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The Pharmacology of an Agonist Medication to Treat Stimulant Use Disorder

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The Pharmacology of an Agonist Medication to Treat Stimulant Use Disorder

A dissertation submitted in partial fulfillment of the requirements for the degree of
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by

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List of Abbreviations

µg = Microgram

µL = Microliter

µm = Micrometer

5-HT = Serotonin

aCSF = Artificial cerebrospinal fluid

Ag = Silver

ANOVA = Analysis of variance

BE = Benzoylecgnine

Cl = Chloride

cm = Centimeter

DA = Dopamine

DAT = Dopamine transporter

FR = Fixed-ratio

HPLC = High-performance liquid chromatography

hr = Hour

Hz = Hertz

ICSS = Intracranial self-stimulation

inj = Injection

IP = Intraperitoneal

IV = Intravenous

K = Potassium

kDa = Kilodalton

kg = Kilogram

MCR = Maximum control rate

MDPV = Methylenedioxypyrovalerone

Mg = Magnesium

mg = Milligram

min = Minute

M = Molar

ml = Milliliter

mM = Millimolar

mm = Millimeter

msec = millisecond

mV = Millivolt

Na = Sodium

NAc = Nucleus Accumbens

NE = Norepinephrine

NET = Norepinephrine transporter

ng = Nanogram

pg = Picogram

PR = Progressive-ratio

RMTg = Rostromedial tegmental nucleus

SAMSHA = Substance Abuse and Mental Health Administration

sec = Second

SEM = Standard error of the mean

SERT = Serotonin transporter

TO = Time out

VTA = Ventral tegmental area

W = Watt

ABSTRACT

THE PHARMACOLOGY OF AN AGONIST MEDICATION TO TREAT STIMULANT USE DISORDER

Amy R. Johnson, Bachelor of Science

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

Virginia Commonwealth University, 2017

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Cocaine use disorder is a serious public health issue for which no approved pharmacotherapies exist. The development of a pharmacotherapy for cocaine use disorder is a priority for the National Institute on Drug Abuse. Amphetamine maintenance has been shown to be effective to reduce cocaine use in double-blind placebo controlled clinical trials, but has not been approved due to concerns over safety and abuse liability. Development of new pharmacotherapies is facilitated by preclinical testing for effectiveness and identification of new targets for medication development. The first part of this dissertation develops a novel non-human primate cocaine self-administration choice procedure that is modeled after a human laboratory cocaine self-administration choice procedure to improve translational research and facilitate medication development. The second part of this dissertation is devoted to examining the mechanisms of amphetamine maintenance-induced decreases in cocaine use. In the novel non-human primate choice procedure, monkeys chose between injections of

cocaine or food pellets (0, 1, 3 or 10) in a 9-choice discrete trials procedure. The reinforcers were available on concurrent independent progressive-ratio schedules. Monkeys chose between cocaine and food in a dose- and magnitude-dependent manner. Maintenance on 7 days of lisdexamfetamine and amphetamine decreased cocaine choices without decreasing food responding, providing evidence that this model may be able to predict drugs that will have clinical efficacy to decrease cocaine use. The next set of experiments examined the effects of amphetamine maintenance on the abuse-related behavioral (intracranial self-stimulation, ICSS) and neurochemical [nucleus accumbens dopamine (DA) and serotonin (5-HT)] effects of cocaine, methylenedioxypyrovalerone, and methamphetamine in rats. Amphetamine maintenance produced sustained increases in ICSS baseline responding and nucleus accumbens DA levels without affecting 5-HT levels. Amphetamine maintenance also attenuated the behavioral and neurochemical abuse-related effects of cocaine but not those of methamphetamine, and with MDPV, amphetamine maintenance decreased the abuse-related neurochemical effect of MDPV, but not the abuse-related behavioral effect. This suggests that amphetamine would likely be most effective against cocaine, least effective against methamphetamine and between the two for MDPV. These data suggest targets that selectively release DA will be the most effective against cocaine use disorder.

Chapter I

Introduction

Pharmacology of Monoamine Transporter Ligands

Stimulants are a diverse class of drugs that typically increase locomotion, attention, wakefulness, heart rate, and blood pressure (Kirkpatrick *et al*, 2012; Rush *et al*, 2009). Most drugs in this class share a common mechanism of action; they interact with monoamine transporters to increase levels of the neurotransmitters dopamine (DA), serotonin (5-HT), and norepinephrine (NE). DA, 5-HT and NE are modulatory neurotransmitters with wide-reaching networks of neurons throughout the brain. The DA system includes 4 major pathways: the mesocortical (ventral tegmental area to the cortex), mesolimbic (ventral tegmental area to the nucleus accumbens), nigrostriatal (substantia nigra to the striatum), and the tuberoinfundibular (arcuate nucleus of the hypothalamus to the pituitary gland); these pathways regulate movement, executive function, learning, and addiction (Iversen and Iversen, 2007). Serotonergic pathways in the brain start in the raphe nuclei and project to almost all areas of the brain, including the cortex, hippocampus, amygdala, and ventral tegmental area (Beliveau *et al*, 2017; Charnay and Leger, 2010). This widespread network of neurons regulates appetite, feeding behavior, mood, and sleep (Jenkins *et al*, 2016; Yadav *et al*, 2009). There are several small nuclei containing cell bodies of noradrenergic neurons in the brain, and these nuclei send projections throughout the brain and spinal cord (Bruinstroop *et al*,

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2013; Rinaman, 2011; Sara and Bouret, 2012). Norepinephrine is important in regulating attention, arousal, and memory (Berridge *et al*, 2013).

These neurotransmitters are packaged into vesicles and released from neuron terminals in response to an action potential. Once the transmitters are released into the synapse, monoamine transporters are the primary mechanism for clearing monoamines from the extracellular space. Disrupting the function of the transporters greatly prolongs the amount of time the neurotransmitters spend in the synapse (Giros and Caron, 1993; Gowrishankar *et al*, 2014). The monoamine transporters consist of 12 membrane-spanning domains and have phosphorylation sites on the c-terminus and n-terminus (both located on the intracellular side of the membrane) as well as glycosylation sites on extracellular loop 2 (McHugh and Buckley, 2015). These transporters use the electrochemical gradient maintained by the Na^+/K^+ pump to transfer a molecule of neurotransmitter along with 2 Na^+ and 1 Cl^- (for DA) or 1 Na^+ and 1 Cl^- (for NE and 5-HT) from the extracellular space to the cytoplasm of the cell (Giros and Caron, 1993; Rudnick, 1977).

Some stimulants bind to the transporter and prevent it from functioning properly, allowing the neurotransmitters to accumulate in the extracellular space and to continue to activate pre- and post-synaptic receptors. Evidence for this comes from synaptosome preparations where these drugs block uptake of radiolabeled ligands into the synaptosomes (Boja and Kuhar, 1989; Giros and Caron, 1993; Rothman *et al*, 2001) and from microdialysis studies showing increased extracellular DA and 5-HT after administration of cocaine (Andrews and Lucki, 2001). These drugs are referred to as uptake inhibitors; cocaine and methylenedioxypirovalerone (MDPV) are examples of

drugs that work in this way (Rothman *et al*, 2001; Schindler *et al*, 2016). Other drugs in this class act as substrates at the transporter and are shuttled into the cell through the transporter (Fleckenstein *et al*, 2007). These drugs then cause release of calcium from internal stores (Goodwin *et al*, 2009), bring depolarizing currents into the cell that may cause activation of voltage-gated calcium channels (Cameron *et al*, 2015), interact with vesicular monoamine transporter 2, collapse the pH gradient of the vesicles, and prevent loading of monoamines into the vesicles, causing the neurotransmitters to accumulate in the cytoplasm of the cell (Fleckenstein *et al*, 2007). This results in efflux of the neurotransmitter into the synaptic space through the transporter (Kahlig *et al*, 2005). Drugs that work in this way are called releasers; amphetamine and methamphetamine fall into this category of stimulants.

Stimulants also vary in their selectivity at the DA, 5-HT, and NE transporters (DAT, SERT, NET, respectively). Some drugs are relatively nonselective and will interact with all 3 transporters at relatively equal doses; cocaine and metheylenedioxymethamphetamine are examples of relatively nonselective drugs (Rothman *et al*, 2001). Others may have a preference for one or two transporters, such as MDPV (which is a highly selective DAT and NET inhibitor) and amphetamine (which is a selective substrate at DAT and NET > SERT) (Rothman *et al*, 2001; Schindler *et al*, 2016). Still other drugs can have mixed action between different transporters. For example, N-ethyl 4-methylamphetamine is an uptake inhibitor at DAT, but a releaser at NET and SERT (Solis *et al*, 2017).

The abuse liability of monoamine uptake inhibitors and releasers has been studied extensively and has been found to be correlated with the selectivity of the

compound for DAT versus SERT. One of the first experiments to observe this effect studied a series of cocaine analogs with different DAT versus SERT selectivity in drug self-administration under a progressive-ratio schedule of reinforcement in rats (Roberts *et al*, 1999). The rats were trained to self-administer cocaine under the progressive-ratio schedule, and then the novel compounds were substituted for the cocaine. Compounds selective for SERT did not support break points different from saline substitution, but drugs selective for DAT supported break points similar to or greater than cocaine. Another series of experiments examined the effects of amphetamine analogs with similar potencies at DAT but with varying potencies at SERT in drug self-administration under fixed-ratio and progressive-ratio schedules of reinforcement in non-human primates (Wee *et al*, 2005). In this study, all compounds were self-administered, but the drug that was the most selective at SERT was self-administered at lower rates than the other compounds and supported lower break points than the other more DAT-selective compounds. A later study found that another more SERT-selective amphetamine analog increased both DA and 5-HT in rats and did not function as a reinforcer in drug self-administration under a fixed-ratio schedule of reinforcement in non-human primates (Rothman and Baumann, 2006). Similarly, in 2 intracranial self-stimulation studies, compounds that were more potent at DAT than SERT produced greater abuse-related effects than compounds that were more potent at SERT rather than DAT (Bauer *et al*, 2013; Suyama *et al*, 2016).

Stimulants like cocaine (relatively equal potency to increase DA and 5-HT; Rothman *et al*, 2001), methamphetamine (~40 times more potent to increase DA than 5-HT; Rothman *et al*, 2001), and MDPV (at least 100 times more potent to increase DA

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versus 5-HT; Baumann *et al*, 2013) have been shown to have abuse liability in the human population. According to the National Survey on Drug Use and Health, in the United States in 2016, approximately 1.9 million people were current users of cocaine (had used in the past month), and 889,000 reported a cocaine use disorder within the past year (SAMSHA, 2017). In the same survey, methamphetamine current use was somewhat lower than cocaine at 664,000 past-month users, but past-year methamphetamine use disorders came in at 684,000 people, providing evidence that methamphetamine use is also problematic (SAMSHA, 2017). MDPV is a synthetic cathinone known as a "bath salt"; the number of MDPV users is not well-established (SAMSHA does not ask specifically about MDPV or other bath salts), but there is evidence from emergency department visits, deaths, and online sites where users record their experiences that MDPV is used and abused by humans (Center for Disease Control, 2011; Karila *et al*, 2017; Wright *et al*, 2013).

Overview of Treatments for Stimulant Use Disorders

Currently, there is demand for a treatment for abuse of and addiction to stimulant drugs. A Cochrane review found cognitive behavioral therapy and contingency management to be better than no treatment on some end points such as treatment retention and continuous abstinence, but only contingency management was able to retain the abstinence effect at follow up (Minozzi *et al*, 2016). Another intervention strategy that has been tried is physical exercise. Studies have seen reduced cocaine use, reduced methamphetamine use, and changes in DA-receptor availability in methamphetamine users after exercise. However, one recently completed study found

no difference in percent of days abstinent from stimulants between an exercise and a control group, even though both groups achieved high abstinence rates (Trivedi *et al*, 2017). Despite the clinical efficacy of these treatment methods, relapse rates are still high, and there is need for better treatments or pharmacotherapies that can be used in conjunction with psychotherapy or other interventions to increase treatment success and decrease relapse.

The National Institute on Drug Abuse has made finding a pharmacotherapy for cocaine abuse a priority for over 25 years. Hundreds of clinical trials have been funded to investigate pharmacotherapies for cocaine or methamphetamine use disorder. However, there is not currently a pharmacotherapy approved for cocaine or methamphetamine use disorder by the Food and Drug Administration.

Pharmacotherapies can be sorted into 2 general categories: antagonist- or agonist-based. Antagonist pharmacotherapies oppose the effects of the drug of abuse or prevent the drug from reaching its target. For stimulants, the antagonist therapies would include DA receptor antagonists, drugs that decrease levels of DA, drugs that increase the metabolism of DA, or vaccines that prevent the abused stimulants from crossing the blood-brain barrier. The direct DA-receptor antagonists (e.g. haloperidol, chlorpromazine) have been tried and have failed in clinical trials for cocaine use disorder; compliance and dropout rate are often major issues with these drugs because of the undesirable side effects that they cause (Grabowski *et al*, 2000; Kishi *et al*, 2013). This type of therapy has received new life with the current interest in studying 5-HT2c receptor agonists and 5-HT2A receptor antagonists with the goal of reducing DA neuron firing and thereby decreasing DA release in the nucleus accumbens (NAc) (Howell and

Cunningham, 2015). To date, these therapies have shown promise in some pre-clinical models of drug addiction (Gerak *et al*, 2016; Harvey-lewis *et al*, 2016), and are currently in clinical trials for cocaine and methamphetamine use disorders. However, they did not reduce cocaine or methamphetamine choice in non-human primates (Banks, 2016; Banks and Negus, 2016).

Agonist pharmacotherapies are drugs that produce pharmacodynamic effects similar to those of the drug of abuse and that may substitute for or prevent withdrawal from the abused drug. One drawback to agonist therapies is they often have abuse potential because they mimic the effects of the drug of abuse. To combat these effects, an ideal agonist pharmacotherapy would have a slow onset of effects and long duration of action, two pharmacokinetic factors that will decrease abuse liability of the pharmacotherapy (Negus and Henningfield, 2015; Rush and Stoops, 2012). For abused stimulants, agonist pharmacotherapies have focused on DA, 5-HT, and to some extent NE. In general, this type of therapy has been largely ineffective in clinical trials, with only amphetamine and bupropion being identified as drugs that have potential to decrease cocaine-taking by a Cochrane review (Castelles *et al*, 2016). Other potential agonist pharmacotherapies, such as methylphenidate (DA and NE), fluoxetine (and other selective serotonin reuptake inhibitors), and tricyclic antidepressants (which increase 5-HT and NE) have not yielded consistent results in clinical trials. Amphetamine maintenance has been tested against cocaine use disorder in double-blind placebo controlled clinical trials and has consistently performed better than placebo.

Amphetamine Maintenance for Cocaine Use Disorder

Clinical Trials. Amphetamine was first tested against cocaine use disorder in a double-blind placebo-controlled clinical trial (Grabowski *et al*, 2001). In this study, subjects meeting the DSMIV criteria for cocaine dependence were administered oral d-amphetamine over a 3-month period. There were 3 groups of subjects in this study: a placebo group, a low-dose d-amphetamine group who received 15 mg/day for the first month and then 30 mg/day for the final 2 months, and a high-dose d-amphetamine group who received 30 mg/day in the first month and 60 mg/day in the final 2 months. The main measure of efficacy in this study was the proportion of urine samples positive for benzoylecgnine (BE, a cocaine metabolite). In month 3, significant differences were noted between the placebo and high-dose groups, with the high-dose group providing lower proportion of BE-positive urines. However, these findings must be interpreted with caution, however, as individuals in the high dose-group succumbed to high rates of attrition during the study.

After the initial clinical trial that suggested d-amphetamine maintenance may have some clinical utility in treating cocaine use disorder, several additional trials have been conducted to evaluate the effects of amphetamine in combination with other drugs. d-Amphetamine was again studied in combination with methadone in a group of dual cocaine and heroin users (Grabowski *et al*, 2004a). d-Amphetamine doses were the same as in the previous study (placebo, low dose 15-30mg/day, and high dose 30-60mg/day); however, all groups received the same dose of methadone treatment in addition to the d-amphetamine or placebo treatments. Similar to the previous study, the high-dose d-amphetamine group had a decreased proportion of BE-positive urine samples as compared to placebo in months 2 – 4 of the study. Another combination that

was tried was d-amphetamine with the low-potency DA uptake inhibitor modafinil (Schmitz *et al*, 2012). Neither modafinil alone (400 mg) nor the combination of d-amphetamine (30 mg) plus modafinil (200 mg) was effective at reducing cocaine use, but the amphetamine-alone (60 mg) and placebo groups saw small reductions in the proportion of BE-positive urines. In line with the previous studies, the amphetamine-alone group had a lower proportion of BE-positive urines as compared to the placebo group for the first few months, however, by the end of the trial period these differences were no longer present. Another combination study was done with extended-release mixed amphetamine salts and the anticonvulsant topiramate compared to placebo, but the drugs were not tested separately (Mariani *et al*, 2012). The combination doses of amphetamine and topiramate were determined individually for each subject by gradually increasing the dose up to a daily maximum of 60 mg amphetamine and 300 mg topiramate. Reductions in dose were made for any intolerable adverse side effects. The group getting amphetamine and topiramate was more likely to achieve 3 consecutive weeks of abstinence, defined as BE-negative urine for 3 weeks in addition to self-reports of no cocaine use during that 3 week period, as compared to the group getting placebo.

Two other recent clinical trials have examined the effects of amphetamine alone as a treatment for cocaine use disorder. One used a sub-population of cocaine users with attention-deficit/hyperactivity disorder (ADHD). As several effective ADHD medications are indirect DA agonists like cocaine, it is conceivable that cocaine users who have ADHD are using cocaine to self-medicate. If this is true, administration of an FDA-approved ADHD medication should decrease ADHD symptoms and decrease

cocaine use in this population. Consistent with that hypothesis, there was improvement in ADHD symptoms as well as reductions in cocaine use (assessed by urine BE levels and self-report) in the groups treated with extended-release mixed amphetamine salts (60 or 80 mg) as compared to groups getting placebo treatment (Levin *et al*, 2015). The other clinical trial was conducted as a multi-site trial in the Netherlands in a heroin-dependent population who also met criteria for cocaine dependence and had made at least two attempts to quit cocaine use previously (Nuijten *et al*, 2016). The participants were maintained on oral methadone (up to 150 mg) and allowed supervised use of pharmaceutical-grade diacetylmorphine (up to 1000 mg). Extended release d-amphetamine (60 mg) was superior to placebo in decreasing number of days of cocaine use, increasing average number of consecutive abstinence days (both assessed by self-report), and decreasing proportion of BE-positive urines in the final 4 weeks of the study.

Meta-analyses of clinical trials have also concluded that amphetamine treatment may have some utility for treatment of cocaine dependence (Castelles *et al*, 2016). Even though treatment effects are generally small, about 30% of amphetamine-treated participants are able to achieve 3 weeks continuous abstinence from cocaine as compared to 6%-13% of patients getting placebo (Mariani *et al*, 2012; Nuijten *et al*, 2016). This treatment effect is comparable to that of the most effective pharmacotherapies approved to treat abuse of substances in other drug classes. For example, in one clinical trial of treatments for opioid addiction, about 30% of patients treated with a high dose of methadone were able to achieve 4 weeks of continuous abstinence as compared to 8% of patients who got a low dose of methadone (Johnson

et al, 2000). Similarly, in a clinical trial for nicotine dependence, 26-38% of patients achieved 12 weeks continuous abstinence when treated with nicotine patches or varenicline as compared to 13% of patients who received placebo (Anthenelli *et al*, 2016). Taken together, these data suggest that, as measured by abstinence from the drug of abuse, amphetamine maintenance appears to be as effective to treat cocaine use disorder as other approved pharmacotherapies are to treat other substance use disorders. This is in contrast to other potential pharmacotherapies for cocaine abuse including antidepressants, anticonvulsants, and antipsychotics, which have not demonstrated efficacy to reduce cocaine use in double-blind placebo-controlled clinical trials (for reviews see: Indave *et al*, 2016; Minozzi *et al*, 2011; Pani *et al*, 2011).

Human Laboratory Studies. The effect of amphetamine maintenance on cocaine-taking behavior in human laboratory experiments has been examined a few times. In one study, subjects were maintained on placebo and 40 mg/day d-amphetamine (treatment condition order was counterbalanced across subjects) for 7 days and given a choice between 4 mg cocaine (placebo) and 4, 10, 20, or 30 mg intranasal cocaine (Rush *et al*, 2010). All drugs were administered under double-blind conditions. Amphetamine maintenance attenuated choice of the 20 mg dose of cocaine as compared to the choice under placebo treatment. Amphetamine maintenance has also been studied on the effects of cocaine + hydromorphone (speedball) administration in the human laboratory (Greenwald *et al*, 2010). Subjects were maintained on 8 mg/day buprenorphine as well as 0, 30, and 60 mg/day sustained release d-amphetamine presented in ascending dose order under double-blind conditions. Amphetamine maintenance was found to decrease break points for cocaine (8 mg

intranasal) but not for hydromorphone (2 mg intramuscular) alone or cocaine + hydromorphone (speedball).

The results from these human laboratory experiments are consistent with the outcomes of clinical trials. In both types of experiments, amphetamine maintenance decreased cocaine self-administration. This adds to the data collected from clinical trials in which researchers are collecting data on measures related to cocaine self-administration (e.g. urine BE levels), but are not measuring the cocaine-taking behavior directly.

Preclinical. The preclinical work on the effect of amphetamine maintenance on the effects of cocaine-taking behavior started with a cross-tolerance experiment in which the researchers gave twice daily injections of amphetamine and looked at effects on cocaine discrimination as well as self-administration (Peltier *et al*, 1996). The highest dose of amphetamine maintenance (3.2 mg/kg/injection) produced cross-tolerance to the discriminative stimulus properties of cocaine, decreased cocaine break points, and produced an approximately 3-fold rightward shift in the FR-based cocaine self-administration dose-effect curve. The next preclinical study did not occur until after the 2001 clinical trial showing some potential clinical utility for amphetamine. A series of studies showed that amphetamine maintenance decreased response rates, injections, and breakpoints for cocaine self-administration in nonhuman primates (Negus and Mello, 2003a, 2003b). They also showed that this effect was relatively selective for cocaine- versus food-maintained responding and that amphetamine effects on food-maintained behavior were less consistent and smaller in magnitude than the effects of amphetamine on cocaine-maintained behavior. In a parallel study, a choice procedure

was used to determine the effects of amphetamine maintenance on cocaine preference versus an alternative food reinforcer in non-human primates (Negus, 2003). Amphetamine maintenance decreased cocaine preference as compared to food in this choice procedure. This study showed that amphetamine maintenance was able to decrease responding for cocaine and at the same time reallocate behavior toward an alternative food reinforcer, further suggesting that the rate-decreasing effects of amphetamine maintenance are selective for cocaine and not due to non-selective amphetamine effects on motor behavior.

A rodent cocaine self-administration experiment provided additional information about the effects of amphetamine on cocaine-taking behavior. This study replicated the previous findings that amphetamine maintenance decreases cocaine-taking behavior; however, the effect was dependent on cocaine dose (Chiodo et al, 2008). Self-administration of low doses of cocaine was attenuated by amphetamine maintenance, but self-administration of high cocaine doses (0.75 and 1.5 mg/kg/inj) were unaffected. Additionally, cocaine had to be actively self-administered during amphetamine maintenance in order for the cocaine-decreasing effect to be expressed. Follow up studies in nonhuman primates (Czoty et al, 2010) and in rodents (Zimmer et al, 2014) underscored the importance of active cocaine self-administration while amphetamine is in the system to be able to detect the effect of amphetamine maintenance on cocaine-taking behavior.

