Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Elizabeth Gwynn Pitts Date

Neurobehavioral effects, and therapeutic-like potential, of MDMA and cortico-striatal trkB

By

Elizabeth Gwynn Pitts
Doctor of Philosophy

Graduate Division of Biological and Biomedical Science
Neuroscience

Shannon Gourley, Ph.D.
Advisor

Leonard Howell, Ph.D.
Advisor

Gary Bassell, Ph.D.
Committee Member

Mary Torregrossa, Ph.D.
Committee Member

David Weinshenker, Ph.D.
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

Neurobehavioral effects, and therapeutic-like potential, of MDMA and cortico-striatal trkB

By

Elizabeth Pitts
B.S., Davidson College, 2011

Advisors: Shannon Gourley, Ph.D. and Leonard Howell, Ph.D.

An abstract of a dissertation submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Neuroscience
2017

Abstract

Neurobehavioral effects, and therapeutic-like potential, of MDMA and cortico-striatal trkB

By Elizabeth Gwynn Pitts

Cocaine addiction is a major public health concern with limited treatment options and no FDA approved pharmacological treatments. Addiction is a disorder characterized by maladaptive decision making, habit-like drug seeking, and social impairment. Brain-derived Neurotrophic Factor (BDNF) and its high-affinity receptor tyrosine kinase receptor B (trkB) play an important, but complex, role in mediating cocaine-reinforced behaviors and flexible decision making. Broadly, this thesis examines the role of BDNF-trkB in the extended circuitry of the orbitofrontal prefrontal cortex (oPFC), a brain region necessary for goal-directed decision making and implicated in the etiology of addiction. Additionally, this thesis examines the potential of neurotrophin-based therapeutics in enhancing goal-directed action selection and social behavior. I first show that trkB in the oPFC is necessary for goal-directed decision making, and that trkB in the dorsal striatum bi-directionally regulates flexible decision making. Next, I examine the long-term effects of adolescent cocaine exposure on decision-making and show that 7,8-dihydroxyflavone (7,8-DHF), a novel trkB agonist, blocks cocaine-induced habits, rescuing goal-directed action selection. I also find that 3,4-methylenedioxymethamphetamine (MDMA), a ring-substituted phenethylamine, selectively increases oPFC BDNF and enhances action-outcome decision making in an oPFC trkB-dependent manner. Finally, I examine the pharmacological mechanisms mediating the affiliative social effects of MDMA, finding that increases in social behaviors are 5-HT_{2A}, but not 5-HT_{1A}, receptor dependent. Together, these findings highlight the importance of BDNF-trkB in the oPFC, and connected regions, in flexible decision making and support the therapeutic potential of neurotrophin-based treatments for cocaine addiction.

Neurobehavioral effects, and therapeutic-like potential, of MDMA and cortico-striatal trkB

By

Elizabeth Pitts
B.S., Davidson College, 2011

Advisors: Shannon Gourley, Ph.D. and Leonard Howell, Ph.D.

A dissertation submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Neuroscience
2017

Acknowledgements

This thesis is dedicated to my sister, Laura, and my parents, Rachel and Conrad. Laura has always been my biggest cheerleader, providing much needed advice and encouragement. I do not know where I would be without her love and support and the fun she brings to my life. My mother, Rachel, made sure I always knew that I could do anything I wanted, and then worked to make that true with her constant support and guidance. I am eternally grateful for all that she has done for me. I would also like to thank my father, Conrad, for always driving me to be better, for providing endless help, and for showing me, through example, what can be accomplished with hard work and a little charm. Being raised by two such amazing and caring people and having a best friend and role-model in my sister has shaped my life forever and for the best and I cannot thank them enough for everything they have taught me and done for me. I would also like to thank David Oberlin for being there through the late nights and long hours and for making every day better with his humor and encouragement.

I would like to thank Dr. Mark Smith and Dr. Julio Ramirez for first engaging my interest in neuroscience. Dr. R's passion for neuroscience is contagious and Mark's knowledge and mentorship developed my interest in drug abuse research and continues to be invaluable. I would also like to thank the entire Emory neuroscience community for growing my knowledge and passion. A special thank you to the leadership, particularly Drs. Yoland Smith, Sean Hochman, and David Weinshenker, for your incredible commitment to making this community so special and to Drs. Malu Tansey and Victor Faundez, who are always so kind and helpful.

I would like to particularly thank my committee members for their invaluable guidance and for all the time and attention they devoted to my training. Dr. Gary Bassell provided an important cellular and molecular expertise and brought new perspectives to my work. Thank you to Dr. Mary Torregrossa; your expertise in habits and decision making has enhanced my work and I have benefitted greatly from your insight. Dr. David Weinshenker has helped me immensely, not only

as a committee member, but as my advisor in my first laboratory rotation in graduate school and as a (patient) mentor for my first attempt at grant writing. Thank you for helping me grow and develop as a scientist and for driving me to consider important questions and wider views of the field.

Thank you to Dr. Leonard Howell for agreeing to be my co-advisor for my graduate training. In addition to being a leader in the field and an endless source of information, Leonard's kindness and passion made working for him immensely rewarding and fun. I was extraordinarily lucky to learn from by such a talented scientist and his mentorship in addiction, pharmacology, and life will continue to guide me. Lastly, I would like to thank Dr. Shannon Gourley. I felt an instant connection to Shannon during my graduate school interviews, drawn by her passion for science, her deep knowledge, and her unique approach to translation-focused research, she was a major reason for my matriculation to Emory University. I am continually in awe of her drive, intelligence, and humor and so thankful for her constant support and guidance. I have grown immensely as a communicator and scientist because of her instruction and example and I feel so lucky to have had her as a mentor.

Table of Contents

Figure Index

Chapter 1:

Prefrontal cortical BDNF-trkB: A regulatory key in cocaine- and food-reinforced behaviors

1.1 Context, Author's Contribution, and Acknowledgement of Reproduction

The following chapter examines the role of BDNF in the PFC in cocaine- and food-based behaviors. The document was written by the dissertation author, Dr. Gourley, and Dr. Jane Taylor. The chapter is reproduced with minor edits from Pitts EG, Taylor JR, and Gourley SL (2016) Prefrontal cortical BDNF: A regulatory key in cocaine- and food-reinforced behaviors. *Neurobiology of Disease.*

1.2 Abstract

Brain-derived neurotrophic factor (BDNF) affects synaptic plasticity and neural structure and plays key roles in learning and memory processes. Recent evidence also points to important, yet complex, roles for BDNF in rodent models of cocaine abuse and addiction. Here we examine the role of prefrontal cortical (PFC) BDNF in reward-related decision making and behavioral sensitivity to, and responding for, cocaine. We focus on BDNF within the medial and orbitofrontal PFC, its regulation by cocaine during early postnatal development and in adulthood, and how BDNF in turn influences responding for drug reinforcement and responding in reinstatement models. When relevant, we draw comparisons and contrasts with experiments using natural (food) reinforcers. We also summarize findings supporting, or refuting, the possibility that BDNF in the medial and orbitofrontal PFC regulates the development and maintenance of stimulus-response habits. Further investigation could assist in the development of novel treatment approaches for cocaine use disorders, a major focus of this dissertation.

1.3 Introduction

Substance use disorders are profound public health concerns, with significant costs for affected individuals and the economy as a whole (Miller & Hendrie, 2008). In 2013, 1.5 million Americans aged 12 and over were current cocaine users (SAMHSA, 2014) and in 2010, cocaine

was the leading cause of emergency room visits involving illicit drug usage (SAMHSA, 2012). Despite this, there is still currently no FDA-approved pharmaceutical treatment for cocaine dependence.

Substance use disorders are characterized by drug use despite negative consequences. Altered plasticity and aberrant changes in so-called “reward” and learning and memory circuits are thought to underlie, in part, maladaptive reward-related decision making in addiction (Everitt & Robbins, 2005; Hyman et al., 2006; Robbins et al., 2008; Dong & Nestler, 2014; Everitt, 2014). Understanding the mechanisms mediating drug-induced neurobiological changes that drive behaviors interpreted as drug-seeking in rodents could provide avenues to novel therapeutics that could break compulsive drug use in humans.

Brain-derived neurotrophic factor (BDNF) is involved in neural organization and synaptic plasticity during development and in adulthood (Huang & Reichardt, 2003; Binder & Scharfman, 2004; Park & Poo, 2013). Through activation of its high affinity receptor, tyrosine kinase receptor B (trkB), BDNF activates signaling cascades that affect gene transcription and synaptic structure and plasticity (Atwal et al., 2000; Binder & Scharfman, 2004; Park & Poo, 2013). Genetic polymorphisms associated with reduced BDNF signaling (Egan et al., 2003) appear to increase the risk for the development of stimulant addiction (Cheng et al., 2005; Su et al., 2014). Additionally, blood serum BDNF levels rise during early periods of cocaine withdrawal (Von Diemen et al., 2014; Viola et al., 2014; Corominas-Roso et al., 2013a,2015). Individuals with higher serum BDNF have been shown to relapse later than those with lower levels (Corominas-Roso et al., 2015); however, higher BDNF levels can also correlate with greater craving and loss of behavioral control (Corominas-Roso et al., 2013b).

Abundant pre-clinical research has aimed at better understanding how brain BDNF is affected by cocaine exposure and how BDNF affects drug-seeking and decision-making behaviors. This chapter focuses on BDNF in the prefrontal cortex (PFC). The PFC plays an important role in learning and memory, decision-making processes, and in both the expression

and inhibition of cocaine-reinforced behaviors (Robbins et al., 2008; Torregrossa et al., 2008; Peters et al., 2009; Lucantonio et al., 2012; Moorman et al., 2014). We first briefly summarize the neuroanatomy of the rodent PFC, as well as tasks commonly used to model aspects of drug abuse and addiction in rodents. We then review evidence that acute and repeated cocaine exposure affects BDNF and *Bdnf* expression in the PFC. Next, we consider whether drug-induced changes in BDNF levels in the PFC play a role in the development of behaviors interpreted as drug-seeking or is instead "protective." We summarize the effects of direct manipulation of PFC BDNF expression on food- and drug-reinforced responding and on PFC-dependent decision making and discuss evidence for the "therapeutic-like" potential of manipulating trkB systems. Finally, we briefly summarize the rationale for, and findings of, the studies presented in the subsequent chapters of this dissertation.

This chapter is particularly focused on the effects of BDNF in PFC neurocircuits – including both medial and orbitofrontal regions of the PFC – and we refer readers to Russo et al. (2009), McGinty et al. (2010), Ghitza et al. (2010), Schmidt et al. (2013), Barker et al. (2014), and Li & Wolf (2015) for additional discussions regarding drug-mediated regulation of BDNF expression and activity *throughout* the multiple corticolimbic regions implicated in substance use disorders.

1.3.1 The rodent PFC: A brief overview

Broadly speaking, the rodent PFC can be divided into a lateral region, the orbitofrontal cortex (oPFC), and a medial region, referred to as the medial prefrontal cortex (mPFC). The mPFC can be further subdivided into the anterior cingulate cortex, prelimbic cortex (PL), infralimbic cortex (IL), and the medial oPFC (Ongur & Price, 2000)(Fig.1-1). The mPFC as a whole receives projections from multiple areas of the limbic systems involved in encoding reward salience and value, including the hippocampus and amygdala (*e.g.*, Jay & Witter, 1991; McDonald, 1991; Gabbott et al., 2006; Mátyás et al., 2014; Zingg et al., 2014). This allows it to integrate information from multiple sources into decision-making processes, and to coordinate

motor output via downstream structures. For instance, the PL innervates the nucleus accumbens (NAC) core and the basolateral and lateral nuclei of the amygdala, while the IL innervates the NAC shell and the basal, central, and medial amygdala (Sesack et al., 1989, McDonald et al., 1996; Heidbreder & Groenewegen, 2003). These two highly-studied structures (the PL and IL) regulate reward-related decision making, often with opposing influences (reviewed Moorman et al., 2014).

The medial oPFC is positioned ventrally to the PL and IL, at the base of the medial wall (Fig.1-1). It innervates a thin strip of the dorsomedial striatum (DMS) immediately adjacent to the ventricles, with projections extending ventrally to the NAC (*e.g.*, see Schilman et al., 2008; Rodriguez-Romaguera et al., 2015). The medial oPFC has received less attention than the more dorsal regions of the mPFC, but despite this, recent reports indicate that the medial oPFC regulates the expression of conditioned fear and repetitive stereotyped behavior (Ahmari et al., 2013; Rodriguez-Romaguera et al., 2015). Further, inactivation of the medial oPFC induces perseverative-like responding for food reinforcers (Gourley et al., 2010), and this may be due to an inability to retrieve outcome-related information to guide response strategies (Bradfield et al., 2015). Medial oPFC inactivation also attenuates cocaine-primed reinstatement, an animal model of relapse (Fuchs et al., 2004). Together, these findings suggest that the medial oPFC regulates aspects of reward-related decision making in both food- and drug-related contexts.

The more lateral regions of the oPFC are essential for stimulus-dependent reward-related decision making and for integrating reward salience and expectancies to allow for “on-the-fly” response selection (Lucantonio et al., 2012; Stalnaker et al., 2015). The oPFC receives projections from the basal amygdala, and it innervates the amygdala and centrolateral and ventral striatum (Ongur & Price, 2000; Schilman et al., 2008; Hoover & Vertes, 2011; Gremel & Costa, 2013; Zingg et al., 2014).

There is much debate regarding what the oPFC does and does not do (*cf.*, Stalnaker et al., 2015). This may be complicated by assuming homogeneity of oPFC projections. The

ventrolateral subregion of the oPFC, situated between the lateral oPFC and the medial oPFC, has overlapping, as well as distinct, projection properties, relative to the other oPFC subregions. For example, the ventrolateral oPFC innervates the dorsal striatum but largely spares the NAC; it also innervates the basolateral amygdala (BLA) but to a lesser degree than the lateral oPFC (Schilman et al., 2008; Rodriguez-Romaguera et al., 2015; Zimmermann et al., 2017b). These distinctions likely position the lateral and ventrolateral oPFC to differentially regulate reward-related behaviors.

1.3.2 Animal models of drug seeking and habit formation

In order to study the molecular- and circuit-level changes associated with cocaine exposure and addiction, researchers must model drug seeking and related behaviors using tractable experimental conditions. We will briefly summarize behavioral tasks relevant to this dissertation. First, **conditioned place preference (CPP)** can be used to examine the development and extinction of a Pavlovian association between a previously neutral context and cocaine administration. Preference for a cocaine-paired context, and how long it lasts when cocaine is withheld, is thought to reflect a subject's sensitivity to the drug and its ability to acquire and extinguish the context-drug association.

Cocaine self-administration studies allow subjects to control drug intake and can be used to quantify the acquisition and maintenance of a drug-reinforced response, binge-like behavior, and the reinstatement of drug seeking following extinction. In this case, subjects perform an operant response (*e.g.,* lever press or nose poke) to receive a cocaine reinforcer. The reinforcer is most commonly a direct intravenous infusion, although cocaine can also be self-administered orally (*e.g.,* Macenski et al., 1998; Miles et al., 2003; Gabriele et al., 2009). Cocaine delivery is often paired with a stimulus, such as a light or a tone. Thus, self-administration studies typically involve components of action-outcome conditioning — associating an operant response with reinforcer delivery — and stimulus-outcome conditioning — associating a cue with the drug.

To assess the **reinstatement of cocaine seeking**, an experimenter-administered cocaine injection, presentation of the drug-paired stimulus, or acute stressor (termed drug, cue, and stress-induced reinstatement, respectively) is used to reinstate operant responding. The amount of responding in extinction (no reinforcer is delivered during reinstatement) is considered a marker of drug seeking (for further discussion, see Marchant et al., 2013).

Another pair of tasks can be used to assess whether rodents use action-outcome (goal-directed) or stimulus-response (habitual) response strategies. Although these tasks typically use food as the reinforcers, they are relevant to issues of drug abuse because stimulus-elicited habits are considered etiological factors in the development and maintenance of addiction (Jentsch & Taylor, 1999; Everitt & Robbins, 2005; Schwabe et al., 2011; Torregrossa et al., 2011). In these tasks, the value of a reinforcer is reduced via prefeeding or transient LiCl-induced gastric malaise (**outcome devaluation**). Alternatively, the contingency between a trained response and the reinforcer is violated (**action-outcome contingency degradation; discussed further in chapter 2 and 3**). Animals using goal-directed behavioral response strategies will adjust (decrease) their responding. Animals that have developed habits, however, will continue responding, as previously (for further review of these tasks, see Yin et al., 2008; Balleine & O'Doherty, 2010).

1.3.3 Cocaine rapidly regulates mPFC BDNF and Bdnf, and acute BDNF infusion can decrease cocaine-related responding

BDNF is a member of the neurotrophin family that, in mammals, includes nerve growth factor, neurotrophin-3, and neurotrophin 4/5. BDNF is initially synthesized as a 32-kD pro-peptide (referred to as "pro-BDNF") and is then cleaved into a 14-kD mature form. pro-BDNF preferentially binds and activates the p75 pro-apoptotic receptor. Meanwhile, mature BDNF preferentially stimulates the trkB receptor. Ligand binding and trkB receptor autophosphorylation initiate multiple intracellular signaling cascades through the MAP kinase, PI3-kinase, and the PLCγ

pathways. Through these, BDNF regulates neuronal activity and synaptic and structural plasticity during both pre- and postnatal development, and in the mature brain (Reichardt, 2006; Lu et al., 2014).

Cocaine dynamically regulates trkB, BDNF protein, and *Bdnf* mRNA expression in the mPFC (summarized in Table 1-1). In mature rodents, *acute* experimenter-administered cocaine can increase *Bdnf* mRNA within ~2 hours of exposure, and this is associated with an increase in expression of the mature form of BDNF 24 hours after injection (Le Foll et al., 2005; Fumagalli et al., 2007,2009). In contrast, 22-72 hours after *repeated* exposure to cocaine, either experimenter- or self-administered, *Bdnf* or BDNF levels can drop (McGinty et al., 2010; Fumagalli et al., 2007,2013). Accordingly, BDNF replacement in the PL *suppresses* cocaine-related responding in extinction and in reinstatement tests using cocaine-associated cues or a cocaine prime (Berglind et al., 2007,2009; Whitfield et al., 2011; McGinty et al., 2010)(Table 1-1). Blockade of mPFC trkB activity occludes these effects, indicating that local BDNF-trkB binding can, at least in part, account for suppressive effects on cocaine seeking (see McGinty et al., 2010; Whitfield et al., 2011). Within the ventromedial PFC, local infusions of BDNF into the IL enhance the extinction of cocaine-CPP (Otis et al., 2014). IL BDNF infusions also rescue cocaine-induced deficiencies in fear extinction recall (Kabir et al., 2013).

The PL preferentially innervates the NAC core, and as with PL BDNF, trkB activity in the NAC core appears to oppose cocaine-seeking behaviors. Specifically, siRNA-mediated knockdown of the trkB receptor increases cue-induced responding when rats are tested immediately following a period of cocaine self-administration (Li et al., 2013). This is significant because cortical projections provide a primary source of BDNF in the striatum, which contains little *Bdnf* mRNA (Altar et al., 1997). Accordingly, PL-selective knockdown of *Bdnf* decreases BDNF protein expression in the striatum (Gourley et al., 2009a,2012a). Conversely, BDNF infusion increases BDNF expression in the NAC, and levels of phosphorylated (active) ERK1/2 also increase (Berglind et al., 2007; McGinty et al., 2010). Infusions of BDNF into the mPFC also

normalize levels of extracellular glutamate and activity of the vesicular trafficking protein synapsin in the NAC following cocaine exposure (Berglind et al., 2009; Sun et al., 2014). These findings are particularly provocative given that the reinstatement of drug seeking after extinction is thought to reflect, at least in part, disturbances in glutamatergic neurotransmission in a mPFC-NAC pathway, specifically, depleted levels following repeated cocaine exposure, followed by robust up-regulation after re-exposure to cocaine or cocaine-related cues (Kalivas, 2009).

1.3.4 A history of cocaine exposure increases mPFC BDNF and Bdnf

In the aforementioned studies of McGinty and colleagues, in which BDNF was infused into the PL of cocaine self-administering rats, BDNF was largely infused immediately following a period of cocaine self-administration, coinciding with low BDNF levels. In other experiments, infusions later in the drug abstinence period were ineffective (Berglind et al., 2007), suggesting the possibility that enhancing mPFC BDNF signaling (*i.e.*, by replacing BDNF tone) has protective benefits during a quite narrow time window. This may be because endogenous BDNF protein and *Bdnf* mRNA appear to increase in the days and weeks following cocaine exposure, eventually *exceeding* typical levels (Hearing et al., 2008; McGinty et al., 2010; Sadri-Vakili et al., 2010; Zhang et al., 2015, but see Fumagalli et al., 2013)(summarized Table 1-1).

A history of early-life experimenter-administered cocaine exposure also progressively increases mPFC BDNF, leading to increased mPFC BDNF expression in adulthood (Lu et al., 2010; Giannotti et al., 2014). Notably, however, a recent study by Simchon-Tenenbaum et al., 2015 did not replicate this finding. This discrepancy may be due to differences in tissue dissection strategies, since Simchon-Tenenbaum et al. appeared to extract the whole PFC for analysis, which would include lateral (oPFC), in addition to medial, subregions. As will be discussed below, the oPFC may respond differently to cocaine exposure, which could preclude the detection of elevated mPFC BDNF in tissue samples containing both the mPFC and oPFC.

1.3.5 Chronic deviations in typical mPFC BDNF-trkB tone influence locomotor sensitization and cocaine- and food-reinforced behaviors

The prolonged augmentation of mPFC BDNF expression following cocaine exposure (see above) could conceivably be associated with increased behavioral sensitivity to the drug. Consistent with this notion, *TrkB* knockdown broadly throughout the mPFC modestly blunts the motoric response to cocaine in sensitized mice (Lu et al., 2010). Additionally, the development of cocaine-induced locomotor sensitization is delayed in *Bdnf*+/- mice (Horger et al., 1999). Locomotor sensitization can also be blocked by a combination of dopamine D_1/D_2 receptor and 5-HT_3 receptor antagonists, which interferes with cocaine-induced increases in mPFC BDNF (Zhang et al., 2015). These studies suggest that mPFC BDNF-trkB could support the development and expression of cocaine-induced locomotor sensitization.

Drug-induced mPFC BDNF over-expression could also conceivably increase cocaine-seeking behaviors. In one study, cocaine increased mPFC levels of *Bdnf* exon IV, and aerobic exercise normalized *Bdnf* exon IV levels and reduced cocaine self-administration in tandem, suggesting that mitigating drug-related increases in mPFC *Bdnf* could be associated with cocaine resilience (Peterson et al., 2014). In a similar vein, PL-targeted *Bdnf* knockdown can blunt cocaine-CPP (Choi et al., 2012).

Despite these findings, blocking drug-related increases in mPFC BDNF does not necessarily protect against cocaine vulnerabilities in all contexts. For example, mPFC-targeted *Bdnf* knockdown *increases*, rather than decreases, cocaine self-administration on a progressive ratio schedule of reinforcement (Sadri-Vakili et al., 2010), even while decreasing responding for food reinforcement (Gourley et al., 2012b). Also, inhibiting *Bdnf* enhances the cytotoxic properties of cocaine in cultured cells (Yan et al., 2007), and PL-targeted *Bdnf* knockdown failed in one report to block biases towards habit-based decision making induced by adolescent cocaine exposure (Hinton et al., 2014).

As discussed, BDNF is subject to anterograde transport, such that selective BDNF overexpression in the dorsomedial PFC induces BDNF over-expression in the downstream amygdala (McGinty et al., 2010), and selective *Bdnf* knockdown in the oPFC *reduces* BDNF levels in the amygdala (Gourley et al., 2013a; Zimmermann et al., 2017b). Interestingly, inhibition of BDNF-trkB signaling in the amygdala [another structure subject to cocaine-induced increases in BDNF expression (Grimm et al., 2003)] interferes with the extinction of cocaine-CPP (Heldt et al., 2014). Together, these findings further suggest that BDNF in certain PFC circuits supports key behavioral inhibitory functions.

These and other findings have led to the perspective that cocaine-induced increases in mPFC BDNF may have some adaptive properties (*e.g.*, discussed by Fumagalli et al., 2013). Indeed, the male offspring of cocaine self-administering rats are cocaine-resilient and also have higher levels of *Bdnf* and BDNF in the mPFC than control counterparts (Vassoler et al., 2013). Cocaine resilience in these rats is entirely blocked by administration of a trkB antagonist, providing evidence that mPFC BDNF-trkB systems can have protective properties. Additionally, typical rats that learn to approach reward-related stimuli (sign-trackers), instead of the location of food reinforcer delivery (goal-trackers), in a Pavlovian conditioned approach task have lower levels of PFC BDNF (Morrow et al., 2015). Sign-trackers also exhibit greater drug-seeking behavior in reinstatement tests (Saunders & Robinson, 2010,2011; Yager & Robinson, 2013), again suggesting that PFC BDNF may be “protective” against certain drug-reinforced behaviors.

Increases in BDNF following cocaine abstinence are linked to cocaine-induced long-term potentiation in the mPFC, and mechanistically, this may occur via the reduction of cell-surface ionotropic $GABA_A$ receptors (Lu et al., 2010). This discovery led to subsequent investigations utilizing viral-mediated gene silencing of the predominant $GABA_A$ subunit, $GABA_A\alpha1$, in the mPFC. Particularly when knockdown was initiated early in life, $GABA_A\alpha1$ silencing induced a deferral to habit-based responding in food-reinforced operant conditioning tasks, mimicking the effects of cocaine (Butkovich et al., 2015). $GABA_A\alpha1$-deficient mice were also delayed in acquiring

a cocaine-reinforced response, but even when cocaine exposure was controlled, $GABA_A\alpha1$ silencing had no effects on the reinstatement of cocaine seeking following extinction (Butkovich et al., 2015). These findings suggest that chronic changes in dorsomedial PFC $GABA_A\alpha1$ systems (linked to changes in BDNF or other factors) do not obviously account for relapse in cocaine addiction.

1.3.6 Does mPFC BDNF influence habit-based behavior?

Several independent groups have reported using reinforcer devaluation and instrumental contingency degradation tasks that a history of repeated cocaine or amphetamine exposure can induce outcome-insensitive habits (Schoenbaum & Setlow, 2005; Nelson & Killcross, 2006,2013; Nordquist et al., 2007; LeBlanc et al., 2013; Corbit et al., 2014; Hinton et al., 2014). Further, instrumental responding for cocaine can quickly become dominated by habit-like strategies (Miles et al., 2003; Zapata et al., 2010). Additionally, acute cocaine exposure can disrupt the consolidation of new action-outcome associative learning and memory, resulting in a deferral to habit-based response strategies (Gourley et al., 2013b), and pairing cocaine with a food-reinforced response also results in behavioral insensitivity to the devaluation of the food reinforcer (Schmitzer-Torbert et al., 2015). Thus, cocaine exposure biases response strategies towards habits.

The relationship between cocaine-induced augmentation of mPFC BDNF and the development and maintenance of cocaine-related habits is, in our view, opaque. Firstly, PL-directed BDNF infusion in mice can induce habit or habit-like behaviors, similar to the effects of cocaine (Graybeal et al., 2011; Gourley et al., 2012b). This may be because mPFC *Bdnf* increases during the initial acquisition of a food-reinforced instrumental response, but then decreases with proficiency (Rapanelli et al., 2010). Aberrant drug-induced elevations in mPFC BDNF that persist after task proficiency has been achieved could conceivably disrupt typical intracellular signaling essential for goal-directed action selection, causing mice to defer to habit-based decision making.

One caveat to this model is that experiments using BDNF infusions may rely on BDNF concentrations that exceed physiological levels (Li & Wolf, 2015). Additionally, PL-targeted *Bdnf* knockdown, which would presumably interfere with cocaine-induced increases in mPFC BDNF, failed in one report to block habits caused by adolescent cocaine exposure (Hinton et al., 2014). The suggestion that drug-related mPFC BDNF overexpression induces reward-seeking habits is also at odds with evidence that stressor exposure blocks cocaine-induced *Bdnf* up-regulation (Fumagalli et al., 2009) and at the same time facilitates habit formation in both rodents and humans (Dias-Ferreira et al., 2009; Schwabe, 2013; see also Gourley et al., 2012b). Further, the presence of the met allele at codon 66 of the *BDNF* gene in humans increases, rather than decreases, the likelihood that individuals will rely on habit-based strategies in spatial navigation tasks (Banner et al., 2011). These findings challenge a model in which cocaine-induced BDNF over-expression in the mPFC induces biases towards habit-based decision making.

1.3.7 Effects of trkB stimulation

To summarize, mPFC BDNF-trkB significantly impacts behavioral sensitivity to cocaine, and in ways that can appear contradictory. For example, mPFC BDNF-trkB appears to enhance locomotor sensitivity to cocaine (Lu et al., 2010), but also *facilitate* the extinction of a cocaine-reinforced response (Berglind et al., 2007) [even while interfering with the extinction of food-reinforced responding (Gourley et al., 2009a)]. Adding to this already complicated picture is evidence that drug-induced BDNF over-expression in certain subcortical structures is implicated in drug-seeking behaviors (reviewed Li & Wolf, 2015).

These properties may suggest that BDNF-trkB has limited utility as a therapeutic target. Nonetheless, a bioactive, high-affinity trkB agonist that causes receptor dimerization and auto-phosphorylation was recently characterized (Jang et al., 2010), resulting in studies assessing the behavioral effects of this putative trkB agonist, 7,8-dihydroxyflavone (7,8-DHF). Systemic administration of 7,8-DHF dose-dependently attenuates methamphetamine-induced locomotor

sensitization (Ren et al., 2014) and normalizes drug-induced impairments in prepulse inhibition (Ren et al., 2013). 7,8-DHF additionally interferes with cocaine seeking in mice that self-administered cocaine, were then subject to forced abstinence, and finally, were re-exposed to the cocaine-associated context (DePoy et al., 2016). 7,8-DHF also blocks stimulus-response habits induced by response over-training (Zimmermann et al., 2017b). This effect is reversed by co-administration of a trkB antagonist, raising the possibility that trkB-targeting manipulations could mitigate habits caused by drugs of abuse. Additionally, local infusions of 7,8-DHF into the IL enhance the extinction of cocaine-CPP (Otis et al., 2014), while systemic 7,8-DHF treatment has apparently no effects on the *acquisition* of cocaine-CPP in typical rodents (Tzeng et al., 2013). Another trkB agonist, LM22A-4, decreases compulsive-like alcohol consumption in mice (Warnault et al., 2015; see for further discussion, Logrip et al., 2015). Seemingly contradictory evidence from Verheij et al. (2016) found that a trkB antagonist decreases drug-seeking behavior in a cue-induced cocaine reinstatement task. However, this novel trkB antagonist paradoxically *increases* trkB phosphorylation in the PFC, supporting the potentially protective effects of PFC trkB stimulation. These findings together highlight the possible utility of pairing trkB-based interventions with therapies for drug use disorders, though further research is certainly necessary.

