1B (B), 5 mg/kg MDPV (filled squares)(N=8) or saline vehicle (open circles)(N=5) were administered via the intraperitoneal route across five treatment sessions separated by 24 hr intervals. Across the five treatment sessions, animals receiving 1 mg/kg MDPV (experiment 1A) or 5 mg/kg MDPV (experiment 1B) displayed more quarter turns compared to rats receiving saline vehicle (p's <0.05). For experiment 1A sensitization tests using 0.5 mg/kg MDPV, there were no differences in quarter turns between rats with a history of 1 mg/kg MDPV vs saline vehicle. For experiment 1B sensitization tests, there was a significantly greater number of quarter turns in rats with a history of saline vs 5 mg/kg MDPV ($p<0.05$).

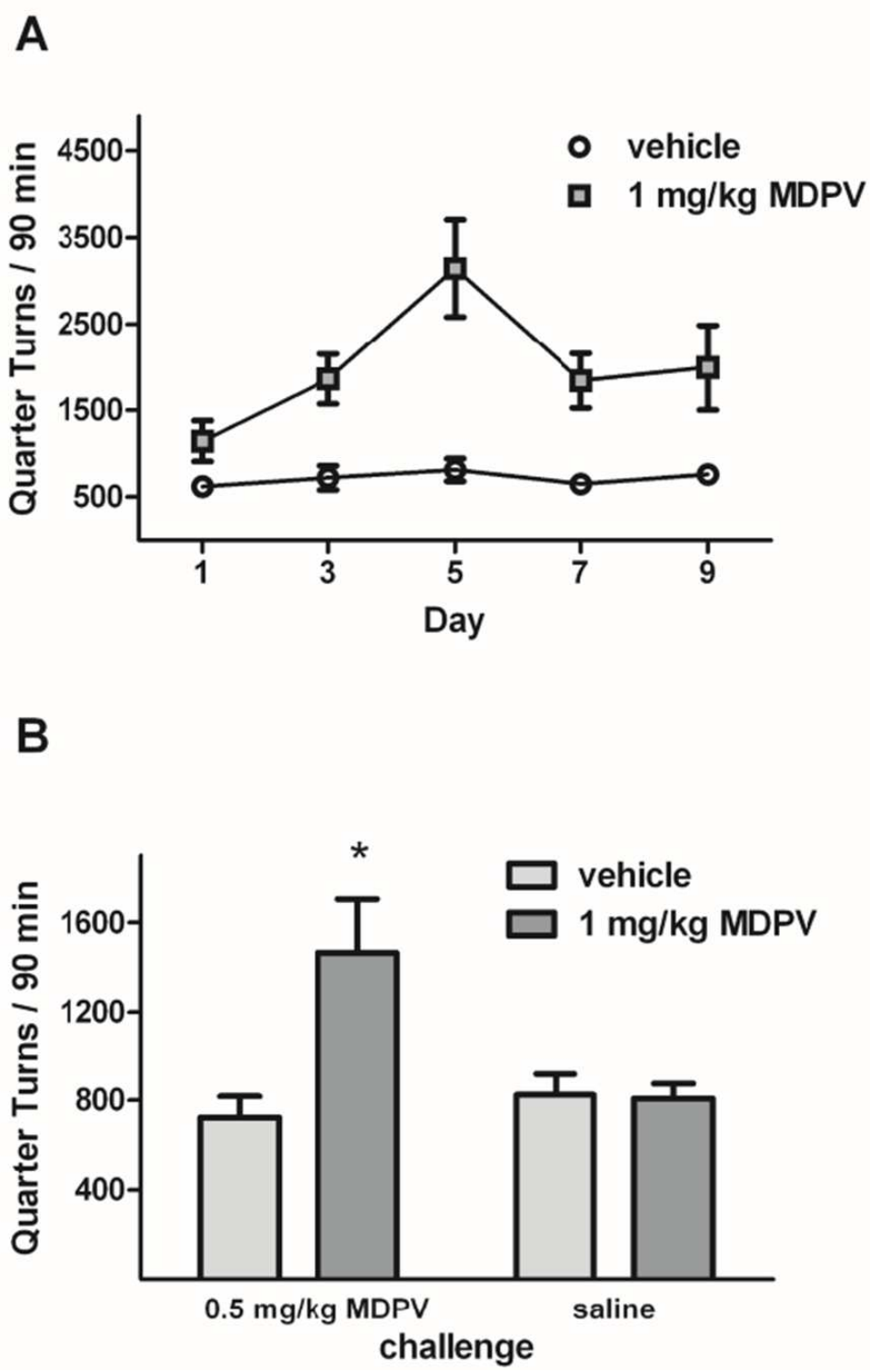

A
Quarter Turns / 90 min
4500
3500
2500
1500
500
vehicle
1 mg/kg MDPV
1
3
5
7
9
Day
B
Quarter Turns / 90 min
1600
1200
800
400
*
vehicle
1 mg/kg MDPV
0.5 mg/kg MDPV
saline
challenge

Figure 17. 48 hr MDPV sensitization. Effects of five repeated MDPV administrations separated by 48 hrs on locomotion and MDPV behavioral sensitization. For experiment 2, 1 mg/kg MDPV (filled squares)(N=10) or saline vehicle (open circles)(N=8) was administered via the intraperitoneal route across five treatment sessions separated by 48 hr intervals. Across the five treatment sessions (A), animals receiving 1 mg/kg MDPV displayed more quarter turns compared to rats receiving saline vehicle ($p<0.05$). For saline sensitization tests (B), there were no differences in quarter turns between rats with a history of 1 mg/kg MDPV vs saline vehicle. For sensitization tests using 0.5 mg/kg MDPV (B), there was a significantly greater number of quarter turns in rats with a history of 1 mg/kg MDPV vs saline vehicle (* $p<0.05$).

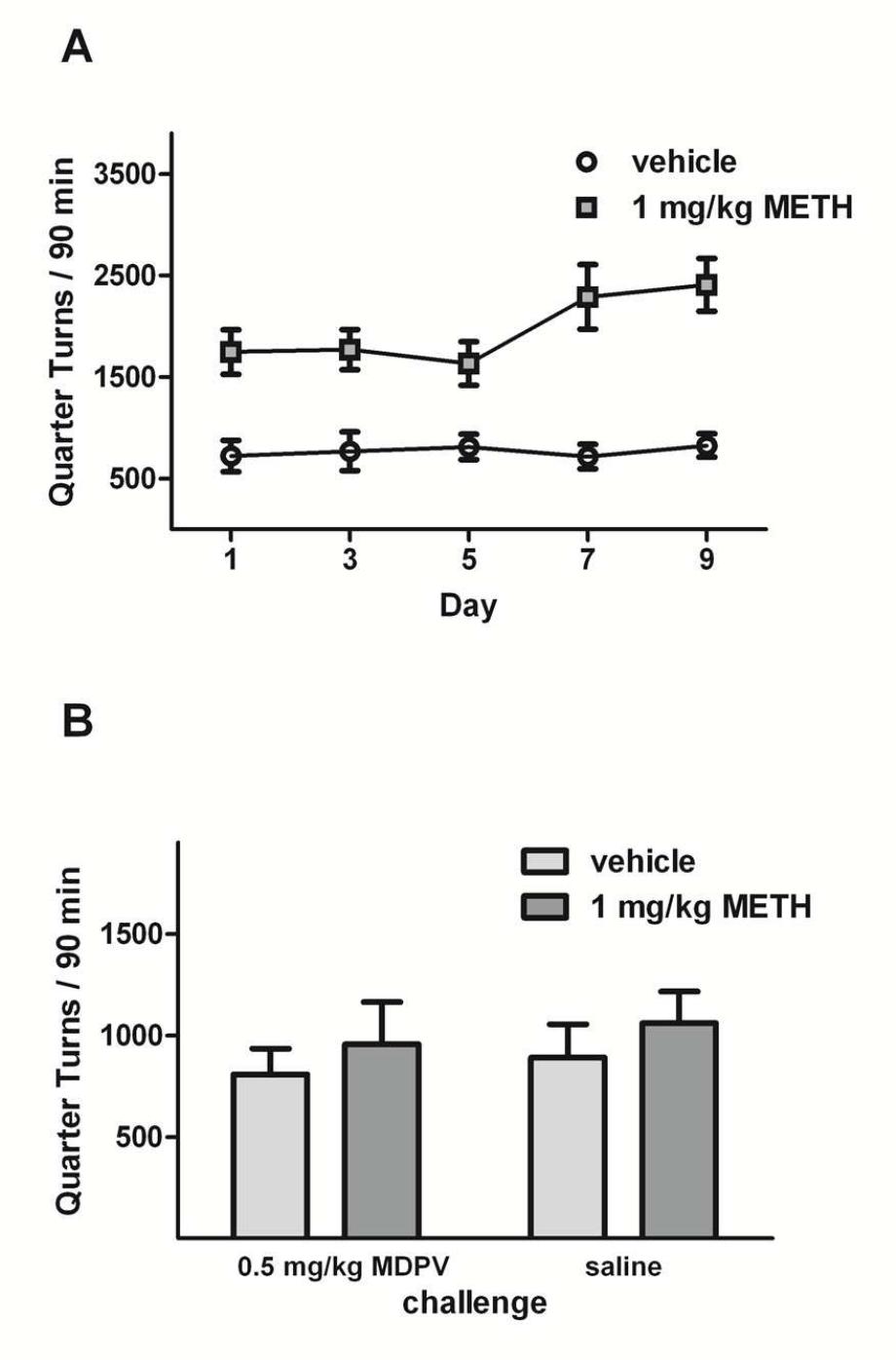
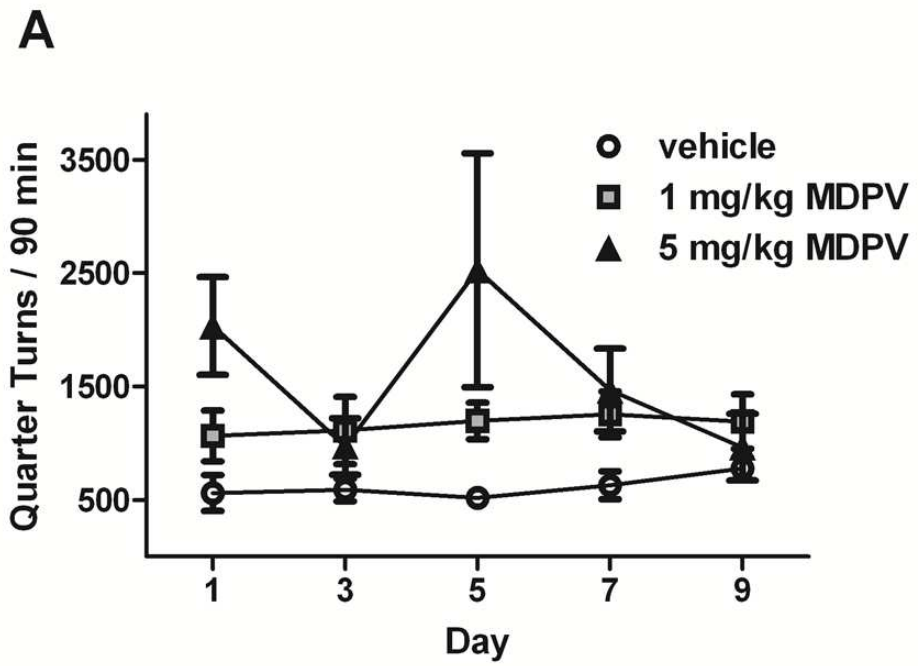

A
vehicle
1 mg/kg METH
Quarter Turns / 90 min
3500
2500
1500
500
1
3
5
7
9
Day
B
vehicle
1 mg/kg METH
Quarter Turns / 90 min
1500
1000
500
0.5 mg/kg MDPV
saline
challenge

Figure 18. 48 hr METH-MDPV cross-sensitization. Effects of five repeated METH administration separated by 48 hrs on locomotion and MDPV cross-sensitization. For experiment 3, 1 mg/kg METH (filled squares)(N=16) or saline vehicle (open circles)(N=16) was administered via the intraperitoneal route across five treatment sessions separated by 48 hr intervals. Across the five treatment sessions (A), animals receiving 1 mg/kg METH displayed more quarter turns compared to rats receiving saline vehicle ($p<0.05$). For saline sensitization tests (B), there were no differences in quarter turns between rats with a history of 1 mg/kg METH vs saline vehicle. For sensitization tests using 0.5 mg/kg MDPV (B), there also were no differences in quarter turns between rats with a history of 1 mg/kg METH vs saline vehicle.