Other studies in nonhuman primates and in rodents have also identified cocaine dose as a variable that is important in determining the effects of amphetamine on cocaine-taking behavior. Larger doses of cocaine are resistant to reductions in choice

and rate of responding by amphetamine maintenance. This effect may be somewhat species-dependent, as rodents saw no benefit from increased length of amphetamine exposure or of a higher amphetamine dose in decreasing high-dose cocaine self-administration (Chiodo and Roberts, 2009). Consistent with those findings, a rodent choice procedure showed that neither 0.1 nor 0.32 mg/kg/hr amphetamine showed the ability to decrease the preference for the highest cocaine dose, even though preference for lower cocaine doses was decreased (Thomsen *et al*, 2013). On the other hand, non-human primates showed decreased cocaine preference at the high cocaine dose after 14, but not 7, days of 0.1 mg/kg/hr amphetamine maintenance (Banks *et al*, 2013). Additionally, a study showed that cocaine-taking behavior could be decreased in all monkeys in the experiment, although a higher cocaine self-administration dose (0.1 vs. 0.03 mg/kg/inj) in one monkey took a higher amphetamine dose (0.056 vs. 0.01 – 0.03 mg/kg/inj) to decrease the cocaine-taking behavior(Czoty *et al*, 2011).

Amphetamine Maintenance for Methamphetamine Use Disorder

Amphetamine maintenance has been less successful to treat methamphetamine use disorder than cocaine use disorder. Early retrospective studies examined the effects of oral dexamphetamine for amphetamine (including methamphetamine) use and saw up to 70% of users were able to stop taking street drugs (Charnaud and Griffiths, 1998; White, 2000). However, those studies were not placebo-controlled, and a later open-label placebo-controlled study found that both placebo and amphetamine groups decreased amphetamine use with no between-group differences in retention or amphetamine use (Shearer *et al*, 2001). A double-blind placebo-controlled clinical trial

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found greater retention in the amphetamine group, and both groups decreased methamphetamine use over the course of the trial; however, there were no between-group differences in methamphetamine use by self-report or by hair-sample analysis (Longo *et al*, 2010). Another double-blind placebo controlled clinical trial found no difference in methamphetamine-positive urines between the placebo group and the amphetamine group (Galloway *et al*, 2011); however, user-rated withdrawal severity and methamphetamine cravings were lower in the amphetamine group.

Amphetamine maintenance did not decrease methamphetamine self-administration in a human laboratory self-administration experiment, but did decrease cardiovascular effects of methamphetamine as well as subject-rated subjective effects of methamphetamine (Pike *et al*, 2014). In a non-human primate methamphetamine-versus-food choice procedure, 7 days of amphetamine maintenance was not effective to decrease methamphetamine choice in group data; however, in 2 of the 4 monkeys methamphetamine choice was completely eliminated while methamphetamine choice was increased in the other 2 monkeys (Schwienteck and Banks, 2015). This demonstrates the individual variability of amphetamine effects on methamphetamine-taking behavior and matches the effects seen in the clinical trials and self-administration studies.

Limitations to Amphetamine Maintenance for Stimulant Use Disorders

Taken together, the body of literature suggests clinical utility for amphetamine maintenance in treating cocaine use disorder, but not methamphetamine use disorder. Most clinical trials show a benefit of amphetamine maintenance over placebo on

measures such as abstinence from cocaine and proportion of cocaine-positive urines. Preclinical and human laboratory data support these findings and show that amphetamine maintenance decreases rates of cocaine self-administration, break points in progressive ratio procedures, and cocaine preference in choice procedures.

However, there are limitations to the efficacy of amphetamine. Clinical trials show a greater decrease in cocaine use in amphetamine-maintained patients as compared to placebo, but only approximately 30% of amphetamine-maintained subjects remain abstinent from cocaine for 3 continuous weeks (Mariani *et al*, 2012). The clinical and pre-clinical data reviewed above suggest that amphetamine dose, active cocaine self-administration while amphetamine is on board, length of treatment, and cocaine dose are relevant factors in how amphetamine will work against cocaine use disorder.

In addition to its limited effectiveness for treatment of cocaine use disorder, amphetamine maintenance has not demonstrated success in decreasing methamphetamine use. This limitation shows that the therapeutic effects of amphetamine maintenance in cocaine use disorder do not generalize to all other stimulants. With the relatively recent emergence of new abused stimulants such as synthetic cathinones (De Felice *et al*, 2014; Karila *et al*, 2017; Schindler *et al*, 2016), it is important to know whether amphetamine maintenance may be successful against use disorders involving stimulants other than cocaine and what factors may predict whether amphetamine maintenance will be effective against a substance use disorder. Understanding the mechanisms responsible for reductions in cocaine use associated with amphetamine maintenance may also lead to development of new therapeutic targets for potential pharmacotherapies.

Dissertation Goals

In view of the promising but limited effectiveness of amphetamine maintenance to treat cocaine use disorder, the present dissertation project had two goals. First, the discovery of improved pharmacotherapies will rely in part on preclinical-to-clinical translational studies of treatment effectiveness in assays of abuse-related cocaine effects. A working hypothesis by our research group has been that this translational research effort would benefit from development of drug self-administration models that are highly homologous in animal and human subjects and thereby minimize the potential for procedural variables to confound preclinical-to-clinical translation of results. Funding was acquired under an R01 grant to test this hypothesis, and my role was to develop a drug self-administration procedure in rhesus monkeys homologous to a similar procedure being developed by colleagues at the University of Kentucky for use in humans. The development of this procedure and evaluation of its sensitivity to maintenance on amphetamine and the amphetamine prodrug lisdexamfetamine are described in Chapters 2 and 3. A second goal of this dissertation was to examine potential mechanisms of selective amphetamine effectiveness as a maintenance medication for treatment of addiction to cocaine but not methamphetamine. These studies were conducted using parallel behavioral and neurochemical procedures in rats, and results and implications of these studies are described in Chapter IV of the dissertation. The dissertation concludes with a discussion of overall implications and potential future directions.

Translational Methods Development. Mechanistic studies are often performed using pre-clinical research with the ultimate goal of finding a new target for future

development of candidate pharmacotherapies. Clinical trials are expensive, and the medications tested must be approved for human use or be extensively evaluated preclinically to determine safe doses for testing in humans. Subsequently, pre-clinical models are practical alternatives for use in testing mechanisms of amphetamine maintenance-induced decreases in drug-taking behavior.

However, the pre-clinical model needs to be predictive of clinical efficacy of the potential pharmacotherapy. This presents a challenge for stimulants because there are no approved pharmacotherapies that can be used to validate pre-clinical models. Nonetheless, the efficacy of amphetamine maintenance to decrease cocaine use in double-blind placebo-controlled clinical trials suggests that amphetamine maintenance can be used as a positive control in preclinical studies, whereas treatments that have failed in the clinic can be used as negative controls. One pre-clinical model that has consistently displayed sensitivity to amphetamine maintenance effects on cocaine use has been self-administration using choice procedures. In choice procedures, subjects have simultaneous access to both a drug of interest (e.g. cocaine) and a non-drug alternative reinforcer (e.g. food), and data are collected both on the allocation of behavior between the drug and non-drug reinforcers and on the overall rate of behavior emitted for both reinforcers (Banks *et al*, 2015b; Banks and Negus, 2012). In these procedures, the optimal outcome of a candidate pharmacotherapy is a reallocation of behavior away from drug choice and toward choice of the alternative without a change in the overall rates of behavior. Conversely, poor outcomes in choice procedures include increases in cocaine choice or no change in cocaine choice up to doses that produce decreases in overall rates of responding. This effectiveness of choice in

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identifying potentially efficacious pharmacotherapies carries over to the human laboratory, where choice studies (usually drug versus money) have been implemented to study the effects of potential pharmacotherapies. Drugs that have been effective to decrease preference of the drug versus the alternative reinforcer in the lab are also typically effective at reducing drug use in the clinic (Czoty *et al*, 2016).

In the pharmacotherapy development process, if a drug has shown effectiveness in the pre-clinical choice model, it would then be warranted to test the drug in a human laboratory choice setting to learn more about its effectiveness in people and its potential side effects before proceeding to a clinical trial. Chapter II & III of this dissertation will deal with development of a novel cocaine-versus-food choice procedure in non-human primates based on a choice procedure from the human laboratory. This procedure has the advantage of being modeled after the human laboratory choice studies and so may minimize any impact of differences in procedural variables in predicting what will work in the human laboratory. This procedure was validated with amphetamine maintenance and tested with lisdexamfetamine, a clinically available amphetamine prodrug that is also under consideration as an agonist pharmacotherapy for cocaine use disorder. At the same time, a collaborator developed the homologous choice procedure in human subjects and conducted parallel studies with amphetamine maintenance.

Mechanisms of Amphetamine Maintenance Effects. Amphetamine is often thought of as an agonist-type medication for stimulant use based on its neurochemical, behavioral, and discriminative stimulus effects (Herin *et al*, 2010; Negus and Henningfield, 2015; Rush and Stoops, 2012). However, cocaine interacts with the monoamine transporters differently than amphetamine and methamphetamine. The end

result of all three drugs is accumulation of extracellular DA, NE, and 5-HT, but there are

other end points on which these drugs can oppose each other. Cocaine can block

effects of amphetamine in vitro (presumably blocking access to the transporter binding

site for amphetamine (Kahlig *et al*, 2005); amphetamine brings a depolarizing current

through the transporter in oocyte preparations, conversely, cocaine causes a

hyperpolarizing current (Cameron *et al*, 2015). Uptake inhibitors and releasers also tend

to have opposite effects on monoamine transporter expression, with uptake inhibitors

increasing expression of the transporters and releasers decreasing expression of the

transporters (Kahlig and Galli, 2003; Kittler *et al*, 2010). This evidence suggests that the

difference in the interaction at the transporter between cocaine and methamphetamine

may be a potential factor in whether amphetamine maintenance will be effective in

reducing a drug's abuse liability.

Another factor that may play a role in the differential effect of amphetamine

maintenance on use of cocaine versus methamphetamine is the DAT versus SERT

selectivity profile of the two drugs. Cocaine is relatively non-selective between DAT and

SERT, whereas methamphetamine is more DAT-selective. As much evidence has

supported the selectivity of compounds for DAT versus 5-HT as a key factor in abuse

liability, it could be that the DA-selective profile of methamphetamine is harder to treat.

The DAT-selective uptake inhibitor, MDPV, provides a way to test between these two

possibilities. If amphetamine maintenance decreases abuse-related effects of MDPV, it

is more likely that amphetamine maintenance will work against abuse of other

monoamine uptake inhibitors. If amphetamine maintenance does not decrease the

abuse-related effects of MDPV, the selectivity profile is potentially an explanation. There

is no clinical or pre-clinical data on the effect of amphetamine maintenance on MDPV, so these effects remain to be seen.

Intracranial self-stimulation (ICSS) is a pre-clinical procedure that can be used to evaluate the abuse-related effects of drugs in rats (Negus and Miller, 2014). An electrode is implanted into the medial forebrain bundle, and the rats are allowed to press a lever to receive electrical stimulation of that brain area. The frequency of the electrical brain stimulation can be manipulated through the behavioral session to provide a dynamic range of low- to high-rate behavior to study. When drugs of abuse such as cocaine, methamphetamine, or MDPV are given prior to an ICSS session, a leftward shift of the frequency-rate curve is produced, and this is indicative of an abuse-related effect. One previous study has found that implanting rats with osmotic minipumps filled with either 0.32 mg/kg/hr amphetamine shifted the ICSS frequency-rate curve to the left for the duration of the treatment and attenuated the abuse-related effects of a high cocaine dose (10 mg/kg) on ICSS (Bauer *et al*, 2013), which aligns with the other clinical and pre-clinical data on amphetamine maintenance for cocaine. Chapter IV of this dissertation will expand upon those data in several ways. First, a wider range of cocaine doses was tested during maintenance on a range of amphetamine doses. Second, the abuse-related effects of a range of methamphetamine and MDPV doses were also tested during amphetamine maintenance. Effects of methamphetamine and MDPV have not been tested previously during amphetamine maintenance in this procedure, and testing these drugs will give valuable information on the validity of ICSS as a model for pharmacotherapy development. If amphetamine attenuates the abuse-related effect of methamphetamine, this may not be a good

predictive model of clinical effects. Alternatively, if amphetamine fails to attenuate the abuse-related effects of methamphetamine ICSS may be a good predictive model, and the results from MDPV tests will provide some answers about the mechanism of amphetamine effects. If the amphetamine effect on the abuse-related effects of MDPV looks more like the effect on cocaine, the interaction at the transporter is likely a key factor in determining amphetamine effects on these drugs. On the other hand, if the amphetamine effect on the abuse-related effects of MDPV looks more like the effect on methamphetamine, the DAT versus SERT selectivity is probably an important factor in amphetamine effects on stimulants.

An increase in NAc DA levels is a neurochemical abuse-related effect of drugs that can be measured using rats. Cocaine, methamphetamine and MDPV all increase NAc DA levels (Andrews and Lucki, 2001; Baumann *et al*, 2012; Schindler *et al*, 2016) Since the selectivity ratio of a drug to interact with DAT versus SERT is important in modulating the abuse-related effect (Bauer *et al*, 2013; Bonano *et al*, 2014), a 2nd experiment in Chapter IV presents the effects of amphetamine maintenance on modulation of DA and 5-HT levels by cocaine, methamphetamine, and MDPV. These experiments continue to probe the mechanism of amphetamine effects on cocaine and other stimulants, with the hypothesis that amphetamine maintenance would attenuate the cocaine- and MDPV-mediated DA increases, but will not affect the methamphetamine increases. Additionally, because amphetamine itself is relatively selective as a substrate at DAT vs. SERT, we also hypothesized that amphetamine maintenance would not alter 5HT increases produced by cocaine or methamphetamine.

Lastly, the effects of amphetamine maintenance on striatal dopamine transporter (DAT) binding were assessed. There is evidence that large doses of releasers or chronic treatment with releasers may decrease DAT binding (Fleckenstein *et al*, 2007). This may be indicative of a neurotoxic effect, so it is important to know whether regimens of amphetamine maintenance that decrease cocaine choice and attenuate abuse-related effects of cocaine will cause a decrease in DAT binding.

Chapter II

Development of a Translational Model to Screen Medications for Cocaine Use

Disorder: Choice Between Cocaine and Food in Rhesus Monkeys

(Drug Alcohol Depend 165:103-110, 2016)

Introduction

Cocaine use disorder remains a significant clinical challenge for which there are no medications currently approved by the Food and Drug Administration. Research to evaluate the efficacy and safety of new medications for drug abuse or other disorders benefits from a translational path from preclinical to clinical studies, and a key step along this path occurs at the transition from research in animals to human subjects (Comer *et al*, 2008; Haney and Spealman, 2008a; Mello and Negus, 1996; Rush and Stoops, 2012). A change in species is unavoidable at this transition; however, the fidelity of translation may benefit from both (1) use of nonhuman primates as animal subjects due to their high degree of homology with humans, and (2) use of analogous experimental procedures that minimize discrepancies in variables other than species (Czoty *et al*, 2016; Foltin *et al*, 2015; Weerts *et al*, 2007; Yu, 2011).

In view of these considerations, the goal of this project and the companion study conducted in humans (Lile *et al*, 2016) was to develop homologous drug self-administration procedures in nonhuman primates and humans as a platform for more efficient and reliable translational research on candidate medications to treat drug

abuse. In particular, these studies sought to harmonize three sets of procedural variables: (1) the route and doses of self-administered cocaine, (2) the schedule of reinforcement that governed availability of cocaine and an alternative non-drug reinforcer, and (3) the treatment regimen for delivery of a candidate medication. With regard to the schedule of self-administration, previous human-laboratory studies have identified concurrent independent progressive-ratio schedules of choice between drug and money as a sensitive tool for medication evaluation (Jones and Comer, 2013; Moeller and Stoops, 2015; Stoops *et al*, 2012; Sullivan *et al*, 2006). Accordingly, cocaine self-administration was established in rhesus monkeys and human subjects under nearly identical concurrent independent progressive-ratio schedules of choice between cocaine and a species-specific non-drug alternative reinforcer (food in monkeys; money in humans). The cocaine dose and magnitude of the non-drug alternative were then systematically manipulated in each species, with the same unit doses of cocaine being used in both species. Results are reported here for the study in nonhuman primates and in a companion paper for the study in human subjects (Lile et al., 2016; see companion paper in this issue). We hypothesized that comparable patterns of cocaine choice could be demonstrated in rhesus monkeys and humans, and that specific parameters of cocaine dose and alternative reinforcer magnitude could be identified for subsequent evaluation of candidate medications in both species.

The present study also evaluated effects of lisdexamfetamine as a representative candidate medication. Lisdexamfetamine is an amphetamine prodrug approved for treatment of ADHD and compulsive eating disorder (Blick and Keating, 2007; Hutson *et al*, 2014), and it was selected for initial testing because preclinical and clinical research

suggests that it might also be useful for treating cocaine use disorder (Banks *et al*, 2015a; Mooney *et al*, 2015). Furthermore, maintenance on its metabolite, d-amphetamine, has been shown to decrease cocaine self-administration across a broad range of experimental conditions in rats, rhesus monkeys, human-laboratory studies, and placebo-controlled, double-blind clinical trials (Herin *et al*, 2010; Negus and Henningfield, 2015; Nuijten *et al*, 2016). Each lisdexamfetamine dose was tested using a subchronic, 7-day treatment regimen, because medications to treat drug use disorders are administered chronically in humans, and it has been argued that preclinical animal- and human-laboratory studies should also evaluate effects of repeated treatment delivery to more accurately predict clinical effectiveness(Banks *et al*, 2015b; Czoty *et al*, 2016; Haney and Spealman, 2008b; Mello and Negus, 1996). We hypothesized that 7-day treatment with lisdexamfetamine would produce a dose-dependent decrease in cocaine choice and a reciprocal increase in choice of the food alternative in this concurrent independent progressive-ratio choice procedure.

Methods

Subjects. Studies were conducted in four adult male rhesus monkeys (*Macaca mulatta*). Two of the monkeys had a history of exposure to monoaminergic compounds (e.g. cocaine and amphetamine), and two had a history of exposure to mu opioid compounds (e.g. oxycodone and naloxone).   Each monkey had a surgically implanted venous catheter with a single lumen (Braintree Inc., Braintree, MA) or double lumen (STI Components, Roanoke, VA). Monkeys could earn 1g banana-flavored pellets (5TUR Grain-based Precision Primate Pellets; Test Diets, St. Louis, MO) during daily experimental sessions. In addition, monkeys received daily food rations (Lab Diet High

Fiber Monkey Biscuits; PMI Feeds, St. Louis, MO), and the biscuit ration size was individually determined for each monkey to maintain a healthy body weight. Biscuit rations were delivered in the afternoons after behavioral sessions to minimize the effects of biscuit availability and consumption on food-maintained operant responding. Animals also received fresh fruit 7 afternoons per week. Water was continuously available in each monkey's home chamber, which also served as the experimental chamber. A 12h light/dark cycle was in effect (lights on from 0600 to 1800 h). Environmental enrichment (foraging devices, novel treats, movies and music) was also provided after behavioral sessions. Facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The Institutional Animal Care and Use Committee approved all experimental protocols.

Apparatus. Each home cage was equipped with an operant response panel, which had two response levers with three stimulus lights above each lever. The lights over the left and right levers were white and red, respectively. Additionally, the cages were equipped with a pellet dispenser that delivered food pellets to a receptacle within the cage. The externalized section of the intravenous catheter for drug self-administration was routed through a jacket and tether system (Lomir Biomedical, Quebec, Canada) to the rear of the cage and connected to a peristaltic fluid pump (Cole-Parmer, Chicago, IL). Catheter patency was periodically evaluated with intravenous (IV) ketamine (4 mg/kg) administration, and the catheter was considered patent if IV ketamine administration produced overt loss of muscle tone within 20 sec.

Single Alternative Training. Initial training for food-maintained responding proceeded in a series of incremental steps, during which only one lever and associated

stimulus lights were active (the "food-associated lever," counterbalanced between monkeys). Under the terminal progressive-ratio (PR) schedule, daily 5 hr behavioral sessions consisted of 10 discrete 30-min trials. The first trial was a "sample" trial, in which subjects received non-contingent delivery of 10 pellets. The remaining 9 trials were "response" trials, in which food pellets were available under the PR schedule. Stimulus lights were illuminated over the lever at the start of each trial, and completion of the ratio requirement produced food pellet delivery, initiated a time out (TO) for the remainder of the trial, and incremented the ratio for the next trial. If a monkey failed to complete the ratio requirement within 30 min, the trial terminated without reinforcement, the response counter reset to "0," a 1-min TO period ensued, and the ratio requirement did not increment for the next trial. The starting ratio was 200 in 2 monkeys and 400 in the other 2 monkeys, and the increment after each completed ratio was 100 for all monkeys (i.e. PR values were 200, 300, 400…1000 for two monkeys; 400, 500, 600…1200 for the other 2 monkeys). The lower starting ratio was used in two monkeys because they failed to complete ≥8 trials with higher starting ratios. Once monkeys reliably completed ≥8 trials for the 10-pellet reinforcer magnitude under the terminal schedule, a pellet magnitude-effect curve was determined at magnitudes of 0, 1, 3 and 10 pellets. During these studies, the designated pellet magnitude was delivered non-contingently during the sample trial of each daily session, and responding under the PR schedule produced this pellet magnitude during subsequent response trials. Each pellet magnitude was presented for a minimum of 7 consecutive days and until responding stabilized (number of trials completed for the last 3 days within 1 of the running mean, with no increasing or decreasing trends). Responding maintained by 10 pellets was

determined first in all monkeys, and the remaining pellet magnitudes were studied in a mixed order across monkeys.

Once the pellet magnitude-effect curve was completed, an intravenous catheter was surgically implanted using aseptic procedures, and cocaine training began. The training regimen for cocaine self-administration was identical to that for food-maintained responding with the exception that the other lever and associated stimulus lights were active (the "cocaine-associated lever"), and responding produced intravenous cocaine injections. Training proceeded until responding maintained by 0.43 mg/kg/injection cocaine was stable under the same terminal schedule used for food in that monkey (i.e. starting ratio of 200 in 2 monkeys and 400 in the other 2 monkeys, with an increment of 100 in all monkeys). Subsequently, a cocaine dose-effect curve was determined at doses of 0, 0.043, 0.14 and 0.43 mg/kg/injection cocaine using test durations and stability criteria identical to those used for the pellet magnitude-effect curve. The cocaine doses were selected to match approximate unit cocaine doses used in the parallel human-laboratory study (i.e. 0.043, 0.14 and 0.43 mg/kg/injection unit doses in monkeys are equivalent to doses of 3, 10 and 30 mg for a 70 kg human subject; Lile et al., 2016; see companion paper in this issue). Responding maintained by 0.43 mg/kg/injection was determined first in all monkeys, and the remaining doses were studied in a mixed order across monkeys.

Cocaine vs. Food Choice Procedure. After determination of magnitude-effect functions for food and cocaine alone, concurrent-choice studies were initiated to assess cocaine choice dose-effect curves during concurrent availability of 1, 3 or 10 pellets. Choice session were identical to sessions under the terminal schedule for food or

cocaine alone with the following exceptions: (1) a single pellet magnitude and a single cocaine dose were concurrently available, (2) both the food and drug reinforcers available during that session were delivered non-contingently at the start of the sample trial, with food delivered first, and cocaine delivered 5 min later, (3) both food- and cocaine-associated levers were active at the start of each choice trial, and lights above both levers were illuminated, (4) the first response during each trial locked in choice for that reinforcer during that trial, deactivated the alternative lever, and extinguished lights above the alternative lever, and (5) completion of a ratio produced the chosen reinforcer and incremented the ratio requirement only for that reinforcer in the next trial. If a monkey failed to complete a ratio requirement within 30 min, then the trial terminated without reinforcement, the response counter reset to "0" for both levers, a 1-min TO period ensued, the ratio did not increment for either reinforcer for the next trial, and the trial was counted as an "omission." Each combination of pellet magnitude and cocaine dose was in effect for 7 consecutive days, and all cocaine doses were tested in combination with a single pellet magnitude before proceeding to a different pellet magnitude. Both the order of cocaine doses within a pellet magnitude and the order of pellet magnitudes were randomized across monkeys.