1.3.8 oPFC BDNF regulates reward-related and goal-directed decision making

Studies utilizing viral-mediated gene silencing strategies to assess the role of *Bdnf* in the oPFC in complex decision making and cocaine-related behaviors indicate that *Bdnf* knockdown enhances the acquisition, and impairs the extinction, of cocaine-CPP (Gourley et al., 2013a). Additionally, oPFC-selective *Bdnf* knockdown induces stimulus-response habits that occur at the expense of goal-directed decision making (Gourley et al., 2013a; Zimmermann et al., 2017b), mimicking the effects of cocaine exposure (discussed above).

Despite these and other findings, whether the oPFC regulates goal-directed action selection *vs.* habit behavior remains a contentious topic. Ostlund and Balleine (2007) generated

large lesions encompassing the lateral and ventrolateral oPFC in rats and reported that oPFC damage did not impact behavioral sensitivity to reinforcer devaluation. In other words, oPFC damage apparently did not cause habits. Meanwhile, certain forms of Pavlovian (stimulus-outcome) conditioning were impaired, consistent with an historical focus on oPFC involvement in stimulus-outcome learning and memory (discussed Ostlund & Balleine, 2007). In 2013, however, Gremel and Costa placed lesions in the ventrolateral oPFC of the mouse, causing behavioral insensitivity to reinforcer devaluation, suggesting that oPFC damage causes habit-based decision making. They also found, using multi-site multi-electrode recordings, neural ensembles in the oPFC that encoded action-value information (Gremel & Costa, 2013). In the same year, Gourley et al. (2013a) reported that oPFC-selective knockdown of *Bdnf* and lesions disconnecting the oPFC from the dorsal striatum also induce habits. We additionally find that habit biases can be attributed to failures in consolidating or retaining action-outcome memory (Zimmermann et al., 2017a), and we have reported that viral-mediated knockdown of *Gabra1* and *Fmr1* in the oPFC also induce a deferral to habit-based responding (Swanson et al., 2015; Gross et al., 2015). These findings suggest that the healthy oPFC is important for goal-directed (action-outcome) response selection.

How might we reconcile these findings with the early findings of Ostlund and Balleine (2007)? Key differences include species and sex, since Ostlund and Balleine (2007) utilized female rats, while Costa, Gremel, and Gourley have primarily utilized male mice. Another possible factor is the training history of the experimental animals. The rats used in the report of Ostlund and Balleine (2007) were first trained for 8 days to associate distinct auditory stimuli with two different outcomes (pellets *vs.* sucrose). Then, 11 days of instrumental conditioning followed, in which rats were trained to respond for the same two outcomes. Thus, rats had ample opportunity to form multiple stimulus-outcome and action-outcome associations prior to test. By contrast, the mice used by Gremel and Costa (2013) and Gourley et al. (2013a) responded for a single outcome and were not subject to explicit reward-associated stimuli. Further, the mice in the Gourley report

also generated much lower response rates overall, which could minimize the opportunity to strongly encode or retain action-outcome information.

It may be that the oPFC is involved in early phases of forming or retaining action-outcome associations, but that with sufficient task experience, this information can be encoded and retained in the absence of a healthy oPFC. Consistent with this notion, mice with unilateral oPFC *Bdnf* knockdown and contralateral amygdala lesions "disconnecting" these structures are insensitive to instrumental contingency degradation, deferring to habit-based response strategies (Zimmermann et al., 2017b). These same mice can, however, ultimately develop sensitivity to changes in instrumental contingencies with repeated training, suggesting that oPFC insult delays, but does not fully block, learning or retaining new information about action-outcome contingencies (Zimmermann et al., 2017b). This may also account for instances in which mice with oPFC damage fail to develop sensitivity to instrumental contingency degradation, but when tested in a reinforcer devaluation task following additional training, responding is intact (Gourley et al., 2013a; Swanson et al., 2015). Additional experiments would, however, be required to explicitly test this model.

Experiments using inhibitory Gi-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in the ventrolateral oPFC further suggest that the oPFC is involved in behavioral sensitivity to action-outcome relationships. Gremel and Costa (2013) found that activating Gi-DREADDs in the oPFC before outcome revaluation testing induced habit-based responding. Additionally, in a recent experiment, Gi-DREADDs were stimulated immediately following modifications in familiar action-outcome contingencies, during the presumptive consolidation of new learning. During a subsequent drug-free probe test, all mice were initially able to select responses that were more, *vs.* less, likely to be reinforced, a goal-directed response strategy. However, response preference rapidly decayed in mice expressing Gi-DREADDs in the oPFC, such that these mice ultimately deferred to habit-based strategies (Zimmermann et al.,

2017a). These studies further suggest that the oPFC is involved in retaining action-outcome-based memory.

1.3.9 Does cocaine impact BDNF expression in the oPFC?

As discussed, mPFC BDNF levels increase following repeated cocaine exposure, including cocaine exposure during early-life development (Lu et al., 2010; Giannotti et al., 2014). In contrast, early-life exposure to atomoxetine, which, like cocaine, inhibits the norepinephrine transporter, *decreases Bdnf* expression in the adult oPFC (Sun et al., 2012). In mature rodents, tissue collected by whole frontal cortex dissection failed in one report to reveal changes in *Bdnf* expression at several time points following cocaine self-administration; this could conceivably be attributable to differential sensitivities to cocaine in the different subregions (*e.g.*, mPFC *vs.* oPFC) of the frontal cortex (Liu et al., 2006; see also Simchon-Tenenbaum et al., 2015). *Bdnf* specifically in the oPFC increased following repeated cocaine self-administration in another report, and interestingly, this up-regulation was only detectable in rats that were re-exposed to the cocaine self-administration context prior to euthanasia (Hearing et al., 2008). Context-specific changes in oPFC *Bdnf* are consistent with the existence of projections from hippocampal structures to the oPFC (*e.g.*, Morecraft et al., 1992). Further studies are needed to fully understand the impact of drug exposure on oPFC BDNF and its potential role in drug-induced impairments in decision making.

1.3.10 Regulation of neuron structure

In addition to synaptic plasticity, BDNF-trkB interactions regulate the shape and structure of neurons. For example, stimulation of trkB promotes neurite outgrowth in several biological systems and acts at several steps to suppress p75 signaling (Reichardt, 2006). This is relevant because p75 activity can otherwise inhibit neural outgrowth via activation of the RhoA GTPase and substrates such as Rho-kinase. Accordingly, we have discovered that systemic

administration of a Rho-kinase inhibitor corrects impulsive-like food-reinforced responding following oPFC *Bdnf* knockdown in female mice (DePoy et al., 2013) and habit-based responding following oPFC *Bdnf* knockdown in male mice (Zimmermann et al., 2017b). These findings highlight another possible point of intervention in combatting cocaine seeking – that is, the regulation of cell shape and structure. This idea is reinforced by evidence that cocaine cue-induced neuroplasticity in the PL regulates changes in dendritic spine head size – a metric of synaptic strength – in the NAC following the presentation of cocaine-related cues (Gipson et al., 2013), and that inhibiting the activity of the cytoskeletal regulatory elements Arg kinase and β1-integrin in the oPFC and forebrain, respectively, greatly exaggerates cocaine-induced locomotor sensitization (Gourley et al., 2009b; Warren et al., 2012).

Determining whether BDNF-trkB influences cocaine-induced cellular structural modifications throughout the corticolimbic structures implicated in drug abuse and addiction may be a fruitful topic of future research. Under certain circumstances, the putative trkB agonist 7,8-DHF can induce dendritic spine *proliferation* in the hippocampus and oPFC (Zeng et al., 2012; Zhang et al., 2014a,b; Zimmermann et al., 2017b), while cocaine decreases dendrite complexity and dendritic spine density in the oPFC (Gourley et al., 2012a; DePoy et al., 2014,2017; Radley et al., 2015). Notably, one study found no changes in oPFC dendritic spine density following extended-access cocaine self-administration (Ferrario et al., 2005). It is possible that different methods in imaging and dendritic branch selection could explain the different results between studies, given that the Ferrario report (2005) utilized Golgi-Cox staining and sampled spines from third-order terminal tips or greater, while other studies used fluorescence imaging and examined segments within 150 μm of the soma (Gourley et al., 2012a; Radley et al., 2015). Whether 7,8-DHF can block cocaine-induced spine loss in the oPFC has not, to our knowledge, been tested.

In another study using mice lacking *Fmr1*, dendritic spines aberrantly proliferated in the hippocampus, and 7,8-DHF *reduced* densities to typical levels (Tian et al., 2015). Together, these findings suggest that 7,8-DHF promotes homeostatic dendritic spine plasticity, rather than simply

increasing or decreasing spine numbers. This is notable given that cocaine and other psychostimulants can both increase and decrease dendritic spine densities, depending on the brain region sampled (reviewed Kolb & Muhammad, 2014; DePoy & Gourley, 2015). Strategies that normalize structural and synaptic plasticity throughout multiple regions may be particularly attractive strategies for treating drug use disorders.

1.4 Conclusions

BDNF is involved in a wide range of brain functions, including neuronal differentiation and neurite outgrowth during development and synapse structure and plasticity throughout development and adulthood (Binder & Scharfman, 2004; Park & Poo, 2013). BDNF is also crucial for multiple forms of learning and memory (Yamada & Nabeshima, 2003; Lu et al., 2008) and is implicated in several psychiatric disorders, including depression, addiction, and obsessive-compulsive disorder (Binder & Sharfman, 2004; Autry & Monteggia, 2012). Studies reviewed here indicate that mPFC and oPFC BDNF systems are dynamically regulated by cocaine exposure, and in turn impact cocaine-related learning and memory and decision making. There is interest in treating substance use disorders with therapies that enhance flexible goal-directed decision-making processes, as opposed to, for example, mitigating the reinforcing effects of, or craving for, cocaine (see Everitt & Robbins, 2005; Pierce & Vanderschuren, 2010). The effects of BDNF on synapse formation and learning and memory could conceivably complement therapies aimed at strengthening goal-directed decision making or extinguishing connections between drug cues and craving. The complex effects of cocaine exposure on BDNF, as well as the complicated role of BDNF in reward-related decision making in general, may limit its therapeutic potential, but preliminary studies with systemic administration of 7,8-DHF (Ren et al., 2013,2014; Zimmermann et al., 2017b) provide some evidence of utility. Further understanding the intricacies of how site-specific, and global, stimulation of BDNF-trkB activity affect behavior could open avenues for the development of novel pharmacotherapies.

1.5 Dissertation Overview

Given the important and complex role of BDNF in cocaine-reinforced behaviors and flexible decision making, and the relatively understudied role of the oPFC in goal-directed (action-outcome) decision making, this dissertation examines the role of BDNF-trkB in the oPFC and connected regions on action selection, and the potential of neurotrophin-based therapeutics in stimulating goal-directed behavior. This dissertation builds on the important finding that oPFC-specific *Bdnf* knockdown impairs goal-directed action selection (thereby increasing habits)(Gourley et al., 2013a; Zimmermann et al., 2017b). However, cortical BDNF is anterogradely transported (Conner et al., 1998), and oPFC *Bdnf* knockdown decreases BDNF in connected brain regions involved in goal-directed action, such as the striatum and the amygdala (Gourley et al., 2013a; Zimmermann et al., 2017b). Therefore, the region of action for BDNF-trkB binding is still unknown. In chapters 2 & 3, I show that inhibiting trkB activity selectively in the oPFC is sufficient to occlude goal-directed action. I also find, using viral vector and chemogenetic techniques, that trkB in the dorsal striatum bi-directionally regulates decision making and that a *Bdnf*-dependent oPFC-BLA circuit is necessary for action-outcome memory formation.

Next, I examine the potential of neurotrophin-based pharmaco-manipulations in enhancing action-outcome decision making. I focus in particular on decision-making strategies following cocaine exposure in adolescence. Adolescence is a time of vulnerability to the long-term effects of cocaine; for example, drug exposure during this period decreases the likelihood of treatment seeking across the lifespan (Kessler et al., 2001) and causes a bias towards habits in rodents (DePoy et al., 2016,2017). In chapter 3, I report that 7,8-DHF, a trkB agonist, blocks habits resulting from cocaine exposure in adolescence, thereby rescuing goal-directed decision making.

Given the complex and often opposing effects of BDNF on decision making and cocaine-reinforced behaviors (discussed above; Li & Wolf, 2015), a drug that increases BDNF-trkB more

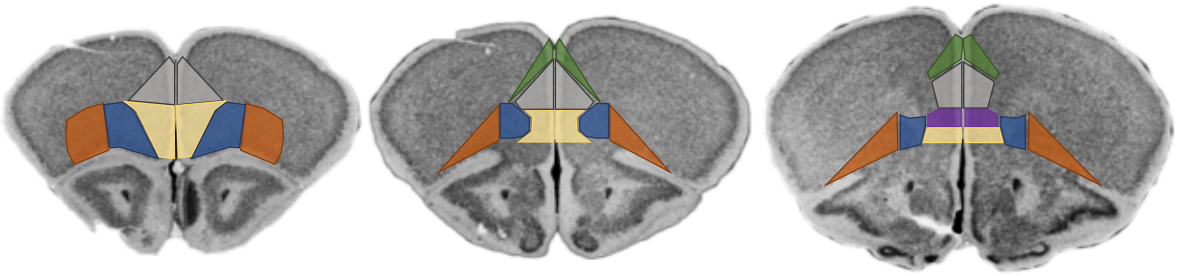
selectively in the PFC, instead of broadly throughout the brain, may prove a better therapeutic. This hypothesis led us to 3,4-methylenedioxymethamphetamine (MDMA), a ring-substituted phenethylamine that increases PFC *Bdnf* (Mouri et al., 2017). Recently, phase II clinical trials have found that MDMA is an effective therapeutic adjunct for treatment-resistant post-traumatic stress disorder (PTSD)(Mithoefer et al., 2011,2013). In rodents, MDMA stimulates fear extinction conditioning in a BDNF-dependent manner (Young et al., 2015). I hypothesized that MDMA may facilitate the consolidation or retention of *appetitive* memories, and that this would also depend on MDMA-induced elevations in BDNF-mediated activation of trkB. In chapter 3, I confirm that MDMA increases BDNF in the oPFC (as in gross PFC dissections; see Mouri et al., 2017); that MDMA enhances action-outcome memory formation, blocking inflexible habits caused by oPFC-selective *Bdnf* knockdown and cocaine; and, finally, that MDMA-mediated rescue of goal-directed decision making is dependent on oPFC trkB.

Another therapeutic-like effect of MDMA is the stimulation of prosocial behaviors (see Kamilar-Britt & Bedi, 2015). Social impairments, such as increased social isolation and decreased social value, are common in addiction (Volkow et al., 2011b; American Psychiatric Association, 2013). In the laboratory, cocaine addicts demonstrate diminished social engagement and impaired social cognition, and these deficits are associated with smaller real-life social networks (Preller et al., 2014a,b). This may impact treatment and recovery in patients, since social network support is an important component in abstinence maintenance (Mutschler et al., 2013). Because of this, therapeutics that increase social behaviors and so-called “social reward,” including MDMA, have been posited as treatments for addiction (McGregor & Bowen, 2012; Jerome et al., 2013; Lee et al., 2016), and a clinical trial focusing on the use of MDMA to treat alcohol addiction has recently been approved in the United Kingdom (Devlin, 2017). In chapter 4, I utilize a nonhuman primate model to examine the pharmacological mechanisms of the prosocial effects of MDMA. Squirrel monkeys are a highly social organism with complex social behaviors and vocalizations and a pharmacokinetic profile for MDMA similar to humans (Mueller et al., 2009). I show that

MDMA stimulates social behaviors and vocalizations in group-housed squirrel monkeys, while a structurally similar monoamine-releaser, methamphetamine, does not. Additionally, I show that MDMA-induced increases in social behavior are 5-HT_{2A}, but not 5-HT_{1A}, receptor-dependent.

Given the therapeutic-like effects of MDMA in increasing goal-directed action and social behaviors, I conclude by discussing the history and present use of MDMA as a therapeutic compound in chapter 5. I also discuss the potential therapeutic value of the *R*(–) enantiomer of MDMA and future directions for research related to the therapeutic effects of MDMA and their mechanisms.

Figure 1-1: *Regions of the rodent prefrontal cortex.*



The mouse PFC can be divided into the anterior cingulate cortex (green), PL (gray), IL (purple), medial oPFC (yellow), ventrolateral oPFC (blue), and lateral oPFC (orange). The agranular insula, often studied in concert with the lateral oPFC, is lateral to the lateral oPFC. Regions outlined on coronal images from the Mouse Brain Library (Rosen et al., 2000).

Table 1-1: *Postnatal cocaine exposure regulates mPFC BDNF systems, and PL BDNF regulates appetitive conditioning: A summary.*

Cocaine regulates *Bdnf* and BDNF (Tissue samples collected from the mPFC except those marked "*," connoting samples collected from the frontal cortex)	
Brief synopsis	***Reference***
Acute cocaine (20 mg/kg) increases *Bdnf* 2-3 hours following exposure, and expression is typical by 5 hours. Methamphetamine has similar effects.	Le Foll et al., 2005
Acute cocaine (40 mg/kg) increases *Bdnf* exon I and IV 4 hours following exposure.	Liu et al., 2006*
Acute cocaine (5 mg/kg) increases *Bdnf* mRNA 2-24 hours after exposure; expression of mature BDNF protein is increased at the 24 hour time point.	Fumagalli et al., 2007
Acute cocaine (10 mg/kg) increases *Bdnf* , *TrkB* (full-length), synaptic trkB, and ERK1/2 phosphorylation within 2 hours of injection. Chronic stressor exposure blocks these effects.	Fumagalli et al., 2009
Repeated cocaine self-administration (1 hr/day; 10 days) and experimenter-administered cocaine (20 mg/kg/day; 10 days) does not impact *Bdnf* expression as measured 1, 30, or 90 days (self-administration) or 4 hours (experimenter-administered) after cocaine.	Liu et al., 2006*
Repeated cocaine exposure (non-contingent; 5 mg/kg/day; 5 days) increases *Bdnf* and CREB expression and phosphorylation 2 hours after the last exposure. However, both pro-BDNF and mature BDNF protein levels are *reduced* 2 and 72 hours after repeated cocaine exposure.	Fumagalli et al., 2007
Repeated cocaine self-administration (2 hr/day; 10 days) increases *Bdnf* expression when assessed 22 hours following the last infusion, but only if a cocaine-associated cue is present. Following 15 days of abstinence *Bdnf* is upregulated regardless of cue presence.	Hearing et al., 2008
Repeated cocaine self-administration reduces *Bdnf* expression within 22 hours of a final infusion, and then BDNF expression levels increase above control within 21 days.	McGinty et al., 2010
Repeated cocaine self-administration (2 hr/day; 14 days) increases *Bdnf* (exon IV) and BDNF levels when measured 1 week after the last exposure. Cocaine increases the association of phosphorylated CREB with *Bdnf* exon IV.	Sadri-Vakili et al., 2010
Repeated cocaine self-administration *or yoked exposure* (14 days) increases mature BDNF and *Bdnf* exon I within 24 hours of the last session, but *Bdnf* exon IV is reduced and *Bdnf* exon VI is unchanged. One week later, BDNF protein levels are unchanged.	Fumagalli et al., 2013
Repeated cocaine self-administration (24 hr/day; 4 trials/hr; 10 days) increases *Bdnf* exon IV when tested 14 days following the last session.	Peterson et al., 2014

Repeated cocaine self-administration (6 hr/day; 10 days) does not modify *Bdnf* or BDNF when tested 45 days after exposure.	Li et al., 2013
Repeated cocaine exposure (non-contingent; 25 mg/kg/day; 5 days) increases BDNF and trkB expression 25 days after administration. Protein levels were assessed following a cocaine prime (7.5 mg/kg) given one day prior to euthanasia.	Zhang et al., 2015
The male offspring of cocaine self-administering rats are cocaine-resilient and have increased mPFC *Bdnf* exon IV, and BDNF. Resilience can be blocked with a trkB antagonist, which *augments* cocaine self-administration.	Vassoler et al., 2013
Sign-tracking rats, known to have higher rates of cocaine-seeking behavior in reinstatement, have lower levels of BDNF.	Morrow et al., 2015*
Early-life cocaine exposure (10 mg/kg/day; postnatal days 28-42) increases *Bdnf* exon IV, pro-BDNF, mature BDNF, and synaptic trkB. This is detectable 48, but not 3, days following exposure. Concurrently, levels of *tPA*, the enzyme responsible for the cleavage of pro-BDNF into mature BDNF, are upregulated. Phosphorylation of Akt, mTOR, and S6K also increases.	Giannotti et al., 2014
Early-life cocaine exposure (15 mg/kg/day; postnatal days 18-24) increases BDNF expression at 8 and 14 days following exposure (but not 1 or 3 days). No changes to trkB.	Lu et al., 2010
***Bdnf* and BDNF in the PL regulate appetitive decision making**	
Brief synopsis	***Reference***
Acute **BDNF infusion** suppresses cue- and cocaine-induced reinstatement of cocaine seeking and normalizes ERK phosphorylation in the downstream NAC, but not dorsal striatum. No effects on the reinstatement of food seeking.	Berglind et al., 2007
Acute **BDNF infusion** suppresses the reinstatement of cocaine seeking and normalizes extracellular glutamate levels in the NAC.	Berglind et al., 2009
Acute **BDNF infusion** suppresses the reinstatement of cocaine seeking, and effects are associated with local trkB-ERK1/2 activation.	Whitfield et al., 2011
Acute **BDNF infusion** immediately following repeated cocaine self-administration can enhance the extinction of a cocaine-reinforced response. Effects are most robust during initial training.	Berglind et al., 2007
Viral-mediated ***Bdnf* knockdown** enhances the extinction of a food-reinforced operant response; effects are most robust during initial training. **BDNF infusion** has no effects at a concentration that decreases adrenal gland weight.	Gourley et al., 2009a
Viral-mediated ***Bdnf* knockdown** *increases* cocaine-reinforced responding on a progressive ratio schedule of reinforcement. No effects on response acquisition.	Sadri-Vakili et al., 2010

Viral-mediated ***Bdnf* knockdown** *decreases* food-reinforced responding on a progressive ratio schedule of reinforcement.	Gourley et al., 2012b
Viral-mediated ***Bdnf* knockdown** interferes with cocaine-CPP.	Choi et al., 2012
Acute **BDNF infusion** induces habit-like behavior in typical mice.	Gourley et al., 2012b
Viral-mediated ***Bdnf* knockdown** is unable to protect against habits induced by adolescent cocaine exposure.	Hinton et al., 2014

Report synopses are provided at left, with the corresponding references at right. These studies highlight the temporally dynamic regulation of BDNF or *Bdnf* following acute (gray cells) *vs.* repeated (white cells) cocaine. Epigenetic factors (dark green cells) and effects of early-life cocaine exposure (light green cells) are also reported. The bottom half of the table addresses the effects of direct manipulations of PL BDNF on reinstatement (beige cells); cocaine- *vs.* food-reinforced responding (blue cells); and cocaine-CPP and habits (orange cells).

Chapter 2:

Bidirectional coordination of actions and habits by trkB

2.1 Context, Author's Contribution, and Acknowledgement of Reproduction

The following chapter examines the role of trkB in the oPFC and the dorsal striatum in flexible decision making. The dissertation author contributed to the chapter by designing and conducting the majority of the experiments, analyzing data, and writing the manuscript under the guidance of Dr. Gourley.

2.2 Abstract

The orbitostriatal circuit is important for flexibly shifting between goal-directed and habitual behaviors. Certain disorders, such as addiction, are characterized by impairments in decision making. Understanding mechanisms of flexible decision making could assist in the development of novel therapeutics. Here, we overexpress a truncated, inactive form of the tyrosine kinase receptor B (trkB), the high-affinity receptor for Brain-derived Neurotrophic Factor (BDNF), in the orbitofrontal cortex (oPFC) and dorsomedial striatum (DMS), and also habit-associated dorsolateral striatum (DLS). First, we found that overexpression of truncated trkB in the oPFC blocked goal-directed action selection. Then, we found that trkB in the DMS is also necessary for goal-directed decision making, while trkB in the DLS impacts habit formation. Together, these findings indicate that orbitostriatal trkB bi-directionally influences whether mice select actions based on their consequences or based on habitual strategies and thus, is a molecular trigger allowing organisms to balance actions and habits.

2.3 Introduction

Flexible action requires shifting between familiar and novel behavioral response strategies. Extensive response training and exposure to stressors and certain drugs of abuse can lead to a bias towards habit-based behaviors that are by contrast inflexible and associated with

illnesses characterized by decision making and impulse control deficits, such as addiction and obsessive compulsive disorder (Burguiere et al., 2015; Everitt & Robbins, 2016).

During the initial acquisition of an instrumental behavior, typical organisms are sensitive to the predictive relationship between actions and their outcomes, and goal-directed action selection strategies dominate. With continued training, reward-related stimuli can gain control over behavior, and behavioral response strategies become habitual (Yin et al., 2008; Balleine & O'Doherty, 2010; Graybiel & Grafton, 2015). The DMS and interactions with the oPFC are necessary for goal-directed actions, while the DLS controls habits (Yin et al., 2008,2009; Gourley et al., 2013a; Gremel & Costa, 2013). The primary neurotrophin BDNF is a key cortical substrate coordinating goal-directed action selection, given that oPFC-selective *Bdnf* knockdown causes a bias toward habit-based behaviors (Gourley et al., 2013a; Zimmermann et al., 2017b). Where, precisely, stimulation of the high-affinity BDNF receptor, trkB, is important remains unclear, however, given that BDNF is subject to anterograde transport. For example, oPFC-selective *Bdnf* knockdown reduces BDNF protein in the dorsal striatum (Gourley et al., 2013a). Here we utilized viral vector strategies to overexpress a truncated, inactive form of trkB (*TrkB.t1*) within the oPFC, DMS, and DLS in order to clarify the role, if any, of trkB within these regions in reward-related action-outcome decision making.

2.4 Materials and Methods

2.4.1 Subjects

Experiments used male wild-type C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) at least 7 weeks of age. Mice were maintained on a 12-hour light cycle (on at 0800) and were experimentally naïve. Mice had *ad libitum* access to water and food, except during instrumental conditioning when body weights were maintained at ~90% of baseline. Procedures were approved by the Emory University Institutional Animal Care and Use Committee.

2.4.2 Intracranial surgery

Mice were anaesthetized with ketamine/dexdomitor (80 mg/kg/0.5 mg/kg i.p.) and then mounted onto a digital stereotaxic apparatus (Stoelting, Wood Dale, IL). Lentiviral vectors expressing *TrkB.t1* and an HA tag or Green Fluorescent Protein (GFP) under a CMV promotor were generated by the Emory University Viral Vector Core and are described in Rattiner et al. (2004). Viral vectors were infused at a rate of 0.1 μL/minute, with a total volume of 0.5 μL, and the microsyringe left in place for 5 minutes following infusion. In experiments targeting the oPFC, viral vectors were infused at +2.6 mm anteroposterior (AP), -2.85 mm dorsoventral (DV), and +/-1.2 mm mediolateral (ML). Viral vectors targeting the DMS were delivered to +0.74 mm AP, -3.0 mm DV, and +/-2.2 mm ML. DLS coordinates were +0.5 mm AP, -3.5 mm DV, and +/-2.7 mm ML.

2.4.3 Action-outcome contingency degradation

Mice were trained to nose poke for food pellet reinforcers (20 mg grain-based pellets; Bioserv, Frenchtown, NJ) in Med-Associates (Georgia, VT) operant conditioning chambers. Mice were trained to nose poke on 2 available apertures using a fixed ratio 1 (FR1) schedule of reinforcement for 5 sessions (1/day). Next, mice were trained for 2 additional sessions (1/day) using a random interval 30 second (RI30) schedule of reinforcement. Sessions lasted for 70 minutes or until the maximum 60 pellets (30 per nose poke) had been delivered.

Next, mice were tested for sensitivity to action-outcome contingencies using a modified version of classical action-outcome contingency degradation. During the ‘non-degraded’ session, one nose poke aperture was occluded, and responding on the other nose poke was reinforced using a variable ratio 2 (VR2) schedule of reinforcement. The next day, during the ‘degraded’ session, pellets were delivered non-contingently at a rate yoked to the reinforcement rate from the previous session. Responses were recorded, but had no programmed consequences. The location of the ‘degraded’ aperture was counterbalanced across subjects. Mice that decrease their response rates during the ‘degraded’ session, when the contingency between response and

outcome is violated, are considered goal-directed. Equivalent response rates during the 'non-degraded' and 'degraded' sessions are thought to reflect habitual responding (Fig.2-1A; Balleine & O'Doherty, 2010).

In the experiment focused on the striatum, following the first contingency degradation test, mice were trained for an additional 4 sessions (1/day) using an RI60 schedule of reinforcement. Then, mice were again tested for sensitivity to action-outcome contingency degradation, as above.

2.4.4 Histology

Mice were anesthetized by isoflurane and euthanized by rapid decapitation. Brains were stored for 48 hours in 4% paraformaldehyde and then transferred to a 30% w/v sucrose solution. Brains were then sectioned at 50 μM. To confirm infusion sites, sections were immunostained for the HA tag on the *TrkB.t1* virus, or GFP was imaged. To visualize HA, sections were blocked, then incubated with the primary antibody (anti-HA; 1:1000; Sigma-Aldrich, St. Louis, MO) overnight at 4°C. The next day, sections were incubated with secondary antibody (Alexa Fluor 488 or 594 anti-rabbit; 1:500; Jackson ImmunoResearch Laboratories, West Grove, PA) and then mounted with Permount (Fisher Scientific, Hampton, NH) for fluorescence imaging.