A
Quarter Turns / 90 min
3500
2500
1500
500
1
3
5
7
9
Day
vehicle
1 mg/kg MDPV
5 mg/kg MDPV

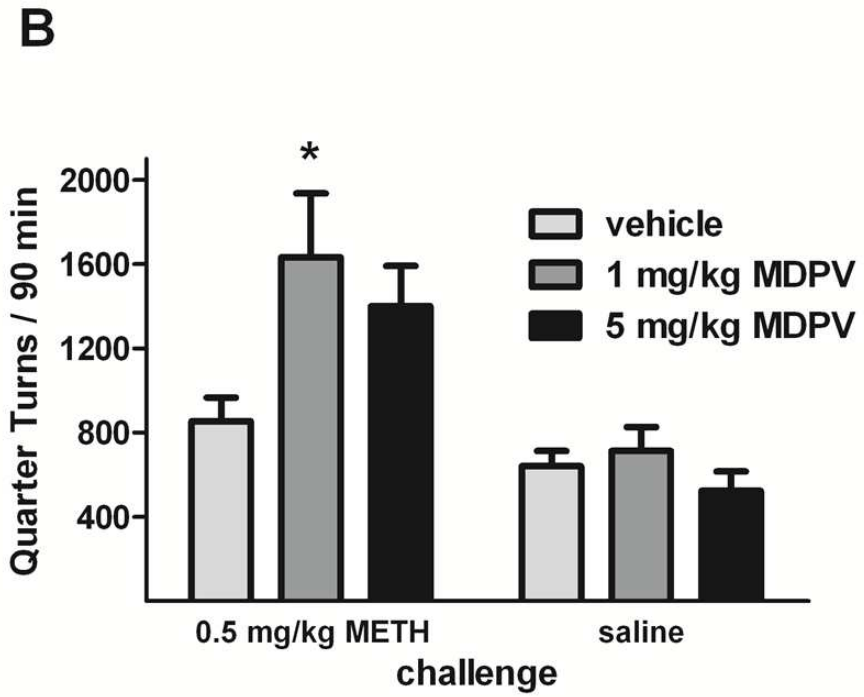

B
Quarter Turns / 90 min
2000
1600
1200
800
400
*
0.5 mg/kg METH
saline
challenge
vehicle
1 mg/kg MDPV
5 mg/kg MDPV

Figure 19. 48 hr MDPV-METH cross-sensitization. Effects of five repeated MDPV administrations separated by 48 hrs on locomotion and MDPV-METH cross-sensitization. For experiment 4, 1 mg/kg MDPV (filled squares)(N=6), 5 mg/kg MDPV (filled triangles)(N=10), or saline vehicle (open circles)(N=8) was administered via the intraperitoneal route across five treatment sessions separated by 48 hr intervals. Across the five treatment sessions (A), animals receiving 1 mg/kg MDPV and 5 mg/kg MDPV displayed more quarter turns compared to rats receiving saline vehicle ($p<0.05$). For saline sensitization tests (B), there were no differences in quarter turns between rats with a history of 1 mg/kg MDPV, 5 mg/kg MDPV or saline vehicle. For sensitization tests using 0.5 mg/kg METH (B), there was a significantly greater number of quarter turns in rats with a history of 1 mg/kg MDPV vs saline vehicle (* $p<0.05$).

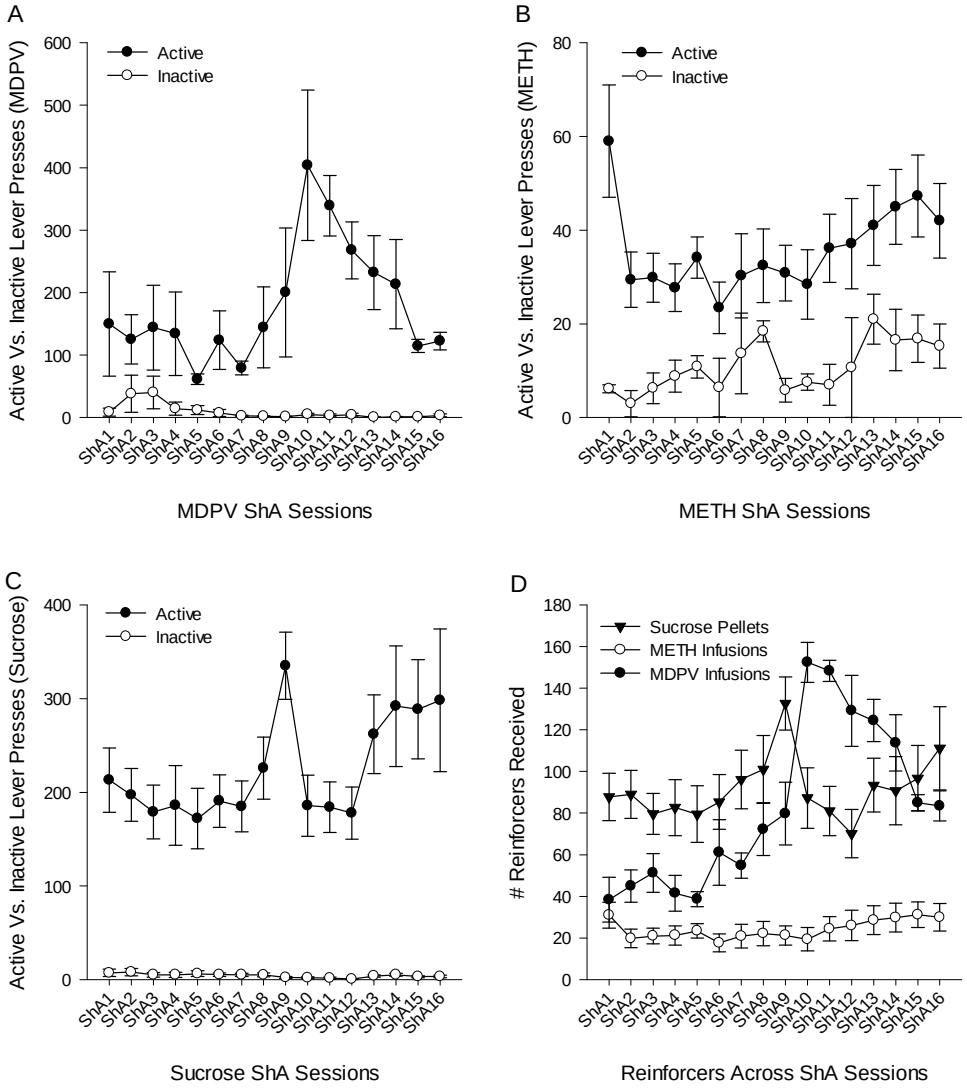

A
Active
Inactive
Active Vs. Inactive Lever Presses (MDPV)
MDPV ShA Sessions
B
Active
Inactive
Active Vs. Inactive Lever Presses (METH)
METH ShA Sessions
C
Active
Inactive
Active Vs. Inactive Lever Presses (Sucrose)
Sucrose ShA Sessions
D
Sucrose Pellets
METH Infusions
MDPV Infusions
Reinforcers Received
Reinforcers Across ShA Sessions

Figure 20: Self-administration of MDPV, METH, and sucrose for Experiment 1. Data presented are active and inactive lever presses across the first 16 days of ShA of IVSA procedures for (a) MDPV (0.05 mg/kg per infusion, N = 7) (b) METH (0.05 mg/kg per infusion, N = 7) (c) sucrose (45 mg pellets, N = 8). All groups displayed successful lever discrimination across all ShA trials. (D) Data represent the total number of reinforcers received for each reinforcer group.