Effects of Lisdexamfetamine. Prior to testing lisdexamfetamine, choice performance was first re-established between 0.14 mg/kg/injection cocaine and 10 pellets (see Results for rationale). Each lisdexamfetamine dose (0.32, 1.0, 1.8, and 3.2 mg/kg/day) was tested for 7 consecutive days, and baseline choice performance was re-established over a period of at least 4 days between each 7-day lisdexamfetamine dose test. On test days, lisdexamfetamine was administered by slow IV infusion over a

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period of 30 min beginning 1 h before the start of the daily choice session. The dose of 1.8 mg/kg/day lisdexamfetamine fully suppressed responding in one of the four monkeys, and as a result, 3.2 mg/kg/day lisdexamfetamine was not tested in this subject. The order in which lisdexamphetamine doses were tested was randomized across monkeys.

Data Analysis. The primary dependent variables were the mean numbers of cocaine choices, food choices, and omissions per session. Data from the last three days of each test condition were first averaged within a monkey and then averaged across monkeys to generate group means. Data were analyzed by one- or two-way repeated-measures ANOVA, as appropriate, and a significant ANOVA was followed by either a Dunnet's or Holm-Sidak post hoc test. The criterion for significance was p<0.05.

Drugs. ($-$)-Cocaine HCl (NIDA, Rockville, MD) and lisdexamfetamine mesylate (B. E. Blough, Research Triangle Institute) were dissolved in sterile saline for IV injection.

Results

Responding maintained by food or cocaine alone. Training took an average of 2.5 months to reach the terminal schedule of food presentation (range = 43 - 142 days). Food pellets maintained a magnitude-dependent increase in responding (Figure 2-1A). When 0 pellets were available, subjects completed an average of approximately 1 ratio requirement. As the number of pellets available increased, subjects increased the number of trials completed ($F_{3,9} = 17.96$, $p < 0.001$), such that an average of approximately 8 trials were completed when 10 pellets were available.

Cocaine self-administration training took an average of 34 days to reach the terminal schedule (range = 24 - 48 days), and cocaine also maintained a dose-dependent increase in responding (Figure 2-1B). When saline was available, subjects completed an average of approximately 1 ratio requirement. As the dose of cocaine increased, the number of trials completed increased ($F_{3,9}$ = 53.42, p < 0.0001), such that an average of at least 8 trials were completed during availability of 0.14 and 0.43 mg/kg/injection cocaine.

Choice between food and cocaine. Figure 2-2 shows the mean numbers of completed cocaine trials, completed food trials, and omissions during the final three days for each cocaine dose at each pellet magnitude. Data within each panel were analyzed by two-way ANOVA [cocaine dose (0, 0.043, 0.14 and 0.43 mg/kg/injection) x trial outcome (cocaine choice, food choice, or omission)], and this analysis revealed a significant interaction at each pellet magnitude (Panel A: $F_{6,18}$ = 9.03, p < 0.001; Panel B: $F_{6,18}$ = 10.82, p < 0.0001; Panel C: $F_{6,18}$ = 17.02, p < 0.0001). Across all 3 pellet magnitudes, cocaine maintained a dose-dependent increase in the number of cocaine trials completed, and doses of 0.14 and 0.43 mg/kg/injection were always chosen in significantly more trials than saline, as denoted by asterisks over open bars in Figure 2-2A-C. Similarly, across all three pellet magnitudes, the mean number of food trials completed tended to decrease as cocaine dose increased; however, this trend was significant only during availability of 3 and 10 pellets. Under those conditions, the number of food choices was higher during concurrent availability of saline than during concurrent availability of 0.14 and 0.43 mg/kg/injection cocaine, as denoted by asterisks over closed bars in Figure 2-2B,C. Omissions tended to be highest when low

magnitudes of the food and cocaine reinforcers were concurrently available (e.g. during concurrent availability of 1 pellet and saline injections in Figure 2-2A), and the mean number of omissions tended to decrease as cocaine dose increased. This tendency attained significance during the availability of 1 pellet, when the number of omissions was higher during availability of saline than during availability of 0.14 and 0.43 mg/kg/injection cocaine, as denoted by asterisks over gray bars in Figure 2-2A).

The analysis of choice results as shown in Figure 2-2 also permitted evaluation of preference between food and cocaine at each combination of pellet magnitude and cocaine dose (see dollar signs in Figure 2-2A-C). Both 0.14 and 0.43 mg/kg/injection cocaine were preferred to 1 pellet (Figure 2-2A). During availability of 3 pellets, food was preferred to saline injections, whereas 0.14 and 0.43 mg/kg/injection cocaine were preferred to food (Figure 2-2B). During availability of 10 pellets, food was preferred to saline and the lowest dose of 0.043 mg/kg/injection cocaine, whereas the highest dose of 0.43 mg/kg/injection cocaine was preferred to food (Figure 2-2C). During the availability 10 pellets, preference for the 0.14 mg/kg/injection cocaine dose was not significant.

Effects of lisdexamfetamine treatment. Choice between 0.14 mg/kg/injection cocaine and 10 pellets was selected as the baseline for the experiment with lisdexamfetamine because (1) it yielded a trend albeit non-significant toward cocaine preference (approximately 6 cocaine and 3 food trials completed) with few omissions, and (2) a reduction in cocaine dose produced reallocation of choice that resulted in significant preference for this food magnitude, again with few omissions. Thus, behavior maintained by this pair of reinforcer magnitudes was likely to be sensitive to

reductions in the relative reinforcing efficacy of cocaine during pharmacological

treatment. As a prelude to presentation of lisdexamfetamine effects, Figure 2-3A shows

four hypothetical changes in choice between 0.14 mg/kg/injection cocaine and 10

pellets that could be observed during candidate medication treatment. Outcome #1 is

interpreted as therapeutically desirable and consists of a decrease in cocaine trials

completed with a reciprocal increase in food trials completed. This outcome indicates a

reallocation of behavior from cocaine choice to food choice and a decrease in the

relative reinforcing efficacy of cocaine in comparison to food. Outcomes #2-4 show

three other possible outcomes interpreted as therapeutically undesirable. Specifically,

outcome #2 shows a concurrent decrease in both cocaine and food trials with an

increase in omissions, suggestive of non-selective behavioral suppression; outcome #3

shows an increase in completed cocaine trials with a reciprocal decrease in food trials,

suggestive of increased relative reinforcing efficacy of cocaine; and outcome #4 shows

no treatment effect. Of course, graded outcomes between these extremes are also

possible.

Figure 2-3B shows choice between 0.14 mg/kg/injection cocaine and 10 pellets

during 7-day treatments with different lisdexamfetamine doses (0, 0.32, 1.0, 1.8, and 3.2

mg/kg/day) Data were analyzed by two-way ANOVA (trial outcome x lisdexamfetamine

dose), which revealed a significant interaction ($F_{6,18} = 4.82$, $p < 0.01$). Lisdexamfetamine

doses of 0.32 and 1.0 mg/kg/day did not significantly alter cocaine or food trials

completed or the number of omissions. A dose of 1.8 mg/kg/day lisdexamfetamine

decreased cocaine trials completed, had no effect on completed food trials, and

increased omissions. The high dose of 3.2 mg/kg/day lisdexamfetamine was tested in

only 3 monkeys and produced a profile of effects similar to 1.8 mg/kg/day lisdexamfetamine.

Lisdexamfetamine time course effects are shown in Figure 4, and saline substitution effects are included for comparison. Under baseline conditions, preference between 0.14 mg/kg/day cocaine and 10 pellets was relatively stable across all 7 days (Figure 2-4A). Saline substitution decreased the number of trials completed on the cocaine-associated key and produced a reciprocal increase in food trials completed. This reallocation of behavior was evident on day 1 and sustained throughout the 7-day experiment. (Figure 2-4B). Lisdexamfetamine produced a dose-and time-dependent decrease in cocaine trials completed while having smaller and more transient effects on completed food trials. Thus, the decline in cocaine choice was associated with sustained food choice and an increase in trial omissions (Figure 2-4C-F).

Individual subject data during 1.8 mg/kg/day lisdexamfetamine treatment are shown in Figure 2-5. This dose of lisdexamfetamine decreased the number of completed cocaine trials in all four monkeys, but the degree to which this decrease in cocaine choice was accompanied by a reciprocal increase in food choice varied across monkeys. Monkey 1501 showed the most robust behavioral reallocation from cocaine to food choice without an increase in omissions (Figure 2-5A). Monkeys 1498 and 1416 showed smaller increases in food trials completed together with small increases in omissions (Figure 2-5B-C). Finally, in Monkey 1524, lisdexamfetamine decreased both cocaine and food trials completed together with an increase in omissions (Figure 2-5D). The lower dose of 1.0 mg/kg/day lisdexamfetamine produced little change in cocaine vs. food choice in this monkey.

Discussion

The goal of this study was to develop a cocaine-vs.-food choice procedure in rhesus monkeys homologous to a cocaine-vs.-money procedure in humans as an experimental tool to facilitate translational research for the development of medications to treat cocaine use disorder (Lile *et al*, 2016). There were three main findings. First, rhesus monkeys could be trained to choose between cocaine and food under a concurrent progressive-ratio schedule very similar to that used in humans to study choice between cocaine and money. Second, for rhesus monkeys as in humans, the allocation of behavior between cocaine and the alternative reinforcer varied systematically as a function of cocaine dose and magnitude of the alternative reinforcer. In particular, when the highest magnitude of 10 pellets was available as the alternative to cocaine in rhesus monkeys, there were few omissions, and preference for the 0.14 mg/kg/injection cocaine dose was no longer significant. Lastly, repeated 7-day treatment with the candidate medication lisdexamfetamine produced a dose-and time-dependent decrease in cocaine choice in all monkeys, and did not significantly impact food choice. These results illustrate the use of the procedure to study a candidate medication and provide qualified support for further consideration of lisdexamfetamine maintenance to treat cocaine use disorder.

Choice between cocaine and food. This study extends the range of conditions under which cocaine-vs.-food choice has been established in rhesus monkeys (Foltin *et al*, 2015; Nader and Woolverton, 1991; Negus, 2003; Paronis *et al*, 2002; Woolverton

and Balster, 1981). Specifically, this study used a concurrent independent progressive-ratio procedure to mimic drug-vs.-money choice procedures used previously in human laboratory studies in general (Jones and Comer, 2013; Moeller and Stoops, 2015; Stoops *et al*, 2012; Sullivan *et al*, 2006) and to match the cocaine-vs.-money choice procedure used in the companion human laboratory study in particular (Lile *et al*, 2016). As such, this study represents an example of back-translation, in which a procedure originally developed for use in humans was modified for use in laboratory animals. Back-translation is one approach that has been used in other disciplines to strengthen the procedural concordance between animal and human studies and improve the predictive power of forward animal-to-human translational research (Insel *et al*, 2013; Keeler and Robbins, 2011). This approach of back-translation has also been recommended as a strategy to strengthen translational research on medications development for cocaine abuse (Czoty *et al*, 2016). To our knowledge, this is the first demonstration of cocaine vs. food choice by rhesus monkeys under this type of schedule, although both food and cocaine-maintained responding have been established separately under progressive-ratio schedules in rhesus monkeys(Bedford *et al*, 1978; Negus and Mello, 2003a; Rowlett *et al*, 1996; Stafford *et al*, 1999).

Previous studies have demonstrated that choice between cocaine and food is sensitive to manipulation of both the cocaine dose and food-reinforcer magnitude in both rhesus monkeys (Nader and Woolverton, 1991; Negus, 2003) and rats (Thomsen *et al*, 2013). In the present study, similar effects were obtained. In general, increasing the magnitude of the available cocaine dose resulted in increased cocaine choice and decreased food choice, whereas increasing the magnitude of the food reinforcer

increased food choice and decreased cocaine choice. The reciprocal effects of reinforcer magnitude on preference were especially apparent when cocaine dose was manipulated during concurrent availability of 10 pellets. Under these conditions, increasing cocaine doses produced a systematic shift from robust food preference to robust cocaine preference, and omissions were rare. These results in the monkey cocaine-vs.-food choice procedure closely approximate the shift from money preference to cocaine preference produced by increasing cocaine doses in the human cocaine-vs.-money choice procedure described in the companion manuscript (Lile *et al*, 2016). This concordance in results from monkey and human cocaine choice procedures provides one source of evidence to support utility of these homologous procedures for translational research on determinants of cocaine choice.

It is also notable that the dose-dependent increases in cocaine-vs.-food choice in rhesus monkeys observed here and in a previous study (Foltin *et al*, 2015) were obtained under discrete-trial procedures that limited the frequency of cocaine injections. These findings contrast with a recent report suggesting that cocaine vs. saccharin preference could be established in rats when intervals between choice opportunities were short (0 or 1 min) but not when inter-trial intervals were longer (10 min) (Vandaele *et al*, 2015). The reasons for this discrepancy are not clear and may be related to various procedural differences including species and identity of the non-drug alternative reinforcer; however, in the present study using discrete 30-min trials, the highest cocaine dose (0.43 mg/kg/inj) was preferred to food at all food magnitude alternatives.

Effects of 7-day lisdexamfetamine treatment on cocaine vs. food choice.
Results of the present study confirm and extend previous reports that cocaine vs. food

choice can be reduced by maintenance either on lisdexamfetamine in rhesus monkeys (Banks *et al*, 2015a) or on its primary metabolite amphetamine in rhesus monkeys or rats (Banks *et al*, 2013; Negus, 2003; Thomsen *et al*, 2013). Amphetamine maintenance also decreased cocaine self-administration maintained under other, non-choice schedules of reinforcement in rhesus monkeys and rats (Chiodo *et al*, 2008; Czoty *et al*, 2010; Negus and Mello, 2003a, 2003b), as well as cocaine choice in human laboratory studies and metrics of cocaine use in clinical trials (Grabowski *et al*, 2001; Levin *et al*, 2015; Nuijten *et al*, 2016; Rush *et al*, 2010; Stoops and Rush, 2013). The present proof-of-concept study used intravenous lisdexamfetamine to permit precise control of the administered dose, but lisdexamfetamine is formulated for oral administration in humans and would likely be tested using oral administration in human laboratory studies. Future translational studies with candidate medications might benefit from use of the same route of administration for treatment drugs in monkeys and humans to parallel use of the same route of administration for cocaine.

A recent double-blind, placebo-controlled pilot clinical trial found that lisdexamfetamine maintenance was not significantly better than placebo in reducing cocaine use by a group of 43 cocaine-dependent individuals (Mooney *et al*, 2015). However, four caveats warrant mention in comparing that clinical trial in humans to the present study in monkeys. First, subjective reports of craving were significantly reduced, and cocaine use was significantly reduced by lisdexamfetamine in a secondary analysis that examined the subset of patients that completed the 14-week study. Thus, there was some evidence for modest effectiveness of the lisdexamfetamine doses tested. Second, the highest dose evaluated in that clinical trial was 70 mg/day, which is

approximately equivalent to the dose of 1 mg/kg/day in monkeys. Both 70 mg/day

lisdexamfetamine in humans and 1 mg/kg/day lisdexamfetamine produced similar, small

and non-significant decreases in metrics of cocaine use. Thus, there was evidence for

concordance in effects produced by similar lisdexamfetamine doses in humans and

monkeys. Third, the authors of the clinical trial appreciated the impact of regulatory

constraints on the doses they could test, and they noted that "Evaluation of higher

doses of lisdexamfetamine may provide clearer evidence of its efficacy in treating

cocaine dependence." Results of the present study illustrate how preclinical studies

might be useful to inform decisions on whether to pursue testing of higher doses in

humans. Specifically, this study found that cocaine choice was significantly reduced by

a higher dose of 1.8 mg/kg/day lisdexamfetamine in monkeys (equivalent to 126 mg/day

in a 70 kg human), and this supports the speculation by the clinical trial authors that

higher lisdexamfetamine doses might also be more effective to decrease cocaine use in

humans. Lastly, the clinical trial revealed individual differences in some adverse events,

in medication adherence, and in study retention. The present study also identified

individual differences in undesirable lisdexamfetamine effects in monkeys. Specifically,

although lisdexamfetamine significantly reduced choice of 0.14 mg/kg/inj cocaine doses

in all subjects, the degree to which this decrease in cocaine choice was accompanied

by a reciprocal increase in food choice varied across subjects. This variability in

lisdexamfetamine effectiveness to promote behavioral reallocation to food choice

observed in the present study may be related to the individual differences in the adverse

effects of lisdexamfetamine in humans, which would further support the concordance

between non-human primate data using these procedures and clinical trial results.

Taken together, the results of the present study with lisdexamfetamine illustrate one strategy for medication evaluation in this procedure. These results provide a preclinical treatment profile that can be compared to results with other candidate medications as they are tested in the future. In particular, it would be of interest to identify treatments that not only reduce cocaine choice, but that also produce a more robust and reliable reallocation of responding to food choice than was produced here by lisdexamfetamine. Additionally, these results provide an outcome in monkeys that could be directly compared to results obtained in the complementary cocaine-vs.-money choice procedure in humans. A comparison of treatment effects with lisdexamfetamine and other candidate medications on cocaine choice in rhesus monkeys and humans will be important for continued validation and refinement of this platform for translational research.
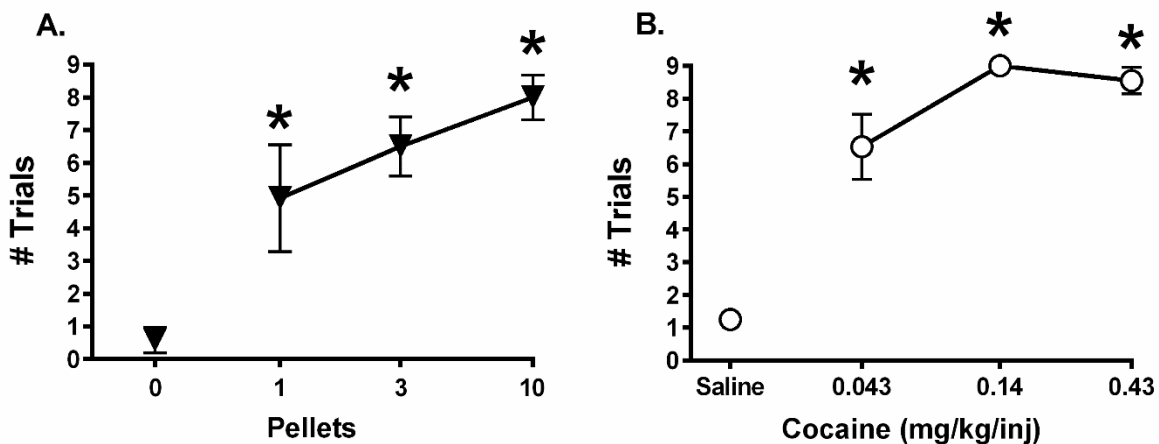
Figure 2-1. Effects of reinforcer magnitude under the discrete trials progressive ratio procedure. Abscissae: Reinforcer magnitude in units of pellet number (A) or cocaine dose (mg/kg/injection; B) available during each trial. Ordinates: Number of trials completed. Each condition was presented for a minimum of 7 days and until stable responding was observed. All points show mean±SEM for the final 3 days in 4 monkeys. Asterisks (*) indicate statistical significance ($p < 0.05$) compared to 0 pellets (A) or saline (B).
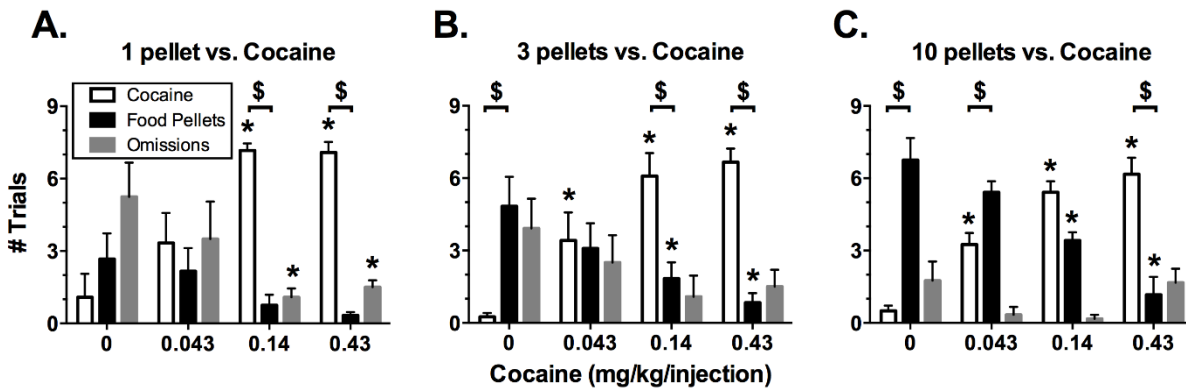
Figure 2-2. Trials completed for either cocaine or food when 1, 3 or 10 food pellets were available as the alternative to cocaine. Abscissae: Unit dose of cocaine available during each trial (mg/kg/injection). Ordinates: Number of cocaine and food trials completed, or number of omitted trials. Each combination of cocaine dose and pellet reinforcer magnitude was available for 7 days. All bars show mean ± SEM for the final 3 days in 4 monkeys. Asterisks (*) indicate statistical significance ($p < 0.05$) within a trial outcome (cocaine choice, food choice, or omission) compared to the 0 cocaine data. Dollar signs ($) indicate statistical significance ($p<0.05$) within a cocaine dose between the numbers of cocaine vs. food trials completed.
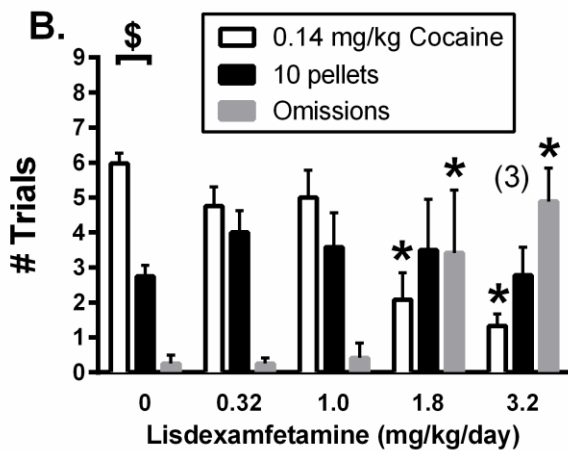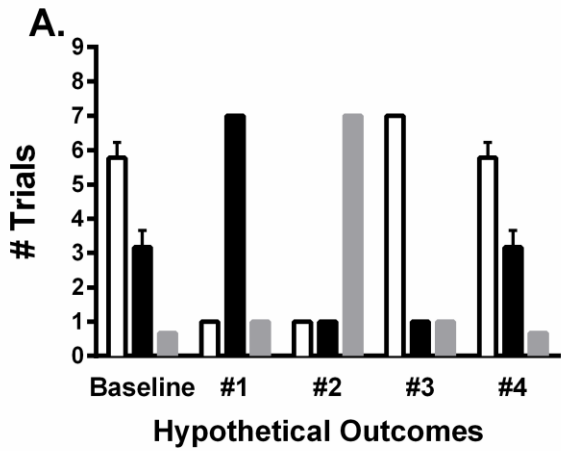
Figure 2-3. Treatment effects on choice between 0.14 mg/kg/injection cocaine and 10

pellets. Abscissae: (A) Hypothetical treatment outcome # (see text for details) or (B)

lisdexamfetamine dose (mg/kg/day). Ordinates: Number of cocaine and food trials

completed, or number of omitted trials. All bars in Panel A show hypothetical data, and

all bars in Panel B show mean ± SEM for the final 3 days in 4 monkeys (0-1.8

mg/kg/day lisdexamfetamine) or 3 monkeys (3.2 mg/kg/day lisdexamfetamine).