2.4.5 Western blotting

Behaviorally-naïve mice received oPFC-targeted infusions of lenti-*TrkB.t1* or GFP. Approximately 3 weeks following infusion, matching with the onset of behavioral studies, mice were rapidly decapitated and brains were stored at -80°C and sectioned into 1-mm thick sections. The oPFC was dissected by a single experimenter using a 1 mm tissue core. Tissue was homogenized in lysis buffer [200 μL; 137 mM NaCl, 20 mM tris-HCl (pH=8), 1% igepal, 10% glycerol, 1:100 Phosphatase Inhibitor Cocktails 1 and 2 (Sigma-Aldrich), and 1:1000 Protease

Inhibitor Cocktail (Sigma-Aldrich)], and protein concentrations were determined by a Pierce BCA Protein Assay kit (Thermo Fisher Scientific). 15 μg of each sample was separated by SDS-page on a 7.5% gradient Tris-glycine gel (Bio-Rad Laboratories, Inc., Hercules, CA). Next, samples were transferred to a PVDF membrane (Bio-Rad) and blocked with 5% nonfat dry milk for 1 hour. The membrane was incubated overnight at 4°C in primary antibodies. Primary antibodies were trkB (1:375; Cell Signaling Technology, Danvers, MA) and HSP70 (1:6000; Santa Cruz Biotechnology, Dallas, TX). Following 1 hour of incubation in secondary antibodies [goat anti-mouse and anti-rabbit peroxidase labeled IgG (Vector Laboratories, Burlingame, CA)], immunoreactivity was assessed using a chemiluminescence substrate (Thermo Fisher Scientific) and a ChemiDoc MP Imaging System (Bio-Rad).

2.4.6 Statistical analyses

All mice were randomly assigned to condition, and sample sizes were in line with prior reports using the same approaches (*e.g.*, Zimmermann et al., 2017a). Behavioral response rates were compared by two-factor mixed-design ANOVA and Bonferroni post-hoc comparisons in case of significant interactions. For western blotting experiments, densitometry values were normalized to a loading control (HSP70) value in the same lane, and then to the control sample mean on the same gel to accommodate fluorescence variance *across* gels. Group means were then compared by a 2-tailed unpaired *t*-test. Values >2 standard deviations above or below the mean were considered outliers and excluded. Statistical analyses were performed in SPSS with $\alpha \leq 0.05$.

2.5 Results

2.5.1 trkB in the oPFC is necessary for goal-directed action selection

First, we overexpressed a truncated, inactive form of trkB, *TrkB.t1*, in the oPFC, sequestering BDNF and interfering with trkB-mediated signaling (Rattiner et al., 2004). We

infused lenti-*TrkB.t1*, lenti-GFP (a control), or a half-and-half mixture of the two in order to generate multiple lenti-*TrkB.t1* "doses" (Fig.2-1B,C). While all groups initially acquired the food-reinforced nose poke responses, mice with full-titer lenti-*TrkB.t1* generated lower response rates (interaction $F_{12,90}$=5.565, p<0.001)(Fig.2-1D), as can also occur with oPFC-selective *Bdnf* knockdown (Gourley et al., 2013a; Zimmermann et al., 2017b; but see chapter 3) and oPFC damage more generally (Stalnaker et al., 2015). This profile is also consistent with impaired action-outcome decision making and a bias towards habit-based behavior (Corbit & Balleine, 2003). Accordingly, lenti-*TrkB.t1* interfered with the ability of mice to select actions based on their outcomes following instrumental contingency degradation (Fig.2-1E). In contrast, GFP control and low-titer ('Half *TkrB*.t1') mice decreased responding in a goal-directed fashion when a behavior was unlikely to be reinforced (interaction $F_{2,15}$=6.191, p=0.011)(Fig.2-1E). We also confirmed that full-titer *TrkB1.t1* overexpression appreciably increased trkB.t1 protein levels, detectable even in gross oPFC tissue punches containing both infected and uninfected tissues (t_7=-2.769, p=0.028)(Fig.2-1F).

2.5.2 trkB in the dorsal striatum bi-directionally regulates decision making

Obstructing oPFC-striatal interactions causes the same impairments in goal-directed action (Gourley et al., 2013a) and also interferes with an organism's ability to modify instrumental behaviors when reward value changes (Gremel & Costa, 2013). The striatum contains very little *Bdnf* mRNA (Hofer et al., 1990), but abundant BDNF protein anterogradely transported from cortical sources (Conner et al., 1998). We thus next examined whether trkB activity in the dorsal striatum was similarly important for flexible action selection. In this case, we overexpressed *TrkB.t1* selectively in the DMS or DLS (Fig.2-2A,B). Response rates did not differ between groups during training (Fig.2-2C). *TrkB.t1* overexpression in the DMS, however, induced failures in response inhibition, despite non-contingent delivery of food pellets (interaction $F_{2,18}$=8.14,

p=0.0003)(Fig.2-2D). Thus, trkB activity in the oPFC and downstream DMS is essential for goal-directed decision making.

Next, we *induced* habit behavior using a random interval schedule of reinforcement (Fig.2-2A). Following this training, both control and DMS *TrkB.t1* mice generated inflexible habit-based responding as expected. By contrast, *TrkB.t1* overexpression in the DLS interfered with habit formation – these mice remained sensitive to changes in action-outcome contingencies (interaction $F_{2,17}$=4.198, *p*=0.033)(Fig.2-2E). Thus, *TrkB.t1* is essential to the functions of both the DMS (supporting goal-directed action) and DLS (supporting habits).

2.6 Discussion

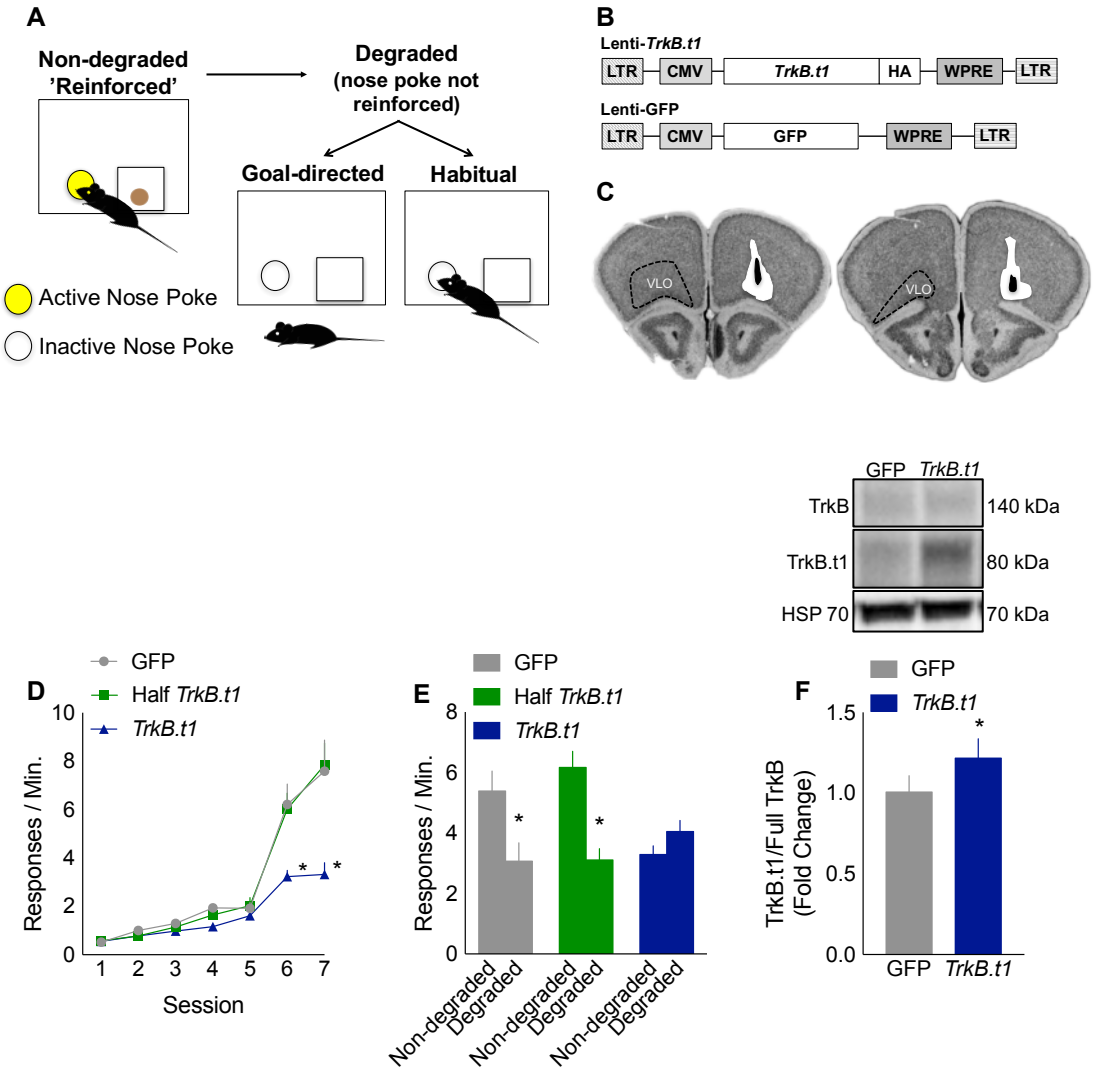
Habits can be beneficial in certain situations because they allow for fast and automatic responding to familiar situations, increasing efficiency and reserving higher-order attention for more demanding tasks. Meanwhile, adjusting behaviors in reaction to changes in reward value or contingencies is essential for appropriate action selection. Therefore, the ability to modify behavior according to goal expectancies in some situations, while responding efficiently (habitually) in others is advantageous. The switch from goal-directed action to habits is thought to reflect a transition in the coordinated control of response strategies by multiple corticostriatal interactions, including oPFC-striatal interactions, to a predominantly DLS-controlled output (Yin et al., 2009; Gourley et al., 2013a; Gremel & Costa, 2013). Reducing *Bdnf* in the oPFC interferes with goal-directed action selection, causing a deferral to habits (Gourley et al., 2013a; Zimmermann et al., 2017b). However, the site where BDNF binding to its high-affinity receptor trkB impacts goal-directed action has previously been unclear, since BDNF is anterogradely transported (Conner et al., 1998), such that oPFC-specific *Bdnf* knockdown decreases BDNF in connected regions, including the striatum (Gourley et al., 2013a). We addressed this issue by over-expressing *TrkB.t1* in the oPFC, impairing goal-directed action and causing a deferral to

habit-based behavior, indicating that BDNF binding to trkB locally within the oPFC is necessary for goal-directed action.

We also find that trkB in the dorsal striatum bi-directionally regulates flexible decision-making strategies, with overexpression of *TrkB.t1* in the DMS causing a premature bias towards habits (as with the oPFC) and *TrkB.t1* in the DLS impairing habit formation. Although trkB is expressed in both the DMS and DLS (Altar et al., 1994), these patterns were nevertheless unexpected, given that systemic administration of a trkB agonist blocks habits induced by extensive response training (Zimmermann et al., 2017b), rather than facilitating this DLS-dependent behavior. trkB stabilizes dendritic spine densities and morphologies throughout multiple brain regions (Bennett & Lagopoulos, 2014) and is essential for corticostriatal long-term potentiation (Park et al., 2014). Thus, broad-spread trkB stimulation (*i.e.*, due to systemic injection of a trkB agonist) may energize goal-directed action by stimulating multiple corticostriatal structures competing with the DLS for control over behavior.

Further understanding the molecular mechanisms mediating the balance between actions and habits could shed light onto treating disorders characterized by impairments in flexible decision making, such as addiction (Everitt & Robbins, 2016). These data provide an initial indication that neurotrophin-based therapeutics that activate orbitostriatal trkB may be beneficial for enhancing action-outcome decision making.

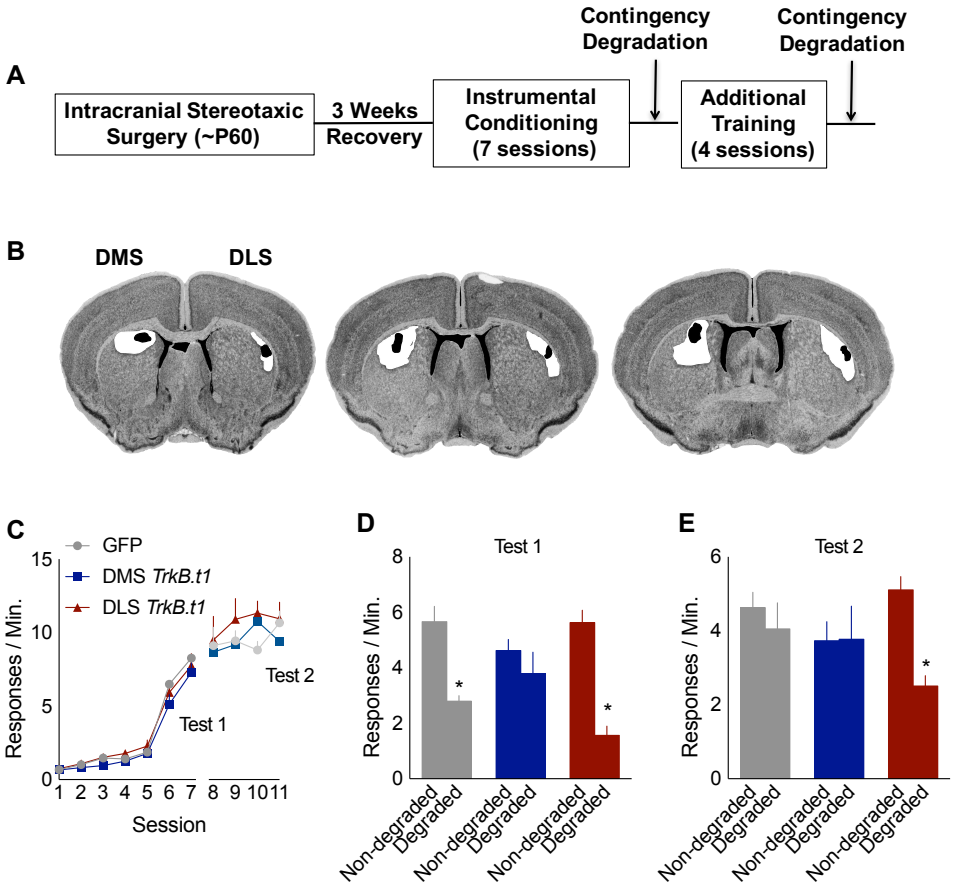
Figure 2-1: *TrkB.t1 overexpression in the oPFC blocks goal-directed action selection.*



(A) Mice are trained to nose poke for food reinforcers. Then, the probability of reinforcement is reduced ('degraded'). Decreasing responding following a change in contingency is considered goal-directed, while insensitivity to change is considered habitual. (B) Viral vector constructs. (C) A lentivirus expressing *TrKB.t1*, GFP, or a half-and-half mixture of both was infused bilaterally into the oPFC. Representative viral vector spread is represented on images from the Mouse Brain Library (Rosen et al., 2000). White represents the maximal spread and black the smallest. (D) Mice were trained to respond for food reinforcers. *TrkB.t1* overexpression reduced response

rates, and (E) mice with a full-titer of lenti-*TrkB.t1* were insensitive to action-outcome contingency degradation, deferring to habit-based behavior. (F) Full-titer *TrKB.t1* overexpression in the oPFC increased trkB.t1 in gross tissue dissections, as expected. Bars and symbols represent means+SEMs, *$p<0.05$.

Figure 2-2: *Striatal trkB bi-directionally regulates decision-making strategies.*



(A) Lenti-*TrkB.t1* or GFP was infused into the DMS or DLS, then sensitivity to action-outcome contingency was tested. (B) Viral vector infusions are represented, with white representing the largest infusion and black the smallest. (C) There were no group differences during food-reinforced instrumental conditioning. (D) Overexpression of *TrkB.t1* in the DMS, however, caused a bias towards inflexible habits, indicated by insensitivity to action-outcome contingencies. (E) Additional nose poke training induced habits in control mice, but overexpression of *TrkB.t1* in the DLS blocked these habits. Bars and symbols represent means+SEMs, *p<0.05.

Chapter 3:

Blockade of cocaine-induced habits by MDMA is trkB-dependent

3.1 Context, Author's Contribution, and Acknowledgement of Reproduction

The following chapter describes the effects of adolescent cocaine exposure on decision-making strategies in adulthood and tests novel strategies for normalizing decision-making behavior. The majority of experiments were performed by the dissertation author, with assistance from Elizabeth Barfield, Kristie Garza, Hayley Arrowood, Sherod Haynes, and Kelly Vogel. The data were analyzed and the document was written by the dissertation author, under the guidance of Dr. Gourley.

3.2 Abstract

Cocaine exposure during adolescence decreases the likelihood that individuals will seek treatment for problematic drug use at any point in life. Further, the majority of cocaine addicts initiate use during adolescence. Therefore, interventions that normalize decision-making abnormalities following developmental cocaine exposure may have significant impact in the treatment of substance use disorders. Mice were exposed to cocaine during adolescence, then tested for whether they engaged in instrumental behaviors according to action-outcome, or habit-based, response strategies. We next determined whether a naturally occurring flavone active at the tyrosine kinase receptor B (trkB), 7,8-dihydroxyflavone (7,8-DHF), or the substituted methylenedioxyphenethylamine, 3,4-methylenedioxymethamphetamine (MDMA), could block cocaine-induced habits. Using viral vector and chemogenetic strategies, we then determined neurotrophin-dependent circuit-level mechanisms by which 7,8-DHF and MDMA block cocaine-induced habits. Adult mice with a history of adolescent cocaine exposure preferentially engaged in habit-based response strategies at the expense of goal-directed actions. trkB stimulation blocked, while over-expression of a truncated trkB receptor in the orbitofrontal prefrontal cortex (oPFC) induced, habitual behavior. MDMA increased levels of the trkB ligand, Brain-derived Neurotrophic Factor (BDNF), in the oPFC and blocked cocaine-induced habits in a trkB-

dependent manner. Finally, we found that a neurotrophin-sensitive oPFC-amygdala circuit is essential to goal-directed action because it supports action-outcome memory, thereby occluding habit-based behavior. Stimulation of oPFC neurotrophin systems, *e.g.*, by 7,8-DHF or MDMA, enhances action-outcome memory. As such, oPFC trkB may be a viable target for new intervention strategies that combat maladaptive habits following developmental cocaine exposure.

3.3 Introduction

Cocaine dependence is characterized by compulsive drug use and maladaptive decision making. Adolescents are particularly vulnerable to the effects of cocaine; for example, cocaine exposure during adolescence reduces treatment seeking in individuals with substance use disorders *across the lifespan* (Kessler et al., 2001). It is thus critically important to understand how developmental cocaine exposure has long-term impact on complex decision making, and to develop strategies to normalize these phenomena.

The oPFC is thought to build "task spaces," allowing organisms to select optimal actions based on expected outcomes (Stalnaker et al., 2015). As such, oPFC inactivation impairs goal-directed action selection, resulting in a deferral to familiar, habit-based behaviors (Gremel & Costa, 2013; Rhodes & Murray, 2013; Zimmermann et al., 2017a). The oPFC is also implicated in addiction etiology (Volkow & Fowler, 2000; Lucantonio et al., 2012). For example, the oPFC is hypoactive in cocaine users during protracted withdrawal (Volkow et al., 1992). In rodents, cocaine reduces dendritic spines – the primary sites of excitatory plasticity in the brain – in the oPFC (Gourley et al., 2012b; Radley et al., 2015; DePoy et al., 2017). When cocaine is administered during adolescence, dendritic spine and dendrite arbor losses in the oPFC are detectable in adulthood (Gourley et al., 2012b; DePoy et al., 2014,2017).

Given that disruptions of neuronal development may mediate adolescent vulnerabilities to cocaine, drugs that target neurotrophin systems, which regulate neuronal structure and synaptic

plasticity (Park & Poo, 2013), may be therapeutically useful. A novel drug that stimulates the high-affinity receptor for BDNF, 7,8-DHF, corrects decision-making impairments following oPFC-selective *Bdnf* knockdown (Zimmermann et al., 2017b). Here, we examined whether 7,8-DHF would also block cocaine-induced habits. We also explored the utility of MDMA in blocking cocaine-induced habits. We were motivated by evidence (reported here) that MDMA increases oPFC BDNF and corrects decision-making strategies in mice with oPFC-selective *Bdnf* knockdown. Using viral vector strategies to over-express a truncated, inactive form of the trkB receptor, we next determined that the enrichment of goal-directed action selection by MDMA was trkB-dependent. Finally, we discovered that neurotrophin-dependent oPFC-BLA interactions encode action-outcome associations, enabling goal-directed action selection. These findings suggest that oPFC BDNF-trkB is necessary for goal-directed action selection and that neurotrophin-based therapeutics may be useful for enhancing goal-directed decision making.

3.4 Materials and Methods

3.4.1 Subject

Male and female C57BL/6 mice or mice homozygous for a floxed *Bdnf* gene (exon V) bred on a mixed BALB/c background (Jackson Laboratories, Bar Harbor, ME) were used. Mice were kept on a 12-hour light/dark cycle (0800 on) and given *ad libitum* access to water and food, except during instrumental conditioning when animals were food restricted to ~90% of their baseline weight. All studies were approved by the Emory University Institutional Animal Care and Use Committee.

3.4.2 Drugs

Cocaine HCl (10 mg/kg, i.p., in saline, Sigma-Aldrich, St. Louis, MO) was delivered from postnatal days (P) 31-35 and/or during cocaine self-administration testing (described below). 7-8-DHF (3-5 mg/kg, i.p., in 17% DMSO and saline; TCI Chemicals, Portland, OR) or MDMA HCl

(5.6 mg/kg, i.p., in saline, generously provided by NIDA) was delivered in adulthood (>P56). Drugs were administered in a volume of 1 mL/100 g.

3.4.3 Intravenous cocaine self-administration

First, mice were trained to nose poke for food reinforcement (20 mg grain-based pellets; Bioserv, Frenchtown, NJ) in standard Med-Associates (Georgia, VT) operant conditioning chambers for mice equipped with 3 nose poke recesses and a food magazine. Mice were trained to nose poke on a single recess for food reinforcers using a fixed ratio 1 (FR1) schedule of reinforcement over 7 sessions. Sessions lasted for 70 minutes or until 60 reinforcers were acquired.

Mice were next anesthetized with ketamine/dexdomitor (80 mg/kg/0.5 mg/kg i.p.). A Silastic catheter was implanted into the right jugular vein and exited on the dorsal surface of the scapula. Mice were allowed to recover for $\geq$7 days. Heparinized saline (30 USP/mL; 0.05 mL/infusion) was infused daily to maintain patency, which was tested by a ketamine (15 mg/mL; 0.03 mL/infusion) challenge prior to cocaine self-administration acquisition and the dose-response curve. Any mice that failed to respond to the ketamine challenge, defined by loss of muscle tone within 10 seconds, were excluded.

Next, mice were trained to self-administer cocaine using an FR1 schedule. Nose pokes resulted in cocaine (1.25 mg/mL; 20 μL infusion) and a 20-sec timeout, during which the house light was extinguished and nose pokes were not reinforced. Sessions ended when mice acquired 30 reinforcers or at 120 minutes. This response acquisition period lasted $\geq$5 sessions until responding was considered stable, defined as 2 consecutive days of ≥20 mg/kg cocaine, with ≥70% responding on the active nose poke and ≤20% variability in response rate (Butkovich et al., 2015).

Next, a dose-effect curve was determined. During each session, cocaine (0.125, 0.225, 0.4, 0.7, 1.25, or 2.25 mg/mL; 20 μL infusion) or saline was available according to a FR1 schedule

of reinforcement. Doses were tested in a pseudo-random order, and sessions ended at 120 minutes with no reinforcer limit. For an average 25 g adult mouse, the above would represent 0.1, 0.18, 0.32, 0.56, 1, and 1.8 mg/kg/infusion.

After intravenous cocaine self-administration response acquisition and testing a dose-effect curve, responding was extinguished in a novel context. Mice were placed in a chamber with new flooring, lights, and odors and were attached to a catheter swivel, but nose pokes had no programmed responses. Extinction criterion was ≤25% of maintenance day responding (Butkovich et al., 2015). Mice were trained for at least 5 sessions, and sessions lasted 120 minutes.

Following extinction, mice were re-exposed to the cocaine self-administration context, and responding was recorded, but had no programmed consequences. The following day, mice were again placed in the cocaine context and presented with a single non-contingent cue (house light extinguishes, pump is activated). Nose pokes on the previously reinforced aperture produced additional cue presentations, but no drug infusion. Reinstatement sessions lasted 60 minutes.

3.4.4 Oral cocaine self-administration

Mice self-administered cocaine during adolescence in standard Med-Associates (Georgia, VT) operant conditioning chambers for mice (16 X 14 X 12.5 cm) equipped with 2 nose poke recesses and a retractable lever. Oral cocaine self-administration was used in adolescent mice due to challenges associated with placing catheters in small adolescent mice and was performed as in DePoy et al. (2016). First, mice were “pre-trained” to lever-press during 2 sessions in which the lever was extended throughout the session and one press resulted in presentation of a dipper filled with 10% w/v sucrose. Next, mice were trained using a chained response requirement to receive oral cocaine or sucrose solution. A nose poke in the active recess (FR1 schedule) resulted in the extension of the lever for 10 s. A lever-press resulted in presentation of a dipper filled with 10% w/v sucrose or 75 µg/mL cocaine in sucrose solution. Sessions lasted for 60 minutes or until

30 reinforcers were acquired. Lever-press training was initiated on P29 and mice self-administered cocaine or sucrose from P31-47 (16 days).

3.4.5 Intracranial surgery

General

Mice were anesthetized with ketamine/dexdomitor. With needles centered at Bregma on a leveled skull, coordinates were located using a digitized stereotaxic frame (Stoelting, Wood Dale, IL). Throughout, infusions were delivered at 0.1 μL/minute, and the microsyringe was left in place for 5 (0.25 and 0.5 μL) or 12 (1.5 μL) minutes before withdrawal and suturing. Following surgery, mice were left undisturbed for 2-3 weeks.

oPFC infusions

Lentiviral vectors expressing GFP, Cre recombinase (Cre), or *TrkB.t1* under the CMV promoter were generated by the Emory University Viral Vector Core. Viral vectors were delivered to +2.6 mm anteroposterior (AP), -2.8 or -2.85 mm dorsoventral (Gourley et al., 2009b), and +/-1.2 mm mediolateral (ML) to Bregma in a volume of 0.25, 0.5, or 1.5 μL/side, as indicated.

BLA infusions

rAAV8-CamKIIα-hM4D-mCherry and rAAV8-CamKIIα-eGFP were generated by the University of North Carolina Viral Vector Core. Viral vectors were delivered to -1.4 mm AP, -4.9 mm DV, and +/-3.0 mm ML in a volume of 0.25 μL.

3.4.6 Food-reinforced instrumental conditioning

Mice were food-restricted and maintained at ~90% baseline weight for ≥1 week prior to instrumental conditioning. Male mice were initially trained for 5-7 sessions (1/day) using a FR1

reinforcement schedule, with 30 reinforcers available for responding on each of 2 nose poke recesses (60 pellet/session). Next, mice were shifted to a random interval 30-second (RI30) schedule for 2 sessions. Female mice were trained using 10 FR1 sessions because female mice expend less effort for food reinforcers (Seu et al., 2014) and thus can require additional training to develop robust response rates. Meanwhile, female mice also develop food-reinforced habits faster than males (see Fig.3-1: group x day interaction $F_{1,15}$=4.8, p<0.05; also, Quinn et al., 2007), so we eliminated RI training, which can promote habit-based behavior. Sessions lasted for 70 minutes or until 60 pellets were delivered.

Action-outcome contingency degradation was conducted as previously described (Gourley et al., 2012b; DePoy et al., 2017; Zimmermann et al., 2017b). Briefly, in the 'non-degraded' session, one nose poke aperture was occluded, and responding on the other aperture was reinforced according to a variable ratio 2 (VR2) schedule for 25 minutes. In the 'degraded' session, the previously reinforced nose poke aperture was occluded, and pellets were delivered into the magazine non-contingently at a rate matched to the reinforcement rate from the previous session. Responding on the available nose poke recess was recorded, but responding had no programmed consequences, thus violating the contingency between nose poking and reinforcement. Drugs were administered immediately following the 'degraded' session with the goal of enhancing new learning. The location of the 'degraded' aperture was counter-balanced between and within groups.

The next day, both apertures were available for a 5-minute probe test conducted in extinction. Goal-directed decision making is reflected by preferential responding on the 'non-degraded' aperture (*i.e.*, generating the response that was most likely to be reinforced), while equivalent responding is considered a failure in action-outcome conditioning, a deferral to familiar habit-based behavior.

3.4.7 7,8-DHF-cocaine cross-sensitization

Mice were placed in Med Associates locomotor monitoring chambers equipped with 16 photocells. After 1 hour of habituation, mice were injected with either vehicle (17% DMSO in saline) or 7,8-DHF (5 mg/kg, as in the main text). This process was repeated 3 times/week for 14 total sessions. Then, mice were left undisturbed for 10-14 days. To test for potential cross-sensitization between 7,8-DHF and cocaine, mice were then returned to the locomotor monitoring chambers. Locomotor activity was measured for 1 hour for habituation. All mice were then injected with saline and monitored for 1 hour, followed finally by an injection of cocaine (10 mg/kg, i.p., in saline). Again, locomotor activity was monitored for 1 hour.

3.4.8 Histology

Mice were euthanized by rapid decapitation, and brains were immersed in 4% paraformaldehyde for 48 hours and then 30% w/v sucrose until being sectioned at 50 μm. Infusion sites were verified by immunostaining for Cre (as in DePoy et al., 2013, with the primary antibody concentration at 1:750; Sigma-Aldrich) or the HA tag on the *TrkB.t1* virus (as in Heldt et al., 2014, with the primary antibody concentration at 1:1000; Sigma-Aldrich). Alternatively, GFP or mCherry was visualized. Sections were mounted and coverslipped with Permount mounting medium (Thermo Fischer Scientific, Waltham, MA) and imaged.