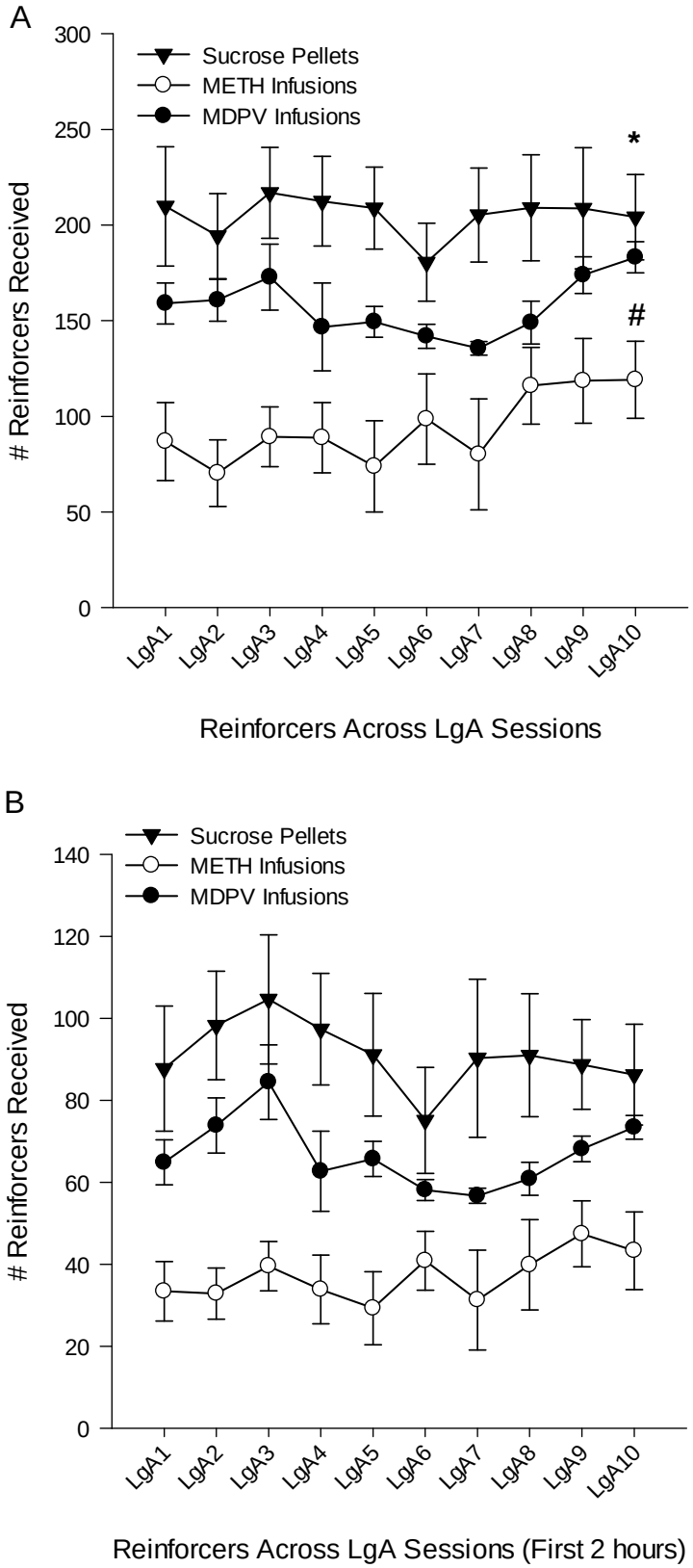
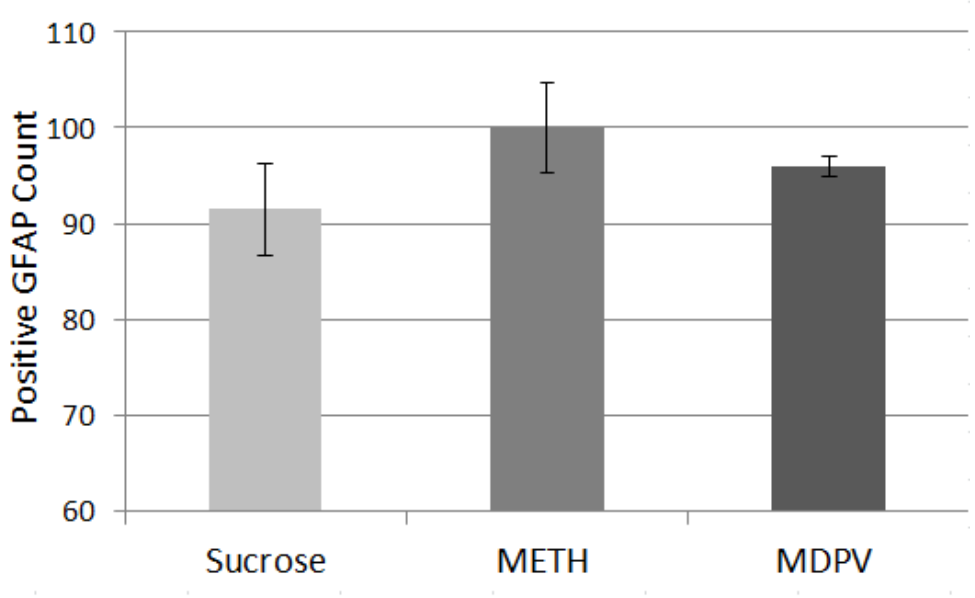
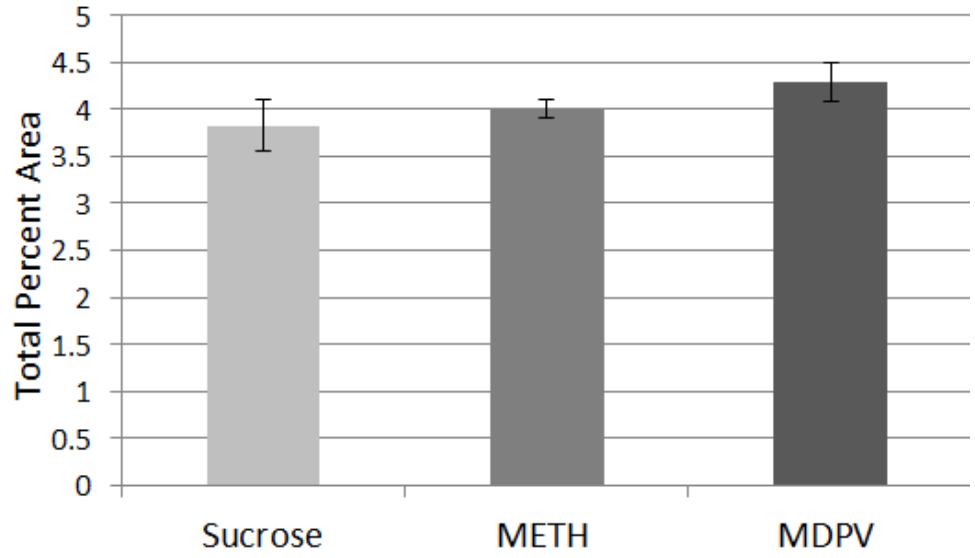

A
Sucrose Pellets
METH Infusions
MDPV Infusions
Reinforcers Received
300
250
200
150
100
50
0
*
#
LgA1
LgA2
LgA3
LgA4
LgA5
LgA6
LgA7
LgA8
LgA9
LgA10
Reinforcers Across LgA Sessions
B
Sucrose Pellets
METH Infusions
MDPV Infusions
Reinforcers Received
140
120
100
80
60
40
20
0
LgA1
LgA2
LgA3
LgA4
LgA5
LgA6
LgA7
LgA8
LgA9
LgA10
Reinforcers Across LgA Sessions (First 2 hours)

Figure 21: Reinforcers obtained during LgA IVSA procedures in Experiment 1. (A) Total number of reinforcers obtained in experiment 1 across long (LgA) sessions for each reinforcer group. Rats in the MDPV and METH group showed a trend toward escalation on LgA session 10 vs. LgA session 1 (p's = 0.052 and 0.061, respectively). (B) Total number of reinforcers obtained in experiment 1 across the first 2 hours of LgA sessions for each reinforcer group. No escalation of intake was noted for any of the groups.

A


110
100
90
80
70
60
Positive GFAP Count
Sucrose
METH
MDPV

B


5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Total Percent Area
Sucrose
METH
MDPV

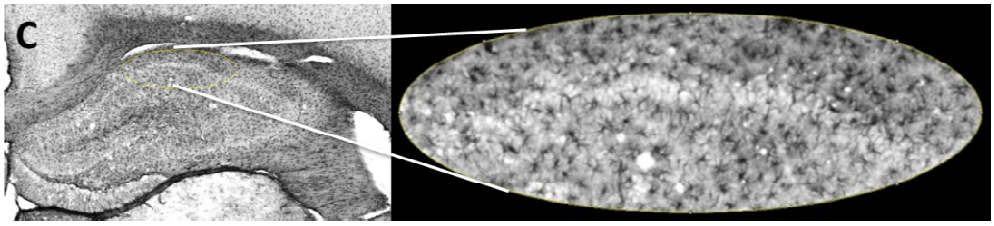

C

Figure 22: GFAP staining in the CA1 region of the hippocampus. (A) Total number of GFAP positive cells and (B) total percentage of the CA1 region of the dorsal hippocampus with positive GFAP staining in rats that self-administered MDPV (N=7), METH (N=7), or sucrose (N=8). (C) Representative 10X magnification photograph of the CA1 region.

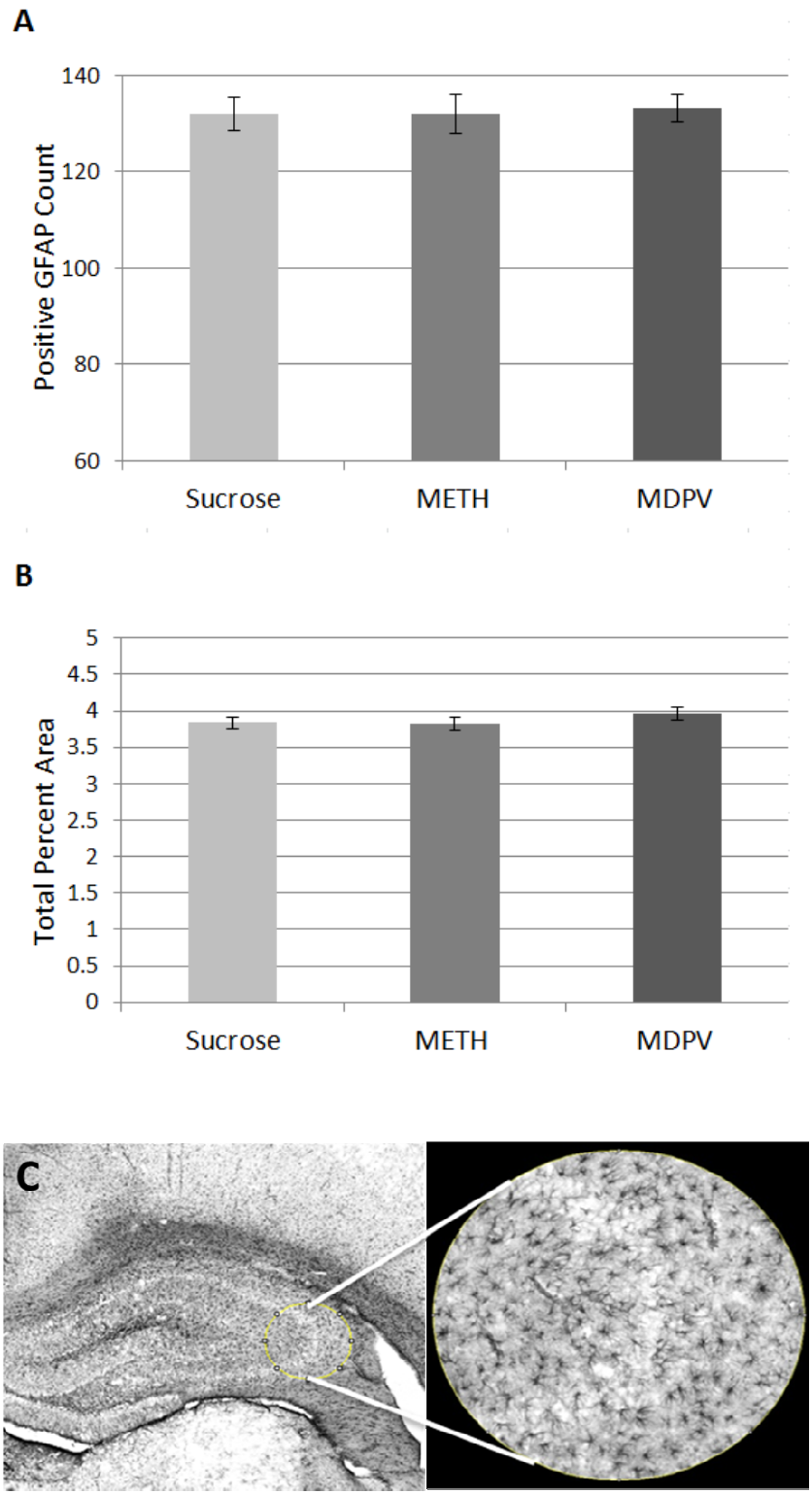
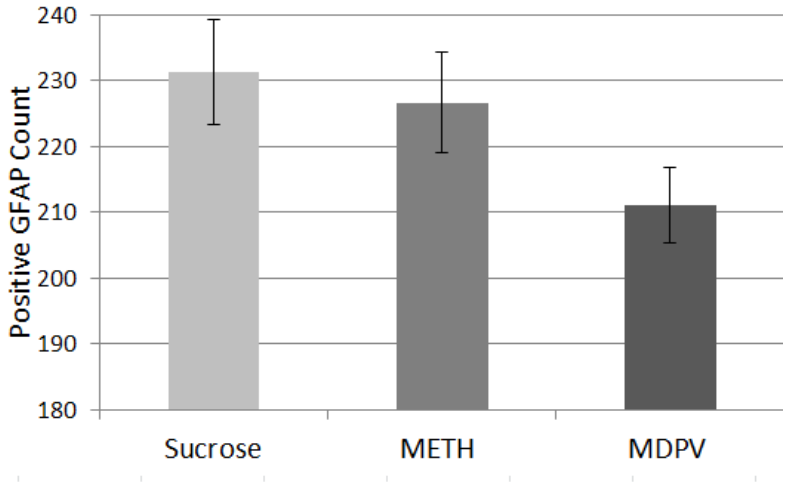
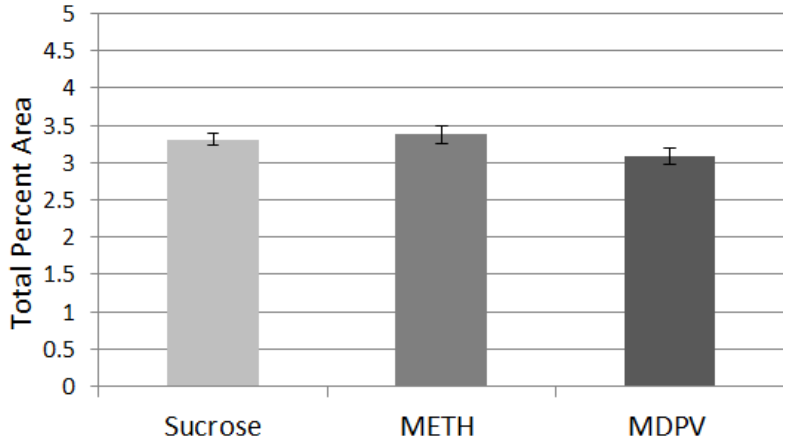