Asterisks (*) indicate statistical significance (p < 0.05) within a trial outcome (cocaine

choice, food choice, omission) compared to the 0 lisdexamfetamine treatment dose in

Panel B. Dollar signs ($) indicate statistical significance (p<0.05) within a

lisdexamfetamine dose between the numbers of cocaine vs. food trials completed.
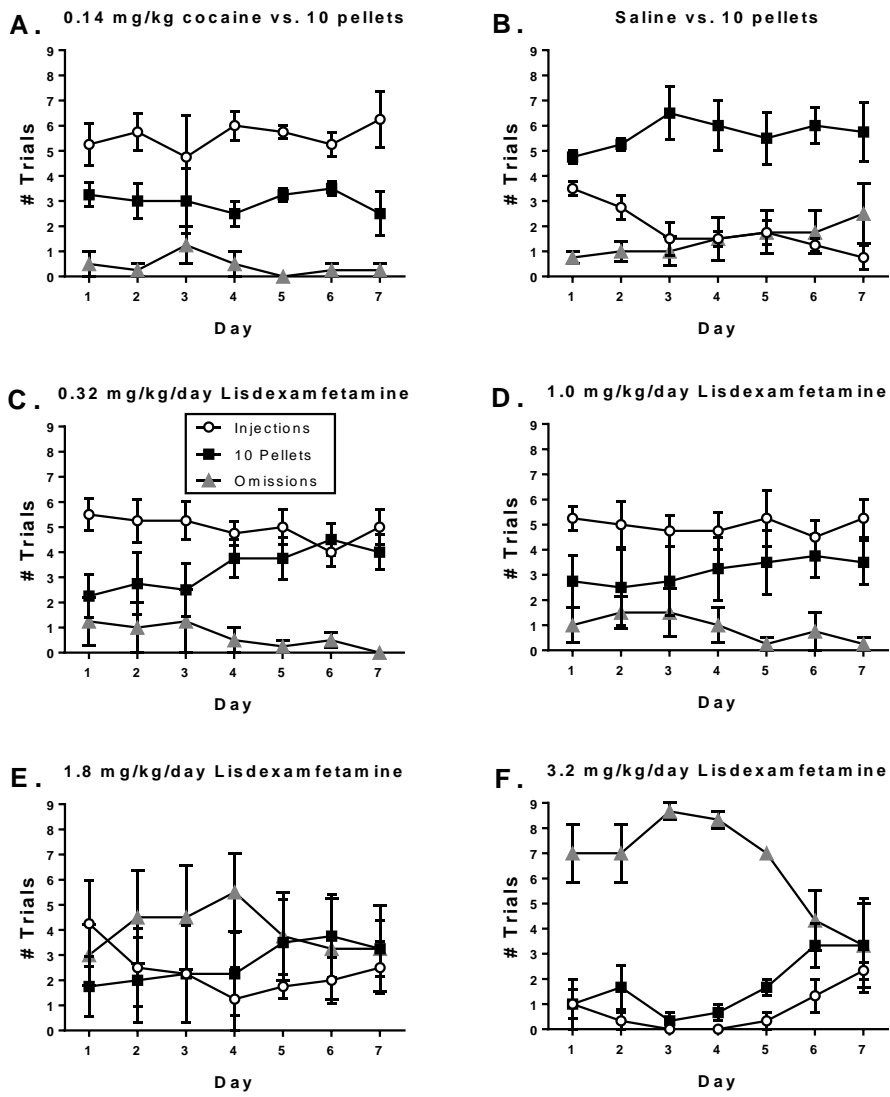
Figure 2-4. Time course of choice between injections and 10 pellets under different

experimental conditions. Abscissae: Experimental condition day. Ordinates: Number of

cocaine and food trials completed, or number of omitted trials. (A) Baseline choice

between 0.14 mg/kg/injection cocaine and 10 pellets. (B) Choice between saline and

10 pellets. (C-F) Choice between 0.14 mg/kg/injection cocaine and 10 pellets during

treatment with increasing lisdexamfetamine doses (0.32-3.2 mg/kg/day). All points

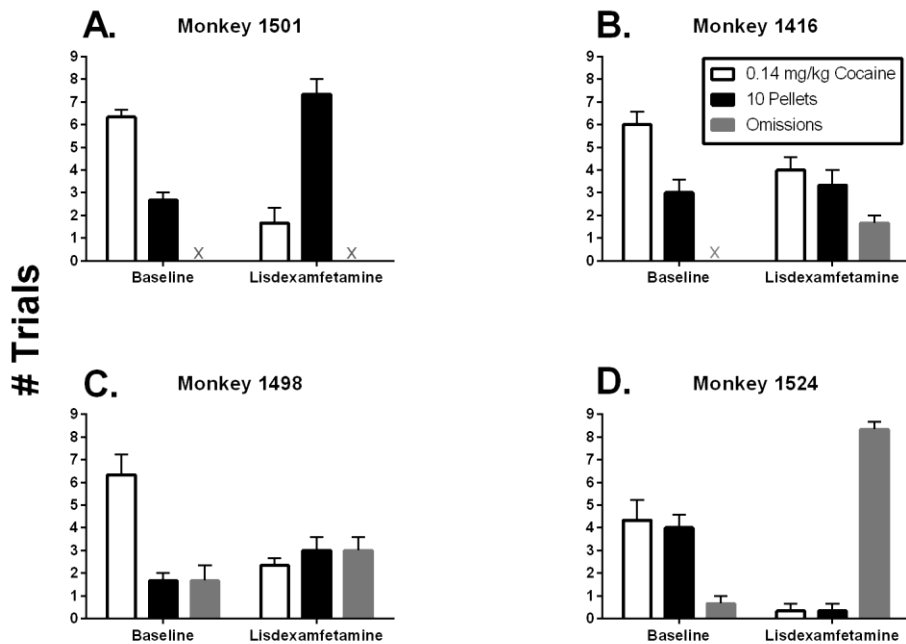show mean±SEM for 4 monkeys except Panel F, where N=3.

Figure 2-5. Individual subject data for 0.14 mg/kg/injection cocaine and 10 pellets under baseline conditions and during 1.8 mg/kg/day lisdexamfetamine treatment. Abscissae: Treatment condition. Ordinates: Number of cocaine and food trials completed, or number of omitted trials. Graphs show data for individual subjects that contributed to mean data shown in Figure 2-3B, and all bars show mean ± SEM for the final 3 days in each subject. The "x" symbol indicates no omissions under the indicated conditions.

Chapter III

The Effects of Amphetamine Maintenance

on Drug versus Food Choice in Rhesus Monkeys

(In preparation for a joint manuscript with colleagues at the University of Kentucky)

Introduction

Amphetamine maintenance remains one of the only treatments to show

consistent decreases in cocaine use in double-blind placebo controlled clinical trials

(Grabowski *et al*, 2001, 2004a; Mariani *et al*, 2012). The results from the previous

chapter indicated that the non-human primate choice procedure is sensitive to the

amphetamine prodrug, lisdexamfetamine. In this experiment, we will test d-

amphetamine to continue the validation of the model and to be able to extend the

findings to the parallel work being done at the University of Kentucky in the human self-

administration choice procedure.

Methods

Subjects. Studies were conducted in 3 adult male rhesus monkeys (*Macaca*

mulatta) surgically implanted with a venous double-lumen catheter (0.03" ±0.01" inner

diameter for each lumen; 0.093" ±0.014" total outer diameter; 0.011" wall diameter and

70±5 durometer; Reiss Manufacturing, Inc, Blackstone, VA). All 3 monkeys had

responded in the cocaine-choice procedure for at least two years and been tested with

lisdexamfetamine as described previously (Johnson *et al*, 2016). Monkeys could earn 1 g banana-flavored pellets (5TUR Grain-based Precision Primate Pellets; Test Diets, St. Louis, MO) during daily experimental sessions. In addition, monkeys received daily rations of fresh fruit and biscuits (Lab Diet High Fiber Monkey Biscuits; PMI Feeds, St. Louis, MO), and these rations were provided after behavioral sessions to minimize their impact on food-maintained responding. Environmental enrichment (foraging devices, novel treats, movies and music) was also provided after behavioral sessions. Water was continuously available in each monkey's home chamber, which also served as the experimental chamber. A 12 h light/dark cycle was in effect (lights on from 0600 to 1800 h). Facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and all procedures were approved by the Institutional Animal Care and Use Committee.

Apparatus. Each home chamber was equipped with an operant response panel, which had 2 response levers and 3 stimulus lights above each lever. The lights over the left and right levers were white and red, respectively. Additionally, the cages were equipped with a pellet dispenser (ENV-203-1000, Med Associates, St. Albans, VT) that delivered food pellets to a receptacle within the chamber. The externalized section of the intravenous (IV) catheter was routed through a jacket and tether system (Lomir Biomedical, Quebec, Canada) to the rear of the chamber and connected to a peristaltic fluid pump (Cole-Parmer, Chicago, IL). Catheter patency was periodically evaluated with IV ketamine (4 mg/kg) administration, and the catheter was considered patent if IV ketamine administration produced overt loss of muscle tone within 20 s.

Choice sessions. Training was accomplished as described previously (Johnson *et al*, 2016). Under the terminal schedule, 5-hr choice sessions were conducted daily from 8:30am-1:40pm and consisted of 10 discrete 30-min trials separated by 1-min time out periods. During the first trial of each day, the unit cocaine dose (0.14 or 0.43 mg/kg/injection, IV) and food reinforcer magnitude (10 pellets) available on that day were delivered non-contingently, with food delivered at the beginning of the trial and the unit cocaine dose administered 5 min later. The remaining 9 trials were response trials, during which cocaine and food pellets were available concurrently under independent progressive-ratio schedules. The starting ratio for both reinforcers was 200 in 2 monkeys and 400 in the other monkey, and the increment after each completed ratio was 100 for all monkeys (i.e. PR values were 200, 300, 400…1000 for two monkeys; 400, 500, 600…1200 for the other monkey). Each response trial began with the stimulus lights illuminated over both the cocaine- and the food-associated levers, and both levers were active. The first response extinguished the stimulus lights over the alternative lever and locked in the choice for that trial. The subject had the remainder of the 30-min trial to complete the response requirement on the chosen lever, and responses on the alternative lever had no programmed consequence. Completion of the response requirement resulted in 1) extinguishing the stimulus lights over the lever, 2) delivery of the reinforcer, 3) incrementing the response requirement only for the chosen reinforcer, and 4) initiation of a TO for the remainder of the trial. If a monkey failed to complete a ratio requirement within 30 min, then the trial terminated without reinforcement, the response counter reset to "0" for both levers, a 1-min TO period

ensued, the ratio did not increment for either reinforcer for the next trial, and the trial was counted as an "omission."

Effects of d-amphetamine. Before starting tests with *d*-amphetamine, stable choice was established between 0.14 mg/kg/injection cocaine and 10 food pellets. These reinforcer magnitudes were selected based on our prior study (Johnson et al. 2016). The criterion for stability was that the number of completed cocaine trials and omissions on each day was within 1 of the running 3-day mean, with no increasing or decreasing trends. Each d-amphetamine dose (saline, 0.019, 0.037, 0.074 mg/kg/hr) was administered IV via continuous infusion 23 hours each day (4pm - 3pm next day) for 13 days through one lumen of the double-lumen catheter. The lower amphetamine doses were selected to match the doses tested in humans (0.019 and 0.037 mg/kg/hr = 30 and 60 mg/day in a 70 kg human). Additionally, to match the human testing regimen, the first 6 treatment days were designated as the acclimation period, and no behavioral sessions were conducted. For the remaining 7 treatment days, choice sessions were conducted to evaluate choice between 0.14 mg/kg/inj cocaine and 10 pellets. Saline treatment conditions were reinstated for at least four days and until stable choice was reestablished before initiating the next treatment condition. d-Amphetamine dose order varied across monkeys. In addition, *d*-amphetamine (0.037 mg/kg/hr) treatment was also tested during choice between a higher unit cocaine dose (0.43 mg/kg/injection) and 10 food pellets. For these studies, choice was evaluated using the 13-day treatment protocol first for saline treatment and then for 0.037 mg/kg/hr amphetamine treatment.

Data analysis. The primary dependent variables were the mean numbers of completed cocaine trials and food trials per session. Data for the last 3 days of each test condition were first averaged within a monkey and then averaged across monkeys to generate group means. Data were analyzed by two-way repeated-measures ANOVA with amphetamine dose and trial outcome (cocaine or food trials completed) as the two factors, and a significant ANOVA was followed by a Dunnett's post-hoc test to compare choice during treatment with different amphetamine doses to choice during saline treatment. Treatment effects on omissions were evaluated by a separate one-way ANOVA. Individual and group data are also shown for all 7 days of choice testing during treatment with saline and 0.037 mg/kg/hr d-amphetamine. The time course of group data were analyzed using a model comparison approach to evaluate the regression coefficient by an extra sum-of-squares F-test (Motulsky and Christopoulous 2003). For all statistical analyses, the criterion for significance was $p < 0.05$.

Plasma Amphetamine Analysis

Specimen preparation and extraction. A freshly prepared seven-point calibration with a range of 10 ng/mL to 1000 ng/mL amphetamine (Cerilliant, Round Rock, TX), a drug-free control (negative control) containing only amphetamine-d11 (Cerilliant, Round Rock, TX), the internal standard (ISTD), and a double negative control that contained neither amphetamine nor ISTD were analyzed with each batch of samples. Amphetamine was extracted from the calibrators, controls, and samples using a previously described method for amphetamines, other phenylisopropylamines and their metabolites (Poklis and Moore, 1995). Briefly, 50 µL (500 ng/mL amphetamine-

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d$_{11}$) of the ISTD was added to 1.0 mL aliquots of calibrators, controls, and specimens, followed by 100 µL of concentrated ammonium hydroxide (Macron Fine Chemicals, Center Valley, PA) and 2.0 mL of n-butyl chloride (Burdick and Jackson, Muskegon, MI). Samples were mixed for 30 sec and centrifuged for 5 min. The n-butyl chloride layer was transferred to a borosilicate test tube (12 x 75 mm) and reduced to 1 mL under a gentle stream of nitrogen at room temperature. One hundred microliters (100µL) of heptafluorobutyric anhydride (Regis Technologies, Morton Grove, IL) was added to the mixture. Samples were than heated at 70°C for 20 min. The n-butyl chloride was then evaporated under a gentle stream of nitrogen at room temperature and reconstituted in 50 µL ethyl acetate (Fisher Scientific, Fairlawn, NJ). One microliter (1 µL) of the extract was injected into the Gas Chromatography Mass Spectrometry (GC/MS) for analysis.

Gas chromatography mass spectrometry method. The identification and quantification of amphetamine was performed using a Shimadzu gas chromatography mass spectrometry QP-2010 with EI ionization (Shimadzu Scientific Inc., Columbia, MD). Chromatographic separation was performed on an Rtx®-5 30m x 0.32mm, 0.5 µm capillary column (Restek Bellefonte, PA). The initial temperature was 70°C with a hold time of 1 min, then a 20°C/min ramp to 320°C and held for 0.5 min. The temperature for the injection port was 250°C; for the ion source 260°C; and for the interface 280°C. The total flow rate was 42.1 mL/min with a column flow of 3.65 mL/min. The retention time for amphetamine was 3.89 min and for amphetamine-d$_{11}$ 3.68 min. The following ions were monitored for amphetamine, 240, 118 and 91 *m/z* and amphetamine-d$_{11}$, 244 and 128 *m/z*. Each calibrator concentration was determined to be within ±15% of the expected value. The linear regression correlation coefficients (r^2) for all calibration

curves were ≥ 0.995. The amphetamine concentrations were determined by linear regression plot based on peak area ratio of the calibrators.

Data analysis. Data from individual monkeys were averaged for each chronic amphetamine treatment dose. Plasma amphetamine levels were analyzed by one-way repeated-measures ANOVA with amphetamine dose as the factor, and a significant ANOVA was followed by a Bonferroni's post-hoc test.

Drugs. (−)-Cocaine HCl (NIDA Drug Supply Program, Rockville, MD) and *d*-amphetamine hemisulfate (Sigma Aldrich; St. Louis, MO) were dissolved in sterile water for IV injection. All drug doses are expressed as the salt forms listed above and all solutions were passed through a 0.2-μm sterile filter (EMD Millipore, Billerica, MA) before IV administration.

Results

Effects of *d*-Amphetamine on cocaine vs. food choice. During saline treatment, monkeys completed a similar number of trials on the 0.14 mg/kg/injection cocaine- and 10-pellet food-associated levers (Figure 3-1). Also, monkeys rarely omitted a trial during saline treatment. d-Amphetamine dose-dependently (0.037 and 0.074 mg/kg/hr) decreased the number of cocaine trials completed, and the largest d-amphetamine treatment dose (0.074 mg/kg/hr) also significantly decreased the number of food trials completed [d-amphetamine dose: $F_{3,6}$ = 12.69, p = 0.005; interaction: $F_{3,6}$ = 5.28, p = 0.04] (Figure 3-1). Increasing d-amphetamine treatment doses also tended to increase trial omissions, but the effect was not statistically significant [d-amphetamine dose: $F_{1.19,2.37}$ = 12.69, p = 0.055]. Increasing the unit cocaine dose available to 0.43

mg/kg/injection as the alternative to 10 food pellets resulted in 4.6±0.6 and 1.9±1.2 trials completed on the cocaine- and food-associated levers, respectively (data not shown). Treatment with 0.037 mg/kg/hr d-amphetamine did not significantly alter cocaine (4.2±0.1) or food (1.6±1.1) trial completions compared to saline treatment conditions (data not shown, p>0.05).

Figure 3-2 shows the time course of saline and 0.037 mg/kg/hr d-amphetamine treatment effects on cocaine and food trials completed in individual monkeys and for the group during concurrent availability of 0.14 mg/kg/injection cocaine and 10 food pellets. During 7-day saline treatment, the number of trials completed for each reinforcer was relatively stable in all monkeys, and the regression coefficients (95% CL) for group data did not differ from 0 for either cocaine (0.04; -0.16 to 0.23) or food choices (0.18; -0.02 to 0.38), indicating no systematic changes in choice over time. During 7-day 0.037 mg/kg/hr d-amphetamine, cocaine but not food choice decreased in all monkeys. For the group, the regression coefficient for cocaine was negative (-0.35; -0.59 to -0.1), indicating a significant decrease in cocaine choice over time, whereas the regression coefficient for food did not differ from 0 (0.04; -0.43 to 0.48).

Mean (± SEM) plasma d-amphetamine levels at the end of 13-day d-amphetamine treatments were 48.7 (±8.7) ng/mL for 0.019 mg/kg/hr d-amphetamine, 128.7 (±30.3) ng/mL for 0.037 mg/kg/hr d-amphetamine, and 348 (±32.6) ng/mL for 0.074 mg/kg/hr d-amphetamine. One-way ANOVA indicated a significant effect of amphetamine dose ($F_{2,4} = 39.31$, $p = 0.002$), and the post hoc test indicated that plasma amphetamine levels were higher during the 0.037 and 0.074 mg/kg/hr treatments as compared to the 0.019 mg/kg/hr d-amphetamine treatment.

Summary

Amphetamine maintenance decreased cocaine choices at 0.037 and 0.074 mg/kg/hr. The highest dose, 0.074 mg/kg/hr, also decreased food choices. These results are consistent with results seen with amphetamine maintenance in preclinical choice procedures (Banks *et al*, 2013; Thomsen *et al*, 2013) and human laboratory choice procedures (Rush *et al*, 2010). Additionally, the amphetamine plasma levels provide a benchmark to which human amphetamine plasma levels can be compared. The amphetamine doses chosen for this study were equivalent to human doses in mg/kg/day of amphetamine exposure, but route of administration and the timing of the doses differ between species. The human subjects will be getting twice daily oral doses of amphetamine while the non-human primates received a continuous IV infusion 23 hr/day, so comparison of plasma amphetamine levels will give more information about the equivalence of doses between the different dosing procedures.

I, along with the help of Katherine Nicholson, collected the amphetamine plasma samples from the monkeys, but did not analyze the samples for amphetamine concentration or learn the techniques associated with determining plasma amphetamine concentration. These analyses were performed by Justin Poklis.
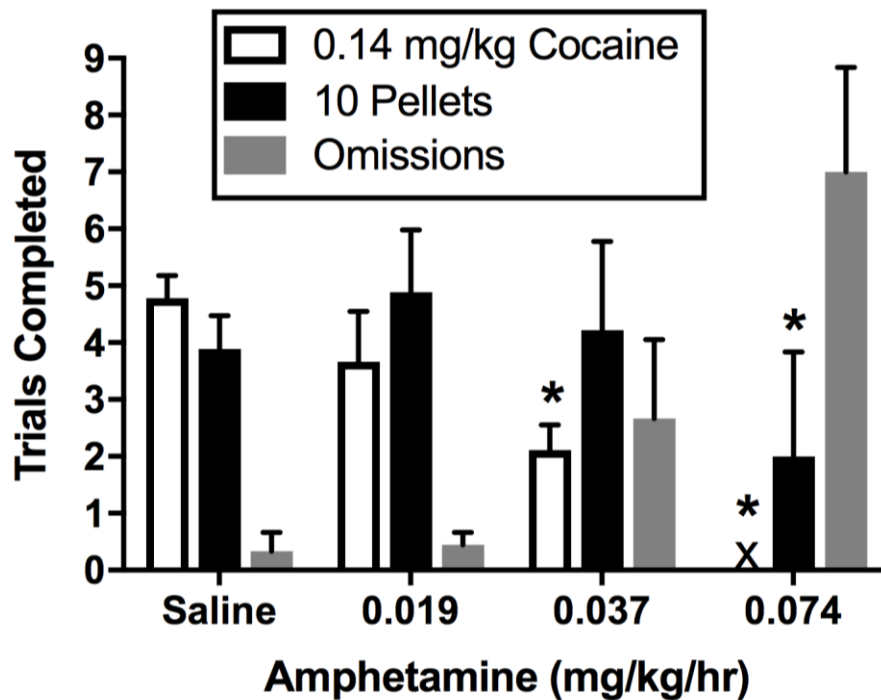
Figure 3-1. Effects of saline or amphetamine treatment on cocaine vs. food choice by rhesus monkeys. Abscissa: amphetamine treatment dose in mg/kg/hr. Ordinate: Number of trials completed for 0.14 mg/kg/inj cocaine or for 10 food pellets. Number of omissions is also shown, and all bars show mean±SEM for the last three days of treatment in three monkeys. Asterisks indicate significantly different from "Saline" as determined by a significant two-way ANOVA followed by Dunnett's post hoc test, $p < 0.05$. Note that "X" for cocaine choices during treatment with 0.074 mg/kg/hr amphetamine indicates that cocaine choices=0, and the bar is contained in the abscissa.
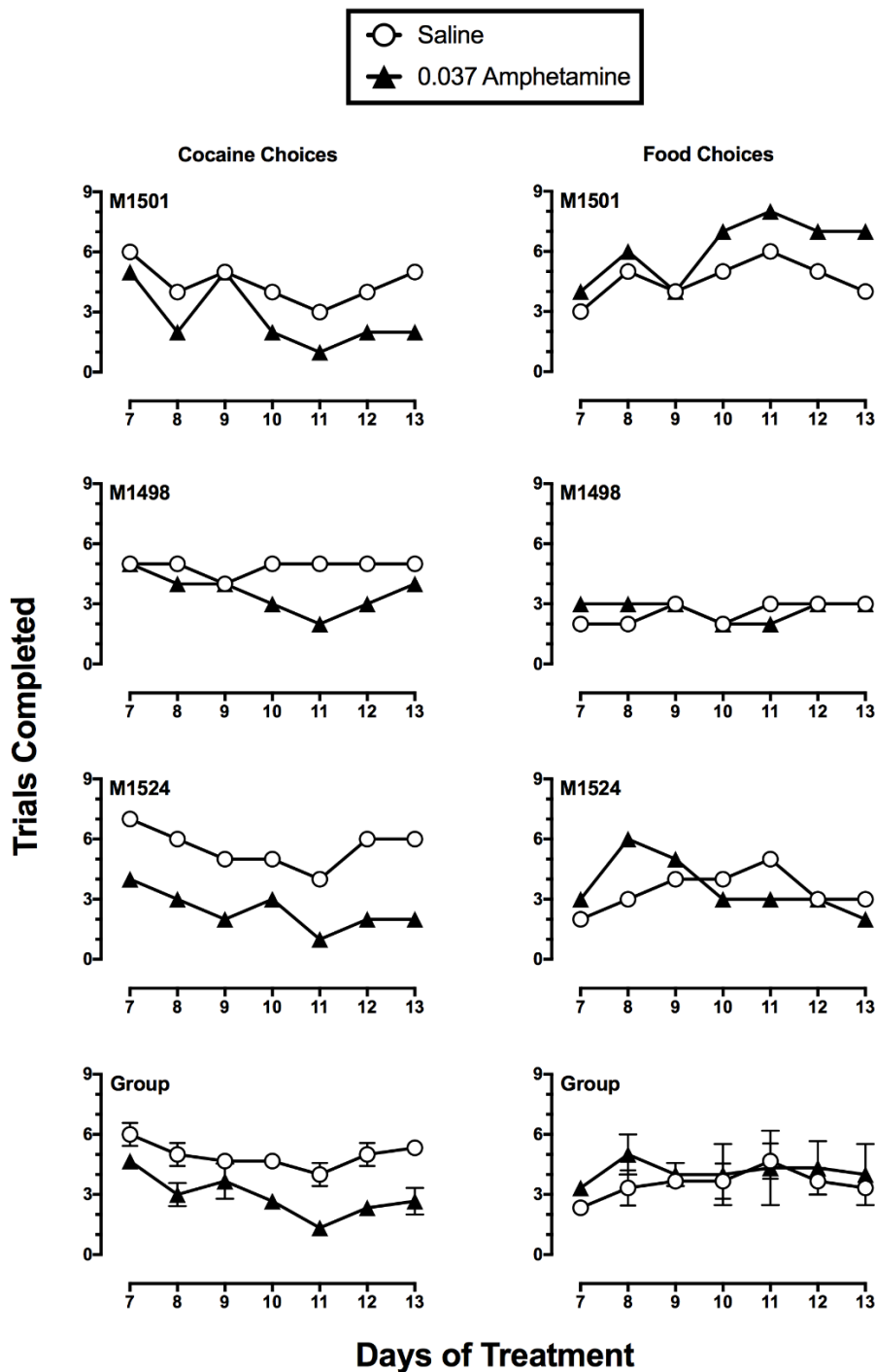
Figure 3-2. Time course of cocaine choice and food choice for individual monkeys and for the group during treatment with saline and 0.037 mg/kg/hr amphetamine. The identification number for individual monkeys is shown in the upper left corner of each panel, and bottom panels show group data. Abscissa: Days of amphetamine treatment.