3.4.9 Quantitative imaging

Sections were immunostained for the HA tag (above) or glial fibrillary acidic protein (as in Gourley et al., 2010, with the primary antibody concentration at 1:1000; DakoCytomation, Carpinteria, California). Sections were imaged on a Nikon 4550s SMZ18 stereo microscope (Nikon Instruments, Melville, NY). All images were collected in the same session with settings held constant. A sampling area was drawn around the infusion site, and the mean integrated intensity was quantified in NIS Elements (Nikon Instruments).

3.4.10 Western blotting

Mice were euthanized by rapid decapitation and brains were collected 30 minutes, 2 hours, or 8 hours following 7,8-DHF or 2 hours following MDMA administration. Brains were flash frozen on dry ice and stored at -80°C. Brains were sectioned into 1-mm thick coronal sections, and a single experimenter dissected tissue from the oPFC, medial prefrontal cortex (mPFC), amygdala, and dorsal striatum using a 1 mm tissue corer. Samples were sonicated in lysis buffer [200 μL; 137 mM NaCl, 20 mM tris-HCl (pH=8), 1% igepal, 10% glycerol, 1:100 Phosphatase Inhibitor Cocktails 1 and 2 (Sigma-Aldrich), and 1:1000 Protease Inhibitor Cocktail (Sigma-Aldrich)]. Protein concentrations were determined using a Pierce BCA Protein Assay kit (Thermo Fisher Scientific) following manufacturer's instructions.

15 μg of each sample were separated by SDS-page on 7.5% or 4-20% gradient Tris-glycine gels (Bio-Rad Laboratories, Inc., Hercules, CA) and then transferred to PVDF membranes (Bio-Rad). Blots were blocked in 5% nonfat dry milk for 1 hour. The primary antibodies used were: ERK1/2 (1:2000; Cell Signaling Technology, Danvers, MA), phospho-ERK1/2 (1:500, Cell Signaling Technology), trkB (1:375; Cell Signaling Technology), phospho-trkB (1:250; Cell Signaling Technology), BDNF (1:250; Abcam), and HSP70 (1:6000; Santa Cruz Biotechnology, Dallas, TX). Membranes were incubated in primary antibody overnight at 4°C and then at room temperature for 1 hour in secondary antibody goat anti-mouse or anti-rabbit peroxidase labeled IgG (Vector Laboratories). Bands were measured using a chemiluminescence substrate (Thermo Fisher Scientific) and a ChemiDoc MP Imaging System (Bio-Rad). Values were normalized to the HSP 70 loading control and then to the control mean on each gel to control for variation across membranes. Phospho-ERK1/2 values were normalized to total ERK1/2.

3.4.11 Enzyme-linked immunosorbent assay (ELISA)

Tissue samples were homogenized as for western blots (above). BDNF was quantified using a BDNF ELISA kit (Promega Corporation, Madison, WI) following manufacturer's instructions, excluding the extraction step. Samples were tested in duplicate and normalized to control samples on each independent plate.

3.4.12 Statistical analyses

Statistical analyses were performed using SPSS with $\alpha \leq 0.05$. Response rates and photobeam breaks were compared by 2 or 3-factor ANOVA with repeated measures when appropriate. ANOVA or *t*-tests were used to compare western blotting values. *t*-tests were used to compare group means from reinstatement, ELISA, marble burying, and open field tests. If data had unequal variance (according to Levene's test for equality of variance), then Welch's *t*-test was used to compare group means. In the case of significant interactions, Bonferroni post-hoc analyses were used. Throughout, values >2 standard deviations from the mean were considered outliers and excluded.

3.5 Results

3.5.1 Adolescent cocaine exposure has long-term behavioral consequences

We first examined the long-term effects of adolescent cocaine exposure on cocaine self-administration. Mice were exposed to cocaine from P31-35, then trained to nose poke for food reinforcers, then for intravenous-delivered cocaine, in adulthood ($\geq$P56)(Fig.3-2A). P31-35 was selected because cocaine exposure during this brief window is sufficient to induce habit-based behavioral biases in adulthood (Hinton et al., 2014; DePoy et al., 2017). Cocaine history did not impact food-reinforced response acquisition ($F<1$)(Fig.3-2B), but elevated response rates when mice were transitioned to cocaine (interaction $F_{1,76}=4.339$, $p=0.05$)(Fig.3-2C). This is likely attributable to familiarity with the interoceptive properties of cocaine, given that mice with a history of cocaine exposure also rapidly inhibited responding when saline replaced cocaine in the *i.v.*

catheter. By contrast, a history of cocaine exposure did not shift responding in a cocaine dose-effect curve (interaction $F_{6,78}$=3.095, p=0.009)(Fig.3-2D), indicating adolescent cocaine exposure did not heighten sensitivity to the reinforcing qualities of cocaine. Additionally, adolescent cocaine exposure did not modify responding in extinction (interaction $F_{4,72}$=1.643, p=0.173)(Fig.3-2E) or context- or cue-induced reinstatement of drug seeking (context: t_{17}=0.952, p=0.354; cue: t_{17}=-0.114, p=0.911)(Fig.3-2F,G).

To further understand how adolescent cocaine exposure modifies reward-related decision making, we tested separate mice in an instrumental contingency degradation procedure. This test can be used to determine whether mice select actions based on their consequences. Mice were first trained to respond equally on two nose poke recesses for food reinforcers, then the likelihood that one familiar response would be reinforced was greatly decreased, while the other response remained reinforced (Fig.3-3A,B). Adolescent cocaine exposure did not impact response acquisition in adult males or females (both F<1)(Fig.3-3C,E), however, cocaine-exposed mice failed to differentiate between responses that were more *vs.* less likely to be reinforced following instrumental contingency degradation, a pattern consistent with stimulus-elicited habits, rather than goal-directed actions (interaction males $F_{1,21}$=4.918, p=0.038; females $F_{1,13}$=5.377, p=0.037)(Fig.3-3D,F).

We also examined action-outcome decision making in mice that *self-administered* cocaine during adolescence. Response rates for orally-ingested cocaine and sucrose increased over time (main effect $F_{15,525}$=14.388, p<0.001), but mice with access to cocaine generated higher response rates (main effect $F_{1,35}$=4.739, p=0.036)(Fig.3-4A). Adolescent cocaine did not impact instrumental response acquisition (main effect and interaction F<1)(Fig.3-4B), but mice that self-administered cocaine during adolescence developed habit-based response strategies (interaction $F_{1,32}$=4.118, p=0.05)(Fig.3-4C), as in the case of experimenter-administered cocaine.

3.5.2 trkB stimulation normalizes decision-making strategies following developmental cocaine

We hypothesized that drugs that stimulate trkB may rescue goal-directed action selection in adult mice exposed to cocaine during adolescence. We first confirmed that 7,8-DHF, a novel trkB agonist, increased trkB phosphorylation in the oPFC within 30 minutes following administration (main effect $F_{1,7}$=6.776, p=0.035)(Fig.3-5A). Phospho-trkB returned to control levels within 2 hours of injection (main effect $F_{2,22}$=1.9, p=0.173). We then administered 7,8-DHF immediately following contingency degradation training, during presumptive formation of new memory (Fig.3-6A). As before, response rates did not differ during training (F<1)(Fig.3-6B). And as hypothesized, 7,8-DHF blocked habit-based responding in cocaine-exposed mice, restoring outcome-sensitive decision making when paired with new learning (interaction $F_{1,44}$=6.612, p=0.014)(Fig.3-6C).

3.5.3 MDMA stimulates oPFC BDNF and blocks cocaine-induced habits

There are some concerns regarding unintended consequences of stimulating trkB therapeutically, *e.g.*, pain hypersensitivity (Merighi et al., 2008). We were thus interested in examining other drugs that might stimulate BDNF-trkB with more brain region specificity. MDMA reportedly increases PFC BDNF (Hemmerle et al., 2012). We found using ELISA that MDMA increased BDNF in the oPFC (t_{14}=-1.95, p=0.036)(Fig.3-7A), but not mPFC or amygdala (t<1)(Fig.3-7B,C). Additionally, MDMA reduced BDNF in the dorsal striatum (t_{15}=3.042, p=0.008)(Fig.3-7D). Using western blotting, we found that MDMA increased both the mature and pro forms of BDNF (mature t_8=-1.984, p=0.042; pro-BDNF $t_{6.38}$=-2.435, p=0.024)(Fig.3-7E,F) and ERK1/2 phosphorylation (t_7=2.333, p=0.05)(Fig.3-7G) in the oPFC.

To determine whether the pro-neurotrophic effects of MDMA could influence decision-making strategies, we next confirmed that MDMA could normalize response abnormalities due to *Bdnf* knockdown. We selectively reduced *Bdnf* in the oPFC using viral-mediated gene silencing, resulting in a ~13% loss of BDNF protein in gross oPFC tissue punches containing both infected and uninfected tissue (t_{16}=2.368, p=0.031)(Fig.3-7H-J). We then trained mice to respond for food

reinforcers (F<1)(Fig.3-7K) and tested sensitivity to instrumental contingency degradation. *Bdnf* knockdown caused a bias towards habit-based behaviors as expected (Gourley et al., 2013a; Zimmermann et al., 2017b), and MDMA blocked these habits (interaction $F_{1,26}$=5.233, p=0.044)(Fig.3-7L).

We next hypothesized that MDMA may block habits in mice exposed to developmental cocaine. We again administered MDMA immediately following contingency degradation training in an effort to normalize action-outcome learning and memory (Fig.3-8A). Again, developmental cocaine exposure did not affect response acquisition (F<1)(Fig.3-8B), and as hypothesized, MDMA rescued goal-directed action selection following developmental cocaine exposure (interaction $F_{1,42}$=4.166, p=0.048)(Fig.3-8C).

3.5.4 Blockade of cocaine-induced habits by MDMA is trkB-dependent

We hypothesized that MDMA acts by increasing oPFC BDNF levels, stimulating trkB. To test this model, we infused a lentivirus expressing a truncated, inactive form of trkB (*TrkB.t1*) and an HA tag (Fig.3-9A) in order to sequester BDNF. We infused two volumes. Histological analyses indicated that viral vector spread did not differ between 0.5 and 1.5 μL groups (t_{14}=-1.053, p=0.31)(Fig.3-9B,C), but the 1.5 μL infusion generated greater HA immunoreactivity (t_{14}=-2.672, p=0.018)(Fig.3-9D), indicating a higher density of viral infection. Levels of GFAP (an astrocytic marker) did not differ, indicating that the 1.5 μL infusion did not cause greater tissue damage (t_{13}=1.258, p=0.23)(Fig.3-9E).

Groups did not differ during instrumental response training (F<1)(Fig.3-9F). As with *Bdnf* knockdown (Fig.3-7L), *TrkB.t1* overexpression blocked outcome-based decision making, causing a deferral to habit-based behavior. MDMA corrected decision-making strategies in the 0.5, but not 1.5, μL group (interaction $F_{2,32}$=7.792, p=0.002)(Fig.3-9G). This pattern suggests that MDMA blocks cocaine-induced habits by stimulating trkB signaling in the oPFC.

3.5.5 Neurotrophin-dependent oPFC-BLA interactions coordinate outcome-based decision making

Lastly, we wanted to examine the extended circuitry involved in neurotrophin-sensitive decision making. We modified classical disconnection procedures in which unilateral lesions would be placed in the contralateral oPFC and BLA, instead reducing *Bdnf* unilaterally in the oPFC and placing inhibitory Gi-coupled DREADDs in the contralateral BLA (Fig.3-10A-C). Mice with ipsilateral infusions or control viral vectors served as controls. All mice acquired the responses, with no differences between groups (male and female $F<1$)(Fig.3-10D,F). The DREADD ligand CNO was then delivered in conjunction with instrumental contingency degradation, inactivating the BLA. Both male and female mice with contralateral infusions were subsequently unable to differentiate between responses that were more, *vs.* less, likely to be reinforced, instead generating habit-based response patterns (interaction males $F_{2,34}=1.415$, $p=0.044$; females $F_{2,18}=3.563$, $p=0.05$)(Fig.3-10E,G). Thus, interfering with BDNF-dependent oPFC-amygdala interactions occludes goal-directed response selection by disrupting action-outcome learning and memory.

We also report that 7,8-DHF does not cross-sensitize with cocaine (interaction $F_{2,12}=6.3$, $p=0.04$; all post-hoc comparisons $p>0.055$)(Fig.3-11). The implications of this experiment are discussed below.

3.6 Discussion

Adolescent drug abuse has long-lasting effects on decision making, for example, decreasing the likelihood that individuals will seek treatment for drug use disorders at any point in the lifespan (Kessler et al., 2001). In rodent models, adolescent mice are more vulnerable than adults to developing cocaine-induced biases towards habit-based behavior (DePoy et al., 2016,2017). This is significant because habits are considered etiological factors in the

development and maintenance of addiction (Everitt & Robbins, 2016). Improving action-outcome decision making following cocaine (thereby *occluding* habits) could be beneficial, for example, in enhancing treatment outcomes in abstinent, cocaine-dependent individuals. Here we report that a novel agonist active at the high-affinity receptor for BDNF, trkB, can block cocaine-induced habits. We also find that MDMA blocks cocaine-induced habits, and in a trkB-dependent fashion. Our findings further suggest that these pharmaco-manipulations act by stimulating neurotrophin-sensitive oPFC-BLA interactions.

3.6.1 Adolescent cocaine exposure has long-term neurobehavioral consequences

Here, we utilized an instrumental contingency degradation task to determine whether mice could select actions based on their consequences, and how cocaine impacted this process. Mice were trained to respond equally on two nose poke recesses for food reinforcers, then the reinforcer associated with one response was provided non-contingently. A goal-oriented response strategy is to preferentially generate the response that remains reinforced in a subsequent probe test, while equivalent responding reflects a deferral to familiar, habit-based response patterns (Balleine & O'Doherty, 2010). Cocaine exposure during early adolescence (P31-35 here) impaired action-outcome decision making (causing habit-based behaviors) in adult male and female mice, consistent with prior investigations (DePoy et al., 2016,2017). Adolescent cocaine exposure also stimulated cocaine-, but not food-, reinforced responding in adulthood, further evidence of long-term consequences despite the relatively brief period of early-life exposure. This effect was likely driven by familiarity with the interoceptive properties of cocaine, rather than heightened sensitivity to its reinforcing consequences, given that developmental cocaine exposure did not shift a dose-response curve. Importantly, our findings do not preclude the possibility that sensitivity to cocaine or cocaine self-administration patterns differ between adolescent and adult mice (Wong et al., 2013), but rather, indicate that even relatively limited cocaine exposure early in adolescence can trigger cocaine-reinforced responding and habit biases in adulthood.

3.6.2 Blockade of cocaine-induced habits

Cocaine alters multiple brain regions involved in complex decision making, including the oPFC. For example, the oPFC atrophies in cocaine abusers (Franklin et al., 2002; Alia-Klein et al., 2011; Volkow et al., 2011a), and in rodents, dendritic spines are eliminated (Radley et al., 2015). Adolescent cocaine exposure causes lasting changes in neuron structure, decreasing dendritic spine densities (Gourley et al., 2012a; DePoy et al., 2017) and simplifying dendrite arbors (DePoy et al., 2014). Cocaine-induced changes in oPFC dendrite structure may be causally related to impairments in decision making, given that drugs that correct cocaine-induced habit biases appear to recruit cytoskeletal regulatory systems (DePoy et al., 2017).

Neurotrophins are potent regulators of neuron structure. For example, BDNF increases dendritic spine size and density by activating its high-affinity receptor trkB (Bennett & Lagopoulos, 2014). oPFC-selective *Bdnf* knockdown induces habit biases, which are reversible by the small-molecule trkB agonist 7,8-DHF, at doses that also stimulate dendritic spinogenesis in the oPFC (Zimmermann et al., 2017b). Based on this profile, we administered 7,8-DHF to cocaine-exposed mice, reinstating goal-directed action selection despite prior cocaine exposure.

There are several concerns, however, regarding the utility of stimulating BDNF-trkB for therapeutic purposes. Specifically, BDNF in different brain regions has opposing effects on cocaine-related behavior (reviewed Li & Wolf 2015; Pitts et al., 2016). On the one hand, BDNF infusions into the mPFC and systemic 7,8-DHF treatment decrease drug seeking (Berglind et al., 2007,2009; Whitfield et al., 2011; DePoy et al., 2016), and we find that 7,8-DHF does not cross-sensitize with cocaine, suggesting that it is unlikely to enhance stimulant usage. In apparent contradiction, however, a trkB *antagonist* blocks cue-induced reinstatement of cocaine seeking in mature rats (Verheij et al., 2016). This outcome may be due to BDNF-trkB activity in the nucleus accumbens, which elicits cocaine self-administration and cocaine-seeking behavior (Graham et al., 2007). Interestingly, however, the novel trkB antagonist utilized by Verheij et al. (2016) to

block reinstatement paradoxically *stimulates* trkB phosphorylation in the PFC, in line with evidence that PFC BDNF-trkB decreases motivation to take cocaine (Sadri-Vakili et al., 2010) and facilitates the extinction of cocaine-conditioned place preference (Gourley et al., 2013a; Otis et al., 2014).

With these patterns in mind, we next determined the effects of MDMA on BDNF in the oPFC. MDMA enhances conditioned fear extinction, a PFC-dependent process (Young et al., 2015) and increases PFC *Bdnf* (Hemmerle et al., 2012; Mouri et al., 2017). Here, MDMA stimulated BDNF in the oPFC but not mPFC (see also Young et al., 2015), indicating that MDMA-induced elevations in PFC BDNF may be driven by oPFC BDNF. MDMA also increased phosphorylation of ERK1/2, a trkB substrate. This is pertinent, given that the BDNF-mediated interference of cocaine seeking depends on trkB activation of ERK1/2 (Whitfield et al., 2011). Interestingly, MDMA also *decreased* BDNF in the dorsal striatum, a provocative finding given that dorsal striatal BDNF augments the reinforcing properties of cocaine (Im et al., 2010).

Next, we reduced *Bdnf* selectively in the oPFC using viral-mediated gene silencing, resulting in a 13% loss of BDNF in gross tissue sections. As in prior reports, *Bdnf* knockdown induced habit-based behavior (Gourley et al., 2013a; Zimmermann et al., 2017b), and MDMA blocked these habits, presumably by stimulating BDNF in non-infected neurons. Surprisingly, *Bdnf* knockdown did not impact response rates during instrumental response training as previously reported (DePoy et al., 2013; Gourley et al., 2013a; Zimmermann et al., 2017b). This is possibly explained by a longer period of food restriction prior to training, which may have allowed mice to habituate to food restriction stress.

MDMA also blocked cocaine-induced habits. While there is some concern regarding the use of MDMA as a therapeutic compound (Parrott 2013), it has clinical efficacy in treating PTSD (Mithoefer et al., 2011,2013), and acute administration does not induce obvious harmful effects or trigger recreational usage (Mithoefer et al., 2013). Importantly, overexpression of an inactive, truncated form of trkB (*TrkB.t1*) in the oPFC interfered with the corrective properties of MDMA,

indicating that the “anti-habit” effects of MDMA are oPFC trkB-dependent. *TrkB.t1* overexpression also itself induced habit biases (as was also reported in separate mice in chapter 2). These findings are significant because oPFC *Bdnf* knockdown induces habits (Fig.4; Gourley et al., 2013a; Zimmermann et al., 2017b), but the site of BDNF-trkB binding was previously unknown because BDNF is subject to anterograde and retrograde transport. The patterns reported here suggest that local oPFC BDNF-trkB binding supports goal-directed action.

3.6.3 oPFC BDNF organizes goal-directed action via the BLA

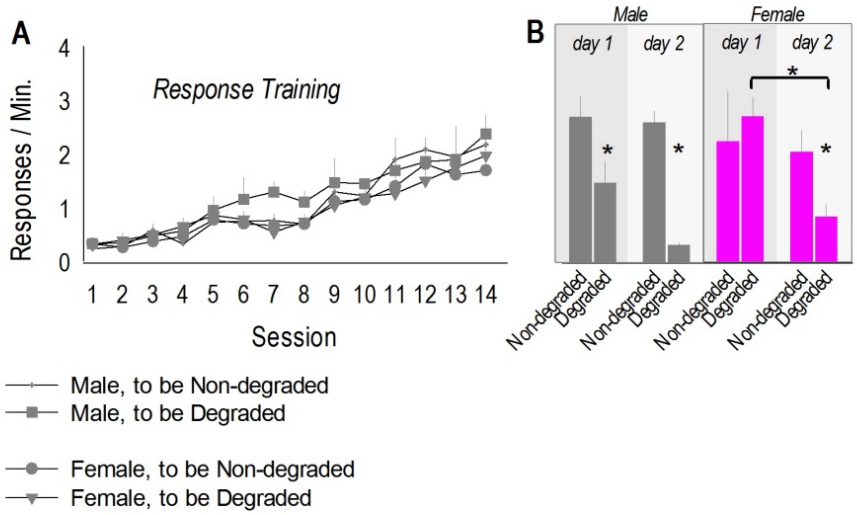
The oPFC is bidirectionally connected with the BLA, and projections originating in the ventrolateral subregion targeted here are largely ipsilateral (McDonald et al., 1996; Zimmermann et al., 2017b). We capitalized on this segregated organization and reduced *Bdnf* unilaterally in the oPFC and infused inhibitory Gi-coupled DREADDs into the contralateral BLA. This “disconnection” approach allowed us to assess the impact of BDNF-dependent oPFC-BLA interactions on response choice in an *inducible* manner. We administered CNO to inactivate the BLA immediately following action-outcome contingency degradation, then tested mice drug-free during the probe test. We were motivated by evidence that the BLA is essential for learning about, but not necessarily expressing, goal-directed response strategies (Wellman et al., 2005; West et al., 2012; Parkes & Balleine, 2013), and that task-related plasticity in the oPFC is essential for action-outcome memory retention (Zimmermann et al., 2017a). Both male and female mice with asymmetric infusions “disconnecting” the oPFC and BLA were unable to select the response most likely to be reinforced, indicating that BDNF-dependent oPFC-BLA interactions are necessary for action-outcome memory encoding in mice.

3.6.4 Conclusions

The oPFC is thought to integrate information about context and reward to predict likely outcomes – particularly when those outcomes are not readily observable – to guide goal-directed

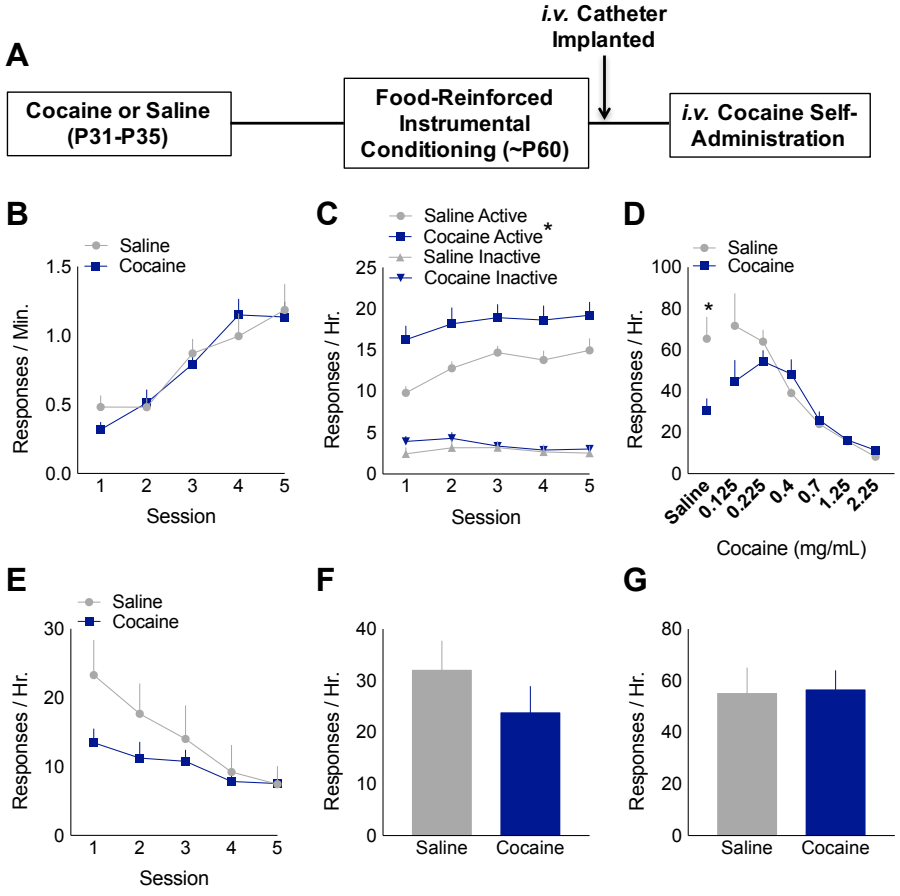
action (Stalnaker et al., 2015). Accordingly, oPFC inactivation can cause outcome-insensitive, habit-based response strategies in rodents (Gourley et al., 2013a; Gremel & Costa, 2013) and non-human primates (Jackson et al., 2016; Fiuzat et al., 2017). Cocaine addicts display habit biases in similar tasks, even when responding for non-drug reinforcers, as with our mice here (Ersche et al., 2016). We report that a BDNF-sensitive oPFC-BLA circuit supports goal-directed action, and pharmacological strategies that stimulate oPFC BDNF-trkB systems rescue flexible decision-making strategies, blocking habits, following cocaine. Thus, neurotrophin-based therapeutics may be effective adjuncts for addicted individuals seeking to maintain a drug abstinent lifestyle, particularly individuals first exposed to cocaine early in life.

Figure 3-1: *Sex differences in sensitivity to instrumental contingency degradation.*



(A) Mice were trained to nose poke for food reinforcers using an FR1 schedule of reinforcement for 2 weeks, with no groups differences. Response acquisition curves are segregated according to sex and whether mice subsequently experienced intact ("Non-degraded") or "Degraded" conditions. Mice experienced only one of these conditions. Note that this design deviates from the protocol used in the subsequent text, in which case mice experienced both conditions followed by a probe test. Methods were otherwise the same. (B) Response rates during the Non-degraded and Degraded sessions are shown. Males in the Degraded condition inhibited responding on the first day of training, while female mice required 2 days of training before inhibiting responding. This pattern is consistent with greater reliance on habit-based response strategies in females relative to males (Quinn et al., 2007). Means+SEMs, *$p<0.05$.

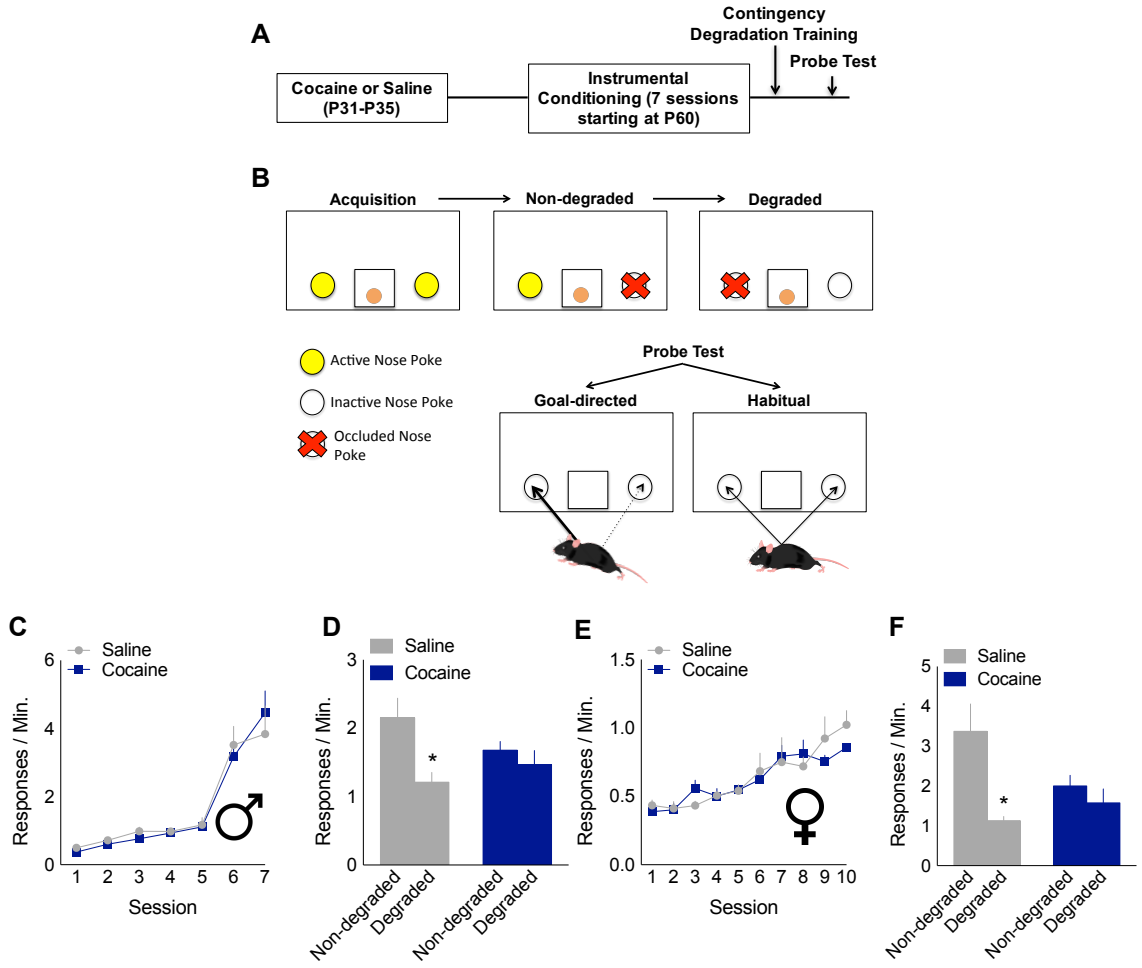
Figure 3-2: *Adolescent cocaine exposure facilitates discriminative responding for cocaine in adulthood.*



(A) Experimental timeline. Mice were exposed to cocaine during early adolescence and then tested in an *i.v.* cocaine self-administration procedure in adulthood. (B) Mice acquired food-reinforced instrumental responses without group differences, but (C) prior cocaine exposure increased response rates during the acquisition phase of the cocaine self-administration procedure. (D) This can likely be attributed to discriminating the interoceptive properties of cocaine, rather than a shift in reinforcing properties, given that mice with a history of adolescent cocaine exposure inhibited responding when saline replaced cocaine in the infusion pump, but largely did not differ from control mice when varied cocaine doses were delivered. (E) Response rates during subsequent extinction testing did not differ, (F) nor did responding during a test of

context-induced reinstatement of cocaine seeking or (G) cue-induced reinstatement of the response. Symbols represent means+SEMs, *$p<0.05$.