A
140
120
100
80
60
Positive GFAP Count
Sucrose
METH
MDPV
B
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Total Percent Area
Sucrose
METH
MDPV
C

Figure 23: GFAP staining in the CA3 region of the hippocampus. (A)Total number of GFAP positive cells and (B) total percentage of the CA3 region of the dorsal hippocampus with positive GFAP staining in rats that self-administered MDPV (N=7), METH (N=7), or sucrose (N=8). Representative 10X magnification photograph of the CA3 region.

A


240
230
220
210
200
190
180
Positive GFAP Count
Sucrose
METH
MDPV

B


5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Total Percent Area
Sucrose
METH
MDPV

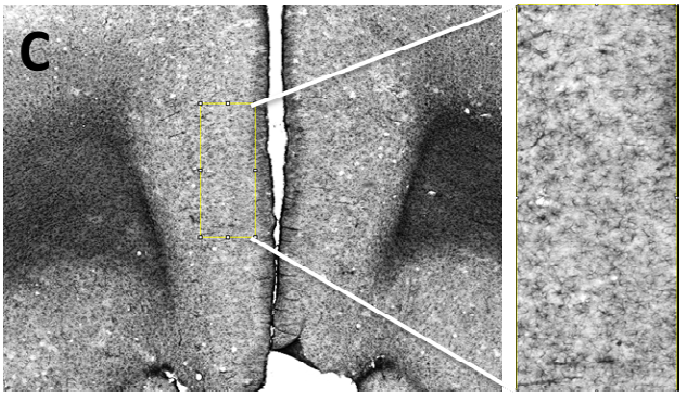

C

Figure 24: GFAP staining in the mPFC. (A) Total number of GFAP positive cells and (B) total percentage of the mPFC with positive GFAP staining in rats that self-administered MDPV (N=7), METH (N=7), or sucrose (N=8). Representative 10X magnification photograph of the mPFC.

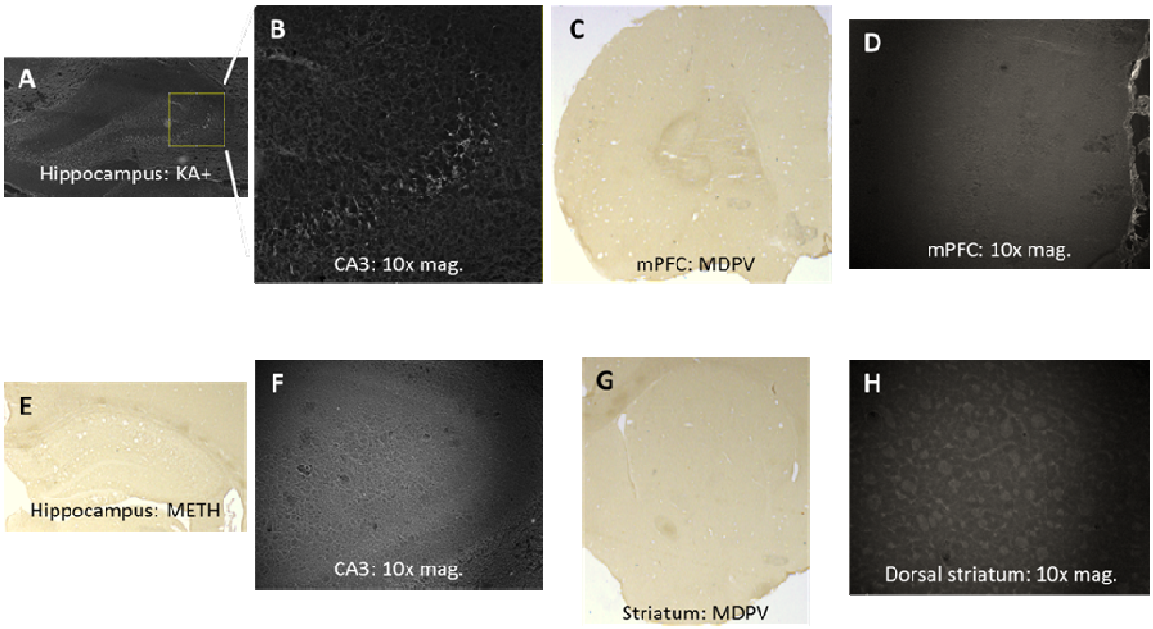

A
Hippocampus: KA+
B
CA3: 10x mag.
C
mPFC: MDPV
D
mPFC: 10x mag.
E
Hippocampus: METH
F
CA3: 10x mag.
G
Striatum: MDPV
H
Dorsal striatum: 10x mag.

Figure 25. Results of FluoroJade C neurodegeneration staining. (A,B) FluoroJade C staining across the entire dorsal hippocampus (A) and at 10X magnification in the CA3 region (B) in a rat injected intracerebroventrically with 1 µg/µl KA. (C) Representative image of the mPFC region of a rat that underwent MDPV self-administration. (D) Representative FluoroJade C staining (10X magnification) of the ILC/PLC from the same subject under fluorescence. (E) Representative image of the hippocampus in a rat that underwent METH self-administration. (F) Representative FluoroJade C staining (10X magnification) of CA3 region in the same rat under fluorescence). (G) Representative image of the striatum of a rat that underwent MDPV self-adminitration. (H) Representative FluoroJade C staining (10X magnification) of the dorsal striatum of the same subject under fluorescence.

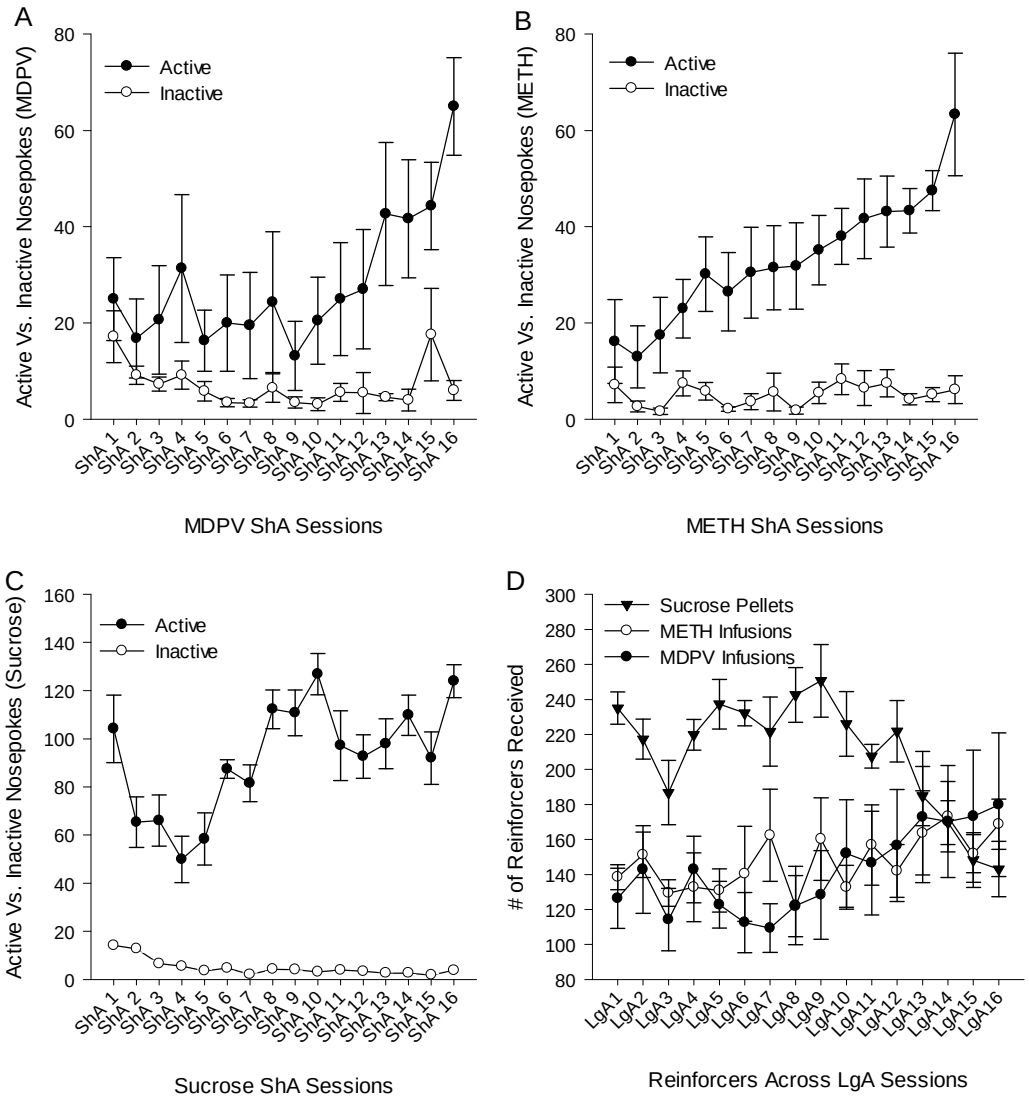

A
Active Vs. Inactive Nosepokes (MDPV)
Active
Inactive
MDPV ShA Sessions
B
Active Vs. Inactive Nosepokes (METH)
Active
Inactive
METH ShA Sessions
C
Active Vs. Inactive Nosepokes (Sucrose)
Active
Inactive
Sucrose ShA Sessions
D
# of Reinforcers Received
Sucrose Pellets
METH Infusions
MDPV Infusions
Reinforcers Across LgA Sessions