Note that that choice sessions were not conducted on Days 1-6 of treatment, so graphs

show data only from Days 7-13. Ordinate: Number of cocaine choices (left panels) or

food choices (right panels) completed on each day

Chapter IV

Amphetamine Maintenance Differentially Modulates Effects

of Cocaine, Methylenedioxypyrovalerone (MDPV), and Methamphetamine

on Intracranial Self-Stimulation and Nucleus Accumbens Dopamine Release

in Rats

(Submitted for publication)

Introduction

There are more than 2 million current (i.e. past month) psychostimulant users in

the United States (SAMSHA, 2017), and no pharmacotherapies are currently approved

by the Food and Drug Administration for treatment of psychostimulant abuse. According

to the 2016 National Survey on Drug Use and Health (SAMSHA, 2017) cocaine and

methamphetamine are the two most commonly abused psychostimulants, and other

drugs such as methylenedioxypyrovalerone (MDPV) have emerged during the last

decade that may also lead to problematic use (Karila *et al*, 2017). Many

psychostimulants produce their effects by interacting with transporters for the

monoamine neurotransmitters dopamine (DA), serotonin (5-HT), and norepinephrine

(NE) (DAT, SERT, and NET, respectively) to increase synaptic monoamine levels, and

drugs that are more potent to increase DA versus 5-HT tend to produce more robust

abuse-related effects (Bauer *et al*, 2013; Rothman and Baumann, 2006; Suyama *et al*,

2016; Wee *et al*, 2005). Moreover, there are 2 broad classifications of monamine

transporter ligands based on their transporter interactions. Uptake inhibitors like cocaine and MDPV bind to the transporters and inhibit their function and promote extracellular accumulation of the associated monoamine. Releasers like methamphetamine and amphetamine are shuttled through the transporters into cells, where they cause a cascade of events that results in monoamine efflux (De Felice *et al*, 2014; Rothman *et al*, 2001).

Although no pharmacotherapies are approved to treat psychostimulant use disorders, amphetamine maintenance decreases cocaine use in double-blind placebo-controlled clinical trials (Castelles *et al*, 2016; Grabowski *et al*, 2001; Greenwald *et al*, 2010; Levin *et al*, 2015; Schmitz *et al*, 2012) and also decreases choice of cocaine over an alternative reinforcer in laboratory studies in humans, non-human primates, and rats (Banks *et al*, 2015a; Rush *et al*, 2010; Thomsen *et al*, 2013). In contrast, amphetamine maintenance is not effective to decrease methamphetamine use in either clinical trials or preclinical studies (Galloway *et al*, 2011; Schwienteck and Banks, 2015), and effects of amphetamine maintenance on abuse-related effects of other psychostimulants like MDPV are unknown. Additionally, the mechanisms that underlie selective amphetamine-maintenance effects on cocaine vs. methamphetamine use remain to be determined. Amphetamine maintenance is thought to function as an agonist-type therapy for cocaine abuse because both drugs produce similar behavioral effects and increase synaptic DA levels in brain-reward areas such as nucleus accumbens (NAc) (Grabowski *et al*, 2004a; Rothman *et al*, 2002); however, it is not clear why such an agonist-type effect would be selective for cocaine but not methamphetamine.

Intracranial self-stimulation (ICSS) is one preclinical procedure that can be used to evaluate effects of candidate medications on abuse-related drug effects (Negus and Miller, 2014), and we reported previously that an amphetamine maintenance regimen sufficient to reduce cocaine-vs.-food choice in rats (Thomsen *et al*, 2013) also blunted cocaine-induced ICSS facilitation in rats (Bauer *et al*, 2014). The present study extended this finding in three ways. First, amphetamine-maintenance effects were compared on ICSS facilitation produced by cocaine, MDPV, and methamphetamine. Second, ICSS facilitation by monoamine transporter ligands correlates with selectivity to increase DA vs. 5-HT levels in NAc (Negus and Banks, 2017; Suyama *et al*, 2016). Accordingly, we also used in vivo microdialysis to compare amphetamine-maintenance effects on cocaine, MDPV, and methamphetamine-induced changes in NAc DA and 5-HT levels. We hypothesized that amphetamine maintenance would block effects of cocaine and the other DA uptake inhibitor MDPV, but not of the DA releaser methamphetamine, on both ICSS and NAc DA levels. Lastly, effects of amphetamine maintenance on striatal DAT density were also determined, because DAT downregulation is one possible mechanism of amphetamine maintenance-induced decreases in cocaine effects (Boudanova *et al*, 2008; German *et al*, 2015).

Methods

Subjects

Studies were conducted in a total of 119 male Sprague-Dawley rats, weighing 300-350 grams at time of surgery. Rats were individually housed on a 12-hr light-dark cycle (lights on from 6 a.m.-6 p.m.) in a facility accredited by the Association for the

Assessment and Accreditation of Laboratory Animal Care. All rats had ad libitum access

to food and water in their home cages. Animal maintenance accorded with The National

Institutes of Health guidelines on care and use of research animals, and experimental

protocols were approved by the Virginia Commonwealth University Institutional Animal

Care and Use Committee.

Surgical Procedures

For implantation of microelectrodes and guide cannulae, subjects were

anesthetized with 3.0-3.5% isoflurane in oxygen until unresponsive to toe-pinch and

placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). For ICSS

studies, the cathode of a stainless steel electrode (0.25mm diameter and insulated

except at the flattened tip; MS303/1-AIU/SPC, Plastics One, Roanoke, VA, USA) was

implanted in the left medial forebrain bundle at the level of the lateral hypothalamus

(2.8mm posterior to bregma, 1.7mm lateral to the midsagittal suture, and 8.8mm ventral

to the skull). For microdialysis studies, guide cannulae (0.5mm outer diameter; CXG-8,

Eicom, San Diego, CA, USA) were implanted bilaterally and terminated 1 mm above the

nucleus accumbens (NAc; 1.5mm anterior to bregma, 1.8mm lateral to midsagittal

suture, 6.0mm ventral to dura). A dummy cannula (CXD-8, Eicom) was inserted into

each guide cannula to maintain cannula patency. Electrodes/guide cannulae were

secured to the skull using screws (Plastics One, Inc., Roanoke, VA, USA) and

orthodontic resin (Butler Schein, Dublin, OH, USA), and for ICSS studies, the anode of

the electrode (0.125mm diameter, uninsulated) was wrapped around one of the screws

to act as a ground. Ketoprofen (5 mg/kg IP) was administered immediately after

surgery and again 24 hr later as a postoperative analgesic, and rats were allowed at least seven recovery days prior to initiation of ICSS training or microdialysis testing.

For minipump implantation, rats were anesthetized with 3.0% isoflurane in oxygen until unresponsive to toe-pinch. An incision was made at the mid-scapular region and a subcutaneous pocket cleared. Osmotic minipumps (2ML2, Alzet, Cupertina, CA, USA) were inserted in the subcutaneous space, and the incision was sutured closed. Ketoprofen (5 mg/kg IP) was administered immediately after surgery as a postoperative analgesic.

Intracranial Self-Stimulation (ICSS)

Apparatus. Operant chambers consisted of sound-attenuating boxes containing modular acrylic and metal test chambers (29.2 × 30.5 × 24.1 cm). Each chamber had a response lever (4.5 cm wide, 2.0 cm deep, 3.0 cm off the floor), three stimulus lights (red, yellow, and green) centered 7.6 cm above the response lever, a 2 W house light, and an ICSS stimulator (Med Associates, St. Albans, VT, USA). Bipolar cables routed through a swivel-commutator connected the stimulator to the electrode (Model SL2C, Plastics One, Roanoke, VA, USA). Med-PC IV computer software controlled all programming parameters and data collection (Med Associates, St. Albans, VT, USA).

Training. Training, testing, and data analysis was conducted using methods described previously (Bauer *et al*, 2013, 2014; Bonano *et al*, 2014; Pereira Do Carmo *et al*, 2009). Briefly, subjects were placed into operant chambers with the house light illuminated and allowed to press a lever to receive delivery of a 0.5-sec train of square-wave cathodal pulses (0.1 msec/pulse) under a fixed-ratio (FR) 1 schedule of

reinforcement. Under the terminal schedule of reinforcement, daily behavioral sessions consisted of 3 10-min components, each consisting of 10 1-min trials. Each trial presented a different frequency of electrical stimulation available for brain stimulation, and the frequency decreased in 0.05 log increments across trials from 158-56 Hz. The first 10 sec of each trial were a time out period, during which lever presses had no scheduled consequences and 5 non-contingent stimulations were delivered. The remaining 50 sec of each trial were a response period, during which lever presses produced brain stimulation and illumination of the stimulus lights over the lever under an FR 1 schedule of reinforcement. Training continued under these conditions until performance stabilized (3 days during which the mean numbers of stimulations per trial and total stimulations per component on each day were within 15% of the running mean across days). This was completed within 12 weeks of surgery for all rats, and the final 3 days of training served to establish the "Pre-pump" baseline for ICSS performance.

Testing. Once pre-pump baseline performance was established, testing was conducted using an 8-day treatment protocol. On Days 0 and 7, rats received a series of increasing IP doses of cocaine (1, 3.2, 10 mg/kg), MDPV (0.1, 0.32, 1.0 mg/kg), or methamphetamine (0.1, 0.32, 1.0 mg/kg). Dose-effect test sessions consisted of 3 baseline ICSS components followed by 3 drug injections administered at 30-min intervals. A pair of ICSS test components began 10 min after each injection. Thus, dose-effect test sessions generated data for daily baseline performance and for effects produced by a 1-log-unit range of increasing test-drug doses. On Day 1, after completion of the first dose-effect test session, rats were surgically implanted with osmotic minipumps containing either saline, 0.1 mg/kg/hr amphetamine, or 0.32

mg/kg/hr amphetamine, and 3-component baseline ICSS sessions were conducted on Days 2-6 before the second dose-effect test session on Day 7. Separate groups of N=6 rats were used to test each minipump treatment in combination with each test drug, and only cocaine was tested with 0.1 mg/kg/hr amphetamine maintenance.

Data Analysis. For all behavioral sessions, the first ICSS component on each day was considered a "warm-up" component, and data were discarded. The primary dependent measure for the remaining components of each session was rate of reinforcement as measured by number of stimulations in each trial. The raw reinforcement rate was normalized for each rat to a percent of maximum control rate (%MCR). The maximum control rate (MCR) was defined for each rat as the average of the maximal number of stimulations in any trial of the 2nd and 3rd components of the 3 pre-pump baseline sessions. The number of stimulations for each trial during the remainder of the study was then converted to a percentage of the MCR using the equation %MCR = (reinforcement rate during a frequency trial / MCR) × 100. Data for the 2nd and 3rd components of the 3 pre-pump baseline sessions were averaged first within each rat and then across rats to generate a group mean pre-pump baseline "frequency-rate" curve in each group to relate log frequency of brain stimulation to rate of reinforcement. For dose-effect test sessions, data from the 2nd and 3rd daily-baseline components and for each pair of test components were averaged within each rat and then across rats to generate group mean frequency-rate curves for baseline and each test drug dose on that day. Frequency-rate curves were compared in two ways. First, repeated measures two-way ANOVA was used, with ICSS frequency as one factor and the experimental manipulation as the second factor. A significant ANOVA was followed

by Holm-Sidak post hoc test with the criterion for significance set at $p < 0.05$. Second,

the EF50 for each ICSS curve was defined as the effective frequency that maintained

50% MCR. EF50 values and 95% confidence limits were interpolated by linear

regression from the linear portion of each ICSS curve, and EF50 values were

considered to be different if 95% confidence limits did not overlap. In some cases, all

points were above 50% MCR; in these cases, interpolation of EF50 values was not

possible, and EF50 is shown as "<1.75" because 1.75 log Hz was the lowest frequency

tested. All analyses were conducted in Prism (Graphpad Software, La Jolla, CA).

Microdialysis

  Procedure. Microdialysis procedures were similar to those described previously

(Suyama et al., 2016). On test days, rats were briefly anesthetized with 3.0% isoflurane

in oxygen, one of the dummy cannulae was removed, and a microdialysis probe (10mm

long, CX-I-8-2, Eicom) with a 2mm artificial cellulose "cuprophan" membrane (50 kDa

molecular weight cutoff) at its tip was inserted into an 8mm guide cannula such that it

extended 2mm beyond the end of the guide cannula and into the NAc. The probe was

connected to a two-channel liquid swivel (TCS2-23, Eicom), and the rat was placed into

an acrylic experimental cage (30 cm^3). Microdialysis probes were perfused with artificial

cerebrospinal fluid (aCSF; 147 mM NaCl, 2.8mM KCl, 1.2mM CaCl2, 1.2mM MgCl2) at

a rate of 1μL/min. Mobile phase consisted of 1.5% methanol (EMD, Gibbstown, NJ,

USA), 100mM phosphate buffer (Sigma Chemicals, St. Louis, MO, USA), 500mg/L 1-

decane sodium sulfonate (TCI America, Montgomeryville, PA, USA), and 50 mg/L

EDTA-2Na+ (Dojindo Laboratories, Kumamoto, Japan). Dialysate samples were

collected into a 50 μL injector loop at 10-min intervals using an online auto-injector (EAS-20s, Eicom) and immediately analyzed for DA and 5-HT concentrations by high-performance liquid chromatography (HPLC) coupled to electrochemical detection (HTEC-500, Eicom). DA and 5HT were separated using a C_{18}-reverse phase column (PP-ODS II, Eicom) and detected using a graphite working electrode and an Ag vs. AgCl reference electrode with an applied potential of +450 mV. DA and 5-HT were identified by characteristic standard solution retention times, and concentrations were quantified by comparison with peak heights of the standard concentration curve (0.01–100 pg per 10 μL) generated prior to drug administration in each microdialysis experiment. The lower neurotransmitter detection limit was 0.1 pg.

Baseline samples were collected until DA and 5-HT levels stabilized (six consecutive baseline samples with <25% variability around the running mean of both neurotransmitters). Subsequently, a test drug dose was administered IP, and dialysate samples were collected for another 100 min. Two sets of studies were conducted. First to determine the dose-dependence of test-drug effects, saline and multiple doses of cocaine (1.0, 3.2, 10 mg/kg), MDPV (0.1, 0.32, 1.0 mg/kg), or methamphetamine (0.1, 0.32, 1.0 mg/kg) were evaluated in rats without a minipump. Each rat was tested no more than four times (no more than twice per cannula; at least one week between re-accessing a given site), and each dose of each drug was tested in 6 rats. Second, to determine effects of amphetamine maintenance on test-drug effects, rats were surgically implanted with a minipump containing either saline or 0.32 mg/kg/hr amphetamine and tested between 7 and 13 days after minipump implantation with either 10 mg/kg cocaine, 0.32 mg/kg MDPV, or 0.32 mg/kg methamphetamine. Each

minipump treatment and test drug group contained 6 rats. The test-drug doses were selected because they were each found to produce similar, approximately 200-250% increases in NAc DA levels during initial dose-effect studies. Some flexibility was instituted in the time of testing relative to minipump implantation to accommodate the occasional need for HPLC equipment repairs, but testing always occurred after at least 7 days (the treatment duration in behavioral studies) and no more than 14 days (the maximum duration of minipump drug delivery).

Data Analysis. The primary dependent variables were extracellular DA and 5-HT concentrations in each dialysate fraction. For dose-effect studies, data were expressed as a percentage of the baseline concentration for each neurotransmitter using the equation % Baseline = (test concentration / baseline concentration) * 100. For minipump studies, data were expressed as a difference (delta) from baseline (test concentration – baseline concentration) and absolute monoamine concentration in each sample. A different approach was used for these studies because saline- and amphetamine-treated rats had significantly different baseline DA levels prior to test-drug administration (see Results). Regardless of the metric, data at each time point were averaged across rats to yield group mean results. Results were analyzed for each drug dose using a repeated-measures one-way ANOVA, with time as a fixed effect and subject as a random effect (JMP Pro 11, SAS, Cary, NC). A significant ANOVA was followed by Dunnett's post hoc test to compare monoamine concentrations at each time point with control monoamine concentrations in the sample evaluated 10 min after drug administration. This sample was selected as the control because preliminary experiments conducted by probe immersion into a known standard DA concentration

indicated a lag time of ~20min for dialysate to traverse the tubing from the probe to the electrochemical detector at the 1 µL/min flow rate. Accordingly, the 10-min sample was collected prior to drug administration, had advanced into the auto-injector tubing at the time of drug injection, and was evaluated after drug injection. Baseline DA and 5-HT levels in saline-treated and amphetamine-treated rats were compared by Student's t-test with Welch's correction. The criterion for statistical significance was set at $p < 0.05$.

[³H]WIN35,428 Saturation Binding

Membrane preparation. Rats that had received no other surgeries or treatments were implanted with minipumps that delivered either saline (N=6) or 0.32 mg/kg/hr amphetamine (N=8). After 7 days of treatment, rats were euthanized by rapid decapitation, and whole striatum, including NAc and caudate/putamen, were dissected on ice and frozen at -80°C until use. On the day of each binding assay, striata were thawed in cold assay buffer (20 mM sodium phosphate buffer, pH 7.9 with 0.32 M sucrose), homogenized with a Polytron homogenizer, and centrifuged at 50,000 x g at 4°C for 10 min. The supernatant was discarded, the pellet was re-suspended by homogenization in assay buffer, and the protein concentration was determined by the Bradford method.

Binding assay. The DA transporter inhibitors WIN35,428 [(–)-2β-carbomethoxy-3β-(4-fluorophenyl)tropane] and RTI-112 [2β-carbomethoxy-3β-(3-methyl-4-chlorophenyl)tropane] were used as the radiolabeled and non-labeled ligands, respectively(Kuhar *et al*, 1999). Six concentrations of [³H]WIN35,428, ranging from approximately 0.4-30 nM, were incubated in assay buffer containing 40 µg membrane

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protein for 90 min at 30°C in a final volume of 0.25 ml. Non-specific binding was determined at each concentration of radioligand in the presence of 30 μM unlabeled RTI-112. The incubation was terminated by rapid filtration under vacuum through GF/B glass fiber filters using a 48-well Brandel harvester and rinsed 3 x with 3 ml ice-cold 50 mM Tris-HCl, pH 7.4. Bound radioactivity was determined by liquid scintillation spectrophotometry at 45% efficiency for ^3H after overnight equilibration of the filters in Econosafe scintillation fluid.

Data analysis. All binding data are reported as specific binding, derived from duplicate determinations from the 6 saline-treated and 8 amphetamine-treated rats. Single-site saturation analysis ($n_H = 1$) was conducted to determine B_{max} and K_D values by iterative curve fitting. B_{max} and K_D values were compared between groups with the two-tailed unpaired Student's t-test. All analyses were conducted in Prism.

Drugs

For behavioral and microdialysis studies, (-) cocaine HCl, (±) 3,4-methylenedioxypyrovalerone HCl, and (+)-amphetamine hemisulfate were obtained from National Institute for Drug Abuse drug supply program (Bethesda, MD, USA). (+)-Methamphetamine HCl was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in bacteriostatic saline. Cocaine, MDPV, and methamphetamine were all administered via intraperitoneal injection at a volume of 1 ml/kg. Amphetamine was delivered subcutaneously via an osmotic minipump (Alzet) at a rate of 5 μl/hr.

For receptor binding studies, [^{3}H]WIN35,428 (82.6 Ci/mmol) was purchased from Perkin-Elmer (Waltham, MA). RTI-112 was kindly provided by Dr. F. Ivy Carroll of Research Triangle Institute (Research Triangle Park, NC). Econosafe scintillation fluid was purchased from Research Products International (Prospect IL). All other chemicals were reagent grade and purchased from Sigma Chemical Co. or Fisher Scientific (Hampton, NH).

Results

Intracranial Self-Stimulation

      Pre-pump baseline performance. Subjects were assigned to one of 7 groups (N=6 per group): cocaine + saline, 0.1 mg/kg/hr or 0.32 mg/kg/hr amphetamine; MDPV + saline or 0.32 mg/kg/hr amphetamine; or methamphetamine + saline or 0.32 mg/kg/hr amphetamine. During pre-pump baseline sessions for all rats in the study, the mean ± S.E.M. maximum control rate (MCR) was 55.06 ± 1.5 reinforcements per trial and the mean EF50 (95% confidence limits) was 2.02 (2.00-2.03) log Hz. One-way ANOVA indicated no difference in MCRs across treatment groups ($F_{(6,35)}$ = 1.35, n.s.), and overlapping confidence limits indicated no difference in pre-pump baseline EF50 values across groups (Table 4-1).