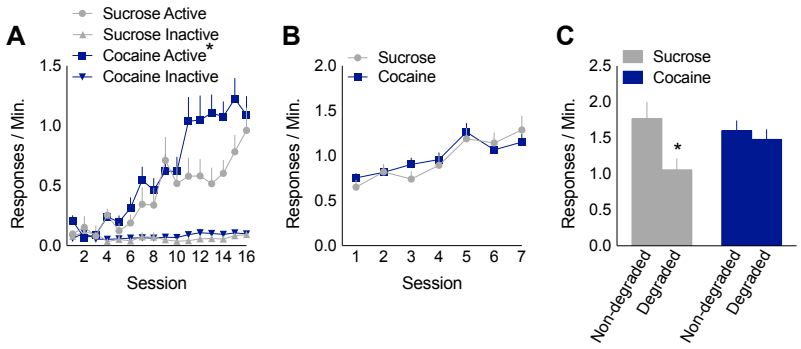
Figure 3-3: *Mice exposed to cocaine as adolescents are insensitive to changes in action-outcome contingencies as adults.*



(A) Mice were exposed to cocaine during early adolescence, then tested for sensitivity to action-outcome contingencies in adulthood. (B) Schematic of task. Mice are trained to respond equally on two distinct apertures. Then, the probability of reinforcement is greatly decreased for one response. Preferential engagement of the response that remains likely to be reinforced ("non-degraded") is interpreted as goal-directed, while equivalent responding is considered habit-based. (C) Male mice with a history of cocaine exposure acquired the nose poke responses. (D) Following instrumental contingency degradation, however, mice with a history of adolescent cocaine exposure were unable to differentiate between responses that were likely, *vs.* unlikely, to be

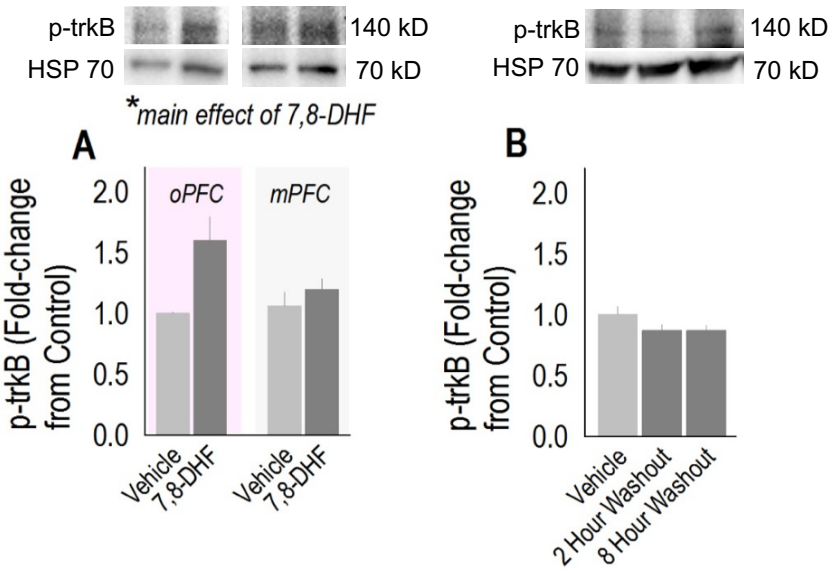
reinforced (“non-degraded” *vs.* “degraded”). Instead, these mice engaged two familiar responses equally. (E) Similarly, female mice with a history of adolescent cocaine exposure acquired nose poke responses, but were (F) unable to modify responding following instrumental contingency degradation. Symbols and bars represent means+SEMs, *$p<0.05$.

Figure 3-4: *Mice that self-administer cocaine in adolescence are insensitive to changes in action-outcome contingencies as adults.*



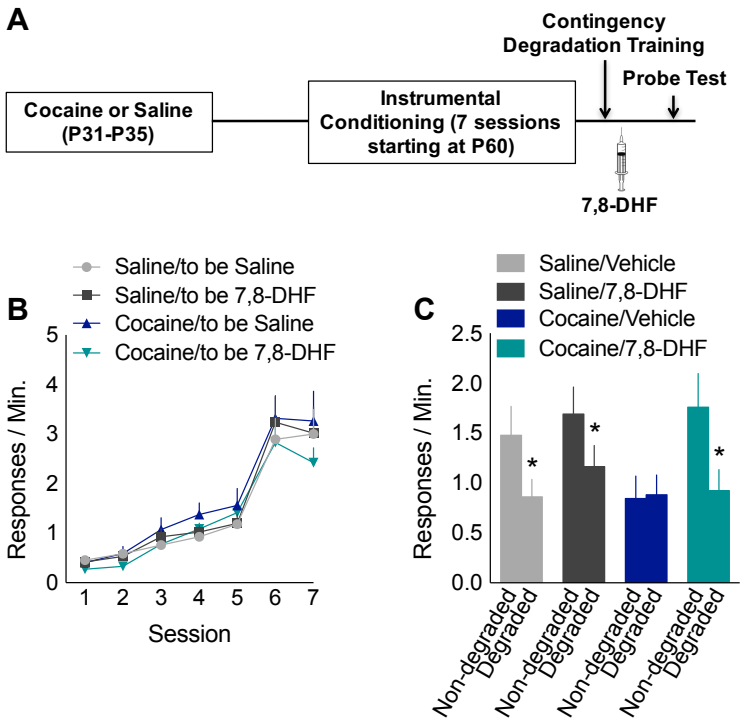
(A) Adolescent mice responded for cocaine (75 μg/mL; *p.o.*) or sucrose. Mice with access to cocaine generated higher response rates than control mice consuming only sucrose. (B) Mice that self-administered cocaine during adolescence acquired food-reinforced nose poke responses in adulthood, but (C) were unable to modify responding following instrumental contingency degradation. Bars and symbols represent means+SEMs, *p<0.05.

Figure 3-5: *7,8-DHF increases trkB phosphorylation.*



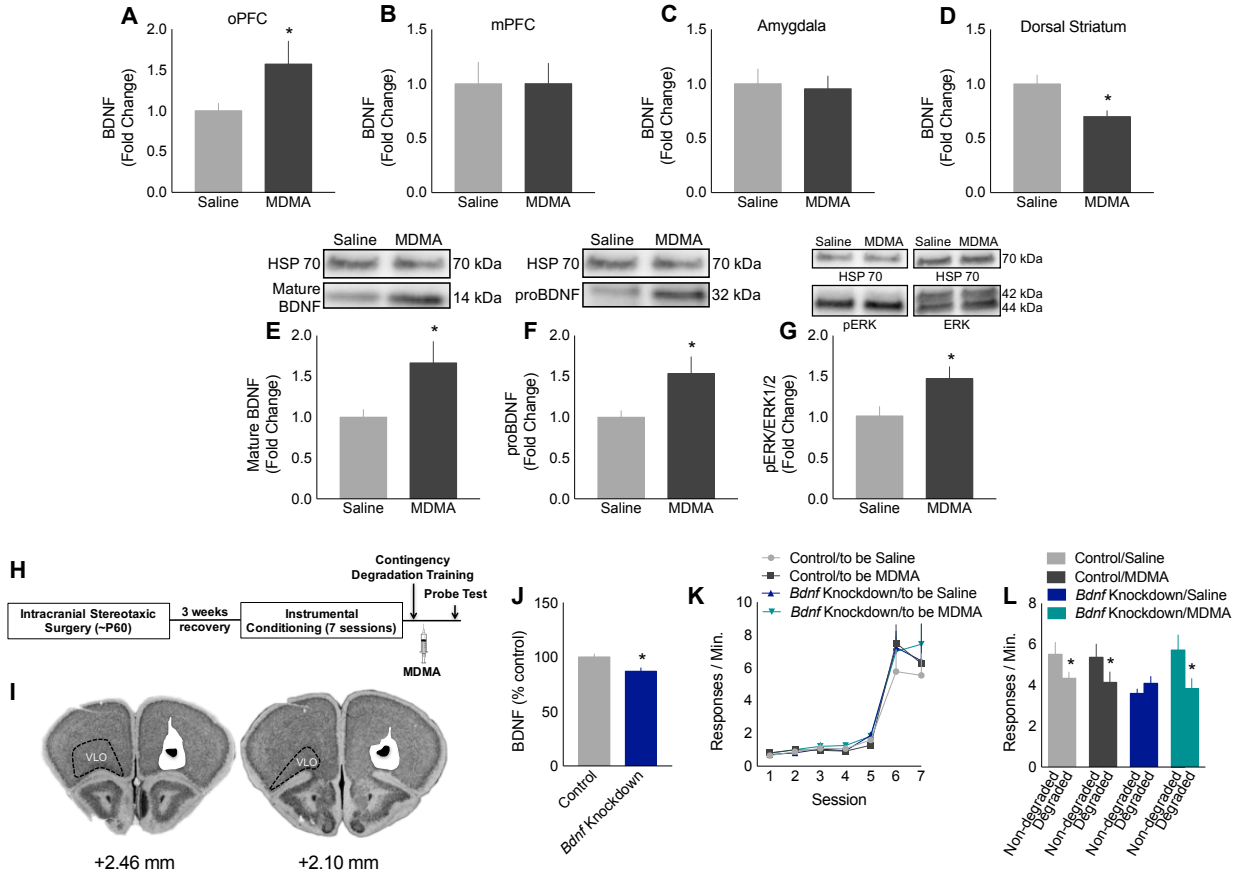
(A) Mice were injected with 7,8-DHF, then brains were collected 30 min later, revealing elevated phospho-trkB (p-trkB) in the oPFC and mPFC. (B) In a separate experiment, we collected whole PFC tissue samples and assessed phospho-trkB at 2 and 8 hours after injection, revealing no effects. There were no changes in trkB, t.trkB, or the HSP 70 loading control throughout (not shown). Bars represent means+SEMs, *$p<0.05$.

Figure 3-6: *7,8-DHF blocks adolescent cocaine-induced behavioral inflexibility.*



(A) We exposed mice to cocaine during early adolescence, then tested for sensitivity to action-outcome contingencies in adulthood. 7,8-DHF was given immediately following instrumental contingency degradation, then response preference was assessed the following day when mice were drug-free. (B) Mice acquired the instrumental responses without group differences. (C) Developmental cocaine exposure induced failures in action-outcome decision making, resulting in non-selective responding, while 7,8-DHF corrected these failures. Symbols and bars represent means+SEMs, *$p<0.05$.

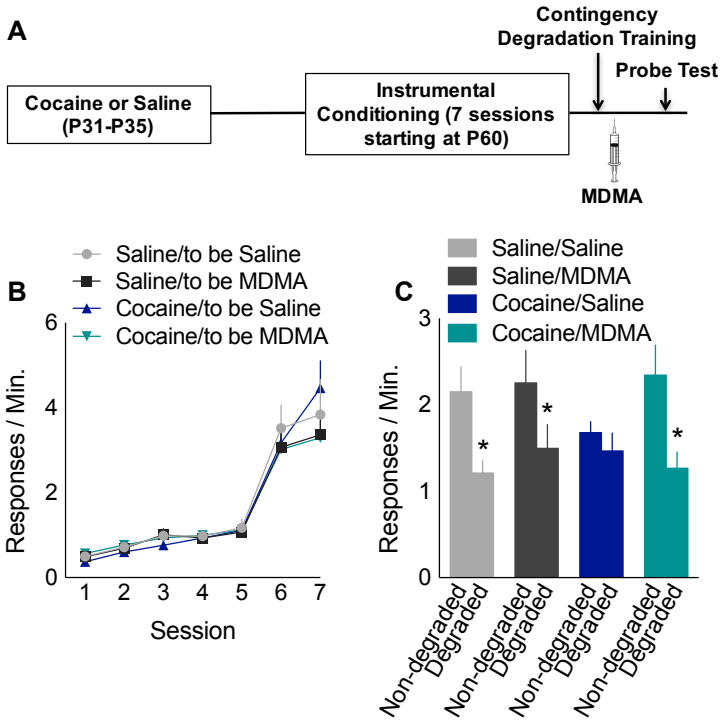
Figure 3-7: *MDMA stimulates oPFC BDNF and blocks Bdnf knockdown-induced behavioral inflexibility.*



(A) MDMA increased BDNF in the oPFC, as quantified by ELISA. (B) MDMA did not affect BDNF levels in the mPFC or (C) amygdala, and (D) MDMA decreased BDNF in the dorsal striatum. (E) Western blotting revealed increased mature BDNF and (F) proBDNF protein in the oPFC, concurrent with (G) elevated phospho-ERK1/2. (H) Experimental timeline. *Bdnf* was reduced using Cre-expressing viral vectors in 'floxed' *Bdnf* mice. Mice were then tested for sensitivity to action-outcome contingencies. MDMA was given immediately following instrumental contingency degradation, then response preference was assessed the following day when mice were drug-free. (I) Representation of viral vector spread on images from the Mouse Brain Library (Rosen et al.,, 2000) are shown, with white representing maximal spread and black the smallest spread. "VLO" refers to the ventrolateral oPFC. (J) Infusion of Cre-expressing viral vectors decreased

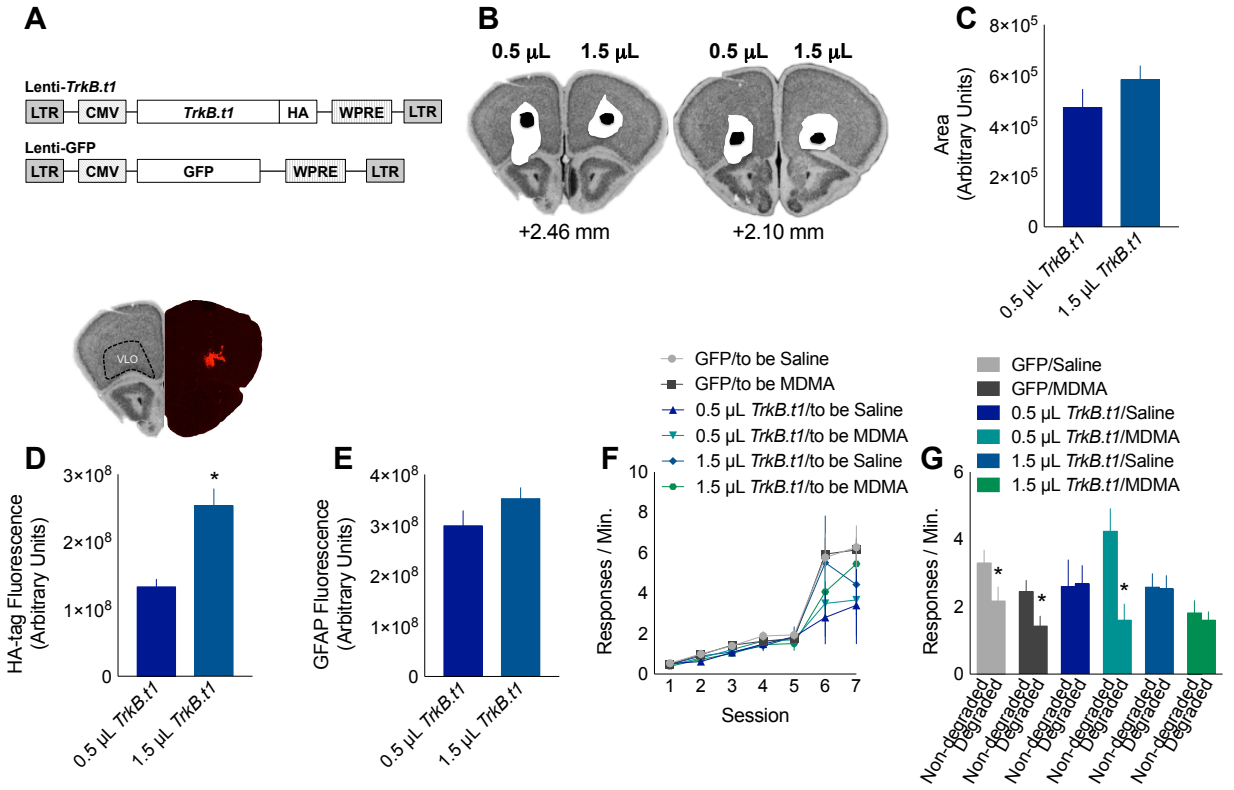
BDNF in gross oPFC tissue punches. (K) Mice acquired the instrumental responses with no group differences. (L) *Bdnf* knockdown induced failures in action-outcome decision making, resulting in non-selective responding, while MDMA corrected these failures. Symbols and bars represent means+SEMs, *$p<0.05$.

Figure 3-8: *MDMA blocks adolescent cocaine-induced behavioral inflexibility.*



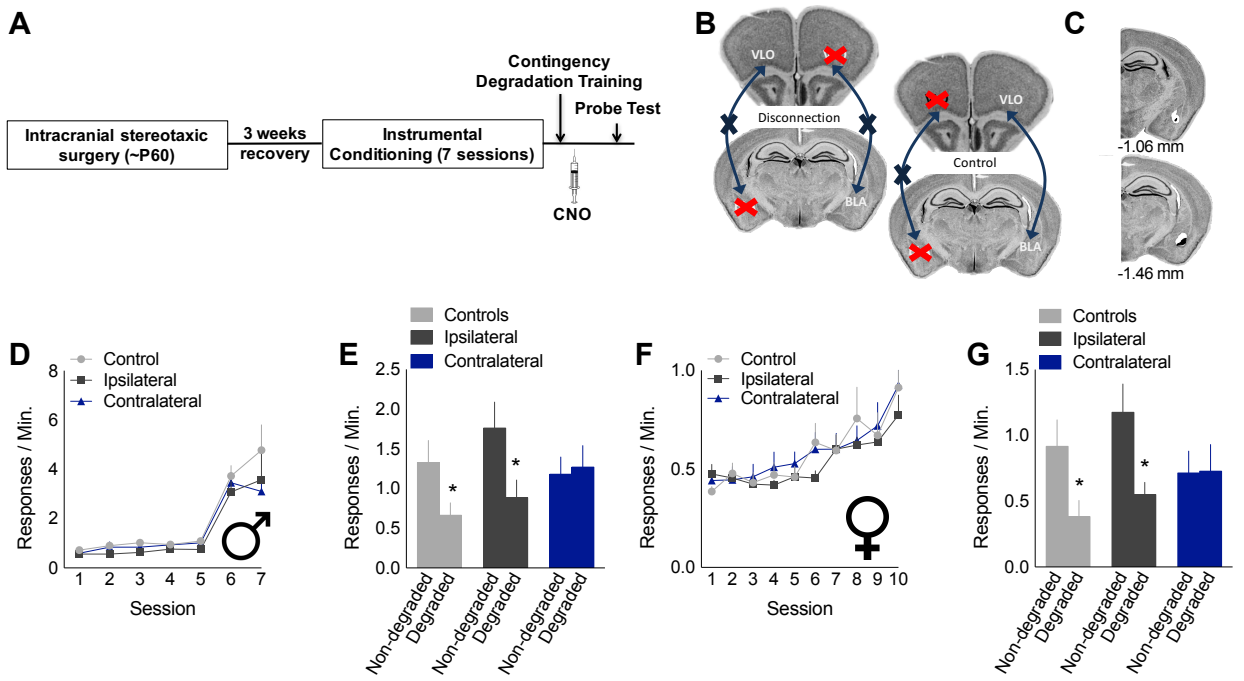
(A) Experimental timeline. As before, mice were exposed to cocaine during early adolescence, then tested for sensitivity to action-outcome contingencies in adulthood. MDMA was given immediately following instrumental contingency degradation, then response preference was assessed the following day when mice were drug-free. (B) Response rates did not differ between groups during instrumental response acquisition. (C) Developmental cocaine exposure induced failures in action-outcome decision making, resulting in non-selective responding, while MDMA corrected these failures. Symbols and bars represent means+SEMs, *p<0.05.

Figure 3-9: *MDMA-mediated enhancement of goal-directed action is oPFC trkB-dependent.*



(A) Constructs for lenti-*TrkB.t1* and lenti-GFP viral vectors. (B) Viral vectors were infused in 0.5 µL or 1.5 µL volumes. (C) We detected no difference in viral vector spread, but (D) the groups differed in HA immunofluorescence, with the larger volume generating greater fluorescence. **Inset:** Representative HA immunofluorescence. "VLO" refers to the ventrolateral oPFC. (E) Groups did not differ in GFAP immunoreactivity, indicating the larger infusion volume did not cause greater tissue damage. (F) Mice acquired the instrumental responses with no group differences. (G) Both small and large volumes of lenti-*TrkB.t1* caused insensitivity to action-outcome contingencies. The habit-based response strategies induced by the smaller *TrkB.t1* volume were blocked by MDMA, but the larger-volume *TrkB.t1* overexpression caused habits to be insensitive to MDMA-mediated blockade. Symbols and bars represent means+SEMs, *p<0.05.

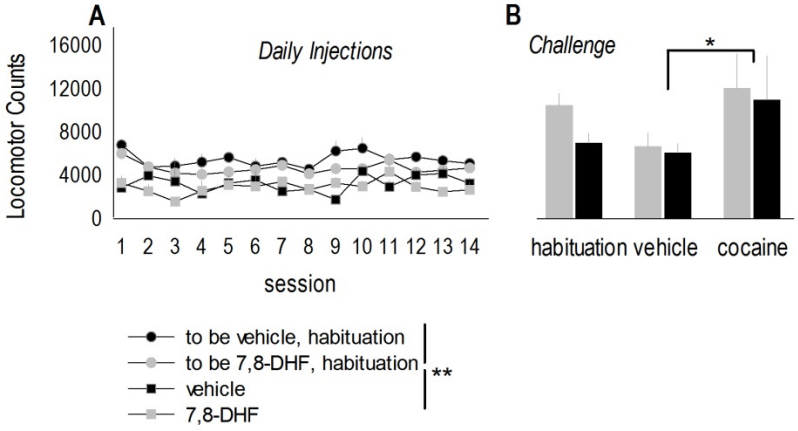
Figure 3-10: *BDNF-dependent oPFC-BLA interactions are necessary for action-outcome decision making.*



(A) Experimental timeline. Viral vectors expressing Cre were infused unilaterally into the oPFC of 'floxed' *Bdnf* mice, while inhibitory Gi-coupled DREADDs were infused unilaterally into the BLA. Mice were then tested for sensitivity to changes in action-outcome contingencies. CNO was given immediately following instrumental contingency degradation, then response preference was assessed the following day when mice were drug-free. (B) Placement of viral vectors in contralateral hemispheres creates a "disconnection," such that BDNF-deficient neurons innervate the intact BLA in one hemisphere, and healthy oPFC neurons innervate Gi-DREADD-infected neurons in the contralateral BLA. Ipsilateral infusions leave one hemisphere intact, and an additional control group expresses only control viral vectors. "VLO" refers to the ventrolateral oPFC. (C) BLA infusion sites represented on images from the Mouse Brain Library (Rosen et al., 2000). White indicates the largest viral vector spread and black the smallest. (oPFC infusion sites were comparable to previously shown infusions.) (D) Male mice acquired the responses without group differences, but (E) mice with contralateral infusions were unable to select responses

according to expected outcomes, instead responding non-selectively following instrumental contingency degradation. (F) Female mice tested in the same task acquired the nose poke responses without group differences, and (G) again, oPFC-BLA disconnection impaired action-outcome decision making, causing a deferral to non-selective, habit-based strategies. Symbols and bars represent means+SEMs, *$p<0.05$.

Figure 3-11: *Repeated 7,8-DHF does not induce locomotor sensitization to cocaine.*



(A) Mice were repeatedly injected with vehicle or 7,8-DHF following a habituation period. Locomotor activity was higher during the habituation periods than following drug or vehicle injection, and we detected no interactions between day and injection. (B) Mice were allowed a washout period, followed by a habituation period, followed by saline and cocaine (10 mg/kg) injections. While cocaine elicited more locomotor activity overall, we found no group differences. In other words, 7,8-DHF did not induce cross-sensitization with cocaine. Means+SEMs, *p=0.03 vehicle vs. cocaine, **p<0.001 habituation vs. post-injection.

Chapter 4:

MDMA increases affiliative behaviors in squirrel monkeys in a serotonin 2A receptor-dependent manner

4.1 Context, Author's Contribution, and Acknowledgement of Reproduction

The following chapter examines the receptor pharmacology of the prosocial effects of MDMA in nonhuman primates. Research was conducted by the dissertation author, Ms. Adelaide Minerva, and Ms. Erika Chandler. Data were analyzed by the dissertation author and Dr. Jordan Kohn. The document was written by the dissertation author, under the guidance of Dr. Howell. The chapter is reproduced with minor edits from Pitts EG, Minerva AR, Chandler EB, Kohn JN, Logun MT, Sulima A, Rice KC, and Howell LL (2017) 3,4-methylenedioxymethamphetamine increases affiliative behaviors in squirrel monkeys in a serotonin 2A receptor-dependent manner. *Neuropsychopharmacology.*

4.2 Abstract

3,4-methylenedioxymethamphetamine (MDMA) increases sociality in humans and animals. Release of serotonin (5-HT) is thought to play an important role in the increase in social behaviors, but the mechanisms underlying these effects are poorly understood. Despite the advantages of nonhuman primate models, no studies have examined the mechanisms of the social effects of MDMA in nonhuman primates. The behavior and *vocalizations* of four group-housed squirrel monkeys were examined following administration of MDMA, its enantiomers, and methamphetamine. 5-HT receptor antagonists and agonists were given as drug pretreatments. Data were analyzed using linear mixed-effects models. MDMA and its enantiomers increased affiliative social behaviors and vocalizations, whereas methamphetamine had only modest effects on affiliative behaviors. Pretreatment with a 5-HT_{2A} receptor antagonist and a 5-HT_{2C} receptor agonist attenuated the MDMA-induced increase in social behaviors, while a 5-HT_{1A} receptor antagonist did not alter affiliative vocalizations and increased MDMA-induced social contact. Nonhuman primates show MDMA-specific increases in affiliative social behaviors following MDMA administration, in concordance with human and rodent studies. MDMA-induced increases

in social behaviors are 5-HT_{2A}, but not 5-HT_{1A}, receptor-dependent. Understanding the neurochemical mechanisms mediating the prosocial effects of MDMA could help in the development of novel therapeutics with the unique social effects of MDMA, but fewer of its limitations. That could be important for expanding clinical treatment to a wide range of psychiatric disorders that may benefit from therapeutic-adjuncts that enhance social behavior, including PTSD, autism, and addiction.

4.3 Introduction

The amphetamine derivative MDMA is the essential active component of the club drug 'ecstasy,' and increased sociability is cited as a main reason for its recreational usage (Sumnall et al., 2006). MDMA increases social interaction, self-reported ratings of social feelings, and the number of social words used in humans, as well as increasing adjacent lying (a passive social interaction) and social conditioned place preference in rodents (see Kamilar-Britt & Bedi, 2015).

Behaviorally-active doses of racemic MDMA release monoamines into the synapse (Rietjens et al., 2012). Recreationally and clinically used MDMA is usually a 1:1 mixture of enantiomers, although stereoselective disposition increases the ratio of *R*(–)-MDMA overtime (Fallon et al., 1999). The stereoisomers have different pharmacological profiles; *S*(+)-MDMA increases dopamine and serotonin (5-HT), while *R*(–)-MDMA less-potently releases 5-HT, but has little effect on dopamine release (Murnane et al., 2010). Additionally, *R*(–)-MDMA binds to specific receptors (*e.g.*, 5-HT_2)(Lyon et al., 1986). The potency for 5-HT release is greater than other psychostimulants (Rothman et al., 2001), which is thought to mediate the unique subjective profile of racemic MDMA (Liechti et al., 2000a). However, additional mechanisms underlying the prosocial effects of MDMA are not well understood.

Rodent studies indicate that activation of the 5-HT_{1A} receptor may be necessary for MDMA-induced adjacent lying (*e.g.*, Thompson et al., 2007). However, human studies using pindolol, a beta-adrenergic antagonist that also partially blocks 5-HT_{1A} receptors, found that 5-

HT_{1A} receptors were not necessary for MDMA-induced social feelings or changes in emotional empathy (van Wel et al., 2012; Kuypers et al., 2014).

Another important 5-HT receptor that could play a role in the unique social effects of MDMA is the 5-HT_{2A} receptor. Several of the effects of MDMA are 5-HT_{2A} receptor-dependent, including hyperlocomotion (Herin et al., 2005), changes in body temperature (Herin et al., 2005), and striatal dopamine overflow (Schmidt et al., 1994). Some human literature supports the role of the 5-HT_{2A} receptor in the social effects of MDMA. Ketanserin, a 5-$HT_{2A/2C}$ antagonist, blocked MDMA-induced positive affect (van Wel et al., 2012) and emotional excitation, but did not decrease ratings of extroversion or positive mood (Liechti et al., 2000b). The mixed 5-$HT_{2A/2C}$ profile of ketanserin might contribute to the conflicting results, given that 5-HT_{2A} and 5-HT_{2C} receptors often have opposing effects on psychostimulant-induced behaviors and dopamine overflow in the striatum (Howell & Cunningham, 2015).

The present study evaluated the social effects of MDMA, its enantiomers, and methamphetamine in squirrel monkeys. Methamphetamine was used to examine the social effects of a similar amphetamine-derivative, but with a higher dopamine to 5-HT release profile (Rothman et al., 2001). Additionally, this study examined the receptor pharmacology underlying MDMA-induced social behaviors. Animal experiments allow for the use of novel, and more selective, antagonists to better understand the pharmacological mechanism of the social effects of MDMA. Further, squirrel monkeys have a pharmacokinetic profile for MDMA similar to humans (Mueller et al., 2009), providing considerable translational relevance, given the concern that different pharmacokinetic processing can alter the effects of MDMA (Green et al., 2012). Despite these advantages, only one study has examined the social effects of MDMA in nonhuman primates (Ballesta et al., 2016), and no studies have used nonhuman primates to analyze the mechanisms underlying the social effects of MDMA. Here, behavioral and vocal changes were examined following administration of MDMA, its enantiomers, or methamphetamine. Additionally, receptor-specific antagonists were used to investigate the role of the 5-HT_{2A} and 5-HT_{1A} receptors

in the social effects of MDMA.