Figure 26: Self-administration of MDPV, METH, and sucrose for Experiment 2. Data presented are active and inactive nosepokes across the first 16 days of ShA of IVSA procedures for (a) MDPV (0.05 mg/kg per infusion, N = 6) (b) METH (0.05 mg/kg per infusion, N = 6) and (c) sucrose (45 mg pellets, N = 12). All groups displayed successful lever discrimination during ShA sessions. (D) Total number of reinforcers obtained in experiment 2 across long (LgA) sessions for each reinforcer group. No escalation of intake occurred acoss LgA sessions for any group of rats.

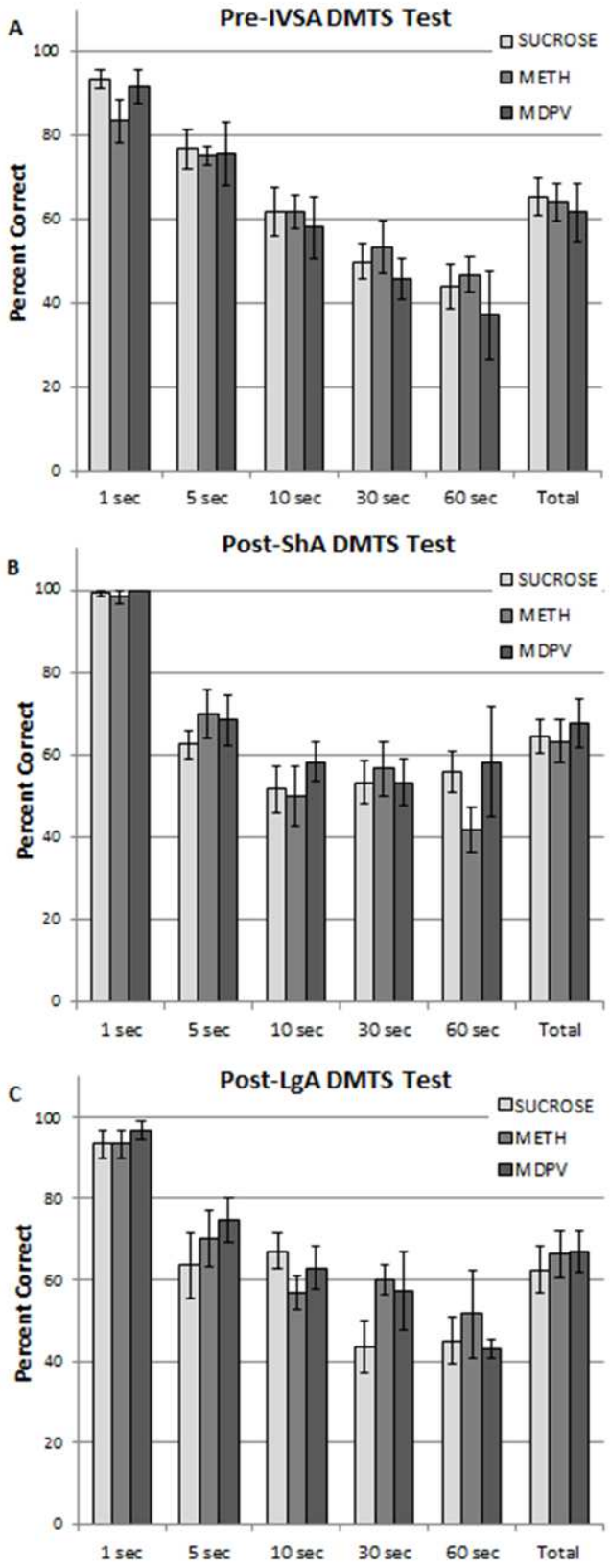

A
Pre-IVSA DMTS Test
SUCROSE
METH
MDPV
Percent Correct
100
80
60
40
20
0
1 sec
5 sec
10 sec
30 sec
60 sec
Total
B
Post-ShA DMTS Test
SUCROSE
METH
MDPV
Percent Correct
100
80
60
40
20
0
1 sec
5 sec
10 sec
30 sec
60 sec
Total
C
Post-LgA DMTS Test
SUCROSE
METH
MDPV
Percent Correct
100
80
60
40
20
0
1 sec
5 sec
10 sec
30 sec
60 sec
Total

Figure 27. Results from DMTP probe tests. Data represent percent correct responses across all five waiting times (1, 5, 10, 30, and 60) and total percent correct across all trials (total) during DMTP tests in groups of rats that underwent self-administration of MDPV (N=6), METH (N=6), or sucrose (N = 12). (A) Percent correct in rats prior to self-administration procedures (Pre-IVSA DMTP test). (B) Percent correct in rats following 15 or 16 days of ShA self-administration procedures (Post-ShA DMTP test). (C) Percent correct in rats following 15 or 16 days of LgA self-administration procedures (Post-LgA DMTP test).

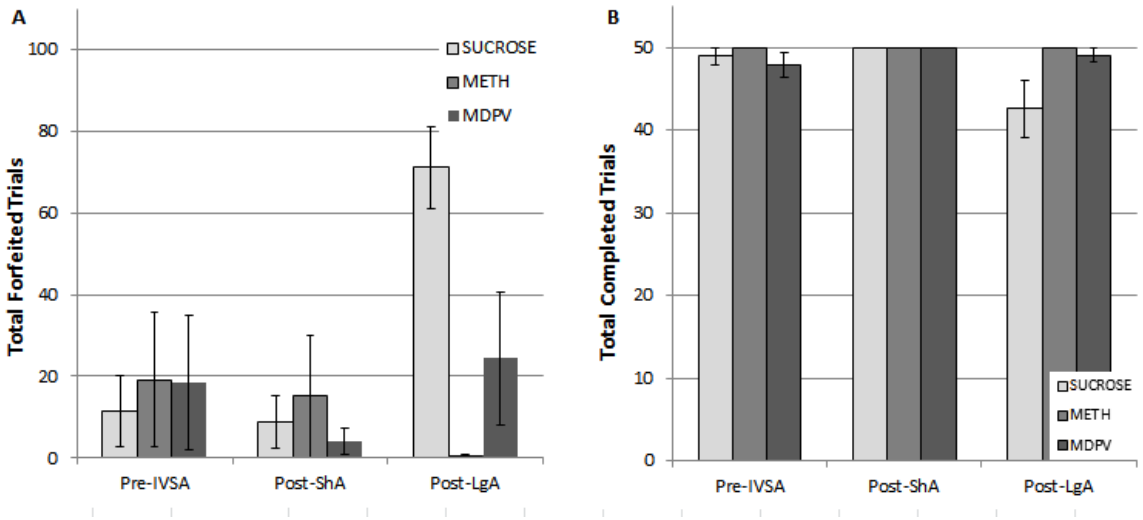

A
Total Forfeited Trials
100
80
60
40
20
0
SUCROSE
METH
MDPV
Pre-IVSA
Post-ShA
Post-LgA
B
Total Completed Trials
50
40
30
20
10
0
SUCROSE
METH
MDPV
Pre-IVSA
Post-ShA
Post-LgA

Figure 28: Total forfeited and completed DMTP probe trials. (A) Total number of forfeited DMTP test trials in groups of rats that underwent self-administration of MDPV (N=6), METH (N=6), or sucrose (N = 12) prior to testing procedures (Pre-IVSA), following 15 or 16 days of ShA self-administration procedures (Post-ShA), or following 15 or 16 days of LgA self-administration procedures (Post-LgA). (B) Total number of completed DMTP test trials (maximum 50) in groups of rats that underwent self-administration of MDPV, METH, or sucrose prior to self-administration procedures (Pre-IVSA), following 15 or 16 days of ShA (Post-ShA), or following 15 or 16 days of LgA (Post-LgA).

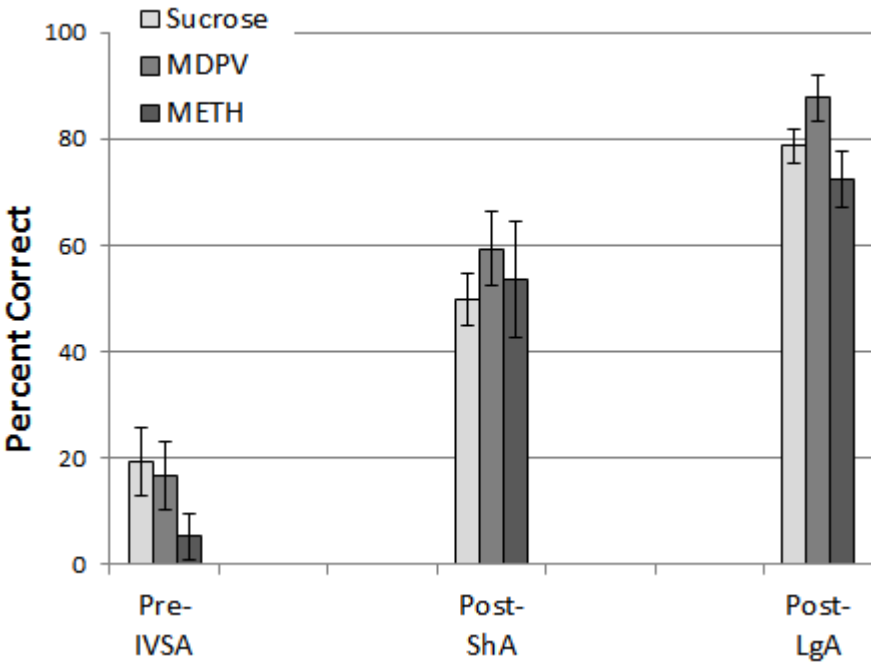

Sucrose
MDPV
METH
Percent Correct
100
80
60
40
20
0
Pre-
IVSA
Post-
ShA
Post-
LgA

Figure 29: Performance on S+/S- reversal tests. Data represent percent correct responses during S+/S- reversal tests in groups of rats that underwent self-administration of MDPV (N=6), METH (N=5), or sucrose (N = 11). (A) Percent correct in rats prior to self-administration procedures (Pre-IVSA), following 15 or 16 days of ShA (Post-ShA). or following 15 or 16 days of LgA (Post-LgA).