      Pre-pump effects of cocaine, MDPV, and methamphetamine. Figure 4-1 and Table 4-1 show the effect of cocaine (1.0 – 10.0 mg/kg), MDPV (0.1 – 1.0 mg/kg), and methamphetamine (0.1 – 1.0 mg/kg) on Day 0, before minipumps were implanted. Data are combined for saline- and amphetamine-treated rats because these data were collected before minipumps were implanted and before treatments had started. Brain

stimulation maintained a frequency-dependent increase in reinforcement rates under baseline conditions, and all three drugs produced dose-dependent leftward/upward shifts in ICSS frequency-rate curves (see figure legends for statistical results). The largest dose of 1.0 mg/kg methamphetamine also decreased high ICSS rates at the highest 2 frequencies. Table 4-1 shows that all 3 drugs also produced dose-dependent decreases in EF50 values. EF50 values could not be determined for the highest doses of MDPV and methamphetamine, because facilitation was so robust that all points on the frequency-rate curves were above 50% MCR.

Effects of saline or amphetamine maintenance on baseline ICSS. Figure 4-2 shows the effects of saline or 0.32 mg/kg/hr amphetamine maintenance on baseline ICSS. In saline-treated rats, the Day 8 baseline frequency-rate curves were not different from the pre-pump baseline in any group. Conversely, 0.32 mg/kg/hr amphetamine maintenance facilitated ICSS in all 3 groups. Additionally, Table 4-1 shows that EF50 values in saline-treated rats were similar to pre-pump baselines; however, 0.32 mg/kg/hr amphetamine produced EF50 values lower than pre-pump baselines and lower than baselines in saline-treated rats. Figure 4-4 shows that a lower maintenance dose of 0.1 mg/kg/hr amphetamine also significantly facilitated ICSS in rats that were subsequently treated with cocaine.

Effects of cocaine, MDPV, and methamphetamine during saline or amphetamine maintenance. Figure 4-3 shows effects of cocaine, MDPV, and methamphetamine in rats treated with saline or 0.32 mg/kg/hr amphetamine, and EF50 values are shown in Table 4-1. Effects of all three drugs during saline maintenance were similar to pre-pump effects. Amphetamine maintenance produced an

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approximately 10-fold decrease in the potency of cocaine to facilitate ICSS. Thus, during saline treatment, cocaine dose-dependently facilitated ICSS at all three doses as indicated both by two-way ANOVA of frequency-rate data (Figure 4-3) and by reductions in EF50 values (Table 4-1). However, during maintenance on 0.32 mg/kg/hr amphetamine, there was only a main effect of cocaine dose and not a frequency x dose interaction. Post-hoc tests revealed that 10 mg/kg cocaine was different than baseline (see figure 4-3 legend for statistics). Moreover, only 10 mg/kg cocaine significantly reduced EF50 values. Figure 4-4 and Table 4-1 shows that maintenance on a lower amphetamine dose (0.1 mg/kg/hr) failed to blunt cocaine-induced ICSS facilitation.

Maintenance on 0.32 mg/kg/hr amphetamine had lesser effects on MDPV-induced ICSS facilitation. In saline-treated rats, the lowest dose of 0.1 mg/kg MDPV significantly increased ICSS at only one frequency (1.95 log Hz) and failed to alter the EF50. Higher doses of 0.32 and 1.0 mg/kg MDPV produced robust ICSS facilitation across a broad range of frequencies and also significantly reduced EF50 values. In rats treated with 0.32 mg/kg/hr amphetamine, 0.1 mg/kg MDPV did not facilitate ICSS at any frequency or reduce the EF50, but higher doses still facilitated ICSS by both measures.

Amphetamine maintenance also had only modest effects on methamphetamine-induced ICSS facilitation. In saline-treated rats, all methamphetamine doses facilitated ICSS both by two-way ANOVA of frequency-rate data and by significant reductions in EF50 values. In rats treated with 0.32 mg/kg/hr amphetamine, the lowest dose of 0.1 mg/kg methamphetamine no longer facilitated ICSS by analysis of frequency-rate curves, but this dose did still produce a significant, if small, decrease in EF50 value.

Moreover, higher doses of 0.32 and 1.0 mg/kg methamphetamine produced robust ICSS facilitation by both measures.

Microdialysis

Effects of cocaine, MDPV, and methamphetamine in rats without minipumps. Figure 4-8 shows that all microdialysis probe placements were in the NAc. Figure 4-5 shows the effects of saline, cocaine, MDPV and methamphetamine on NAc DA and 5-HT levels. Baseline DA and 5-HT levels were 1.46±0.09 and 0.27±0.01 pg/9uL, respectively. After saline injection, DA levels did not significantly change, but 5-HT levels increased slightly at 30 min to 109% of baseline (statistics shown in figure legend). Cocaine produced a dose- and time-dependent increase in both DA and 5-HT levels. 10 mg/kg cocaine increased DA levels across the entire dose range up to a maximum of 233% of baseline after 60 min, and increased 5-HT levels across the same dose range to a maximum of 284% of baseline after 40 min. MDPV produced a dose- and time-dependent increase in DA across the entire dose range but no increase in 5-HT at any dose tested. The 0.32 mg/kg MDPV dose used for subsequent studies increased DA levels to a maximum of 201% of baseline after 100 min. Methamphetamine, like cocaine, produced a dose- and time-dependent increase in DA across the entire dose range and also increased 5-HT levels, but only at the highest 2 doses tested. The 0.32 mg/kg methamphetamine dose used for subsequent studies increased DA levels to a maximum of 238% of baseline after 50 min, and 5-HT levels to a maximum of 147% of baseline after 30 min.

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Effects of saline or amphetamine maintenance on baseline DA and 5-HT

levels. Mean ± SEM DA levels in the NAc were increased by 7 days of 0.32 mg/kg/hr

amphetamine treatment (9.28 ± 0.75 pg/9μL) as compared to 7 days of saline treatment

(1.67 ± 0.52 pg/9 μL; $t_{(17.26)}$ = 7.68, p < .001). Conversely, mean ± SEM 5-HT levels in

the NAc were similar in amphetamine-treated rats (0.38 ± 0.08 pg/9μL) as compared to

the saline-treated rats (0.28 ± 0.06 pg/9 μL; $t_{(28.04)}$ = 1.69, n.s.).

Effects of cocaine, MDPV, and methamphetamine during saline or

amphetamine maintenance. Figure 4-6 shows the effects of cocaine, MDPV, and

methamphetamine on NAc DA and 5-HT levels after 7 treatment days with saline or

0.32 mg/kg/hr amphetamine. Data are expressed as change from baseline (delta) rather

than % baseline due to the significant difference in DA baselines between the groups.

These data are also graphed as absolute concentrations in Figure 4-7 to show the

differences in DA baseline and the effects of drugs relative to those altered baselines.

Cocaine (10 mg/kg) significantly increased NAc DA levels after saline treatment

but not after 7 days of 0.32 mg/kg/hr amphetamine treatment. Conversely, 10 mg/kg

cocaine increased 5-HT levels in both saline- and amphetamine-treated rats. Thus,

amphetamine maintenance selectively blocked cocaine-induced increases in NAc DA.

MDPV (0.32 mg/kg) also significantly increased NAc DA levels in saline-treated rats but

not in amphetamine-treated rats. MDPV did not significantly alter 5-HT levels in either

saline- or amphetamine-treated rats. Thus, amphetamine maintenance also blocked

MDPV-induced increases in NAc DA. Methamphetamine (0.32 mg/kg) increased NAc

DA and 5-HT levels in both saline- and amphetamine-treated rats. Thus,

methamphetamine failed to block methamphetamine-induced increases in NAc DA and 5-HT.

[^{3}H]WIN35,428 Saturation Binding

Mean ± SEM B_{max} values in rats maintained on saline or 0.32 mg/kg/hr amphetamine were 1.52 ± 1.4 pmol/mg and 1.37 ± 0.04 pmol/mg of membrane protein, respectively. Mean ± SEM K_D values in rats maintained on saline or 0.32 mg/kg/hr amphetamine were 14.5 ± 1.8 nM and 14.9 ± 1.5 nM, respectively. Neither the B_{max} nor K_D values differed significantly between the two groups, indicating that amphetamine maintenance at this dose did not affect striatal DAT levels or binding affinity for this radioliagand.

Discussion

This study compared effects of amphetamine maintenance on abuse-related behavioral and neurochemical effects of cocaine, MDPV, and methamphetamine in rats. There were three main findings. First, cocaine, MDPV, and methamphetamine all produced dose-dependent increases in ICSS facilitation and NAc DA levels before treatment, although only cocaine and methamphetamine increased NAc 5HT levels. This is consistent with the previously published effects of these compounds in ICSS (Bauer *et al*, 2013, 2014; Bonano *et al*, 2014) and in microdialysis (Andrews and Lucki, 2001; Baumann *et al*, 2012; Schindler *et al*, 2016). Second, on day 7 of amphetamine maintenance both baseline ICSS and NAc DA levels were elevated, but there was no significant change in baseline 5HT levels or in the density or binding affinity of striatal DAT. Finally, amphetamine maintenance blunted the effects of cocaine on both ICSS

and NAc DA levels while having little effect on methamphetamine-induced increases in ICSS or NAc DA. Conversely, amphetamine maintenance did not block effects of either cocaine or methamphetamine on NAc 5HT levels, and for MDPV, amphetamine maintenance had little effect on ICSS facilitation, but did block increases in NAc DA. Taken together, these results are consistent with the conclusion that amphetamine maintenance attenuates abuse-related behavioral effects of cocaine by reducing cocaine effects on NAc DA while conserving cocaine effects on NAc 5HT. These results also suggest that amphetamine maintenance might be more effective as a pharmacotherapy for cocaine abuse than for MDPV or methamphetamine abuse.

The increase in ICSS baseline during amphetamine maintenance replicated findings from a previous publication (Bauer *et al*, 2014). The increase in baseline NAc DA levels, but not 5-HT levels) during amphetamine maintenance is also consistent with the idea that the behavioral effects seen in ICSS may reflect DA-system functioning, and are also consistent with the *in vitro* selectivity profile of amphetamine as a substrate for DAT>SERT (Rothman *et al*, 2001) and the *in vivo* selectivity e of acute amphetamine to increase NAc DA but not 5-HT (Suyama *et al*, 2016).

The effectiveness of 0.32 mg/kg/hr amphetamine to blunt ICSS facilitation by 10 mg/kg cocaine also replicated findings from a previous study (Bauer *et al*, 2014). This study expands on the cocaine dose range tested in the previous data, showing that 0.32 mg/kg/hr amphetamine is effective to block ICSS facilitation caused by lower cocaine doses and produce an approximate 10-fold rightward shift in the cocaine dose-effect curve for ICSS facilitation. These effects are consistent with decreased choice of cocaine during amphetamine maintenance in laboratory choice studies in humans,

monkeys and rats (Banks *et al*, 2015a; Rush *et al*, 2010; Thomsen *et al*, 2013). The microdialysis data reflect the ICSS data in that the abuse-related effect of cocaine, the NAc DA-increasing effect of 10 mg/kg, is abolished during amphetamine maintenance, while the 5-HT-increasing effect of cocaine is preserved. Selective 5-HT uptake inhibitors do not facilitate ICSS (Rosenberg *et al*, 2013) nor are they self-administered (Roberts *et al*, 1999).

Amphetamine maintenance is less effective to blunt ICSS facilitation by 0.32 or 1 mg/kg methamphetamine than cocaine. This is consistent with the clinical and self-administration data on amphetamine maintenance for methamphetamine use, which suggests that amphetamine is not an effective pharmacotherapy for decreasing methamphetamine use (Galloway *et al*, 2011; Pike *et al*, 2014; Schwienteck and Banks, 2015). The effect of amphetamine maintenance on NAc DA-increasing effect methamphetamine matches the effect seen in the ICSS experiment in that 0.32 mg/kg methamphetamine increased NAc DA and 5-HT levels during 0.32 mg/kg/hr amphetamine treatment. This effect is also consistent with increases in NAc DA versus 5-HT levels reflecting both ICSS facilitation and self-administration of methamphetamine during amphetamine maintenance.

In the ICSS experiment, amphetamine maintenance did not block the effects of 0.32 or 1 mg/kg MDPV. This profile reflects the effects seen with methamphetamine in ICSS and suggests that amphetamine maintenance would be unlikely to be effective to decrease MDPV use in the clinic. The effect of amphetamine maintenance on the NAc DA-increasing effect of MDPV is similar to the effect of amphetamine maintenance on cocaine. Amphetamine maintenance blocks the DA increase after an injection of 0.32

mg/kg MDPV; however, MDPV lacks the 5-HT increasing effects of cocaine, potentially providing an explanation for the different effect of amphetamine maintenance on the abuse-related behavioral effects of cocaine and MDPV. These data suggest that blocking the DA increase caused by MDPV is not sufficient to block the abuse-related effects of this drug. Additionally, these data suggest that retaining the 5-HT-increasing effect of cocaine may be an important factor in the effectiveness of amphetamine maintenance for the treatment of cocaine use disorder. More studies are needed to determine the role of 5-HT in pharmacotherapy effects for MDPV and cocaine, because these drugs also differ in binding affinity at DAT and time course, both of which are factors that could play a role in the effects of a potential pharmacotherapy.

Effects of amphetamine maintenance on DAT density were evaluated because some evidence suggests that amphetamine can reduce DAT function at least in part by promoting DAT internalization and downregulation. However, previous studies have found that in vivo amphetamine treatments sufficient to reduce DAT function did not reduce DAT expression (German *et al*, 2015). The present study extends on these findings by showing that a regimen of amphetamine maintenance sufficient to reduce abuse-related cocaine effects also did not reduce DAT density. These results suggest that amphetamine maintenance does not reduce abuse-related cocaine effects by reducing DAT density, although DAT function may be suppressed despite sustained DAT expression.

Table 4-1. EF50 values (95% confidence limits) in log Hz after administration of
cocaine, MDPV, or methamphetamine in rats treated chronically with either saline or
0.32 mg/kg/hr amphetamine. * indicates significantly different from baseline as
determined by non-overlapping confidence limits. † indicates all points >50% MCR, and
EF50 could not be calculated. This outcome was also considered to be significantly
different from baseline.

Drug	Dose (mg/kg)	Prepump	Saline	Amphetamine
Cocaine	Baseline	2.02 (1.99-2.05)	2.02 (2.02-2.03)	1.90 (1.88-1.92)
	1.0	1.93 (1.91-1.94)*	1.96 (1.93-1.99)*	1.87 (1.83-1.90)
	3.2	1.89 (1.88-1.90)*	1.88 (1.83-1.92)*	1.91 (1.88-1.93)
	10	1.75 (1.49-1.80)*	1.77 (1.57-1.83)*	1.83 (1.82-1.84)*
MDPV	Baseline	2.02 (1.97-2.06)	2.01 (2.01-2.02)	1.86 (1.80-1.89)
	0.1	1.97 (1.96-1.99)	1.98 (1.94-2.02)	1.84 (1.76-1.89)
	0.32	1.80 (1.78-1.82)*	<1.75†	<1.75†
	1.0	<1.75†	<1.75†	<1.75†
Metham-phetamine	Baseline	2.01 (1.99-2.04)	2.03 (2.00-2.05)	1.92 (1.91-1.94)
	0.1	1.95 (1.94-1.95)*	1.93 (1.92-1.94)*	1.89 (1.88-1.90)*
	0.32	1.83 (1.82-1.83)*	1.83 (1.78-1.87)*	1.77 (1.75-1.79)*
	1.0	<1.75†	<1.75†	<1.75†

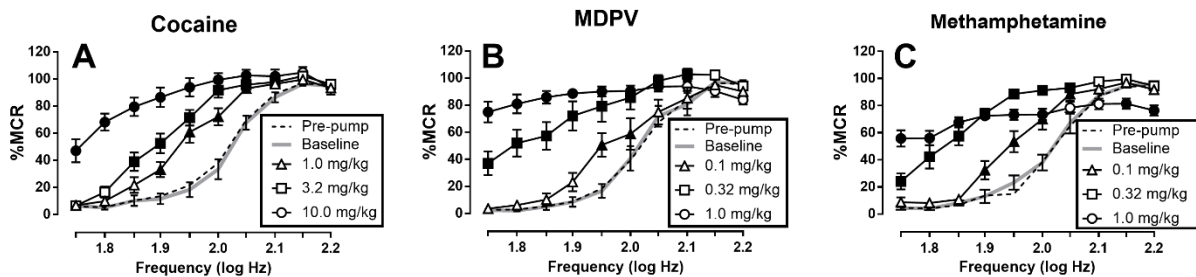**Figure 4-1.** Effects of cocaine (A, n=18), MDPV (B, n=12), and methamphetamine (C, n=12) on ICSS before minipumps were implanted. Abscissae: Brain stimulation frequency in log Hz. Ordinates: % Maximum control rate. All points show mean±SEM, and filled points indicate significantly different from "Baseline" as determined by 2-way ANOVA followed by the Holm-Sidak post hoc test ($p < 0.05$). For each panel, 2-way ANOVA indicated significant main effects of frequency and dose and a significant interaction. Interaction results for each panel are as follows: (A) cocaine ($F_{(27,459)} = 15.28$, $p < 0.0001$), (B) MDPV ($F_{(27,297)} = 12.4$, $p < 0.0001$), and (C) methamphetamine ($F_{(27,297)} = 20.62$, $p < 0.0001$).
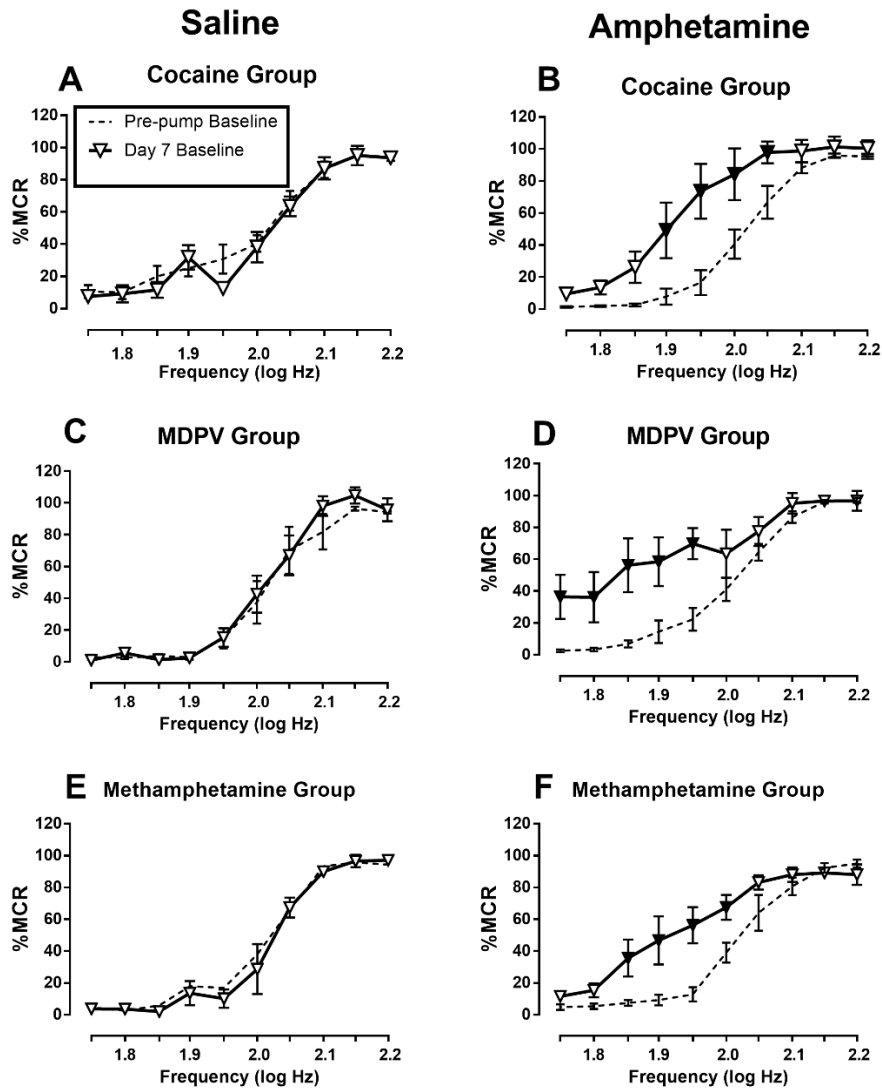
Figure 4-2. Effects of maintenance on saline or 0.32 mg/kg/hr amphetamine on

baseline ICSS performance in rats subsequently tested with cocaine (A,B), MDPV

(C,D), or methamphetamine (E,F). Abscissae: Brain stimulation frequency in log

Hz. Ordinates: % Maximum control rate. All points show mean±SEM from N=6 rats,

and filled points indicate significantly different from "Pre-pump Baseline" as determined

by 2-way ANOVA followed by the Holm-Sidak post hoc test (p < 0.05). In all saline

groups (panels A, C, and E), there was a main effect of frequency, but no main effect of

day and no significant interaction. In all amphetamine groups (panels B, D, and F), there were main effects of frequency and day, as well as a significant interaction. Interaction results are as follows: (B) $F_{(9,45)}$ = 3.45, p = 0.0027, (D) $F_{(9,45)}$ = 4.18, p = 0.0006, (F) $F_{(9,45)}$ = 3.24, p = 0.0041.
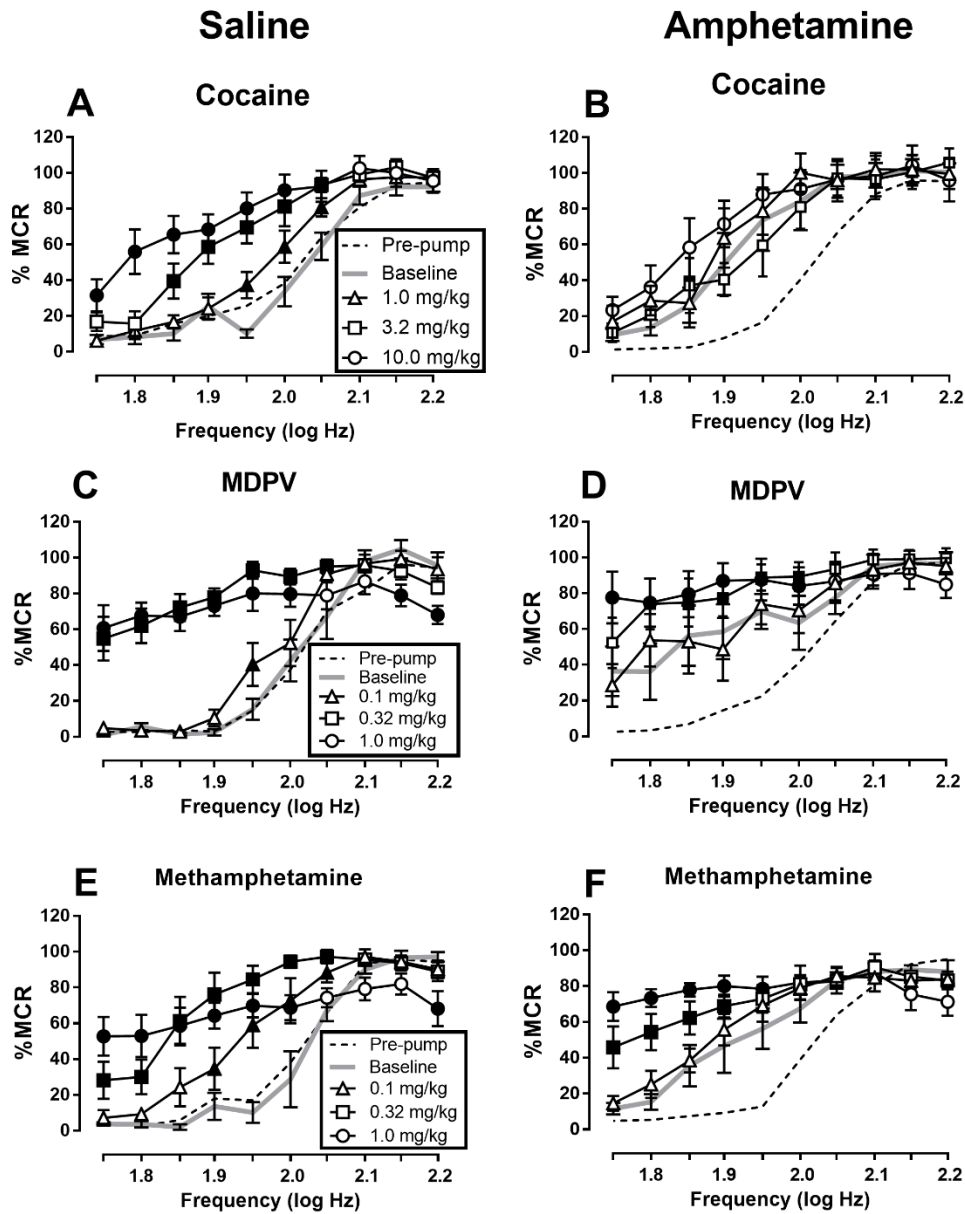
Figure 4-3. Effects of cocaine (A, B), MDPV (C, D), and methamphetamine (E, F) on