4.4 Materials and Methods

4.4.1 Subjects

Four adult male squirrel monkeys (*Saimiri boliviensis*) weighing between 800 and 1300 g and between 11 and 16 years of age were used in all studies. Subjects were group housed in a 1.4x1.8x0.7 meter cage with access to swings and perches. The colony and laboratory were kept at ~23° C. Subjects were fed twice daily (monkey chow: Harlan Teklad, Madison, WI; fresh fruits and vegetables), had *ad libitum* access to water, and received daily enrichment (*i.e.,* foraging opportunities and different toys that were changed daily). All subjects had previous exposure to drugs acting on monoaminergic and/or glutamatergic systems (*e.g.*, Cooper et al., 2014). However, the last drug exposure for all animals was at least two years before the beginning of this study. All studies were conducted in accordance with the National Institute of Health's *Guide for the Care and Use of Laboratory Animals*, the Association for Accreditation of Laboratory Animals Care, and were approved by the Institutional Animal Care and Use Committee of Emory University.

4.4.2 Experimental Protocol

For experimental sessions, the home cage was moved to a laboratory room separated from the colony. Subjects were left alone in the laboratory for two hours before drug administration to habituate to the environment. All subjects were given the same dose of racemic MDMA (0.03-1.0 mg/kg, i.m.), *S*(+)- or *R*(–)-MDMA (0.3-3.0 mg/kg, i.m.), methamphetamine (0.01-0.3 mg/kg, i.m.) or sterile saline. Doses of drugs were given in a randomized order with at least two days in between drug administrations. Pretreatments with M100907 (M100)(0.1 and 0.3 mg/kg, i.m.), a selective 5-HT_{2A} receptor antagonist (Table 4-1), were administered one hour prior to MDMA

administration. Pretreatments with WAY163909 (WAY163)(0.03 and 0.3 mg/kg, i.m.), a selective 5-HT_{2c} receptor agonist (Table 4-1), were administered 45 minutes prior to MDMA administration. These doses and time frame were chosen because they have been shown previously to affect behavior, neuroendocrine response, and neurotransmitter release following stimulant administration (*e.g.*, Fantegrossi et al., 2009). Pretreatments with WAY100635 (WAY100)(0.1 and 0.3 mg/kg, i.m.), a selective 5-HT_{1A} receptor antagonist (Table 4-1), were administered 20 minutes prior to MDMA administration. Dose and timing were based on previous studies in marmosets (Harder & Ridley, 2000) and rodents (Thompson et al., 2007). Saline (sterile 0.9%, i.m.) controls were performed in between drug administration days. Experiments were broken into two testing phases, with baselines collected at the beginning of the experiment and before WAY163 and WAY100 testing. For one hour following drug administration, subjects were videotaped (Samsung F90BN HD camcorder, Suwon, South Korea) and vocalizations were recorded (Seinheiser K6 microphone (Wedemark, Germany) on a Focusrite Scarlett 2i audio interface (High Wycombe, United Kingdom) using Ableton live lite 8 software (Berlin, Germany).

For behavioral outcomes, a reviewer blinded to drug condition watched the video recordings and used a behavioral ethogram to score duration of behaviors (J-Watcher v1.0 software; Sydney, Australia). A single rater, trained to high inter-rater reliability across multiple training videos, scored all videos being compared statistically. The behavioral ethogram used huddling as the main affiliative behavior [squirrel monkeys, unlike other nonhuman primates, do not groom socially (Baldwin & Baldwin, 1981)]. The ethogram also included duration of activity, aggression (e.g., chasing and head grasping), and residual (*i.e.*, not performing other scored behaviors)(Hopf et al., 1974). The focal animal scoring technique (Altmann, 1974) was used to assess duration of behaviors. Each monkey was assessed for 5 minutes within each of three, 20-minute blocks across the hour-long observation period (*i.e.*, each monkey was scored for 15 total minutes across the hour). The order of scoring was randomized across trials, but kept consistent across the 3 blocks within a single hour session.

Auditory files of vocalizations for the entire group were converted to spectrogram files in MATLAB software (The MathWorks, Natick, MA) using software custom-written by Sober and colleagues (Sober & Brainard, 2009). Vocalizations were distinguished based on shape of spectrogram and classified into one of three categories. Vocalizations categorized as affiliative were chucks, purrs, and pulsed calls. These call types are associated with huddling, soliciting contact from a partner, or providing important information to the troop, respectively (Jurgens, 1979; Smith et al., 1982). The other two vocalizations were growls, calls commonly observed in connection with threat displays and aggression, and peeps, observed during exploration and after changes in the environment (Winter, 1968; Jurgens, 1979).

4.4.3 Drugs

Racemic, *S*(+)-MDMA, and *R*(–)-MDMA HCl, methamphetamine HCl (National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC), and WAY100635 HCl [*N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate salt] (Abcam Biochemicals, Cambridge, MA) were dissolved in 0.9% sterile physiological saline. M100907 HCl [(R)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol] was a generous gift from Kenner C. Rice, Ph.D. and was synthesized at the Molecular Targets and Medications Discovery Branch (National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism at the National Institutes of Health). M100 was dissolved in sterile saline and 1.0N hydrochloric acid and returned to a pH of 5-6. WAY163909 HCl [(7b-R,10a-R)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta[b][1,4] diazepino [6,7,1hi] indole] was a generous gift from Pfizer Incorporated (New York, NY) and was dissolved in a 10 mg/ml solution of beta cyclodextrin. Doses were calculated from salt weights.

4.4.4 Data Analysis

The behavioral and vocalization data were analyzed using linear mixed-effects models

(LMM) following log(y+1)-transformation of the dependent variables. This method reliably controls type I error rates and is more parsimonious than generalized linear mixed-effects models (GLMM) that are often applied to non-Gaussian data when testing for significance of regression coefficients (Warton et al., 2016). We conducted all statistical analyses in *R* statistical software (R Core Team, 2014), and LMMs were computed using the *lme4* package (Bates et al., 2012). To evaluate the possibility that more complex GLMMs better describe the relationships between dose, behavior, and vocalizations, we also implemented GLMMs with a log-link function (*i.e*., Poisson regression) and compared pseudo-R-squared values between the GLMM and corresponding LMM using the *MuMIn* package (Barton, 2015). GLMM results did not qualitatively differ from the linear model results and are therefore reported in Table 4-2.

Behavioral data was modeled in seconds, and included dose and bin (within the 1h observation period) as fixed effects, and controlled for random effects of study subject and testing day. Vocalization data (group-wide frequency) were summed into six, 10-min bins across the 1h observation period and were modeled as frequencies (*i.e.,* counts), with dose and bin as fixed effects and the random effect of testing day.

Since dose-response curves are sometimes non-linear, we also tested for polynomial relationships between drug dosage and behavioral and vocalization responses by re-running each LMM with an orthogonal, second-order polynomial (*i.e.,* quadratic) dosage term as a fixed effect. We tested for improvements in fit over the simpler monomial linear models using chi-squared statistics implemented in *lme4*. When results of the likelihood-ratio test suggested an improved fit for the polynomial models, we tested for significance of the fixed effects and report regression coefficients and *t*-statistics from the polynomial models (Table 4-2).

Model residuals were visually inspected for homoscedasticity, and normality was assessed using the one-sample Komlogorov-Smirnov test to examine deviation of standardized residuals from a theoretical standard normal distribution. Model degrees of freedom (df), *t*-statistics, and *p*-values for fixed effects in LMMs were obtained by using residual maximum

likelihood (REML) tests with Satterthwaite approximations of df using the *lmerTest* package (Kuznetsova *et al.,* 2015).

4.5 Results

4.5.1 MDMA and its enantiomers increase affiliative social behaviors

To examine whether MDMA increased affiliative behaviors in nonhuman primates, huddling and activity were scored for one hour following drug administration. No aggressive interactions were observed during testing and stereotypies were not quantified, but no adverse effects were seen. MDMA increased huddling (β_{dose}=18.5, t_{330}=9.98, p<0.001)(Fig.4-1A) and decreased activity (β_{dose}=-15.3, t_{330}=-9.32, p<0.001)(Fig.4-1B). Both enantiomers of MDMA also significantly increased huddling (β_{dose}=24.9, t_{29}=8.40, p<0.001) and decreased activity (β_{dose}=-0.96, t_{27}=-7.40, p<0.001). At lower doses, *S*(+)-MDMA increased huddling ($\beta_{drug*dose}$=-55.5, p=t_{125}=-4.23, p<0.001) and reduced activity levels ($\beta_{drug*dose}$=-1.25, t_{125}=-4.23, p<0.001) relative to *R*(–)-MDMA (Fig.4-1C-F). In contrast to racemic MDMA and its enantiomers, methamphetamine did not significantly increase huddling or change activity levels (Fig.4-1G-H; Table 4-2).

4.5.2 MDMA and its enantiomers increase affiliative vocalizations

MDMA increased the number of affiliative vocalizations in the hour following drug administration (β_{dose}=7.79, t_{19}=4.77, p<0.001)(Fig.4-2A). All three types of affiliative vocalizations increased following MDMA administration, with pulsed calls increasing the most from baseline (~5000%). Purrs also increased from baseline (~1500%), but represent lower total numbers than pulsed calls and chucks (Fig.4-2B). Both the *R(–)* and *S*(+) enantiomers of MDMA also significantly increased affiliative calls (β_{dose}=11.4, t_{19}=8.28 p<0.001), though the *R(–)* enantiomer was associated with more affiliative calls at higher doses compared to the *S*(+) enantiomer ($\beta_{drug*dose}$=-27.2, t_{19}=-5.99, p<0.001)(Fig.4-2C,D). In contrast, methamphetamine was not associated with increased affiliative vocalizations (Fig.4-2E; Table 4-2).

4.5.3 MDMA and its enantiomers affect other vocalizations

Changes to two other main categories of vocalizations, peeps and growls, were also examined following drug administration. MDMA decreased peep call frequency (β_{dose}=-6.47, t_{20}=-5.12, p<0.001)(Fig.4-3A) and increased growl frequency (β_{dose}=5.11, t_{20}=9.85, p<0.001)(Fig.4-3B) during the session. Again, both *R(–)*-MDMA and *S*(+)-MDMA decreased peep calls (β_{dose}=-0.46, t_{21}=-2.61, p=0.016)(Fig.4-3C,E) and increased growls (β_{dose}=7.24, t_{143}=9.58, p<0.001)(Fig.4-3D,F). In comparing the potency and efficacy of the two stereoisomers, *S*(+)-MDMA had stronger effects on peep calls than *R*(–)-MDMA as dosage increased ($\beta_{drug*dose}$=-0.88, t_{21}=-2.73, p=0.012), whereas *R*(–)-MDMA had stronger effects on growls at higher doses ($\beta_{drug*dose}$=-12.9, t_{143}=-5.15, p<0.001). Methamphetamine also significantly decreased peep call frequency (β_{dose}=-6.96, t_{17}=-4.20, p<0.001)(Fig.4-3G), but did not change the frequency of growls emitted (Fig.4-3H; Table 4-2).

4.5.4 MDMA-induced affiliative behaviors are 5-HT$_{2A}$, but not 5-HT$_{1A}$, dependent

To examine the receptor pharmacology underlying the effects of MDMA on nonhuman primate behaviors and vocalizations, we administered 5-HT receptor antagonists or agonists prior to MDMA administration. M100, a selective 5-HT$_{2A}$ receptor antagonist, blocked MDMA-induced huddling ($\beta_{MDMA*M100}$=-23.8, t_{17}=-4.20, p<0.001)(Fig.4-4A). M100 trended (p<0.1) toward reducing MDMA-induced affiliative calls ($\beta_{MDMA*M100}$=-12.3, t_{23}=-1.90, p=0.071) and including the interaction in the model significantly improved model fit (Fig.4-4B). M100 did not significantly attenuate MDMA-induced decreases in activity levels (Fig.4-4C).

Administration of WAY163, a selective 5-HT$_{2C}$ agonist, also significantly attenuated MDMA-induced huddling ($\beta_{WAY163*MDMA}$ =-14.7, t_{190}=-2.11, p=0.036)(Fig.4-4D), but had no effect on affiliative calls following MDMA administration (Fig.4-4E; Table 4-3). WAY163 had no significant effect on MDMA-induced decreases in activity (Fig.4-4F).

WAY100, a selective 5-HT_{1A} receptor antagonist, augmented huddling following MDMA administration ($\beta_{WAY100*MDMA}$=14.9, t_{184}=2.34, p=0.020)(Fig.4-4G), but did not modify MDMA-induced affiliative calls (Fig.4-4H; Table 4-3). Similar to MDMA, WAY100 administration decreased activity (β_{WAY100}=-4.74, t_{184}=-3.80, p<0.001)(Fig.4-4I), but did not moderate the effects of MDMA on activity.

Together, these findings suggest that MDMA-induced affiliative behaviors are 5-HT_{2A} receptor, but not 5-HT_{1A}, receptor-dependent.

4.5.5 Repeated administration of MDMA increases huddling in a subject with initially low MDMA-induced social behaviors

One animal (177) in the group of four squirrel monkeys initially showed low levels of group huddling following MDMA administration (Fig.4-5). However, following repeated, acute administration of racemic MDMA and its enantiomers over the course of the study, 177 developed similar levels of huddling as the other three subjects. There was a significant three-way interaction between phase, dose, and subject ($F_{3, 208}$=3.39, p=0.019) and comparing the least-squares means between phases within each subject showed a significant increase in the average time spent huddling by 177 between phases (phase 1: 35.8 ± 32.8 s; phase 2: 202.1 ± 44.1 s; t_{208}=3.64, p=0.002), but not the other three animals (Fig.4-5). This is not a systematic study of the long-term effects of MDMA, but it is interesting given the long-term effects of MDMA in clinical settings (Mithoefer et al., 2013). Future studies could examine the potential long-term social and group effects of MDMA in a more controlled manner.

4.6 Discussion

The aim of the present study was to examine the affiliative social effects of MDMA in socially-housed squirrel monkeys and to examine the 5-HT receptor pharmacology underlying MDMA-induced social behaviors. MDMA and its enantiomers dose-dependently increased

huddling and the number of affiliative vocalizations emitted by group-housed squirrel monkeys. Additionally, pretreatments with a 5-HT_{2A} receptor *antagonist* or a 5-HT_{2C} receptor *agonist* attenuated MDMA-induced huddling and a 5-HT_{1A} receptor *antagonist* increased MDMA-induced huddling, but did not change affiliative vocalizations.

Studies have shown that MDMA increases feelings of sociability in humans and increases social interaction in rodents (Kamilar-Britt & Bedi, 2015). The only study to examine the social effects of MDMA in nonhuman primates also found that MDMA increases social grooming in long-tailed macaques (Ballesta et al., 2016). In concordance with this research, the present experiments found that MDMA significantly increases huddling and affiliative calls in squirrel monkeys.

MDMA also increased growl calls, vocalizations usually connected with aggression (Jurgens, 1979). This was unexpected given that MDMA decreases aggression in other animal models (see Kamilar-Britt & Bedi, 2015). No aggressive behaviors or other aggressive calls were seen and growl calls, when they occur with chucks, have been seen during huddling (Winter, 1968). This suggests that growls are not exclusively aggressive and may not be indicating aggression in this context, although further studies are necessary.

In contrast with the behavioral changes following MDMA administration, methamphetamine did not significantly increase huddling or affiliative vocalizations. These findings support the unique, robust social effects of MDMA and the use of group-housed squirrel monkeys to further examine those social effects and the underlying mechanisms of MDMA. One limitation of our study was that vocalizations were examined by group, making it impossible to determine if all subjects drove the increase in affiliative vocalizations equally. Future studies could separate calls by subject in order to examine individual-differences in MDMA-induced vocalizations. The effects of MDMA in female and juvenile group-housed squirrel monkeys should also be examined to determine whether sex and age play a role in MDMA-induced social behaviors.

Interestingly, despite its structural similarity to psychostimulant compounds, MDMA significantly decreased activity in nonhuman primates. This finding supports other studies showing decreased activity levels following MDMA (Crean et al., 2006) and no stimulant effects on operant behavior (Fantegrossi et al., 2009). The present study's group-housing design might enhance changes in activity levels as animals switch allocation of behavior from non-social activity towards affiliative social behaviors. Another interpretation, however, could be that increases in huddling are driven by decreases in locomotion. Previous research supports the conclusion that huddling is independent of locomotor effects following drug administration. Drugs that decrease locomotion do not reliably increase huddling (Miczek et al., 1981) and the effects of stimulants on locomotion and social behaviors are not mediated by the same mechanisms (Miczek & Yoshimura, 1982). This is further supported by our findings showing dissociation between the effects of 5-HT receptor ligands on MDMA-induced huddling and locomotion.

In the present study, pretreatment with M100, a selective 5-HT_{2A} receptor antagonist, blocked MDMA-induced affiliative social behaviors. Antagonism of 5-HT_{2A} receptors blocks MDMA-induced striatal dopamine overflow (Schmidt et al., 1994). A potential role of 5-HT-mediated striatal dopamine release in the social effects of MDMA is supported by results indicating that pretreatment with WAY163, a 5-HT_{2C} receptor agonist, also decreases MDMA-induced huddling, because 5-HT_{2C} receptor activation *decreases* striatal dopamine release (Howell & Cunningham, 2015). Additionally, the 5-HT_{2A} receptor is expressed extensively throughout the amygdala (Bombardi & Di Giovanni, 2013) and reduces amygdala-dependent reactivity and anxiety-related behaviors (Weisstaub et al., 2006; Fisher et al., 2009). Given the model that MDMA produces a valence-specific shift in response to social cues, with an increase in recognition of positive social signals and a decrease in response and recognition of negative ones (Kamilar-Britt & Bedi, 2015), the striatal and amygdalar effects of 5-HT_{2A} receptor-activation provide a potential mechanism by which 5-HT_{2A} receptors could mediate increased sociality following MDMA.

One potential caveat with the present study is that MDMA has pronounced effects on body temperature (*e.g.*, Banks et al., 2007) and antagonism of the 5-HT_{2A} receptor attenuates MDMA-induced changes in body temperature in rodents (Herin et al., 2005) and humans (Liechti et al., 2000b). The temperature of the laboratory [near an ambient temperature in which MDMA administration did not change body temperature in nonhuman primates (Banks et al., 2007)] and correlation between affiliative vocalizations and huddling following MDMA (Fig.4-6A) suggest the findings are not driven by changes in body temperature. Additionally, methamphetamine stimulates even more pronounced changes in body temperature (Crean et al., 2006), but did not induce a similar increase in huddling.

The 5-HT_{1A} receptor is also thought to play a role in the social effects of MDMA. In rodents, 5-HT_{1A} receptor stimulation is necessary for MDMA-induced increases in adjacent lying and oxytocin release (Thompson et al., 2007), and activation of oxytocin neurons (Hunt et al., 2011). Further, one study showed positive correlation between plasma oxytocin and social ratings in the laboratory (Dumont et al., 2009). Counter to these findings, pretreatment with WAY100, a selective 5-HT_{1A} receptor *antagonist*, increased huddling following MDMA administration and did not affect MDMA-induced vocalizations. Future studies using a wider range of doses of WAY100 and/or 5-HT_{1A} agonists could provide more definitive evidence on the role of the 5-HT_{1A} receptor in social behaviors.

The present study supports evidence in the human literature showing that blocking 5-HT_{1A} receptors does not change increases in self-reported ratings of sociality or emotional empathy following MDMA (van Wel et al., 2012; Kuypers et al., 2014) and null correlations between plasma oxytocin and social feelings (*e.g.*, Kuypers et al., 2014). The enhancement of MDMA-induced huddling could have been driven by antagonism of 5-HT_{1A} autoreceptors, driving an additional increase in 5-HT release. 5-HT_{1A} receptor antagonism potentiates 5-HT release following administration of selective serotonin reuptake inhibitors (SSRIs)(Hjorth, 1993). Administration of an SSRI increases body contact and grooming behaviors in cynomolgous macaques (Shively et

al., 2014), indicating that 5-HT release alone can increase social contact in nonhuman primates. However, further studies are needed to confirm that 5-HT_{1A} receptor antagonism is enhancing MDMA-induced huddling by blunting autoreceptor feedback.

We propose a model for the unique social effects of MDMA, in which 5-HT release, combined with receptor-selective direct agonist effects, enhances sociality. In this model, 5-HT release alone can enhance some social behaviors [as seen with increased body contact following SSRI administration (Shively et al., 2014)], but the addition of receptor-selective activation enhances these social effects, leading to the unique and robust social behavior caused by MDMA. This model is supported by the dissociation between huddling and affiliative vocalizations following 5-HT_{1A} receptor antagonism, with additional 5-HT release increasing huddling, but not affecting affiliative vocalizations. The differing magnitude of effects of the MDMA enantiomers also provides support for the above model. The *S*(+) enantiomer is a potent releaser of 5-HT and dopamine, while the *R*(–) enantiomer releases less 5-HT, and is ineffective in releasing dopamine (Acquas et al., 2007; Murnane et al., 2010), but has direct agonist effects at the 5-HT_{2A} receptor (Nash et al., 1994). *S*(+)-MDMA increases huddling more strongly than *R*(–)-MDMA at the same dose, as expected given its more potent release of 5-HT. However, in contrast with its more potent monoamine releasing effects, *S*(+)-MDMA is less effective at eliciting affiliative vocalizations at higher doses than *R*(–)-MDMA. Accordingly, the enhanced social effects of *R*(–)-MDMA are possibly caused by the combination of 5-HT release and receptor-selective direct agonism. Future studies could examine this model more directly by co-administering *S*(+)-MDMA or a 5-HT releaser with 5-HT receptor agonists (*e.g.*, DOI) and by further studying the role of agonism at other receptor types, such as adrenergic or dopaminergic receptors, in the effects of both enantiomers.

MDMA is currently being evaluated as a therapeutic-adjunct for the treatment of PTSD (Mithoefer et al., 2011, 2013). MDMA-assisted psychotherapy sessions may produce long-term decreases in PTSD symptoms in treatment resistant patients (Mithoefer et al., 2013). An increase

in therapeutic alliance from increased sociality and openness following MDMA, is thought to play a role in the therapeutic-potential of MDMA (Mithoefer et al., 2011). However, there are still concerns about the potential neurotoxicity and abuse potential that limit its broader clinical appeal (Rietjens et al., 2012). Understanding the neuropharmacological mechanisms of the prosocial effects of MDMA could allow for the development of novel therapeutics that specifically target social behavior, while limiting abuse potential, toxicity, and other side effects. This may be especially advantageous in vulnerable clinical populations with disorders characterized by social impairments, such as addiction. The evidence that R(–)-MDMA increases social behaviors to a level similar to racemic MDMA, without causing large amounts of striatal dopamine release (Acquas et al., 2007; Murnane et al., 2010) or inducing neurotoxic effects (Frau et al., 2013), provides a rationale for its potential advantage in therapeutic settings.

Table 4-1: *Binding affinity (Ki) for 5-HT receptor ligands at various 5-HT, dopamine, and adrenergic receptors.*

K_i (nM)						
	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	D2	D3	α1
M100907	>10,000	0.85	88	2250	6700	128
WAY163909	>1,000[a]	212[a]	10.5[a]	>1,000[a]	>1,000[a]	665[a]
WAY100635	2.2	6,260	>10,000	940	370	19.9
Data from PDSP data base: http://kidb.case.edu/pdsp.php; a: Dunlop et al., 2005						

Table 4-2: *LMM results testing the effects of drug and dosage on social behaviors in adult male squirrel monkeys.*

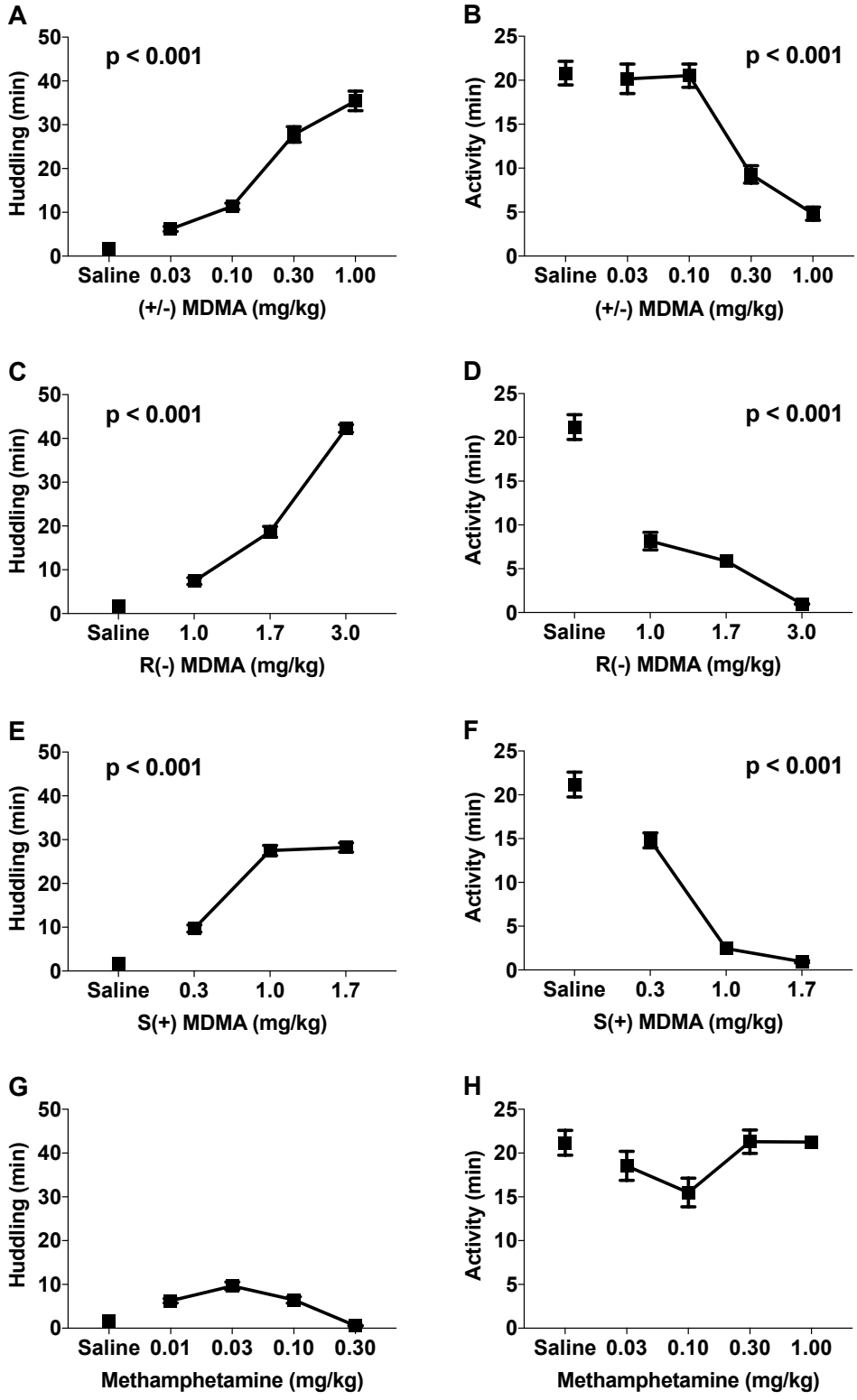
Drug	Outcome Measure	Independent variable	β	SE	*t*	*P*	χ^2 *(P value)*	R^2 (LMM)	R^2 (GLMM)
Racemic MDMA	Affiliative Calls (F)	Time	-0.17	0.05	-3.18	0.002	5.86 (0.016)	44.8%	49.0%
		Dose	7.79	1.63	4.77	< 0.001			
		$Dose^2$	-3.78	1.65	-2.29	0.033			
	Peeps (F)	Time	-0.01	0.04	-0.25	0.804	8.50 (0.004)	53.4%	53.0%
		Dose	-6.47	1.26	-5.12	< 0.001			
		$Dose^2$	3.78	1.26	2.99	0.007			
	Growls (F)	Time	-0.06	0.03	-2.14	0.034	5.86 (0.016)	45.5%	35.5%
		Dose	5.11	0.52	9.85	< 0.001			
		$Dose^2$	1.4	0.53	2.67	0.009			
	Huddling (D)	Time	-0.20	0.01	-3.58	< 0.001	38.3 (< 0.001)	36.3%	37.1%
		Dose	18.5	1.85	9.98	< 0.001			
		$Dose^2$	-12.1	1.88	-6.45	< 0.001			
	Activity (D)	Time	0.02	0.01	3.37	< 0.001	17.7 (< 0.001)	34.8%	34.1%
		Dose	-15.3	1.64	-9.32	< 0.001			
		$Dose^2$	7.14	1.69	4.22	< 0.001			
MDMA Enantiomers	Affiliative Calls (F)	Time	0.12	0.04	2.78	0.006	27.0 (< 0.001)	51.4%	53.2%
		Drug (R vs. S)	-0.94	0.23	-4.01	< 0.001			

	Dose	11.4	1.38	8.28	< 0.001			
	$Dose^2$	-5.01	1.42	-3.52	0.002			
	Drug x Dose	-27.2	4.55	-5.99	< 0.001			
	Drug x $Dose^2$	-16.3	4.53	-3.59	0.002			
Peeps (F)	Time	0.02	0.03	0.77	0.445	2.71 (0.258)	73.5%	80.3%
	Drug (R vs. S)	0.01	0.37	0.01	0.996			
	Dose	-0.46	0.18	-2.61	0.016			
	Drug x Dose	-0.88	0.32	-2.73	0.012			
Growls (F)	Time	0.01	0.03	0.41	0.683	29.2 (< 0.001)	55.8%	61.6%
	Drug (R vs. S)	-0.24	0.13	-1.85	0.066			
	Dose	7.24	0.76	9.58	< 0.001			
	$Dose^2$	-0.4	0.78	-0.51	0.609			
	Drug x Dose	-12.9	2.5	-5.15	< 0.001			
	Drug x $Dose^2$	-14.3	2.49	-5.77	< 0.001			
Huddling (D)	Time	-0.01	0.01	-1.00	0.320	20.7 (< 0.001)	38.8%	40.6%
	Drug (R vs. S)	-0.89	0.37	-2.41	0.017			
	Dose	24.9	2.96	8.40	< 0.001			
	$Dose^2$	0.94	2.89	0.32	0.748			
	Drug x Dose	-55.5	13.1	-4.23	< 0.001			
	Drug x $Dose^2$	-49.8	10.8	-4.6	< 0.001			