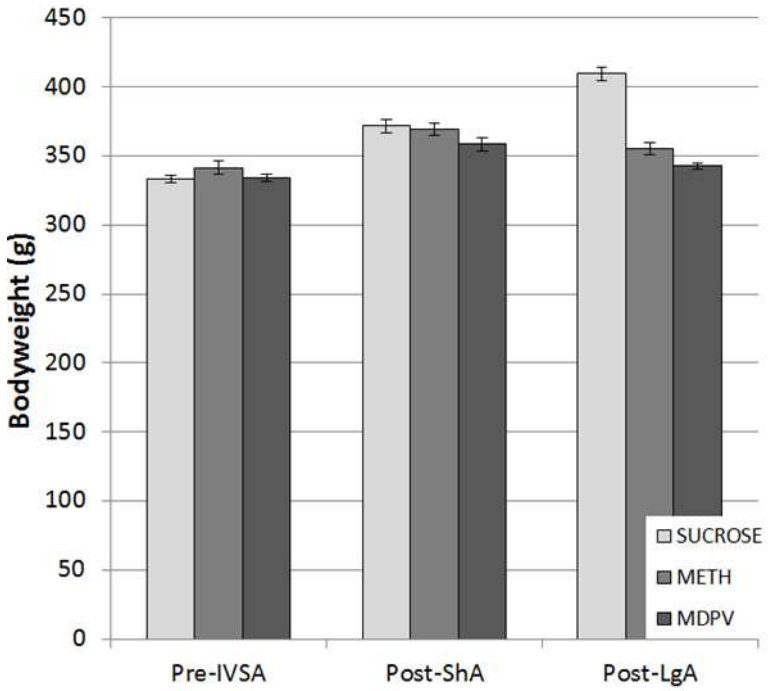

450
400
350
300
250
200
150
100
50
0
Bodyweight (g)
Pre-IVSA
Post-ShA
Post-LgA
SUCROSE
METH
MDPV

Figure 30. Body weights. Body weight (g) in groups of rats that underwent self-administration of MDPV, METH, or sucrose. Weights were recorded the morning of initial cognitive tests (Pre-IVSA), the morning of cognitive tests following 15 or 16 days of ShA (Post-ShA), or the morning of cognitive tests following 15 or 16 days of LgA (Post-LgA).

APPENDIX A

SECURED PERMISSION TO INCLUDE PUBLISHED RESEARCH

I have secured permission from all authors to include published research in the current dissertation.

APPENDIX B

CITATIONS OF PUBLISHED ARTICLES ADAPTED AND/OR USED IN THEIR ENTIRETY IN THIS DISSERTATION

CHAPTER 1: Full citations for published articles adapted for use in the general introduction

Watterson LR, Olive MF. (2014). Synthetic cathinones and their rewarding and reinforcing effects in rodents. *Advances in Neuroscience*, 2014, 1-9.

Watterson LR, Olive MF. (2013). Are AMPA receptor positive allosteric modulators potential pharmacotherapeutics for addiction? *Pharmaceuticals*, 7, 29-45.

Watterson LR, Watterson E, Olive MF. (2013). Abuse liability of novel "legal high" designer stimulants: evidence from animal models. *Behavioural Pharmacology*, 24, 341-55.

CHAPTER 2: Full citation for published article

Watterson LR, Kufahl PR, Nemirovsky NE, Sewalia K, Hood LE, Olive, MF. (2012). Attenuation of reinstatement of methamphetamine-, sucrose-, and food-seeking behavior in rats by fenobam, a clinically utilized metabotropic glutamate receptor 5 negative allosteric modulator, *Psychopharmacology*, 225, 151-159.

CHAPTER 3: Full citation for published article

Watterson LR, Olive MF. (2013). Are AMPA receptor positive allosteric modulators potential pharmacotherapeutics for addiction? *Pharmaceuticals*, 7, 29-45.

CHAPTER 4: Full citation for published article

Watterson LR, Kufahl PR, Nemirovsky NE, Sewalia K, Grabenauer M, Thomas BF, Marusich JA, Wegner S, Olive MF. (2014). Potent rewarding and reinforcing effects of the synthetic cathinone 3,4-methylenedioxypyrovalerone, *Addiction Biology*, 19, 165-174.

CHAPTER 5: Full citation for published article

Watterson LR, Hood LE, Nemirovsky NN, Johnson T, Sewalia K, Grabenauer M, Thomas BF, Marusich JA, Olive MF. (2012). The rewarding and

reinforcing effects of methylone, a drug commonly found in “bath salts”. *Journal of Addiction Research and Therapy*. S9, 1–8.

CHAPTER 6: Full citation for published article

Watterson LR, Burrows B, Hernandez R, Moore KN, Grabenauer M, Marusich JA, Olive MF. (2014). Effects of α-pyrrolidinovalerophenone (α-PVP) and 4-methylethcathinone (4-MEC), two synthetic cathinones commonly found in second-generation “bath salts”, on ICSS thresholds in rats. *International Journal of Neuropsychopharmacology* (In Press).

CHAPTER 7: Full citation for manuscript

Watterson LR*, Kufahl PR*, Taylor S, Nemirovsky NE, Olive MF. Sensitization to the locomotor stimulant effects of 3,4-methylenedioxypyrovalerone (MDPV) and cross-sensitization to methamphetamine in rats. (Under Review). **these authors contributed equally to this work.*

CHAPTER 8: Full citation for manuscript

Watterson LR, Kufahl PR, Taylor S, Nemirovsky NE, Hryciw A. Olive MF. Assessment of neurotoxic and cognitive effects following chronic intravenous MDPV and methamphetamine self-administration. (In prep)

APPENDIX C

CIRRICULUM VITAE

Lucas Richard Watterson

Arizona State University, Tempe Campus
Department of Psychology
950 S. McAllister, Room 371
PO Box 871104, Tempe, AZ 85287 Fax: 480-965-854
Lucas.Watterson@asu.edu Work: 480-727-5550

EDUCATION

Arizona State University

2014 Doctor of Philosophy, Psychology (Behavioral Neuroscience)
Research Focus: Addiction, Neuropsychopharmacology

Dissertation Title: *Methamphetamine and novel "legal high" methamphetamine mimetics: abuse liability, toxicity, and potential pharmacobehavioral treatments.*

University of North Carolina at Wilmington (UNCW)

2010 Master of Arts, Experimental Psychology
Thesis Research Focus: Neuroscience, decision-making
Additional Research: Behavioral pharmacology, working memory

Thesis Title: *The effects of contemplating moral dilemmas on Iowa Gambling Task performance in adolescents.*

Shippensburg University of Pennsylvania

2006 Bachelor of Arts, Psychology
Research Focus: Physiological Psychology
Graduated *Magna Cum Laude*

Research Interests

The overall purpose of my research is to investigate the behavioral and neurological mechanisms underlying psychostimulant abuse.

Methamphetamine
Designer Stimulants (synthetic cathinones)
Abuse Liability
Neuroplasticity
Glutamate
Medications Development

PUBLICATIONS

Publication and Citation Information (Google Scholar)
http://scholar.google.com/citations?user=rP1wALUAAAAJ&hl=en

Total publications: 19
Research Articles: 11
Review Articles: 6
Book Chapters: 2
Total citations: 103
h factor: 7

Peer-Reviewed Manuscripts

Primary Author

Watterson LR*, Kufahl PR*, Taylor S, Nemirovsky NE, Olive MF. Sensitization to the locomotor stimulant effects of 3,4-methylenedioxypyrovalerone (MDPV) and cross-sensitization to methamphetamine in rats. *Drug and Alcohol Dependence* (Under Review). **these authors contributed equally to this work.*

Watterson LR, Burrows B, Hernandez R, Moore KN, Grabenauer M, Marusich JA, Olive MF. (2014). Effects of α-pyrrolidinovalerophenone (α-PVP) and 4-methylethcathinone (4-MEC), two synthetic cathinones commonly found in second-generation "bath salts", on ICSS thresholds in rats. *International Journal of Neuropsychopharmacology* (In Press).

Watterson LR, Olive MF. (2014). Synthetic cathinones and their rewarding and reinforcing effects in rodents. *Advances in Neuroscience*, 2014, 1-9.

Watterson LR, Kufahl PR, Nemirovsky NE, Sewalia K, Grabenauer M, Thomas BF, Marusich JA, Wegner S, Olive MF. (2014). Potent rewarding and reinforcing effects of the synthetic cathinone 3,4-methylenedioxypyrovalerone, *Addiction Biology*, 19, 165-174.

Watterson LR, Olive MF. (2013). Are AMPA receptor positive allosteric modulators potential pharmacotherapeutics for addiction? *Pharmaceuticals*. 7, 29-45.

Watterson LR, Watterson E, Olive MF. (2013). Abuse liability of novel "legal high" designer stimulants: evidence from animal models. *Behavioural Pharmacology*, 24, 341-55.

Yahn SL*, **Watterson LR*,** Olive MF. (2013). Safety and efficacy of acamprosate for the treatment of alcohol dependence. *Substance Abuse: Research and Treatment.* 6, 1-12. **these authors contributed equally to this work.*

Watterson LR, Kufahl PR, Nemirovsky NE, Sewalia K, Hood LE, Olive, MF.(2012). Attenuation of reinstatement of methamphetamine-, sucrose-, and food-seeking behavior in rats by fenobam, a clinically utilized metabotropic glutamate receptor 5 negative allosteric modulator, *Psychopharmacology.* 225, 151-159

Watterson LR, Hood LE, Nemirovsky NN, Johnson T, Sewalia K, Grabenauer M, Thomas BF, Marusich JA, Olive MF. (2012). The Rewarding and reinforcing effects of methylone, a drug commonly found in "bath salts". *Journal of Addiction Research and Therapy*. S9, 1–8.

Co-Author

Baumann MH, Solis E, **Watterson LR**, Marusich JA, Fantegrossi WE, Wiley JL.(2014). Bath Salts, Spice, and Related Designer Drugs: The Science Behind the Headlines. *Journal of Neuroscience* (In Press)

Hoffman AN, Parga A, Paode P, **Watterson LR**, Nikulina EM, Hammer RP, Conrad C. Chronic stress enhanced fear memories are associated with increased amygdala zif268 mRNA expression and are resistant to reconsolidation. (Submitted to *J Neuroscience*)

Kufahl PR, **Watterson LR,** Olive MF. (2013). The development of acamprosate as a treatment against alcohol relapse. *Expert Opinion On Drug Discovery* (in press)

Kufahl PR., **Watterson LR,** Nemirovsky NE, Hood LE, Villa A, Zautra N, Olive MF. (2013) Attenuation of methamphetamine seeking by the mGluR2/3 agonist LY379268 in rats with histories of restricted and escalated self-administration. *Neuropharmacology.*

Kufahl PR, Nemirovsky NE, **Watterson LR**, Zautra N, Olive MF. (2013). Positive or negative allosteric modulation of metabotropic glutamate receptor 5 (mGluR5) does not alter expression of behavioral sensitization to methamphetamine. *F1000Research*. 2:84: 1-12.