ICSS on day 7 of treatment with either saline (A, C, E) or 0.32 mg/kg/hr amphetamine

(B, D, F). Abscissae: Brain stimulation frequency in log Hz. Ordinates: % Maximum

control rate. All points show mean±SEM from N=6 rats, and filled points indicate

significantly different from "Baseline" as determined by 2-way ANOVA followed by the Holm-Sidak post hoc test (p < 0.05). For each panel, 2-way ANOVA indicated significant main effects of frequency, all but panel D had a main effect of dose, and all but panel B had a significant interaction. Interaction results for each panel are as follows: (A) $F_{(27,135)}$ = 3.93, p < 0.0001, (B) $F_{(27,135)}$ = 1.52, n.s., (C) $F_{(27,135)}$ = 11.03, p < 0.0001, (D) $F_{(27,135)}$ = 3.32, p < 0.0001, (E) $F_{(27,135)}$ = 9.13, p < 0.0001, (F) $F_{(27,135)}$ = 6.05, p < 0.0001. Although there was no interaction in panel B, there was a main effect of cocaine dose ($F_{(3,15)}$ = 3.92, p = 0.030), and Dunnett's post-hoc test (p < .05) indicated that 10 mg/kg cocaine was different than baseline.
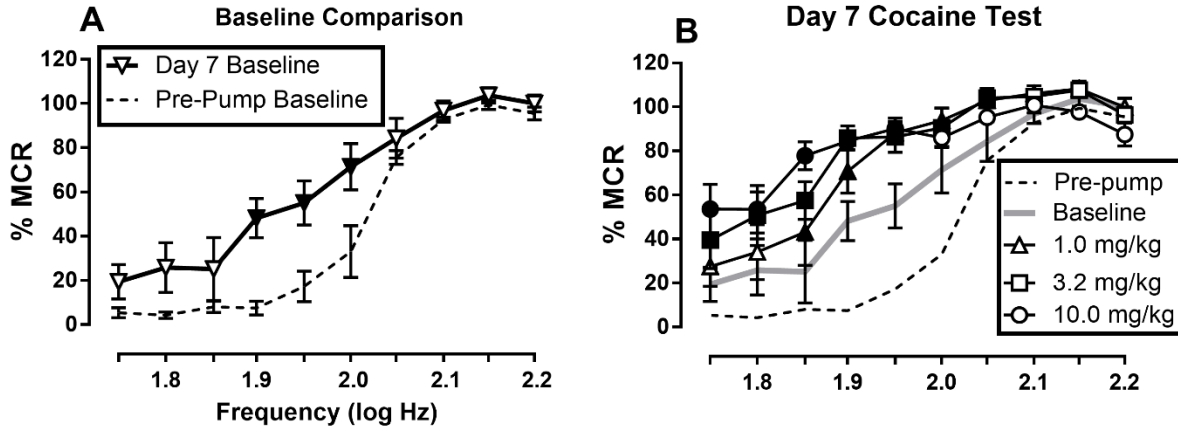
0.1 mg/kg/hr Amphetamine

Figure 4-4. Effect of 0.1 mg/kg/hr amphetamine maintenance on (A) baseline ICSS and (B) cocaine-induced facilitation of ICSS. Abscissae: Brain stimulation frequency in log Hz. Ordinates: % Maximum control rate. All points show mean±SEM in N=6 rats, and filled points indicate significantly different from (A) "Pre-Pump Baseline" or (B) "Baseline" as determined by 2-way ANOVA followed by the Holm-Sidak post hoc test (p < 0.05). F-values only reported for interactions. In panel A, there was a main effect of frequency, main effect of day, and significant interaction ($F_{(9,45)}$ = 3.80, p = .0013). Additionally, mean (95%CL) EF50 values in log Hz differed between the pre-pump baseline [2.03 (2.01-2.05)] and Day 7 baseline [1.92 (1.89-1.94)]. In panel B, there was a main effect of frequency, main effect of cocaine, and a significant dose x frequency interaction ($F_{(27,135)}$ = 5.60, p < .0001). Additionally, cocaine produced dose-dependent and significant decreases in mean (95%CL) EF50 values: Baseline, 1.92 (1.89-1.94); 1.0 mg/kg cocaine, 1.84 (1.80-1.88); 3.2 mg/kg cocaine, 1.80 (1.72-1.83); 10 mg/kg cocaine, 1.74 (1.57-1.80).
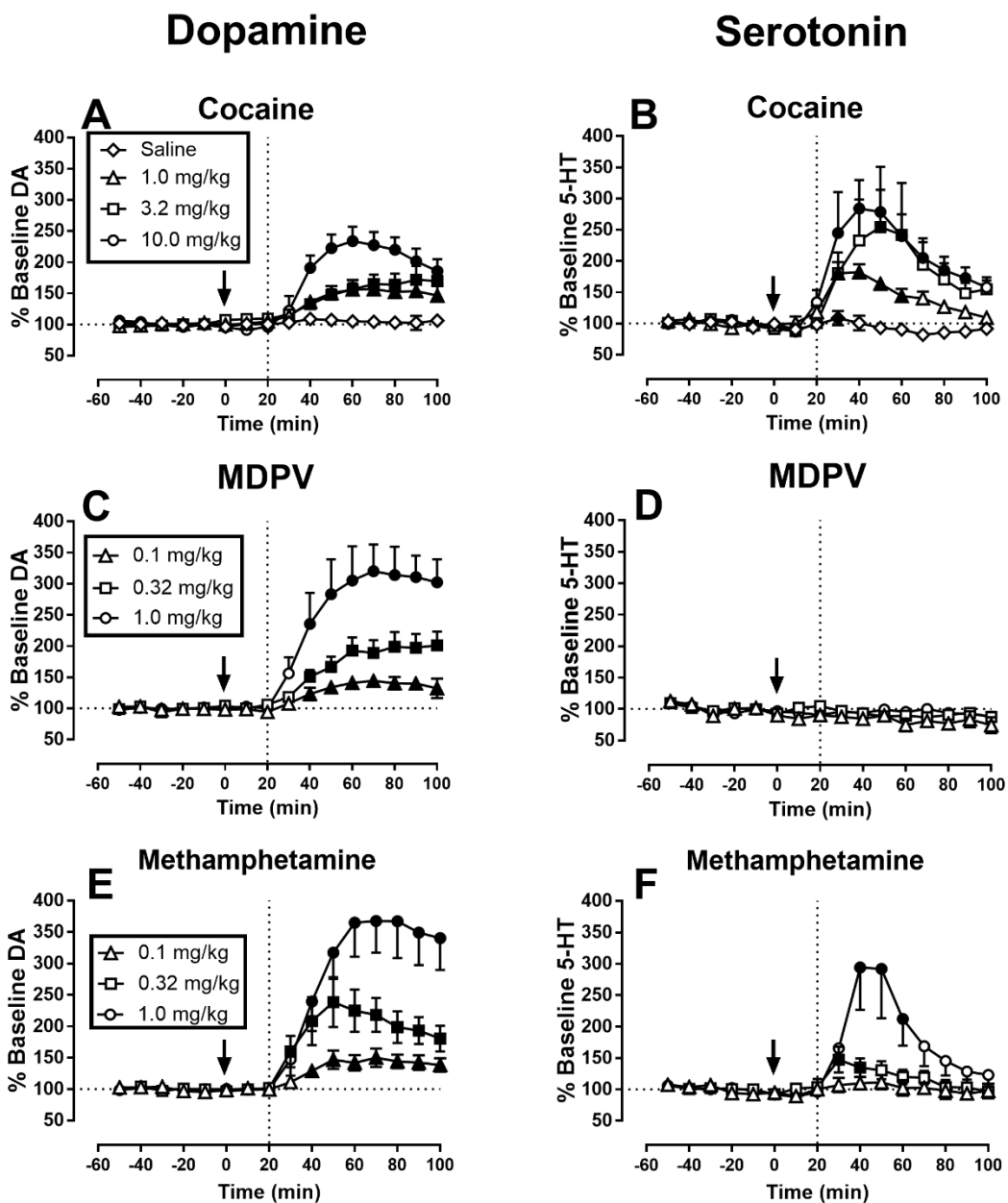
Figure 4-5. Effects of cocaine (A, B), MDPV (C, D), and methamphetamine (E, F) on

NAc DA (A, C, E) and 5-HT (B, D, F) levels. Abscissae: Time in minutes relative to test-

drug injection. Vertical line at 20 min indicates earliest time of drug effect (see

Methods). Ordinates: % of Baseline DA or 5-HT. All points show mean±SEM for N=6

rats, and filled points indicate significantly ($p < .05$) different from the 10-min time point. Statistical results are as follows: (A) saline: not significant, 1.0 mg/kg: $F_{(9,45)} = 10.33$, $p < 0.0001$, 3.2 mg/kg: $F_{(9,45)} = 9.28$, $p < 0.0001$, 10.0 mg/kg: $F_{(9,45)} = 16.62$, $p < 0.0001$; (B) saline: $F_{(9,45)} = 3.97$, $p = 0.0009$, 1.0 mg/kg: $F_{(9,45)} = 7.74$, $p < 0.0001$, 3.2 mg/kg: $F_{(9,45)} = 2.32$, $p = 0.0305$, 10.0 mg/kg: $F_{(9,45)} = 6.91$, $p < 0.0001$; (C) 0.1 mg/kg: $F_{(9,45)} = 13.13$, $p < 0.0001$, 0.32 mg/kg: $F_{(9,45)} = 24.64$, $p < 0.0001$, 1.0 mg/kg: $F_{(9,45)} = 10.33$, $p < 0.0001$; (D) no significant effects; (E) 0.1 mg/kg: $F_{(9,45)} = 9.27$, $p < 0.0001$, 0.32 mg/kg: $F_{(9,45)} = 11.89$, $p < 0.0001$, 1.0 mg/kg: $F_{(9,45)} = 10.26$, $p < 0.0001$; (F) 0.1 mg/kg: not significant, 0.32 mg/kg: $F_{(9,45)} = 4.44$, $p = 0.0003$, 1.0 mg/kg: $F_{(9,45)} = 7.37$, $p < 0.0001$.
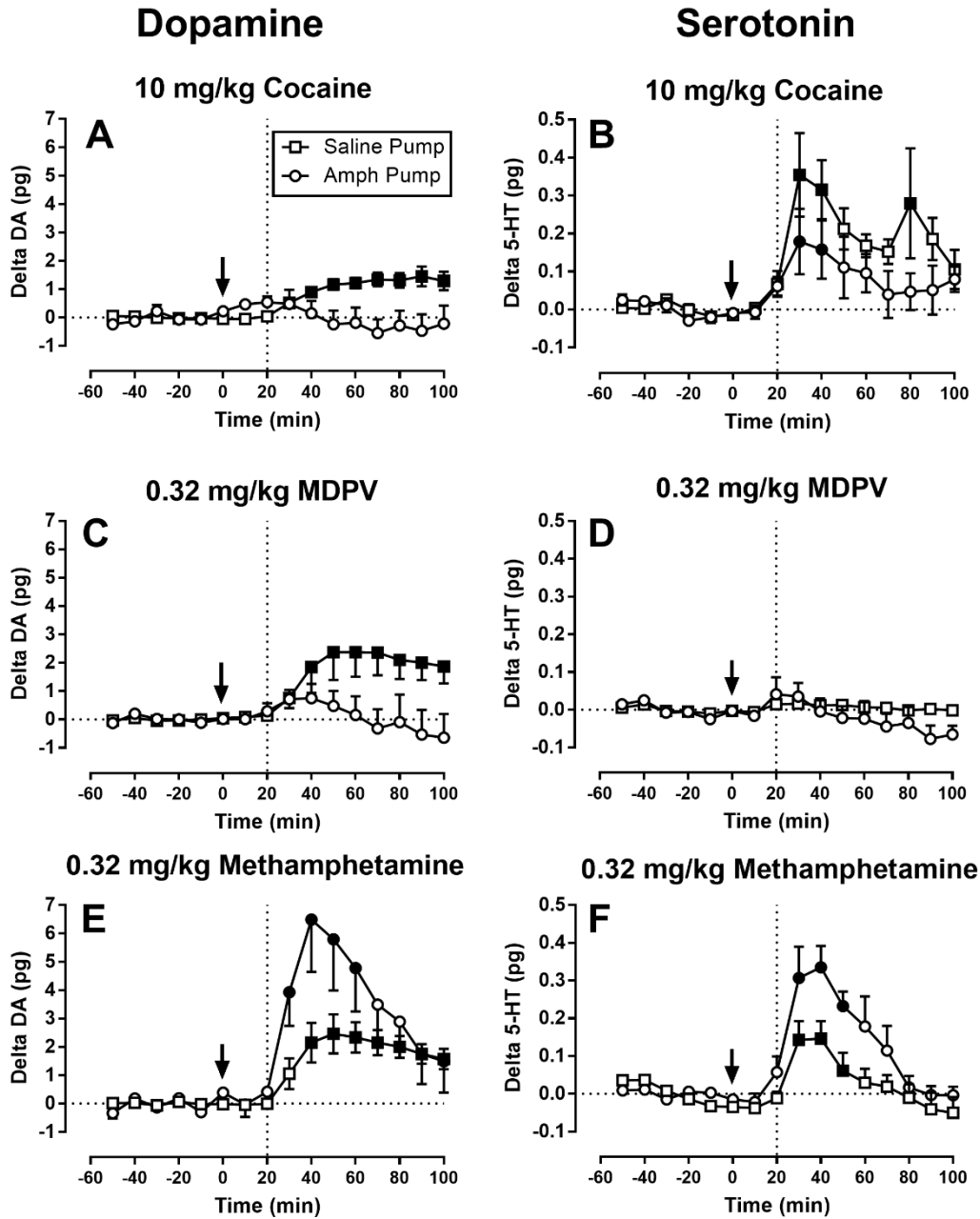
Dopamine

10 mg/kg Cocaine

A

Delta DA (pg)

- □ Saline Pump
- ○ Amph Pump

Time (min)

0.32 mg/kg MDPV

C

Delta DA (pg)

Time (min)

0.32 mg/kg Methamphetamine

E

Delta DA (pg)

Time (min)

Serotonin

10 mg/kg Cocaine

B

Delta 5-HT (pg)

Time (min)

0.32 mg/kg MDPV

D

Delta 5-HT (pg)

Time (min)

0.32 mg/kg Methamphetamine

F

Delta 5-HT (pg)

Time (min)

Figure 4-6. Effects of cocaine (A, B), MDPV (C, D), and methamphetamine (E, F) on

NAc DA (A, C, E) and 5-HT (B, D, F) levels after saline or amphetamine treatment.

Abscissae: Time in minutes relative to test drug injection. Vertical line at 20 min

indicates earliest time of drug effect (see Methods). Ordinates: change from baseline

DA or 5-HT in pg/9μl. Note that there is a different range used in DA panels (A, C, E) versus 5-HT panels (B, D, F) due to lower 5-HT levels in the NAc. All points show mean±SEM for N=6 rats, and filled points indicate significantly (p < .05) different from the 10-min time point. Statistical results are as follows: (A) saline: $F_{(9,45)} = 10.65$, $p < 0.0001$, amphetamine: $F_{(9,45)} = 1.26$, n.s.; (B) saline: $F_{(9,45)} = 3.68$, $p = 0.0016$, amphetamine: $F_{(9,45)} = 2.68$, $p = 0.0224$; (C) saline: $F_{(9,45)} = 5.13$, $p < 0.0001$, amphetamine: $F_{(9,45)} = 1.56$, n.s.; (D) saline: $F_{(9,45)} = 0.90$, n.s., amphetamine: $F_{(9,45)} = 3.16$, $p = 0.0019$; (E) saline: $F_{(9,45)} = 10.31$, $p < 0.0001$, amphetamine: $F_{(9,45)} = 5.88$, $p < 0.0001$; (F) saline: $F_{(9,45)} = 13.79$, $p < 0.0001$, amphetamine: $F_{(9,45)} = 6.63$, $p < 0.0001$.
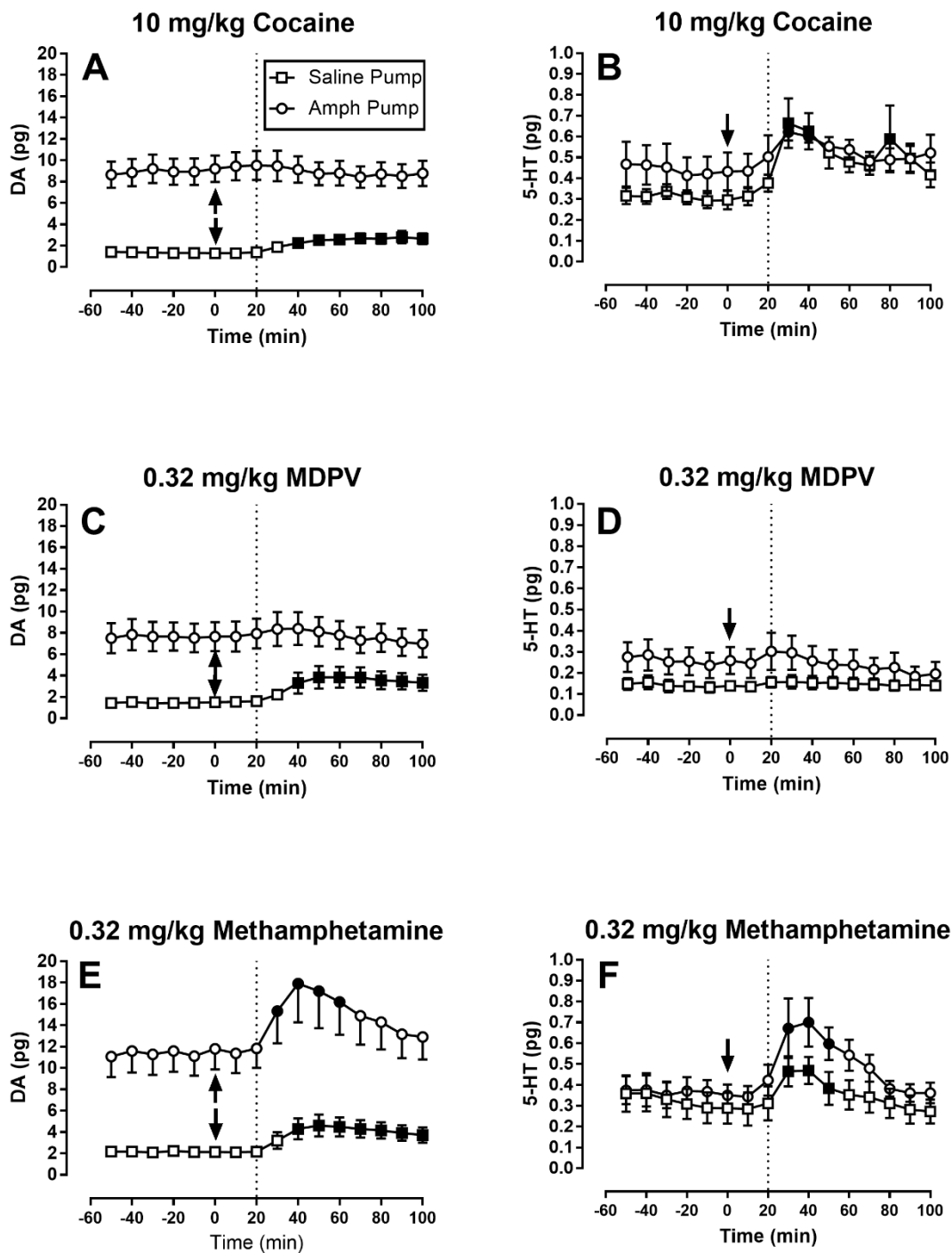
Figure 4-7. Effects of cocaine (A, B), MDPV (C, D), and methamphetamine (E, F) on

NAc DA (A, C, E) and 5-HT (B, D, F) levels after saline or amphetamine treatment.

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Abscissae: Time in minutes relative to test-drug injection. Vertical line at 20 min indicates earliest time of drug effect (see Methods). Ordinates: DA and 5-HT levels in the NAc in pg/9µl. Note that there is a different range used in the DA panels (A, C, E) versus the 5-HT panels (B, D, F) due to lower 5-HT levels in the NAc. All points show mean±SEM in N=6 rats, and filled points indicate significantly (p < .05) different from the 10-min time point. Statistical results are as follows: (A) saline: $F_{(9,45)}$ = 10.65, $p <$ 0.0001, amphetamine: $F_{(9,45)}$ = 1.20, n.s.; (B) saline: $F_{(9,45)}$ = 3.68, p = 0.0016, amphetamine: $F_{(9,45)}$ = 2.46, p = 0.0224; (C) saline: $F_{(9,45)}$ = 5.13, $p <$ 0.0001, amphetamine: $F_{(9,45)}$ = 1.54, n.s.; (D) saline: $F_{(9,45)}$ = 0.91, n.s., amphetamine: $F_{(9,45)}$ = 3.61, p = 0.0019; (E) saline: $F_{(9,45)}$ = 10.31, $p <$ 0.0001, amphetamine: $F_{(9,45)}$ = 5.87, $p <$ .0001; (F) saline: $F_{(9,45)}$ = 13.79, $p <$ 0.0001, amphetamine: $F_{(9,45)}$ = 6.63, $p <$ 0.0001.
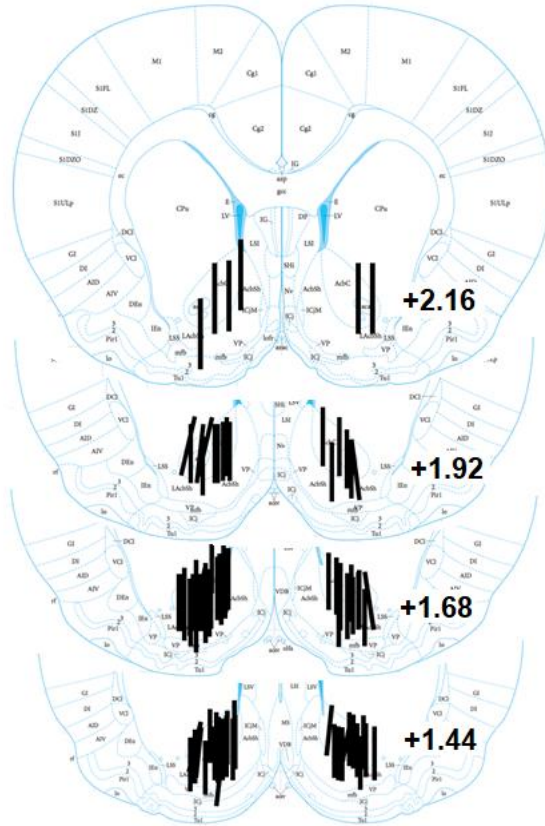
Figure 4-8. Coronal sections showing probe placements in rats used in microdialysis studies. Numbers indicate anterior position of slice relative to bregma. Figures were produced based on comparisons to Paxinos and Watson, 2007.