	Activity (D)	Time	0.02	0.01	4.25	< 0.001	2.96 (0.23)	59.6%	62.9%
		Drug (R vs. S)	0.08	0.20	0.42	0.678			
		Dose	-0.96	0.13	-7.40	< 0.001			
		Drug x Dose	-1.25	0.19	-6.39	< 0.001			
Methamphetamine	Affiliative Calls (F)	Time	0.04	0.04	0.89	0.377	6.31 (0.012)	28.9%	30.8%
		Dose	-1.65	1.23	-1.34	0.199			
		$Dose^2$	-3.09	1.23	2.51	0.023			
	Peeps (F)	Time	0.05	0.04	1.31	0.193	0.517 (0.47)	61.5%	70.0%
		Dose	-6.96	1.66	-4.2	< 0.001			
	Growls (F)	Time	0.01	0.02	0.03	0.979	0.589 (0.44)	11.8%	6.6%
		Dose	0.98	0.59	1.66	0.115			
	Huddling (D)	Time	0.01	0.01	0.44	0.659	0.110 (0.74)	34.4%	62.4%
		Dose	-3.23	2.36	-1.37	0.190			
	Activity (D)	Time	-0.01	0.01	-0.73	0.468	0.205 (0.65)	47.3%	66.5%
		Dose	1.51	2.07	0.73	0.476			

Chi-squared statistic from likelihood-ratio test between monomial and polynomial models is shown (P-value in parenthesis). R^2 represents proportion of total variance explained by each model, including generalized linear mixed-effects models (Poisson GLMM). F: Frequency (per 10 min); D: Duration (sec per 5 min)

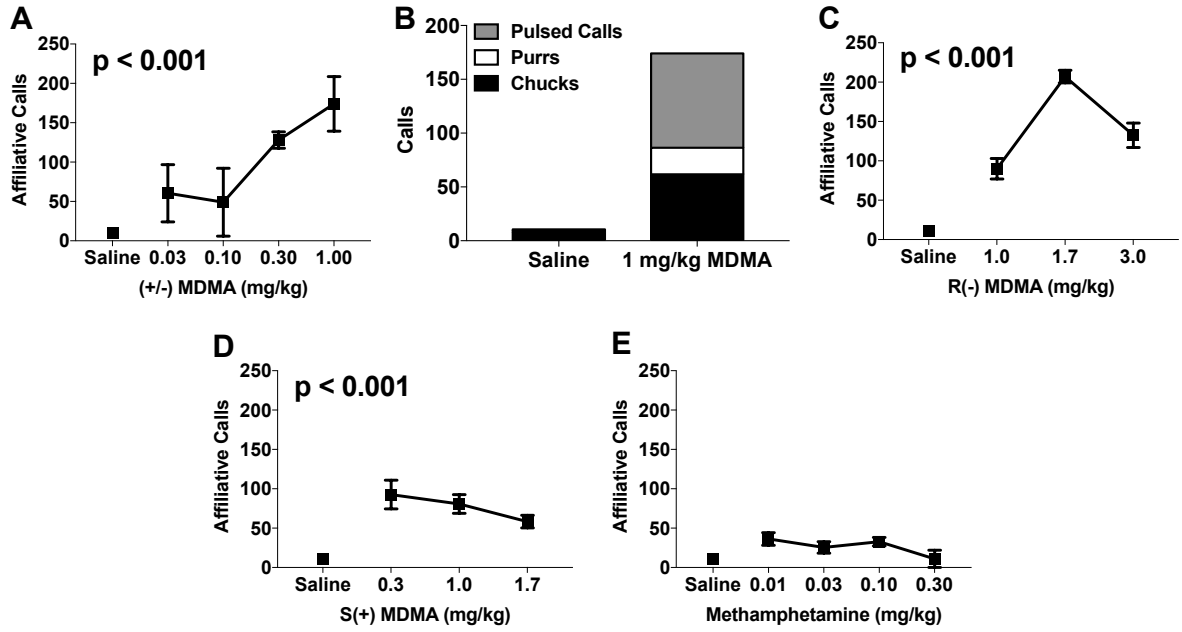
Figure 4-1: *MDMA and its enantiomers, but not methamphetamine, increase huddling.*



(A) MDMA increased the amount of huddling in group housed monkeys and (B) decreased

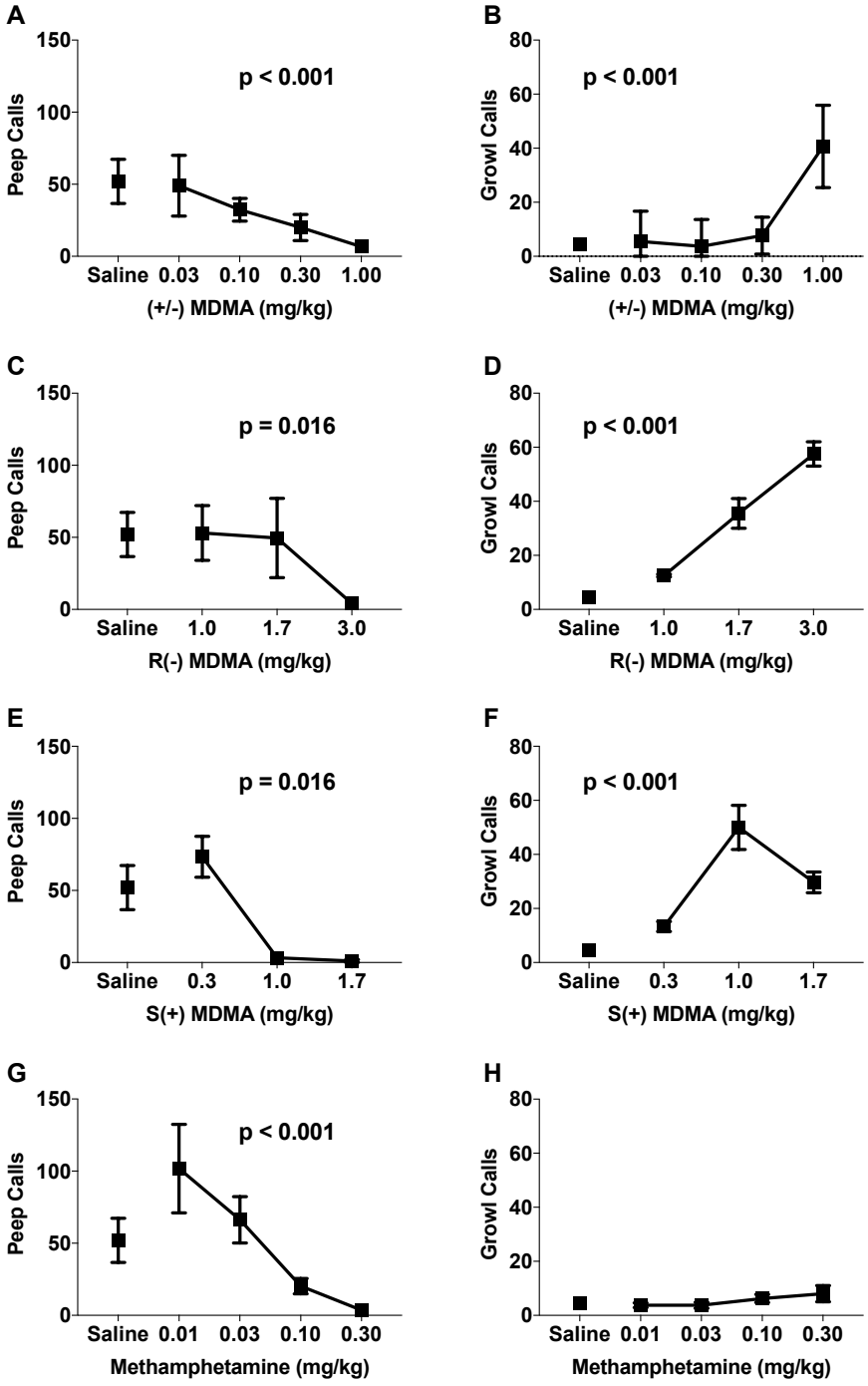
activity. The enantiomers of MDMA had a similar profile of effects as the racemate, with huddling increasing and activity decreasing following (C,D) *R(–)*-MDMA and (E,F) *S*(+)-MDMA. (G) The chemically similar amphetamine-derivative, methamphetamine, did not increase amount of huddling or (H) change activity levels. Symbols represent means+SEMs.

Figure 4-2: *MDMA and its enantiomers, but not methamphetamine, increase affiliative vocalizations.*



(A) MDMA dose-dependently increased affiliative vocalizations in group housed squirrel monkeys. (B) The highest dose of MDMA increased all categories of affiliative calls (chuck, purr, and pulsed calls). (C) Both *R(–)*-MDMA and (D) *S*(+)-MDMA also increased affiliative vocalizations, but (E) methamphetamine did not. Symbols and bars represent means+SEMs.

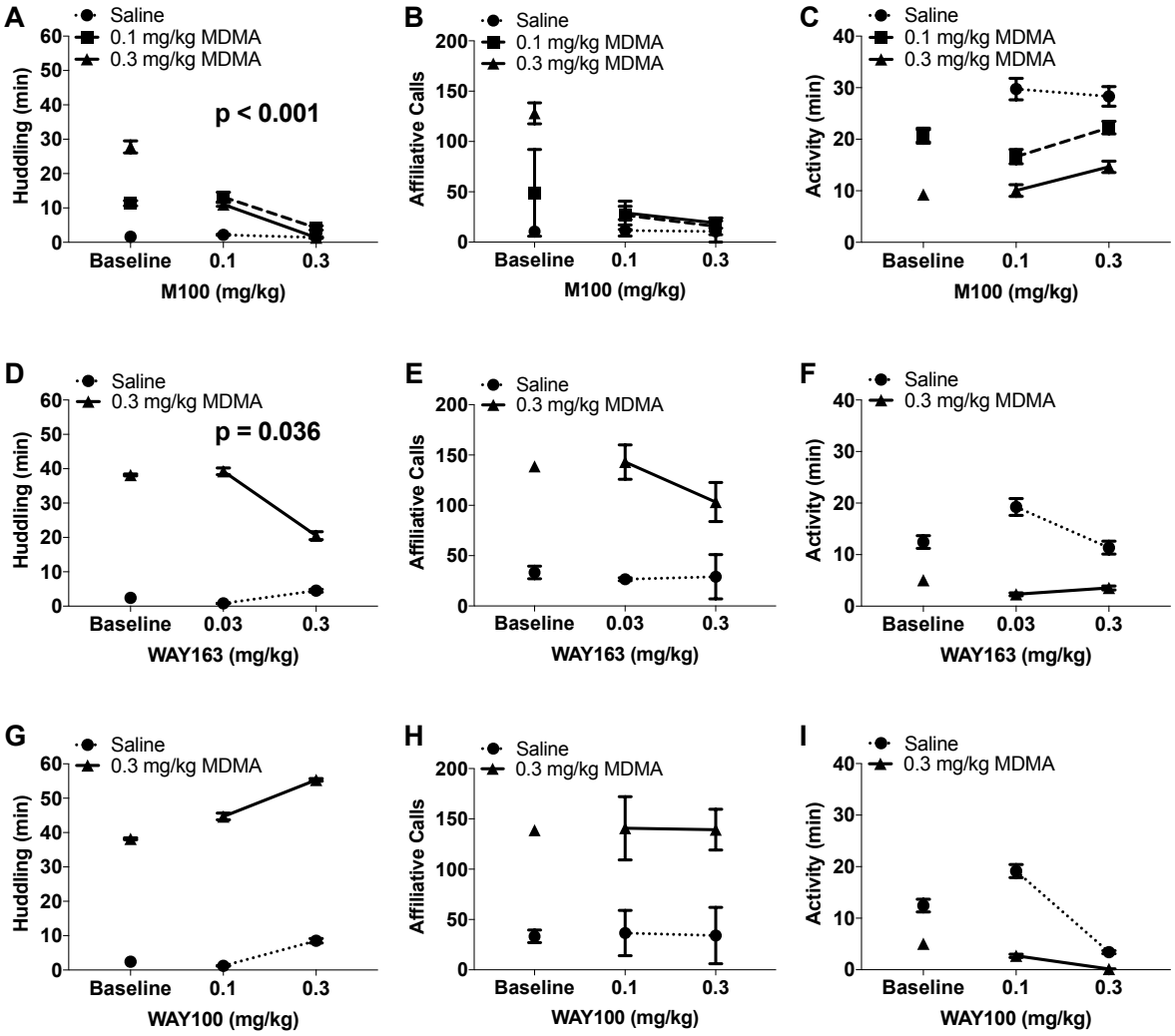
Figure 4-3: *MDMA and its enantiomers decrease peeps and increase growl calls.*



(A) MDMA decreased peep calls and (B) increased growls. The enantiomers of MDMA again produced a similar effect, with (C,D) *R(–)*-MDMA and (E,F) *S*(+)-MDMA also decreasing peep and increasing growl vocalizations. (G) Methamphetamine also significantly decreased peep

calls, but (H) did not alter the number of growls. Symbols represent means+SEMs.

Figure 4-4: *MDMA-induced affiliative behaviors are 5-HT$_{2A}$ receptor-dependent.*



(A) Pretreatment with M100907 (M100), a selective 5-HT$_{2A}$ receptor antagonist, significantly decreased MDMA-induced huddling and (B) trended towards decreasing affiliative vocalizations following MDMA. (C) M100 did not affect MDMA-induced changes in activity. (D) Pretreatment with WAY163909 (WAY163), a selective 5-HT$_{2C}$ receptor agonist, also significantly reduced MDMA-induced huddling, but did not impact MDMA's effects on (E) affiliative vocalizations or (F) activity levels. (G) In contrast, pretreatment with WAY163909 (WAY163), a selective 5-HT$_{1A}$ receptor antagonist did not decrease huddling following MDMA, and in fact increased huddling, likely through inhibition of autoreceptor feedback on serotonin levels. (H) WAY163 did not change

the effects of MDMA on affiliative vocalizations or (I) activity. Symbols represent means+SEMs,

Table 4-3: *LMM results testing the effects 5-HT modulators co-administered with MDMA on social behaviors in adult male squirrel monkeys.*

Drug	Outcome Measure	Independent variable	β	SE	*t*	*P*	R^2 (LMM)	R^2 (GLMM)
5-HT_{2A} Receptor antagonist (M100)	Affiliative Calls (F)	Time	-0.11	0.04	-2.71	0.008	32.9%	31.6%
		MDMA Dose	4.70	1.10	4.25	< 0.001		
		M100 Dose	-0.42	1.19	-0.35	0.726		
		MDMA * M100	**-12.30**	**6.48**	**-1.90**	**0.071**		
	Peeps (F)	Time	-0.06	0.03	-1.68	0.095	51.0%	52.1%
		MDMA Dose	-4.02	1.41	-2.85	0.009		
		M100 Dose	-0.91	1.52	-0.60	0.557		
		MDMA * M100	6.59	8.28	0.80	0.434		
	Growls (F)	Time	-0.01	0.02	-0.42	0.672	11.9%	15.8%
		MDMA Dose	0.47	0.46	1.02	0.317		
		M100 Dose	-1.20	0.50	-2.42	0.024		
		MDMA * M100	3.00	2.70	1.11	0.277		
	Huddling (D)	Time	-0.01	0.01	-2.28	0.023	35.1%	74.1%
		MDMA Dose	6.74	0.96	7.04	< 0.001		
		M100 Dose	0.01	1.02	0.12	0.908		
		MDMA * M100	**-23.80**	**6.06**	**-3.93**	**< 0.001**		

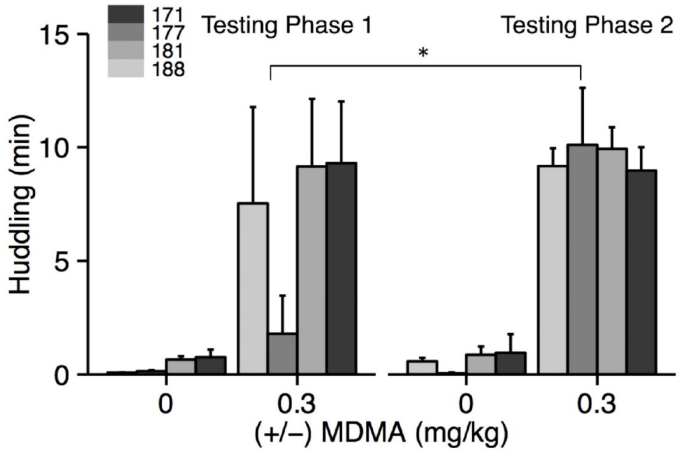
	Activity (D)	Time	0.01	0.01	3.27	0.001	54.4%	74.8%
		MDMA Dose	-5.82	0.75	-7.78	< 0.001		
		M100 Dose	2.37	0.80	2.98	0.003		
		MDMA * M100	**8.46**	**4.72**	**1.79**	**0.074**		
5-HT_{1A} Receptor antagonist (WAY100)	Affiliative Calls (F)	Time	-0.30	0.06	-5.11	< 0.001	50.9%	54.1%
		MDMA Dose	3.60	1.62	2.22	0.046		
		WAY100 Dose	-0.12	2.09	-0.06	0.954		
		MDMA * WAY100	4.73	9.27	0.51	0.619		
	Peeps (F)	Time	0.09	0.05	1.71	0.092	36.7%	34.1%
		MDMA Dose	-4.27	0.98	-4.36	< 0.001		
		WAY100 Dose	-2.00	1.26	-1.59	0.138		
		MDMA * WAY100	4.16	5.60	0.74	0.472		
	Growls (F)	Time	-0.06	0.03	-1.95	0.054	29.5%	22.0%
		MDMA Dose	1.02	0.77	1.32	0.211		
		WAY100 Dose	-0.22	0.99	-0.23	0.825		
		MDMA * WAY100	-1.82	4.4	-0.41	0.686		
	Huddling (D)	Time	-0.03	0.01	-5.10	< 0.001	58.4%	76.1%
		MDMA Dose	9.82	1.11	8.81	< 0.001		

		WAY100 Dose	-0.06	1.43	-0.04	0.968		
		MDMA * WAY100	**14.90**	**6.37**	**2.34**	**0.020**		
	Activity (D)	Time	0.02	0.01	2.86	0.005	48.9%	78.2%
		MDMA Dose	-7.13	0.96	-7.45	< 0.001		
		WAY100 Dose	-4.74	1.25	-3.8	< 0.001		
		MDMA * WAY100	0.82	5.51	0.15	0.882		
5-HT_{2C} Receptor agonist (WAY163)	Affiliative Calls (F)	Time	-0.20	0.05	-3.88	< 0.001	47.5%	48.5%
		MDMA Dose	4.64	1.09	4.25	< 0.001		
		WAY163 Dose	-0.60	1.50	-0.40	0.693		
		MDMA * WAY163	-0.78	6.33	-0.12	0.904		
	Peeps (F)	Time	-0.02	0.04	-0.39	0.700	56.8%	48.0%
		MDMA Dose	-5.43	0.82	-6.66	< 0.001		
		WAY163 Dose	-5.32	1.12	-4.77	< 0.001		
		MDMA * WAY163	**16.6**	**4.72**	**3.52**	**0.004**		
	Growls (F)	Time	-0.05	0.03	-1.55	0.125	13.7%	16.5%
		MDMA Dose	0.78	0.52	1.50	0.157		
		WAY163 Dose	-0.44	0.71	-0.63	0.540		
		MDMA * WAY163	1.95	2.99	0.65	0.526		

	Huddling (D)	Time	-0.03	0.01	-3.16	0.002	38.0%	73.5%
		MDMA Dose	10.8	1.19	9.06	< 0.001		
		WAY163 Dose	-0.55	1.64	-0.33	0.739		
		MDMA * WAY163	**-14.70**	**6.94**	**-2.11**	**0.036**		
	Activity (D)	Time	0.01	0.01	1.87	0.064	41.1%	73.7%
		MDMA Dose	-7.75	0.92	-8.44	< 0.001		
		WAY163 Dose	-0.81	1.26	-0.65	0.519		
		MDMA * WAY163	9.54	5.37	1.78	0.078		

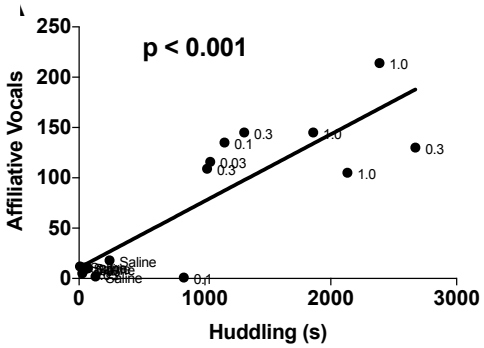
R^2 represents proportion of total variance explained by each model, including generalized linear mixed-effects models (Poisson GLMM). F: Frequency (per 10 min); D: Duration (sec per 5 min)

Figure 4-5: *Multiple MDMA exposures increases MDMA-induced huddling in a low response subject.*



One subject (177) initially showed very little change in huddling behavior following MDMA-administration. Repeated administration of MDMA increased MDMA-induced huddling in this monkey, but did not change huddling rates in the other 3 subjects. Bars represent means+SEMs, *p<0.05.

Figure 4-6: *Huddling is positively correlated with affiliative calls.*



The amount of huddling in each hour session is positively correlated with the number of affiliative calls ($r=0.869$, $p<0.001$), with increasing huddling predicting increases in affiliative vocalizations. Pearson product-moment correlations were calculated for the duration of huddling and the number of affiliative vocalization. Each data point represents the amount of group huddling and affiliative calls during a single session. Dose of MDMA (mg/kg) administered in each session is beside each data point.

Chapter 5:

MDMA: Historical perspectives and future directions

5.1 Summary of Results

The previous chapters describe the role of BDNF-trkB in the oPFC and its connected circuitry in goal-directed action. Further, I find that MDMA, a ring-substituted phenethylamine, increases BDNF selectively in the oPFC, enhances goal-directed decision making in an oPFC trkB-dependent manner, and promotes social behavior.

To summarize each chapter more specifically: In chapter 1, I review current literature pertaining to prefrontal cortical BDNF and its effects on reward-related decision making, comparing and contrasting responding for food vs. drug reinforcers. In chapters 2-4, I describe experimental findings. In chapter 2, I overexpress a truncated, inactive form of trkB, *TrkB.t1*, in an orbitostriatal circuit to generate evidence for region-specific functions of trkB in action-outcome decision making. These findings are significant because previous studies reported that orbitostriatal connections are necessary for goal-directed decision making, such that inactivation of the oPFC, the DMS, or interactions between the two structures causes a deferral to habit-based responding (Gourley et al., 2013a; Gremel & Costa, 2013). Additionally, this circuit may be neurotrophin-sensitive, given that bilateral oPFC *Bdnf* knockdown induces habits and reduces BDNF protein in the dorsal striatum (Gourley et al., 2013a). I expand on these findings by reporting that *TrkB.t1* overexpression in the oPFC causes habits, at the expense of goal-directed decision making, generating the first evidence, to my knowledge, that BDNF binding to trkB *locally within the oPFC* is necessary for flexible decision making.

I additionally found that trkB in the striatum bi-directionally regulates reward-related decision making, with DMS *TrkB.t1* overexpression impairing goal-directed action selection and DLS *TrkB.t1* overexpression impairing habit formation. In other words, trkB is essential to the functions of both the DMS (supporting action selection) and the DLS (supporting habits) (Yin et al., 2009). I am unaware of any other single striatal protein that similarly enables animals to toggle between actions and habits.

In chapter 3, I further explore the role of BDNF in oPFC-subcortical circuits in decision making, finding that inducible “disconnection” of a neurotrophin-dependent oPFC-BLA circuit causes habit-based behavior. Here I used asymmetric oPFC-selective *Bdnf* knockdown and chemogenetic inhibition of the BLA following contingency degradation training. Thus, the BLA was only “off-line” during presumptive memory formation or consolidation, and not during a test for *expression* of new memory. Nevertheless, experimental mice were unable to show evidence of new knowledge of action-outcome contingencies, indicating that BDNF-dependent oPFC-BLA interactions are necessary for action-outcome memory formation or retention. This may occur via “bottom-up” processing, given that BLA damage affects the firing properties of oPFC neurons (Schoenbaum et al., 2003).

In chapter 3, I also examine the long-term effects of adolescent cocaine exposure on action-outcome decision making and determine whether drugs that increase oPFC BDNF or stimulate trkB can moderate the long-term effects of adolescent cocaine exposure. Supporting previous findings (Hinton et al., 2014; DePoy et al., 2016,2017), cocaine exposure in adolescence caused habits in adulthood in mice. I then discovered that 7,8-DHF, a trkB agonist that increases trkB phosphorylation in the oPFC, blocks adolescent cocaine-induced habits. Relatedly, I was interested in the therapeutic-like potential of drugs that stimulate oPFC BDNF-trkB systems with greater brain region specificity relative to a systemically-administered trkB agonist. In our experiments, MDMA increased BDNF in the oPFC, but not the mPFC or the amygdala, and interestingly, decreased BDNF in the dorsal striatum. In concert, MDMA enhanced action-outcome decision making, blocking habit-based behaviors otherwise induced by oPFC-selective *Bdnf* knockdown and adolescent cocaine exposure. Importantly, the MDMA-mediated enhancement of action-outcome decision making was dependent on oPFC trkB.

Both 7,8-DHF and MDMA were administered immediately following contingency degradation training, and response preference was tested the next day, when mice were drug-free. Given the importance of the oPFC in action-outcome memory retention (Jackson et al., 2016;

Zimmermann et al., 2017a,b), neurotrophin-based enhancement of action-outcome memory may be due to oPFC-mediated memory retention. Thus, neurotrophin-based therapeutics may be effective adjuncts to addiction therapy, such as cognitive behavioral or cue exposure therapy, especially for addicts initially exposed to cocaine during adolescence.

Another factor thought to play a role in the maintenance of drug addiction is impaired social decision making. In fact, "social impairment," such as social withdrawal, is one of the four core diagnostic criteria for substance use disorders in The Diagnostic and Statistical Manual of Mental Disorders, 5th edition (American Psychiatric Association, 2013). Addicts often exhibit inappropriate social behaviors, including violence and isolation (Homer et al., 2008; Dawe et al., 2009; Volkow et al., 2011). Cocaine use decreases emotional engagement and measures of social empathy in laboratory settings (Preller et al., 2014a,b). Such social impairments may impact treatment outcomes, given that social support predicts abstinence duration in outpatient treatment programs (Mutschler et al., 2013). Therefore, drugs that promote social engagement may be useful therapeutics for the treatment of addiction. MDMA increases ratings of social feelings, with positive scores in categories such as "sociable," "extroverted," and "close to other people" (see Kamilar-Britt & Bedi, 2015). It also increases desire to socialize with other people (Kirkpatrick et al., 2014). However, mechanisms underlying the unique prosocial profile of MDMA are still poorly understood. In chapter 4, I examine the affiliative prosocial effects of MDMA in nonhuman primates. Squirrel monkeys are highly social, live in large multi-male, multi-female groups in the wild, and have rich vocal communication (Winter et al., 1966; Michel, 1994). I find that MDMA increases social behaviors and vocalizations in group-housed squirrel monkeys, while a structurally similar amphetamine-derivative, methamphetamine, does not. Additionally, MDMA-mediated elevations in social behaviors are 5-HT_{2A}, but not 5-HT_{1A}, receptor dependent.

To summarize, MDMA enhances goal-directed decision making and social behavior, both of which are impaired in cocaine users (Preller et al., 2014a,b; Ersche et al., 2016). Our findings support the potential of MDMA as a therapeutic adjunct in the treatment of addiction. This

conclusion has precedence, given that MDMA has historically been used as a therapeutic adjunct for a wide variety of psychiatric disorders, including drug abuse (*e.g.*, Greer & Tolbert, 1986). DEA regulations, however, have impeded the clinical study of MDMA. Nevertheless, interest in the use of MDMA as a therapeutic compound has seen a resurgence recently, and placebo-controlled clinical trials are finding MDMA to be an effective therapeutic adjunct. I will conclude this dissertation with a discussion focused on MDMA.

5.2 MDMA: Historical Perspectives

MDMA is a ring-substituted phenethylamine that was first discovered by Merck in 1912 and was patented as an intermediate product in a new synthesis pathway for the hemostatic drug Hydrastinine (Freudenmann et al., 2006). However, it was not tested pharmacologically until 1927, was not studied in animal models until the 1950's, when it was re-examined as a potential stimulant and hallucinogen (Hardman et al., 1973; Bernschnedier-Reif et al., 2006), and was not used therapeutically or recreationally until the 1970's (Gaston & Rasmussen, 1972; Pentney, 2001).

MDMA is structurally similar to amphetamine and mescaline, and has mixed stimulant- and hallucinogenic-like subjective effects in humans (Vollenweider et al., 1998; Liechti et al., 2000a, b). MDMA has unique prosocial effects, however, that make it distinct from either of these drug classes. Dr. Nichols (1986) coined the term 'entactogen,' meaning "to touch within," to describe the novel drug class that includes MDMA. Entactogens, including MDMA, increase sociality and feelings of love, empathy, and closeness (see Kamilar-Britt & Bedi, 2015).

Use of MDMA as a therapeutic adjunct first began when Dr. Alexander Shulgin, impressed by the effects of MDMA after personal use, introduced Dr. Leo Zeff to the substance (Benzenhöffer & Passie, 2010). Dr. Zeff then promoted the use of MDMA in therapy throughout the U.S. (Pentney, 2001). Interest in MDMA as a therapeutic adjunct grew quickly and thousands of patients were purportedly treated with MDMA from 1978 through 1985 (Shulgin & Shulgin,

1991). MDMA was said to increase therapeutic alliance and openness, while decreasing anxiety (Greer & Tolbert, 1986; Amoroso, 2015). Anecdotal reports suggest that MDMA-assisted psychotherapy was useful for treating a variety of disorders, including post-traumatic stress disorder (PTSD), phobias, depression, drug addiction, relationship difficulties, and end-of-life anxiety (Downing, 1986; Greer & Tolbert, 1986; Grinspoon & Bakalar, 1986; Adamson & Metzner, 1988; Riedlinger & Riedlinger, 1994). However, no placebo-controlled trials of MDMA were performed during this time.

MDMA's popularity as a therapeutic agent increased in tandem with its use as a recreational drug of abuse, and in 1985 the DEA began formal hearings to determine its legal status. Given the growing concern about the safety and abuse liability of MDMA, the DEA classified MDMA as a schedule I drug, indicating high levels of likelihood of abuse and no medical value (Lawn, 1986).