Overman WH, Pierce A, **Watterson LR,** Coleman JK. (2013). Use of a non-navigational, non-verbal landmark task in children. *International Journal of Behavioral Development*.

Kufahl PR, Hood LE, Nemirovsky NE, Barabas P, Halstengard C, Villa A, Moore E, **Watterson LR,** Olive MF. (2012). Positive allosteric modulation of mGluR5 accelerates extinction learning but not relearning following methamphetamine self-administration. *Frontiers in Neuropharmacology.*

Overman WH, **Watterson LR**, Boettcher L, Hardy C, Walsh K. (2011). The effects of dilemmas and aromas on performance of the Iowa Gambling Task. *Behavioral Brain Research*, 218, 64-72.

Cleva RM, **Watterson LR**, Johnson MA, Olive MF. (2011). Differential modulation of thresholds for intracranial self-stimulation of mGlu5 positive and negative allosteric modulators: implications for effects on drug self-administration. *Frontiers in Pharmacology*, 2, 93. PMCID: PMC3252814

Book Chapters

Burrows BT, **Watterson LR,** Egnatious J, Olive MF. (2014) Behavioral Pharmacology of Synthetic Cannabinoids. In: The Handbook of Cannabis and Related Pathologies: Biology, Diagnosis, Treatment, and Pharmacology. (VR Preedy, ed). Academic Press: Waltham, Massachusetts.

Watterson LR, Olive MF. (2014) Group I mGluR receptors as mediators of learning and memory. In: *Metabotropic Glutamate Receptors: Molecular Mechanisms, Role in Neurological Disorders, and Pharmacological Effects* (MF Olive, ed). Nova Science Publishers: Hauppauge NY.

Cleva RM, Nemirovsky NE, Wischerath KC, **Watterson LR.** (2012) Transgenic rodent tools for manipulating adult neurogenesis In: *Drug Addiction and Adult Neurogenesis* (Olive MF, ed), Research Signpost Publishing, Kerala, India, pp. 15-40.

Invited Talks and Presentations

Watterson LR (2014, November). Abuse liability of synthetic cathinones as revealed by drug self-administration studies in rats. Invited Speaker. Mini-symposium: Bath Salts, Spice, and Related Designer Drugs: The Science Behind the Headlines. To be presented at the Society for Neuroscience conference, Washington, DC.

Watterson LR (2014, July). Novel “legal high” designer stimulants: abuse liability, toxicity, and potential pharmacobehavioral treatments. Invited seminar speaker at Temple University School of Medicine, Center for Substance Abuse Research, Philadelphia, Pennsylvania.

Watterson LR (2014, June). Abuse liability and toxicity of "bath salts" (i.e. synthetic cathinones) as revealed by intravenous drug self-administration and ex-vivo MRI. Presented at the International Behavioral Neuroscience Society, Las Vegas, Nevada.

Watterson LR. (2013, February). Breaking Bath Salts: Deciphering Abuse Liability of Novel Designer Stimulants with Rodent Models of Addiction. Invited colloquium speaker at Northern Arizona University, Psychology and Neuroscience Departments, Flagstaff, Arizona.

Manuscripts in Preparation

Watterson LR, Taylor SB, Kufahl PR and Olive, MF. Effects of combining a memory-retrieval extinction procedure with the novel TrkB agonist 7,8-dihydroxyflavone on reinstatement to methamphetamine following intravenous self-administration.

Watterson LR, Taylor SB, Tomek S, Yahn S, Nemirovsky NE, Olive MF. Toxic effects of chronic intravenous MDPV and methamphetamine self-administration as revealed by ex vivo MRI and immunohistochemistry.

Burrows B*, **Watterson LR*,** Johnson M, Wininger E, Brackney R, Olive MF. Effects of Modafinil and R-Modafinil on brain stimulation reward thresholds; implications for their use as stimulant dependence medications. **these authors contributed equally to this work.*

Kufahl PR, Yahn S, Moore E, **Watterson LR**, Nemirovsky NE, LaCrosse AL, Villa A, Olive MF. Rapid but not gradual intravenous infusion of methamphetamine produces behavioral sensitization.

Kufahl PR, Moore E, Halstengard C, Barabas P, Tomek SE, Nemirovsky NE, Villa A, Yahn S, **Watterson LR,** Hood LE, Day AO, Olive MF. Effects of the mGluR5 Negative Allosteric Modulator MTEP on Ethanol Self-administration and Ethanol Seeking: Roles of Dorsal and Ventral Medial Prefrontal Cortex.

Kufahl PR, Halstengard C, Villa A, Barabas P, Moore E, Tomek SE, Nemirovsky NE, **Watterson LR**, Hood LE, Day AO, Olive MF. Reinstatement of Ethanol Seeking to Cues Learned Before, During and After Withdrawal: Effects of xc- Substrate N-Acetylcysteine.

Kufahl PR, Sewalia K, Halstengard C, Villa A, Barabas P, Moore E,

Watterson LR, Tomek SE, Olive MF. Effects of Ethanol Dependence and Glutamatergic Ligands on Ethanol Intake using the Two-bottle Choice Model.

Taylor SB, **Watterson LR,** Kufahl PR, Tomek ST, Nemirovsky NE, Conrad CD, Olive MF. Chronic variable stress interacts with individual differences to promote methamphetamine self-administration.

Abstracts

First Author

Watterson LR, Taylor SB, Budin F, Ali SF, Kufahl PR, Nemirovsky N, and Olive MF. (2014). Abuse liability and toxicity of synthetic cathinones ("bath salts") as revealed by intravenous drug self-administration, ex vivo MRI, and immunohistochemistry. Presented at the Society for Neuroscience conference, Washington, DC.

Watterson LR, Olive MF (2014). Abuse liability and toxicity of "bath salts" (i.e. synthetic cathinones) as revealed by Intravenous drug self-administration and ex-vivo MRI. Presented at the International Behavioral Neuroscience Society conference, Las Vegas, NV.

Watterson LR, Tomek S, Yahn SL, Kufahl PR, Olive MF. (2013). The effects of 7,8-dihydroxyflavone and a memory-retrieval extinction procedure on reinstatement following methamphetamine self-administration and place conditioning. Presented at the Society for Neuroscience conference, San Diego, CA.

Watterson LR, Kufahl PR, Nemirovsky NE, Sewalia K, Grabenauer M, Thomas BF, Marusich JA, Wegner S, and Olive MF. (2012). Abuse Liability of MDPV and methylone, two common drugs found in "bath salts". Presented at the 4th annual Barrow Neurological Association Conference, Phoenix, AZ.

Watterson LR, Kufahl PR, Nemirovsky NN, Sewalia K, Graenauer M, Thomas BF, Marusich JA, and Olive MF. (2012). Reinforcing and ICSS threshold-lowering effects of the "bath salts" drug MDPV. Presented at the College on Problems of Drug Dependence annual meeting, Palm Springs, CA.

Watterson LR, Kufahl PR, Nemirovsky NE, Sewalia K, Grabenauer M, Thomas BF, Marusich JA, Wegner S, and Olive MF (2012). Evaluation of the abuse potential of MDPV and methylone, two common synthetic cathinones found in "bath salts". Presented at the International Conference on Addiction Research and Therapy, Las Vegas, NV.

Watterson LR, Hood LE, Nemirovsky NE, Kufahl PR, Varney, MA, and Olive, MF. (2012). AMPA receptor positive allosteric modulators facilitate extinction learning and attenuate cue-induced reinstatement of methamphetamine-seeking. Presented at the Neuropharmacology Conference: Cognitive Enhancers, New Orleans, LA.

Watterson LR, Hood LE, Nemirovsky, NE, Kufahl PR, Varney MA, and Olive MF. (2012). Facilitation of extinction learning and suppression of cue-induced reinstatement of methamphetamine-seeking by AMPA receptor potentiators. Presented at The Society for Neuroscience conference, New Orleans, LA.

Watterson LR, Nemirovsky N, Kufahl P, Cleva RM, and Olive MF. (2011). The effects of the metabotropic glutamate 5 receptor antagonist fenobam on reinstatement of methamphetamine-seeking behavior in rats. Presented at The Society for Neuroscience conference in Washington, D.C.

Watterson LR. Kufahl PR., Nemirovsky NE, Sewalia K, and Olive MF. (2011). Potent Reinforcing Effects of the Synthetic Cathinone Methylenedioxypyrovalerone (MDPV) in Rats. Presented at the American College of Neuropsychopharmacology conference in Waikoloa, HI.

Collaborator

Kufahl PR, Taylor SB, **Watterson LR,** Nemirovsky NE, Burrows B, Olive MF. (2014) Self-Administration of both ethanol and methamphetamine increase motivation for methamphetamine. To Be Presented at the Society for Neuroscience conference, Washington, DC.

Kufahl PR, **Watterson LR**, Nemirovsky NM, Taylor SB, Olive MF. (2014) A triple cannula biosensor study of changes in extracellular alcohol, glutamate, and glucose levels during alcohol consumption. Presented at the Monitoring Molecular in Neuroscience conference, UCLA, Los Angeles, CA.

Hoffman AN, Parga A, **Watterson LR,** Paode PR, Nikulina EM, Hammer RP, Conrad CD. (2014) Chronic stress enhanced fear memories are associated with increased amygdala zif268 mRNA expression and are resistant to reconsolidation in an animal model of post-traumatic stress disorder. Presented at The International Behavioral Neuroscience Society conference in Las Vegas, NV.

Mazur GJ, Hoffman AN, Watterson E, **Watterson LR,** and Sanabria F. (2014) Acute and Chronic Nicotine Decreases Response Inhibition Performance

and Enhances the Reinforcing Efficacy of Sucrose. Presented at the Association for Behavior Analysis International conference, Chicago, IL.

Kufahl PR, Moore-Nye E, Halstengard P, Barabas P, Tomek SE, Nemirovsky NE, Villa A, Hood LE, Yahn SL, **Watterson LR,** and Olive MF. (2013) Effects of the mGluR5 negative allosteric modulator MTEP on ethanol self-administration and ethanol seeking: Roles of dorsal and ventral medial prefrontal cortex. Presented at the Society for Neuroscience conference, San Diego, CA.

Hoffman AN, Parga A, Lorson NG, Paode PR, **Watterson LR,** Nikulina EM, Hammer Jr RP, and Condrad CD. (2013). Chronic stress-induced enhanced fear memories are resistant to reconsolidation in an animal model of post-traumatic stress disorder. Presented at the Society for Neuroscience conference, San Diego, CA.

Mazur G, Watterson E, **Watterson LR,** and Sanabria, F. (2013). Acute and chronic nicotine increases premature responding and enhances the reinforcing efficacy of sucrose. Presented at the Association for Behavior Analysis International conference, Chicago, IL.