Chapter V

Discussion

Summary

The overall focus of this dissertation has been on the effectiveness of amphetamine maintenance to treat cocaine use disorder. Amphetamine maintenance has been shown to decrease metrics of cocaine use in humans, nonhuman primates, and rodents (Banks *et al*, 2015a; Rush *et al*, 2010; Thomsen *et al*, 2013). However, amphetamine is not an ideal pharmacotherapy for cocaine use disorder because it possesses abuse liability of its own and it only works for about 30% of users. There is need for a better pharmacotherapy that is more effective and has less abuse liability. Toward that end, chapters II and III of this dissertation focused on development of the non-human primate half of a non-human primate-to-human translational model for testing potential pharmacotherapies for cocaine use disorder. We found that both food pellets and cocaine maintained responding in a dose- and magnitude-dependent manner and that non-human primates would respond for these reinforcers in a session that was set up to mirror the human laboratory procedure. Once choice between the reinforcers was introduced, non-human primates chose between food pellets and cocaine, and their choice behavior was sensitive to changes in cocaine dose as well as food reinforcer magnitude. This procedure was then validated using amphetamine and the amphetamine prodrug, lisdexamfetamine. These drugs decreased cocaine choices

in non-human primates, but did not produce full reallocation of behavior toward the food alternative. The effects seen with amphetamine and lisdexamfetamine provided some preliminary evidence that this procedure may be able to predict treatment effects of new, potential pharmacotherapies for cocaine use disorder.

The experiments in chapter IV of this dissertation focused on elucidating the mechanism of amphetamine effects on cocaine-taking behavior. Amphetamine maintenance was tested against the abuse-related effects of cocaine, MDPV, and methamphetamine in ICSS and microdialysis. In line with the clinical effects, amphetamine maintenance attenuated the abuse-related behavioral and neurochemical effects of cocaine and was less effective in both procedures against the abuse-related effects of methamphetamine. Amphetamine maintenance blocked the abuse-related neurochemical but not behavioral effects of MDPV, suggesting that amphetamine maintenance would be less effective against MDPV use disorders than cocaine use disorders. These experiments provided insight into the mechanism of amphetamine maintenance decreasing cocaine use.

Development of a Novel Translational Non-Human Primate Choice Procedure

These experiments were conducted to develop a model of non-human primate cocaine-versus-food choice that may help streamline the medication development process for cocaine use disorder. The medication development process benefits from a strong preclinical component that can screen out compounds that would be unlikely to be effective in the clinic or that may produce unwanted side effects. Screening out those compounds would reduce risks and costs associated with running unsuccessful human

laboratory drug self-administration or clinical trials. However, procedural differences between preclinical and human laboratory experimental designs could affect this translational process. For example, some preclinical drug self-administration studies train subjects to self-administer cocaine (or some other abused drug) under simple schedules of reinforcement, under which cocaine is the only reinforcer available, and the primary dependent variable is rate of responding or rate of reinforcement (Mello and Negus, 1996; Negus and Banks, 2011). Test drugs can then be evaluated for their effectiveness to reduce rates of cocaine self-administration, and drugs that reduce cocaine self-administration are sometimes suggested as candidate pharmacotherapies that might reduce clinical cocaine use. However, these simplistic preclinical experimental designs differ from clinical patterns of abuse and treatment in numerous respects. Three will be mentioned here. First, clinical drug abuse exists in a complex environment in which other reinforcers are available, and subjects allocate their behavior between these reinforcers. Under these circumstances, cocaine abuse is manifested as excessive use of cocaine at the expense of behaviors maintained by other, more adaptive reinforcers, and a goal of treatment is not only to reduce cocaine use, but also to promote reallocation of behavior away from cocaine use and toward responding maintained by other reinforcers. Second, treatments for drug abuse in general and cocaine abuse in particular are not administered acutely, but rather are administered chronically for weeks, months, or years, and it is well established that treatment effects on drug self-administration can change from acute to chronic treatment. Lastly, treatments can decrease rates of cocaine self-administration not only by reducing sensitivity to the reinforcing effects of cocaine, but also by impairing the

subject's motoric ability to respond, and such non-selective behavioral depression is undesirable in a candidate medication. These types of differences in preclinical research and clinical practice likely contribute to failed translation of results. As one example, acute administration of dopamine receptor antagonists like flupenthixol dose-dependently decreases cocaine self-administration under simple schedules of reinforcement, and this type of result contributed to consideration of these antagonists as treatments for cocaine abuse (Ettenberg *et al*, 1982; Negus *et al*, 1996). However, dopamine receptor antagonists have failed in the clinic, and further preclinical research has suggested that this failure reflects (a) the non-selective effects of DA receptor antagonists to produce general behavioral disruption, (b) the potential for tolerance to develop to the motoric effects of DA receptor antagonists during chronic treatment, and (c) the potential for DA receptor antagonists to increase choice of cocaine over alternative reinforcers when cocaine self-administration is studied using choice procedures (Grabowski *et al*, 2004b).

Keeping those factors in mind, the homologous procedure developed here for testing pharmacotherapies in non-human primates and humans needed to have 2 important properties: 1) to be able to differentiate between decreases in cocaine-taking due to a decrease in the reinforcing properties of cocaine as opposed to a decrease due to non-selective disruption of behavior, and 2) to be able to evaluate the effect of sub-chronic treatment with a candidate pharmacotherapy. Choice procedures provide the ability to distinguish between decreases in cocaine-taking due to general behavioral disruption and effects on the reinforcing properties of the drug. They also have the advantage of being able to detect pharmacotherapies that may promote reallocation of

behavior away from cocaine self-administration and toward alternative non-drug

reinforcers. Choice procedures have also been used to test sub-chronic treatment

regimens of pharmacotherapy dosing in preclinical (e.g. Banks *et al*, 2015a) and human

(Rush *et al*, 2010) drug self-administration choice procedures. To mitigate the risk of

procedural variables confounding results, the non-human primate cocaine self-

administration choice procedure developed in this dissertation was back-translated from

a human laboratory choice procedure. This allowed for homology between the

preclinical and human laboratory self-administration choice procedures and allowed

equivalent IV doses of self-administered cocaine to be tested between the procedures.

There are several unique features to the choice sessions in these studies as compared

to other preclinical choice procedures. The sessions are long (5 hrs), substantial

responding is required for each reinforcer (200-1200 responses depending on how far

the monkey makes it in the progressive-ratio progression), there are few choices per

session (9), the self-administration cocaine doses for the non-human primates are

based on the human doses, maintenance drug doses are based on the doses that can

be tested in humans (although the monkey study may test a broader range of doses of

the maintenance medications), there is a sample trial, and the discriminative stimuli that

indicate availability of the reinforcers do not change based on the dose of cocaine or

magnitude of food pellets available. These variables are all similar to the human

laboratory procedure, and many are different from current preclinical choice procedures.

  Despite these many similarities, there are also several differences between the

preclinical procedure and the human laboratory procedure. The non-drug alternative

reinforcer in the non-human primate studies is food pellets, but in the humans, it is

money. Food does not work well as the alternative reinforcer in humans (Stoops *et al*, 2010), and although it might be possible to set up a token economy as an alternative reinforcer in non-human primate research, the training required would be time- and cost-prohibitive for use in this choice procedure. Another difference lies in the maintenance medication administration. In the human laboratory, patients typically receive oral doses of the maintenance medication at least 2 times a day, while in the non-human primate sessions the doses are given IV, and in the case of amphetamine via continuous infusion. Oral dosing of maintenance medication in non-human primates is possible, but it is difficult to ensure that the monkey receives the full dose of the maintenance. One strategy to evaluate equivalence of dosing in preclinical and clinical studies is to compare plasma levels of drugs and metabolites. Plasma amphetamine levels obtained during amphetamine maintenance in monkeys were reported in Chapter III, and these levels will be compared to those obtained in parallel human studies conducted by our collaborator Dr. Josh Lile at the University of Kentucky.

During initial training, monkeys first responded for food pellets alone and cocaine alone as single reinforcers under the progressive-ratio discrete trials procedure. Both reinforcers maintained behavior greater than vehicle or no pellets, and the largest dose or magnitude maintained completion of all the choices. The monkeys then started choice sessions and chose between cocaine and food in a dose and magnitude-dependent manner. As the dose of cocaine increased, cocaine choices also increased, and as the magnitude of pellets increased, more choices were completed for pellets. This was consistent with the human data collected at the University of Kentucky. High cocaine doses maintained high proportions of cocaine choice, and larger money

amounts increased money choice. The parametric work up of these two procedures showed that the non-human primate and human choices were correlated (Lile *et al*, 2016).

In the non-human primates, lisdexamfetamine and amphetamine maintenance decreased cocaine choices without decreasing food choices. These data are consistent with the published data on amphetamine maintenance decreasing cocaine use (Grabowski *et al*, 2004a; Levin *et al*, 2015). Even so, there is an apparent discrepancy with lisdexamfetamine in that a clinical trial using lisdexamfetamine for cocaine use disorder did not show positive results (Mooney *et al*, 2015). However, the clinical trial was limited by dose and could only test up to doses that were approximately equivalent to the 1.0 mg/kg/day lisdexamfetamine dose in the non-human primate study, which was not effective to decrease cocaine choice in non-human primates either. These results support using this novel self-administration choice procedure in non-human primates to predict with greater accuracy human laboratory and clinical results for potential pharmacotherapies to treat cocaine use disorder.

Mechanisms of Amphetamine Maintenance-Induced Decreases in Abuse-Related Effects of Cocaine

These studies were conducted to gain an understanding of the neurobiological mechanisms underlying the effect of amphetamine on the abuse-related effects of cocaine. In these studies, cocaine, MDPV, and methamphetamine produced facilitation in ICSS and an increase in NAc DA levels, and the potencies of these drugs to facilitate ICSS were similar to their potencies to increase NAc DA; however, only cocaine and

methamphetamine produced increases in NAc 5-HT levels. Amphetamine maintenance also produced an increase in baseline ICSS as well as an increase in baseline NAc DA levels, but not 5-HT levels. These findings support the hypothesis that facilitation in ICSS may reflect increases in DA versus 5-HT levels (Bauer *et al*, 2014; Suyama *et al*, 2016).

Amphetamine maintenance attenuated the facilitation of ICSS by cocaine and blocked the DA-increasing effect, but not the 5-HT-increasing effect, of cocaine. Both results are consistent with the clinical effectiveness of amphetamine to decrease metrics of cocaine use. Amphetamine maintenance did not block the ICSS facilitation or the NAc DA and 5-HT increases caused by methamphetamine. This profile of effects is consistent with the clinical ineffectiveness of amphetamine maintenance on methamphetamine-taking behavior. Amphetamine maintenance did not block the ICSS facilitation by MPDV, did block the NAc DA increase, and did not alter the lack of 5-HT increase by MDPV. These data suggest that amphetamine maintenance would be less effective to treat MDPV use disorder than cocaine use disorder.

Taken together, these data suggest that amphetamine decreases cocaine use by producing a selective increase in baseline DA levels and attenuating the DA, but not 5-HT, increase produced by cocaine. The MDPV data in particular suggest that preserving the 5-HT increasing effect of cocaine may be important in attenuating the abuse-related behavioral effects of cocaine during amphetamine maintenance. Serotonin can regulate DA release, although this relationship is complex because a serotonergic drug can increase or decrease DA depending on the drug used and the 5-HT receptor subtypes targeted (Fischer and Ullsperger, 2017; Howell and Cunningham, 2015).

The disconnect between the lack of DA increase after an injection of MDPV during amphetamine maintenance and the retention of the abuse-related behavioral effects in ICSS is difficult to explain. If DA is not increasing after MDPV administration, what else could be causing ICSS facilitation? Figure 5-1 presents a diagram showing the circuit that could allow this effect to happen. A major difference between ICSS and microdialysis procedures is that in ICSS, brain areas are being electrically stimulated. When the electrode is placed in the medial forebrain bundle at the level of the lateral hypothalamus, the electrode is thought to stimulate descending glutamatergic fibers that synapse onto VTA DA neurons. Under normal circumstances, the activity of these VTA neurons is increased after electrical stimulation. It is my hypothesis that the 5-HT inputs to the VTA and RMTg dampen the firing rate of the VTA neurons after an injection of cocaine during amphetamine maintenance, and the DAT-blocking action of cocaine offsets the decrease in firing rate of the DA neurons, so there is no net effect on DA levels. Without the 5-HT increase, administration of MDPV would not decrease firing rate of VTA neurons, so stimulation of the glutamatergic inputs to the VTA would produce an increase in firing of DA neurons and release of DA and the DAT-inhibiting properties of MDPV would result in an increase in DA in the NAc, which is expressed as facilitation in ICSS. This differential effect would not show up between cocaine and MDPV in microdialysis during amphetamine maintenance because there is no brain stimulation to increase VTA DA neuron firing. This hypothesis could be tested by placing a stimulating electrode into either the medial forebrain bundle or the VTA and a guide cannula in the NAc during amphetamine maintenance. Stimulation of the medial forebrain bundle or the VTA should produce DA increases in the NAc above the high

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DA baseline produced by amphetamine maintenance and MDPV should enhance the effect while cocaine should not.

These data also suggest that maintenance drugs that are more selective for SERT than DAT would be less effective to treat cocaine abuse. Drugs such as selective serotonin reuptake inhibitors have been tested in clinical trials as well as in preclinical procedures and have not been effective to decrease cocaine use, supporting this hypothesis (Pani *et al*, 2011).

The mechanism of amphetamine maintenance effects on cocaine use appears to be in blunting the DA increase of cocaine while maintaining the 5-HT increase of cocaine, but it is still not clear how the cocaine or MDPV DA increase is blunted by amphetamine, but the methamphetamine DA increase is not. Amphetamine maintenance also increased baseline levels of DA. Dopamine levels can be influenced by effects on at least 3 factors: 1) firing rate of the DA neurons, 2) DA uptake rate, and 3) DA release rate (Siciliano *et al*, 2015). Uptake inhibitors such as cocaine are sometimes referred to as "activity-dependent" drugs, meaning that their effects depend on the activity of the DA neurons, so the 3 factors that affect basal DA levels may also be expected to influence the effects of uptake inhibitors. If a consequence of amphetamine maintenance is decreased DA uptake rate, it could explain both the increased DA baseline and the blunted DA-increasing effect of uptake inhibitors (see figure 5-2). The amphetamine maintenance regimen sufficient to blunt the behavioral and neurochemical effects of cocaine was not sufficient to decrease DAT binding sites or change affinity for a DAT ligand in the striatum, so the effect of amphetamine is not mediated by a decrease in DAT. However, it is still a possibility that amphetamine

maintenance has decreased DAT function. Studies have found that acute exposure to releasers such as amphetamine can cause a change in DAT function and conformation such that DATs are more likely to be open toward the intracellular environment than the extracellular environment (Kahlig and Galli, 2003). This decrease in normal DAT function overlaps with the effect caused by cocaine, so adding cocaine will not have any further effect on DA levels and may decrease functional binding sites for cocaine even if DAT expression has not been changed.

It is possible that, during maintenance conditions, amphetamine has reached an equilibrium with DA release, DAT function, and negative feedback mechanisms such as autoreceptor activation or activation of neurons that provide inhibitory input to the VTA neurons, and addition of an uptake inhibitor to the system upsets the balance. An uptake inhibitor would compete with amphetamine for access to the binding site of DAT and would prevent DAT-mediated efflux of DA out of the cell (Kahlig *et al*, 2005). The decrease in DAT-mediated efflux could be enough to offset any increase in DA that would normally be seen when an uptake inhibitor binds to DAT. Another possibility is that amphetamine brings a depolarizing current with it as it is transported into the neurons, possibly resulting in increased neuronal firing rates (Cameron *et al*, 2015); however, when cocaine binds to the transporter, it induces a hyperpolarizing current that may reduce neuronal firing rates. These competing currents could offset each other and result in no change in DA levels. The current studies do not provide any indication of which possibility might be more likely. Studies that evaluate the effects of amphetamine maintenance on stimulated DA release, autoreceptor function, and membrane potential are needed to elucidate these potential mechanisms.

Future Directions

The data contained within this dissertation support the hypothesis that medication development should focus on blunting the DA-increasing effects of cocaine while maintaining the 5-HT-increasing effects of cocaine. The pharmacotherapy studied in this dissertation, amphetamine, produced a sustained increase in baseline DA levels and blocked the DA-increasing effect of 2 uptake inhibitors (cocaine and MDPV) while failing to block the DA increase by a releaser. Drugs that produce sustained decreases in DA levels and blunt the DA-increasing effects of cocaine have not yet been tried in this procedure. Several drugs, when given as an acute injection, can blunt the DA increase by cocaine, but few have been tried using maintenance dosing in microdialysis. Such drugs include lorcaserin (Gerak *et al*, 2016), a 5-HT$_{2C}$ receptor agonist. The localization 5-HT$_{2C}$ receptors has been characterized and they are found in areas of the brain that have the potential to influence DA, so this makes them intriguing targets for candidate pharmacotherapies (Howell and Cunningham, 2015). The current evidence in the literature is that lorcaserin, or the combination of lorcaserin with a 5-HT2A antagonist, decrease cocaine taking by decreasing the rate of DA-neuron firing and attenuate the DA increase by cocaine (Cunningham *et al*, 2013; Gerak *et al*, 2016). There is also evidence that an antagonist at the 5-HT$_{2C}$ receptor (SB242084) can produce increases in NAc DA levels (Devroye *et al*, 2013), but does not facilitate ICSS up to a dose of 1 mg/kg (Bauer *et al*, 2015; Katsidoni *et al*, 2011). This presents a set of drugs that, when administered acutely, can increase or decrease NAc DA levels. These drugs have not been tested in maintenance conditions in microdialysis, so it remains to

be seen what the effects of long-term treatment are on basal NAc DA levels and cocaine-induced NAc DA increase. The findings in this dissertation with amphetamine maintenance suggest that a sustained increase in NAc DA levels is associated with a blunted DA response to cocaine. If the acute DA-increasing effects of the 5-HT$_{2C}$ antagonist drugs maintain over several days of administration, they could present a way to learn if a sustained increase in NAc DA is sufficient to attenuate the abuse-related effects of cocaine.

These drugs could first be tested acutely in rats using microdialysis and ICSS to identify dose ranges. The effects of maintenance with 5-HT$_{2C}$ agonists and antagonists could then be tested against the abuse-related effects of cocaine in ICSS and microdialysis. If a drug is identified in these procedures as attenuating either the abuse-related behavioral or neurochemical effect of cocaine, it would be a good candidate pharmacotherapy to test in the non-human primate choice procedure described earlier in this dissertation. If the drug reduces cocaine choices without producing unwanted side effects in the non-human primate studies, it would then be an excellent choice to move into the human laboratory choice testing.
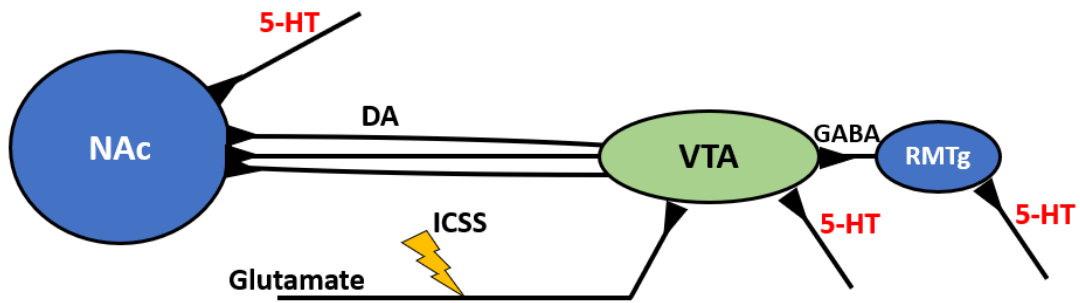
Figure 5-1. Diagram showing the brain circuit affecting ICSS. Stimulation of the

descending glutamate neurons increases activity in the VTA DA neurons, increasing DA

release in the NAc. There are inhibitory 5-HT receptors located on VTA DA cell bodies

and on the DA terminals in the NAc and excitatory 5-HT receptors on cell bodies in the

RMTg that will produce an increase in inhibitory input to the VTA. An increase in 5-HT

levels in the VTA, NAc and RMTg could decrease firing rate of the VTA neurons so as

to offset any increase caused by excitatory input from the ICSS stimulation.
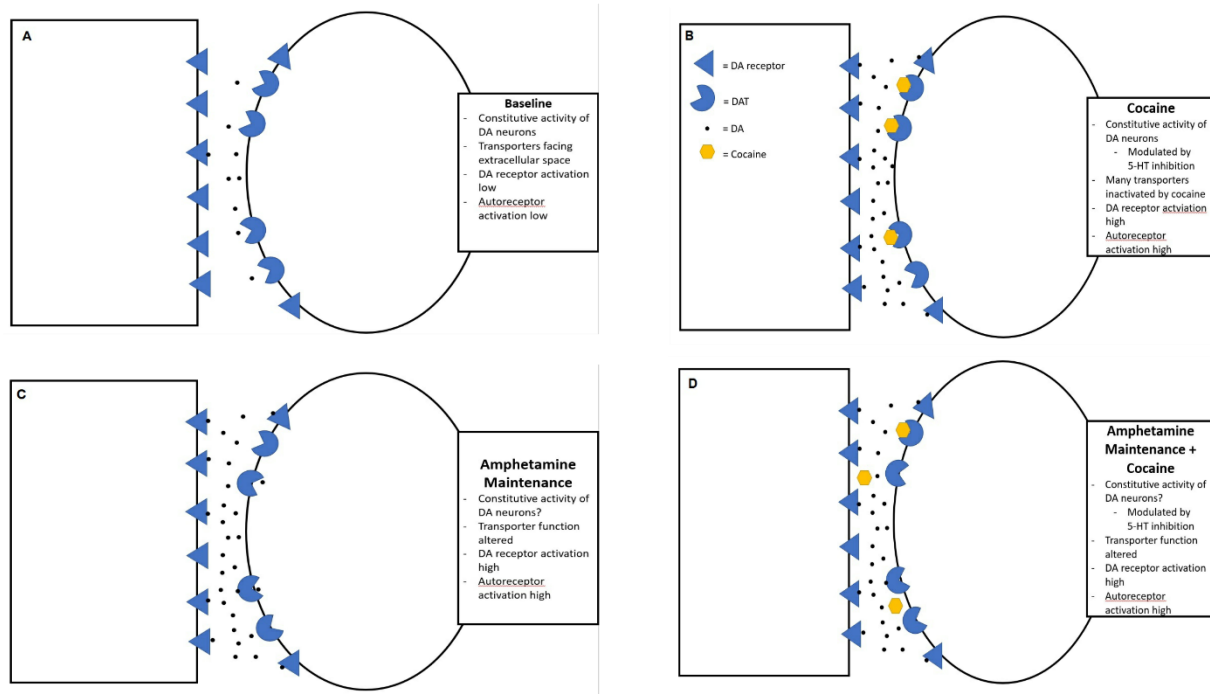
Figure 5-2. A depiction of DA neurons in the NAc at baseline (A), with the addition of cocaine (B), during amphetamine maintenance (C), and during amphetamine maintenance with cocaine (D). During baseline conditions (A), the DA neurons are firing at a basal level, but the functioning DATs keep basal DA levels low. When cocaine is added to this system (B), the DATs are inhibited and DA levels rise, activating post-synaptic DA receptors as well as autoreceptors on the presynaptic DA neuron. During amphetamine maintenance (C), DATs mediate efflux of DA out of the cell rather than bringing DA into the cell. This results in high levels of baseline DA levels and increased activation of postsynaptic DA receptors as well as autoreceptors. Adding cocaine on top of the amphetamine maintenance (D) may produce little change in extracellular DA because DATs are already not functioning at full capacity and DA levels are already high. However, cocaine can still act at SERT to increase 5-HT levels, so it may still be able to decrease DA neuron firing (see figure 5-1 for circuit).

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List of References

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Vita

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