Kufahl PR, **Watterson LR,** Nemirovsky N, Hood LE, Villa A, Zautra N, and Olive MF. (2012). Enhanced sensitivity to attenuation of methamphetamine seeking by the mGluR2/3 agonist LY379268 in rats with a history of extended self-administration. Presented at the College on Problems of Drug Dependence annual meeting, Palm Springs, CA.

Kufahl PR, Hood LE, Villa A, Nemirovsky NE, Halstengard C, Barabas P, Moore E, **Watterson LR,** Olive MF (2012). mGlur5 positive allosteric modulation accelerates extinction learning and relearning following methamphetamine self-administration. Presented at The Society for Neuroscience conference, New Orleans, LA.

Eure R, Deal M, Hawkey S, April LB, **Watterson LR,** Bruce K, and Galizio M. (2011). Effects of number of sample and comparison stimuli on olfactory span. Poster presented at the Association for Behavior Analysis International conference in Denver, CO.

Kufahl PR, **Watterson LR,** Nemirovsky NE, La Crosse AL, and Olive MF. (2011). Rapid but not gradual infusion of methamphetamine produces behavioral sensitization. Presented at The Society for Neuroscience conference in Washington, D.C.

Cleva RM, **Watterson LR,** Wischerath KD, Casterline C, Alonso J, Toledo A, Nemirovsky N, Kufahl PR, and Olive MF. (2011). Enhancement of adult

hippocampal neurogenesis facilitates extinction learning and attenuates cue-induced reinstatement of heroin-seeking behavior. Presented at The Society for Neuroscience conference in Washington, D. C.

Deal M, Poerstel LB, **Watterson LR,** Jacobs K, Goldstein L, and Galizio M. (November 2010). MK-801 (Dizocilpine), but not Chlordiazepoxide, Scopolamine, Methylphenidate, or Morphine selectively affects olfactory memory span in rats. Poster presented at the Society for Neuroscience conference in San Diego, CA.

LeFever TW, Bullard L, **Watterson LR,** Ward A, and Galizio M. (October 2009). Titrating delayed match / non-match-to-sample using olfactory stimuli in rats. Posted presented at the Society for Neuroscience conference in Chicago, IL.

LeFever TW, Ward A, **Watterson LR,** Bullard L, and Galizio M. (October 2009). Effects of alonzapine on fixed and titrating delayed match / non-match-to-sample, using olfactory stimuli in rats. Posted presented at the Southeastern Association for Behavior Analysis conference in Wilmington, NC.

Poerstel LB, Semrau M, Toop E, **Watterson LR**, Deal M, and Galizio M. (October 2009). Dizocilpine (MK-801) and morphine impair olfactory memory span in rats. Posted presented at the Southeastern Association for Behavior Analysis conference in Wilmington, NC.

Overman WH, **Watterson LR**, Ware A, Dressler E, and Gillikin GS. (October 2009). Contemplation of moral dilemmas 24 hours prior to the Iowa Gambling Task enhances performance. Poster presented at the Society for Neuroscience conference in Chicago, IL.

Overman WH, Boettcher L, Hardy C, Walsh K, and **Watterson LR**. (November 2008). Changes in sex differences on the Iowa gambling task are caused by deliberation of dilemmas and presentation of olfactory stimuli. Poster presented at the Society for Neuroscience conference in Washington, D. C.

Herbert J, Traver B, **Watterson LR**, and Hale RL. (March 2005). The orienting response in binge-drinking college students. Poster presented at the Eastern Psychological Association conference in Boston, MA.

HONORS AND AWARDS

2014 Research Travel Grant, ASU, Graduate and Professional Students Association ($950)

2014	Dissertation Completion Fellowship, ASU, Graduate College ($9500)
2014	Robert B. Cialdini Dissertation Project Prize, ASU, Department of Psychology ($850)
2014	Research Travel Grant, ASU, Graduate and Professional Students Association ($500)
2014	Graduate Excellence Award, ASU, College of Liberal Arts and Sciences ($250)
2014	Teaching Excellence Award, ASU, Graduate and Professional Students Association ($750)
2014	International Behavioral Neuroscience Society Travel Award ($700)
2013	Phi Kappa Phi "Love of Learning" Scholarship ($500)
2013	Samuel Leifheit Service Award Nominee, ASU, Psychology Department
2012	Inducted in Phi Kappa Phi (ASU)
2012	Graduate Research Travel Award, ASU, Psychology Department ($200)
2011	Graduate Research Travel Award, ASU, Psychology Department ($200)
2010	Inducted into Phi Kappa Phi (UNCW)
2010	Research Excellence Recruiting Fellowship, ASU, Psychology Department ($10,000)
2009	Research Travel Award, UNCW, Psychology Department ($400)
2008	Research Travel Award, UNCW, Psychology Department ($400)
2006	Graduated *Magna Cum Laude*, Shippensburg University
2006	Research Travel Award, Shippensburg University, Department of Psychology ($500)

2006	Academic Excellence Award, Shippensburg University, Department of Psychology
2004 – 2005	Certificate of Student Research Achievement, Shippensburg University, Department of Psychology
2003 – 2006	Dean's list, Shippensburg University

TEACHING

Teaching Assistant

Spring 2014	325 Physiological Psychology (online) Instructor: Whitney Hanson Arizona State University, Psychology Department
Spring 2013	290 Psychology Research Methods Lab, 24 students Instructor: Dr. Heather Cate Arizona State University, Psychology Department Course evaluation: 1.7 (1 = most positive, 5 = most negative)
Fall 2013	290 Psychology Research Methods Lab, 21 students Instructor: Dr. Eva Szeli Arizona State University, Psychology Department Course evaluations: 1.6 (1 = most positive, 5 = most negative)
Spring 2009	225 Statistics - 20 hours / week Instructor: Dr. Bryan Myers UNCW, Psychology Department
Fall 2008	246 Personality Psychology – 10 hours /week 101 General Psychology – 10 hours / week Instructor: Dr. Len Lecci UNCW, Psychology Department

Guest Lectures

Fall 2013	394 Your Brain on Drugs: Marijuana and Synthetic Cannabinoids
Spring 2014	325 Physiological Psychology (online): Psychopharmacology 325 Physiological Psychology (online lecture): Substance abuse 591 Psychopharmacology: Designer Drugs / "Legal Highs"

Fall 2013	325 Physiological Psychology: Addiction
Spring 2013	591 Psychopharmacology: Targets of Drug Action 591 Psychopharmacology: Substance Abuse and Dependence 591 Psychopharmacology: Psychostimulants 591 Psychopharmacology: Designer Drugs / "Legal Highs"
Fall 2009	556 Physiological Psychology: Executive Functions

MENTORING

Honor's Thesis Graduate Student Mentor:

Trevor Johnson (Defended fall 2012): *Intracranial Self-Stimulation and the Abuse Potential of the Synthetic Cathinones Methylone and α-PVP.*

Megan Johnson (Defended spring 2011): *Intracranial Self-Stimulation and the Abuse Liability of Modafinil, a novel wake-promoting drug.*

Honor's Thesis Graduate Student Mentor and Committee Member:

Stephanie Yahn (Summer 2012): *Methamphetamine Addiction and Adult Neurogenesis: A possible role for novel neuroprotective compounds in the reduction of vulnerability to relapse.*

Scott Wegner (Defended fall 2012): *A Determination of the Hedonic Properties of Synthetic Cathinones 4-MEC and MDPV Through the Use of Intracranial Self-Stimulation.*

Undergraduate Student Research Mentees:

Elizabeth Dressler (M.A. in Experimental Psychology, Boston University, Research Associate at Monterey Technologies, Inc)
Ashley Ware (Currently a Ph.D. student at Texas Children's Hospital)
Lauren Hood (Currently a Ph.D. student at University of Washington)
Stephanie Yahn (Currently a Ph.D. student at University of Miami)
Craig Trevor Johnson (M.A. in Engineering, Arizona State University)
Seven Tomek (Currently a M.A. student at University of North Carolina – Wilmington)
Kaveish Sewalia (Currently a M.A. study at Arizona State University)

Emily Williams, Natali Nemirovsky, Megan Johnson, Evan Armstrong, Lee Benson, Raymundo Hernandez, Joshua Fassett, Spencer Huggitt, Brian Burrows.

POSITIONS HELD

Research Positions

2010 – Present	Graduate Research Assistant – Neurobiology of Addiction laboratory, Dr. Foster Olive Arizona State University
2009 – 2010	Graduate Research Assistant – Behavioral pharmacology and comparative cognition laboratory, Dr. Mark Galizio University of North Carolina - Wilmington
2007 – 2010	Graduate Research Assistant – Decision-making laboratory, Dr. William H Overman, Jr. University of North Carolina - Wilmington
2004-2006	Undergraduate Research Assistant – Alcohol physiology laboratory, Dr. Robert Hale Shippensburg University of Pennsylvania

Applied Clinical Experience

2006- 2007	Position: Vocational Rehabilitation Counselor Supervisor: Linda Mayo, M.S. Occupational Services Incorporated – A non-profit organization specializing in vocational rehabilitation for mental health resource consumers. Duties: Provided one-on-one counseling and guidance for a case-load of 35 mental health resource consumers. Worked within cooperative teams of mental health professionals with the goal of aiding consumers acquire the necessary skills and resources to obtain competitive employment.
2003- 2006	Position: Job Coach Supervisor: Linda Mayo, M. S. Occupational Services Incorporated Duties: Facilitating the acquisition of job skills for consumers of mental health services in order to aid them in obtaining competitive employment. This required one-on-one supervision in the community and employment of behavioral analytic principles.

SERVICE

Community Outreach

2011 – 2013	ASU Homecoming: Psychology Brain Booth

2011 – 2013	Arizona State University Brain Fair for Children

University Service

2013	Invited Speaker: The Path to Graduate School (Psi Chi)
2013 – Present	Grant Reviewer: Graduate and Professional Student Association (GPSA)
2012	Graduate Student Orientation Presenter (GPSA)

Professional Service

Manuscript Peer-Reviewer: *Drug and Alcohol Dependence, Psychopharmacology, Mini Reviews in Medicinal Chemistry, European Journal of Neuropsychopharmacology*

Affiliations

Society for Neuroscience
Association for Behavior Analysis International
Phi Kappa Phi (UNCW, ASU)
Society for Neuroscience; Tempe Chapter
College on Problems of Drug Dependence
International Drug Abuse Research Society
International Behavioral Neuroscience Society
American Society for Pharmacology and Experimental Therapeutics

REFERENCES

1. Dr. M. Foster Olive, Associate Professor
   Arizona State University
   Department of Psychology (Behavioral Neuroscience)
   950 S. McAllister Rd.
   PO Box 871104
   Tempe, AZ 85287-1104.
   (480)727-9557
   Foster.olive@asu.edu

2. Dr. William H. Overman Jr., Professor
   University of North Carolina – Wilmington
   Department of Psychology
   Social & Behavioral Sciences Building, 110H
   601 South College Road, Wilmington, NC 28403
   (910) 962-3379

overmanw@uncw.edu

3. Dr. Mark Galizio, Professor
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4. Dr. Peter Kufahl, Faculty Research Associate
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5. Dr. Sara Taylor
Hendrix College
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