5.3 Current Clinical Use of MDMA

Prohibition effectively halted the therapeutic use of MDMA, but interest in MDMA as a therapeutic adjunct continued. In recent years, MDMA has returned to the clinic, driven by the Multidisciplinary Association for Psychedelic Studies (MAPS), an organization founded to organize and support research into the therapeutic use of MDMA and related drugs (MAPS, 2015). In 2001, MAPS successfully gained FDA approval to begin the first Phase II clinical trial of MDMA for the treatment of PTSD (Doblin, 2002).

Mithoefer et al. (2011) performed the first randomized, placebo-controlled pilot for MDMA as a treatment for PTSD. 20 patients with treatment-resistant PTSD were given 2 8-hour MDMA- or placebo-assisted psychotherapy session, interspersed among several non-drug sessions. MDMA-assisted therapy significantly decreased scores on the Clinician-Administered PTSD Scale (CAPS), a gold-standard measure of PTSD symptom severity. While psychotherapy alone decreased CAPS scores in the placebo control group (average score reduction of 20.5), MDMA-

assisted sessions significantly increased symptom reduction (average reduction of 53.7). The reduction in CAPS scores was maintained through long-term follow ups, conducted 17-74 months (mean of 3.8 years) after the initial study, with 87.5% of patients no longer meeting PTSD diagnostic criteria (Mithoefer et al., 2013). Importantly, no serious adverse side effects or increase in recreational usage were seen following clinically administered MDMA (Mithoefer et al., 2011, 2013). Additional studies by different researchers (Oehen et al., 2013) and in different treatment populations (Danforth et al., 2016) are finding similarly promising effects of MDMA-assisted psychotherapy to decrease PTSD symptom severity. Given these finding, the FDA has recently approved Phase III clinical trials for the assessment of MDMA as a treatment for PTSD (Phillips, 2016).

MDMA is also currently being assessed in a Phase II clinical trial as a treatment for social anxiety in autistic adults (Danforth et al., 2016), and other clinical investigations of MDMA are moving forward at an increasing pace. Between 1970 and 2010, only two Phase II clinical trials were performed, of which only one was completed. In 2016 alone, six studies were completed, and more are underway (Mithoefer et al., 2016). However, all ongoing clinical trials of MDMA in the United States are examining its treatment for anxiety-related disorders, such as PTSD, social anxiety, and anxiety associated with terminal illness (Mithoefer et al., 2016). Despite this, there is evidence that MDMA may be an effective treatment for a wide variety of psychiatric disorders. Earlier work, performed by psychiatrists before the scheduling of MDMA and in non-placebo controlled studies, indicates that MDMA may be useful for the treatment of depression and drug and alcohol addiction (Greer & Tolbert, 1986; Riedlinger & Riedlinger, 1994), and there is recent interest in its therapeutic potential for a wide range of psychiatric disorders, including mood disorders and addiction (Johansen & Krebs, 2009; Jerome et al., 2013; Patel & Titheradge, 2015). In fact, the first clinical trial examining MDMA as a treatment for alcohol abuse has just been approved in the United Kingdom (Devlin, 2017).

5.4 Potential Advantage of *R(–)*-MDMA

Despite increasing interest in the use of MDMA as a therapeutic, there is still considerable debate about the clinical viability of MDMA (Parrot, 2013,2014; Doblin et al., 2014). MDMA is a widely-used drug of abuse (Center for Behavioral Statistics and Quality, 2015). Additionally, repeated use may have long-term consequences, given the evidence that MDMA causes neurotoxicity in animal models and neurotoxicity and cognitive impairments in users, though many of these studies have methodological concerns (Capela et al., 2009; Rogers et al., 2009; Parrott, 2013). Understanding the mechanisms that mediate the social and therapeutic effects of MDMA may allow for the development of novel therapeutics that lack the adverse side effects of MDMA.

One potential alternative is the *R*(–) enantiomer of MDMA. MDMA is a racemic drug that consists of equal amounts of two enantiomers, *S*(+)-MDMA and *R*(–)-MDMA. While no controlled experiments have compared the behavioral effects of the two enantiomers in humans, pre-clinical experiments have found results that are promising for the potential clinical utility of *R*(–)-MDMA. Most importantly, *R*(–)-MDMA appears to be less neurotoxic than racemic and *S*(+)-MDMA, producing less reactive gliosis (Frau et al., 2013) and not causing long-term depletion of 5-HT or 5-HIAA in rats (Schmidt et al., 1987; Johnson et al., 1988). Additionally, *R*(–)-MDMA does not produce hyperthermia (Fantegrossi et al., 2003; Frau et al., 2013), which correlates with degree of MDMA-induced neurotoxicity (Green et al., 2003).

R(–)-MDMA may also have lower abuse liability than racemic or *S*(+)-MDMA. In contrast to *S*(+)-MDMA, *R*(–)-MDMA does not increase striatal dopamine release in rats (Hiramatsu & Cho, 1990; Acquas et al., 2007) or nonhuman primates (Murnane et al., 2010), and does not show significant occupation of the dopamine transporter (Fantegrossi, 2008). Further supporting reduced abuse liability, *R*(–)-MDMA does not reinstate drug-seeking in nonhuman primates trained on amphetamine self-administration (McClung et al., 2010), while S(+)-MDMA did reinstate amphetamine-seeking behavior. Also, when studied in a self-administration paradigm, *R*(–)-MDMA engendered significantly lower breakpoints on a progressive ratio schedule of

reinforcement than racemic and *S*(+)-MDMA (Wang & Woolverton, 2007). However, Fantegrossi et al. (2002) found that both enantiomers and racemic MDMA are equally effective reinforcers on a fixed ratio schedule of reinforcement, suggesting *R*(–)-MDMA still has some abuse liability.

5.5 Future Directions

The reduced liability for toxicity, hyperthermia, and abuse induced by *R*(–)-MDMA could increase the clinical utility of MDMA, especially for drug abuse disorders. However, the risk/benefit ratio does not improve if *R*(–)-MDMA is less therapeutically effective than racemic MDMA. In chapter 4, we find that *R*(–)-MDMA increases social contact and vocalizations in squirrel monkeys to similar levels as racemic MDMA. This is an important first step, given the important role increased sociability is thought to play in the therapeutic effects of MDMA (Mithoeffer et al., 2011). Other studies in the Howell lab find that *R*(–)-MDMA and racemic, but not *S*(+)-MDMA, facilitate the extinction of conditioned freezing – an index of conditioned fear – in mice, further evidence that *R*(–)-MDMA may be an effective therapeutic compound. However, future studies need to examine the effects of *R*(–)-MDMA in a *variety* of pre-clinical assays to further examine this possibility and to understand mechanistic factors. For example, future studies could examine whether *R*(–)-MDMA stimulates BDNF in the oPFC (as with racemic MDMA), whether *R*(–)-MDMA can enhance action-outcome decision making, and if so, whether it is dependent on BDNF-trkB activity in the oPFC. Future studies could also examine whether *R*(–)-MDMA-mediated enhancement of conditioned fear extinction is BDNF-dependent, as with racemic MDMA (Young et al., 2015).

More broadly, future experiments should also examine mechanisms mediating the brain region-specific elevations in BDNF following MDMA (chapter 3). MDMA-mediated changes in brain BDNF and *Bdnf* are likely quite complex. We find that MDMA increases BDNF in the oPFC 2 hours following administration, but BDNF levels did not change in the mPFC or amygdala, and levels were *decreased* in the dorsal striatum (chapter 3). Other studies also find brain region-

specific regulation of *Bdnf* levels, with 2 reports finding that MDMA increases frontal cortex, but decreases hippocampal, *Bdnf* (Martínez-Turillas et al., 2006; Hemmerle et al., 2012). Further, Mouri et al. (2017) found that a single administration of MDMA, using a dose higher than that deployed in our study (10 mg/kg), increases *Bdnf* in the prefrontal cortex and amygdala, but not the striatum or hippocampus. Meanwhile, repeated administration did increase striatal and hippocampal *Bdnf.* Additionally, pairing MDMA with behavioral training can modulate MDMA's effects on BDNF levels. Abad et al. (2014) found that MDMA increases hippocampal BDNF, and that this elevation is greater when mice are also trained on a Morris water maze. Similarly, Young et al. (2015) reported that MDMA stimulates amygdala *Bdnf* only when MDMA is paired with fear extinction training. Thus, MDMA appears to modulate BDNF and *Bdnf* in a brain region-specific manner that is dependent on dose, number of injections, timing, and behavioral experience. Given that BDNF in varied brain regions differentially regulates cocaine- and reward-related behaviors (discussed in chapter 1; Li & Wolf, 2015), understanding the mechanisms by which MDMA influences BDNF could aid in the development of novel therapeutic approaches to drug use disorders.

Also, future studies should examine the broader circuits mediating the effects of MDMA on decision making. Here I show that MDMA decreases BDNF in tissue dissections containing the whole dorsal striatum (chapter 3), important because trkB in the DLS subregion supports habit behavior (chapter 2). These findings raise the possibility that MDMA-induced decrements in striatal BDNF play a role in its ability to enrich action-outcome-based decision making, *i.e.*, by reducing trkB activation within the DLS. Future studies could microdissect the different subregions of the dorsal striatum to test for subregion-selective effects of MDMA on striatal BDNF levels. This could be accomplished using laser capture microdissection in mice; however, these dissections would likely not contain enough protein to generate unambiguous western blots without pooling tissue samples from multiple animals, given the small size of the mouse brain. To address this

issue, MDMA could instead be administered to rats, which have larger brains, allowing for larger dissections that could still be subregion specific.

Future studies could also investigate causal relationships between MDMA-mediated losses in striatal BDNF and action-outcome decision making. For example, mice could be given cocaine or saline in adolescence. Then, in adulthood, MDMA could be administered immediately following contingency degradation training to engage action-outcome learning and memory systems and block cocaine-induced habits (as in chapter 3). In half of the mice, BDNF or 7,8-DHF could be infused into the DLS immediately prior to MDMA administration (2x2x2 experimental design). If decreased BDNF-trkB-mediated intracellular signaling within the DLS is contributing, at least in part, to the ability of MDMA to stimulate goal-directed decision making, then counteracting that deficiency by infusing BDNF or 7,8-DHF should interfere with the behavioral effects of MDMA.

Future studies should additionally attempt to untangle the mechanisms and circuits mediating the various therapeutic-like effects of MDMA and identify their overlap, if any. We find that MDMA-mediated stimulation of action-outcome decision making is dependent on trkB in the oPFC (chapter 3). The oPFC may also be involved in social deficits in cocaine addiction, with blunted activation of the oPFC during social interaction correlating with small social network size in cocaine addicts (Preller et al., 2014a). Thus, future studies could examine whether the oPFC is playing a role in MDMA-mediated prosocial behaviors (chapter 4), in tandem with its decision-making effects.

We also find that MDMA-induced increases in social behaviors are 5-HT_{2A} receptor-dependent (chapter 4). The 5-HT_{2A} receptor may also be involved in the effects of MDMA on BDNF and decision making (chapter 3). Activation of the 5-HT_{2A} receptor increases *Bdnf* in C6 glioma cells (Meller et al., 2002) and a 5-HT_{2A} receptor agonist increases, while a 5-HT_{2A} receptor antagonist decreases, PFC *Bdnf* levels following acute psychological stress in rats (Jiang et al., 2016). Additionally, 5-HT_{2A} receptor activation differentially regulated *Bdnf* levels in the neocortex

and hippocampus of rats, increasing *Bdnf* in the frontal and parietal cortex and decreasing *Bdnf* in the dentate gyrus of the hippocampus (Vaidya et al., 1997). To test the role of the 5-HT_{2A} receptor in the effects of MDMA on BDNF and decision making, M100 (a selective 5-HT_{2A} receptor antagonist) could be administered prior to MDMA. If M100 administration blocked MDMA-induced changes in BDNF and action-outcome decision making, then the 5-HT_{2A} receptor could underlie, in part, both the decision making and social effects of MDMA.

Finally, another set of potential future directions would be to examine the effects of MDMA and other neurotrophin-related therapeutic-like agents on dendritic spine density and morphology. Cocaine exposure decreases dendritic spine densities in the oPFC (Radley et al., 2015), and in the case of adolescent exposure, decrements in dendritic spine density in the oPFC are detectable in adulthood (Gourley et al., 2012; DePoy et al., 2017). Further, certain pharmacological interventions that block cocaine-induced habits also restore typical spine densities (DePoy et al., 2017). Conversely, destabilization of the actin cytoskeleton – the structural lattice that supports dendritic spine shape and motility – within the oPFC *causes* habits (DePoy et al., 2017), indicating a causal relationship between dendritic spine plasticity and action-outcome decision making. BDNF-trkB modulates dendritic spine morphology and plasticity (Park & Poo, 2013; Bennett & Lagopoulos, 2014), and repeated 7,8-DHF treatment stimulates dendritic spinogenesis in the oPFC (Zimmermann et al., 2017b). Further, repeated MDMA increases dendritic spine densities in the mPFC (Ball et al., 2009). Future studies could examine whether MDMA and 7,8-DHF restore typical dendritic spine densities in the oPFC following adolescent cocaine exposure, and whether this structural plasticity mediates the ability of these compounds to block cocaine-induced habits.

5.6 Conclusions

Together, these chapters suggest that BDNF-trkB in the oPFC and connected regions dynamically regulates flexible decision making and that neurotrophin-related pharmaco-therapies,

including MDMA, may be effective therapeutics for disorders characterized by maladaptive decision making. Specifically, MDMA enhances action-outcome decision making and social behaviors, and it may thereby be an effective therapeutic adjunct for the treatment of cocaine addiction and potentially, other substance use disorders. Further understanding the mechanisms mediating these effects may aid in the development of novel therapeutics with wider clinical appeal.

Appendix: Complete list of publications to which the author has contributed during her graduate training

Pitts EG, Taylor JR, and Gourley SL (2016) Role of prefrontal cortical BDNF in reward-related decision making. *Neurobiology of Disease* 91: 326-335.

Pitts EG, Minerva AR, Chandler EB, Logun MT, Kohn JN, Sulima A, Kenner RC, and Howell LL (2017) 3,4-methylenedioxymethamphetamine increases affiliative behaviors in squirrel monkeys in a serotonin 2A receptor-dependent manner. *Neuropsychopharmacology*. Advance online publication. doi: 10.1038/npp.2017.80.

Schmidt KT, Schroeder JP, Foster SL, Squires K, Coleman BM, Pitts EG, Epstein MP, and Weinshenker D (2017) Norepinephrine regulates cocaine-primed reinstatement via α1-adrenergic receptors in the medial prefrontal cortex. *Neuropharmacology*. Advance online publication. doi: 10.1016/j.neuropharm.2017.04.005.

Shapiro LP, Hinton EA, Allen AG, Hinton EA, Pitts EG, Bassell GJ, Gross C, and Gourley SL. "Protective" consequences of reducing PI3K p110β following cocaine. In revision.

Pitts EG, Young MB, Curry DW, and Howell LL. (±)-MDMA and its enantiomers: potential therapeutic advantages of *R*(–)-MDMA. Submitted.

Pitts EG and Gourley SL. Bidirectional coordination of actions and habits by trkB. In preparation.

Pitts EG, Barfield ET, and Gourley SL. Blockade of cocaine-induced habits by MDMA is trkB-dependent. In preparation.

Pitts EG, Minerva AR, Chandler EB, and Howell LL. MDMA causes long-term, off-drug increases in social behaviors in group-housed squirrel monkeys. In preparation.

References

Abad S, Fole A, del Olmo N, Pubill D, Pallas M, Junyent F*, et al.* (2014): MDMA enhances hippocampal-dependent learning and memory under restrictive conditions, and modifies hippocampal spine density*. Psychopharmacology (Berl)* 231: 863-874.

Acquas E, Pisanu A, Spiga S, Plumitallo A, Zernig G, and Di Chiara G. (2007): Differential effects of intravenous R,S-(+/-)-3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and its S(+)- and R(-)-enantiomers on dopamine transmission and extracellular signal regulated kinase phosphorylation (pERK) in the rat nucleus accumbens shell*. J Neurochem* 102: 121-132.

Adamson S and Metzner R. (1988): The nature of the MDMA experience and its role in healing, psychotherapy and spiritual practice*. ReVision* 10: 59-72.

Ahmari SE, Spellman T, Douglass NL, Kheirbek MA, Simpson HB, Deisseroth K*, et al.* (2013): Repeated cortico-striatal stimulation generates persistent OCD-like behavior*. Science* 340: 1234-1239.

Alia-Klein N, Parvaz MA, Woicik PA, Konova AB, Maloney T, Shumay E*, et al.* (2011): Gene x disease interaction on orbitofrontal gray matter in cocaine addiction*. Arch Gen Psychiatry* 68: 283-294.

Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL*, et al.* (1997): Anterograde transport of brain-derived neurotrophic factor and its role in the brain*. Nature* 389: 856-860.

Altar CA, Siuciak JA, Wright P, Ip NY, Lindsay RM, and Wiegand SJ. (1994): In situ hybridization of trkB and trkC receptor mRNA in rat forebrain and association with high-affinity binding of [125I]BDNF, [125I]NT-4/5 and [125I]NT-3*. Eur J Neurosci* 6: 1389-1405.

Altmann J. (1974): Observational study of behavior: sampling methods*. Behaviour* 49: 227-267.

American Psychiatric Association, in *Diagnostic and Statistical Manual of Mental Disorders*. 2013, American Psychiatric Association: Washington, DC.

Amoroso T. (2015): The psychopharmacology of ±3,4 methylenedioxymethamphetamine and its role in the treatment of posttraumatic stress disorder*. J Psychoactive Drugs* 00: 1-8.

Atwal JK, Massie B, Miller FD, and Kaplan DR. (2000): The TrkB-Shc site signals neuronal survival and local axon growth via MEK and P13-kinase*. Neuron* 27: 265-277.

Autry AE and Monteggia LM. (2012): Brain-derived neurotrophic factor and neuropsychiatric disorders*. Pharmacol Rev* 64: 238-258.

Baldwin J and Baldwin J. *The squirrel monkeys, genus saimiri*, in *Ecology and behavior of neotropical primates*, A. Coimra-Filho and R. Mittermeier, Editors. 1981, Academia Brasileira de Ciencias: Rio de Janeiro, Brazil. p. 277-330.

Ball KT, Wellman CL, Fortenberry E, and Rebec GV. (2009): Sensitizing regimens of (+/-)3, 4-methylenedioxymethamphetamine (ecstasy) elicit enduring and differential structural alterations in the brain motive circuit of the rat*. Neuroscience* 160: 264-274.

Balleine BW and O'Doherty JP. (2010): Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action*. Neuropsychopharmacology* 35: 48-69.

Ballesta S, Reymond G, Pozzobon M, and Duhamel JR. (2016): Effects of MDMA Injections on the behavior of socially-housed long-tailed macaques (Macaca fascicularis)*. PLoS One* 11: 1-10.

Banks ML, Sprague JE, Kisor DF, Czoty PW, Nichols DE, and Nader MA. (2007): Ambient temperature effects on 3,4-methylenedioxymethamphetamine-induced thermodysregulation and pharmacokinetics in male monkeys*. Drug Metab Dispos* 35: 1840-1845.

Banner H, Bhat V, Etchamendy N, Joober R, and Bohbot VD. (2011): The brain-derived neurotrophic factor Val66Met polymorphism is associated with reduced functional magnetic resonance imaging activity in the hippocampus and increased use of caudate nucleus-dependent strategies in a human virtual navigation task. *Eur J Neurosci* 33: 968-977.

Barker JM, Taylor JR, De Vries TJ, and Peters J. (2014): Brain-derived neurotrophic factor and addiction: Pathological versus therapeutic effects on drug seeking. *Brain Res*.

Barton K, *MuMIn: Multi-model inference. R package version 1.13.4. http://CRAN.R-project.org/package=MuMIn*. 2015.

Bates D, Maechler M, and Bolker B. (2012): lme4: Linear mixed-effects models using S4 classes. http://CRAN.R-project.org/package=lme4.

Bennett MR and Lagopoulos J. (2014): Stress and trauma: BDNF control of dendritic-spine formation and regression. *Prog Neurobiol* 112: 80-99.

Benzenhöfer U and Passie T. (2010): Rediscovering MDMA (ecstasy): the role of the American chemist Alexander T. Shulgin. *Addiction* 105: 1355-1361.

Berglind WJ, See RE, Fuchs RA, Ghee SM, Whitfield TW, Jr., Miller SW*, et al.* (2007): A BDNF infusion into the medial prefrontal cortex suppresses cocaine seeking in rats. *Eur J Neurosci* 26: 757-766.

Berglind WJ, Whitfield TW, Jr., LaLumiere RT, Kalivas PW, and McGinty JF. (2009): A single intra-PFC infusion of BDNF prevents cocaine-induced alterations in extracellular glutamate within the nucleus accumbens. *J Neurosci* 29: 3715-3719.

Bernschneider-Reif S, Oxler F, and Freudenmann RW. (2006): The origin of MDMA ("ecstasy")--separating the facts from the myth. *Die Pharmazie* 61: 966-972.

Binder DK and Scharfman HE. (2004): Brain-derived neurotrophic factor. *Growth Factors* 22: 123-131.

Bombardi C and Di Giovanni G. (2013): Functional anatomy of 5-HT2A receptors in the amygdala and hippocampal complex: relevance to memory functions. *Exp Brain Res* 230: 427-439.

Bradfield LA, Dezfouli A, van Holstein M, Chieng B, and Balleine BW. (2015): Medial orbitofrontal cortex mediates outcome retrieval in partially observable task situations. *Neuron* 88: 1268-1280.

Burguiere E, Monteiro P, Mallet L, Feng G, and Graybiel AM. (2015): Striatal circuits, habits, and implications for obsessive-compulsive disorder. *Curr Opin Neurobiol* 30: 59-65.

Butkovich LM, DePoy LM, Allen AG, Shapiro LP, Swanson AM, and Gourley SL. (2015): Adolescent-onset GABA alpha1 silencing regulates reward-related decision making. *Eur J Neurosci*.

Capela JP, Carmo H, Remião F, Bastos ML, Meisel A, and Carvalho F. (2009): Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: an overview. *Mol Neurobiol* 39: 210-271.

Careaga MB, Girardi CE, and Suchecki D. (2016): Understanding posttraumatic stress disorder through fear conditioning, extinction and reconsolidation. *Neurosci Biobehav Rev* 71: 48-57.

Center for Behavioral Health Statistics and Quality. (2015): Behavioral health trends in the United States: Results from the 2014 National Survey on Drug Use and Health (HHS Publication No. SMA 15-4927, NSDUH Series H-50). Retrieved from http://www.samhsa.gov/data/.

Cheng CY, Hong CJ, Yu YW, Chen TJ, Wu HC, and Tsai SJ. (2005): Brain-derived neurotrophic factor (Val66Met) genetic polymorphism is associated with substance abuse in males. *Brain Res Mol Brain Res* 140: 86-90.

Choi DC, Gourley SL, and Ressler KJ. (2012): Prelimbic BDNF and TrkB signaling regulates consolidation of both appetitive and aversive emotional learning. *Transl Psychiatry* 2: e205.

Conner JM, Lauterborn JC, and Gall CM. (1998): Anterograde transport of neurotrophin proteins in the CNS--a reassessment of the neurotrophic hypothesis. *Rev Neurosci* 9: 91-103.

Cooper DA, Kimmel HL, Manvich DF, Schmidt KT, Weinshenker D, and Howell LL. (2014): Effects of pharmacologic dopamine beta-hydroxylase inhibition on cocaine-induced reinstatement and dopamine neurochemistry in squirrel monkeys. *J Pharmacol Exp Ther* 350: 144-152.

Corbit LH and Balleine BW. (2003): The role of prelimbic cortex in instrumental conditioning. *Behav Brain Res* 146: 145-157.

Corominas-Roso M, Roncero C, Daigre C, Grau-Lopez L, Ros-Cucurull E, Rodríguez-Cintas L*, et al.* (2015): Changes in brain-derived neurotrophic factor (BDNF) during abstinence could be associated with relapse in cocaine-dependent patients. *Psychiatry Research* 225: 309-314.

Corominas-Roso M, Roncero C, Eiroa-Orosa FJ, Gonzalvo B, Grau-Lopez L, Ribases M*, et al.* (2013a): Brain-derived neurotrophic factor serum levels in cocaine-dependent patients during early abstinence. *European Neuropsychopharmacology* 23: 1078-1084.

Corominas-Roso M, Roncero C, Eiroa-Orosa FJ, Ribases M, Barral C, Daigre C*, et al.* (2013b): Serum brain-derived neurotrophic factor levels and cocaine-induced transient psychotic symptoms. *Neuropsychobiology* 68: 146-155.

Crean RD, Davis SA, Von Huben SN, Lay CC, Katner SN, and Taffe MA. (2006): Effects of (+/-)3,4-methylenedioxymethamphetamine, (+/-)3,4-methylenedioxyamphetamine and methamphetamine on temperature and activity in rhesus macaques. *Neuroscience* 142: 515-525.

Danforth AL, Struble CM, Yazar-Klosinski B, and Grob CS. (2016): MDMA-assisted therapy: A new treatment model for social anxiety in autistic adults. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 64: 237-249.

Dawe S, Davis P, Lapworth K, and McKetin R. (2009): Mechanisms underlying aggressive and hostile behavior in amphetamine users. *Curr Opin Psychiatry* 22: 269-273.

DePoy LM, Allen AG, and Gourley SL. (2016): Adolescent cocaine self-administration induces habit behavior in adulthood: sex differences and structural consequences. *Transl Psychiatry* 6: e875.

DePoy LM and Gourley SL. (2015): Synaptic cytoskeletal plasticity in the prefrontal cortex following psychostimulant exposure. *Traffic*.

DePoy LM, Noble B, Allen AG, and Gourley SL. (2013): Developmentally divergent effects of Rho-kinase inhibition on cocaine- and BDNF-induced behavioral plasticity. *Behav Brain Res* 243: 171-175.

DePoy LM, Perszyk RE, Zimmermann KS, Koleske AJ, and Gourley SL. (2014): Adolescent cocaine exposure simplifies orbitofrontal cortical dendritic arbors. *Front Pharmacol* 5: 228.

DePoy LM, Zimmermann KS, Marvar PJ, and Gourley SL. (2017): Induction and blockade of adolescent cocaine-induced habits. *Biol Psychiatry* 81: 595-605.

Devlin H. *World's first trials of MDMA to treat alcohol addiction set to begin*, in *The Guardian*. 2017.

Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ*, et al.* (2009): Chronic stress causes frontostriatal reorganization and affects decision-making. *Science* 325: 621-625.

Doblin R. (2002): A clinical plan for MDMA (ecstasy) in the treatment of posttraumatic stress disorder (PTSD): Partnering with the FDA. *J Psychoactive Drugs* 34: 185-194.

Doblin R, Greer G, Holland J, Jerome L, Mithoefer MC, and Sessa B. (2014): A reconsideration and response to Parrott AC (2013) "Human psychobiology of MDMA or 'Ecstasy': an overview of 25 years of empirical research". *Hum Psychopharmacol* 29: 105-108.

Dong Y and Nestler EJ. (2014): The neural rejuvenation hypothesis of cocaine addiction. *Trends in Pharmacological Sciences* 35: 374-383.

Downing J. (1986): The psychological and physiological effects of MDMA on normal volunteers. *J Psychoactive Drugs* 18: 335-340.

Dumont GJ, Sweep FC, van der Steen R, Hermsen R, Donders AR, Touw DJ, *et al.* (2009): Increased oxytocin concentrations and prosocial feelings in humans after ecstasy (3,4-methylenedioxymethamphetamine) administration. *Soc Neurosci* 4: 359-366.

Dunlop J, Sabb AL, Mazandarani H, Zhang J, Kalgaonker S, Shukhina E, *et al.* (2005): WAY-163909 [(7bR, 10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indol e], a novel 5-hydroxytryptamine 2C receptor-selective agonist with anorectic activity. *J Pharmacol Exp Ther* 313: 862-869.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, *et al.* (2003): The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112: 257-269.

Ersche KD, Gillan CM, Jones PS, Williams GB, Ward LH, Luijten M, *et al.* (2016): Carrots and sticks fail to change behavior in cocaine addiction. *Science* 352: 1468-1471.

Everitt BJ. (2014): Neural and psychological mechanisms underlying compulsive drug seeking habits and drug memories - indications for novel treatments of addiction. *European Journal of Neuroscience* 40: 2163-2182.

Everitt BJ and Robbins TW. (2005): Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* 8: 1481-1489.

Everitt BJ and Robbins TW. (2016): Drug addiction: Updating actions to habits to compulsions ten years on. *Annu Rev Psychol* 67: 23-50.

Fallon JK, Kicman AT, Henry JA, Milligan PJ, Cowan DA, and Hutt AJ. (1999): Stereospecific analysis and enantiomeric disposition of 3,4- methylenedioxymethamphetamine (ecstasy) in humans. *Clin Chem* 45: 1058-1069.

Fantegrossi WE. (2008): In vivo pharmacology of MDMA and its enantiomers in rhesus monkeys. *Exp Clin Psychopharmacol* 16: 1-12.

Fantegrossi WE, Bauzo RM, Manvich DM, Morales JC, Votaw JR, Goodman MM, *et al.* (2009): Role of dopamine transporters in the behavioral effects of 3,4-methylenedioxymethamphetamine (MDMA) in nonhuman primates. *Psychopharmacology (Berl)* 205: 337-347.

Fantegrossi WE, Godlewski T, Karabenick RL, Stephens JM, Ullrich T, Rice KC, *et al.* (2003): Pharmacological characterization of the effects of 3,4-methylenedioxymethamphetamine ("ecstasy") and its enantiomers on lethality, core temperature, and locomotor activity in singly housed and crowded mice. *Psychopharmacology (Berl)* 166: 202-211.

Fantegrossi WE, Ullrich T, Rice KC, Woods JH, and Winger G. (2002): 3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") and its stereoisomers as reinforcers in rhesus monkeys: Serotonergic involvement. *Psychopharmacology (Berl)* 161: 356-364.

Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, and Robinson TE. (2005): Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. *Biol Psychiatry* 58: 751-759.

Fisher PM, Meltzer CC, Price JC, Coleman RL, Ziolko SK, Becker C*, et al.* (2009): Medial prefrontal cortex 5-HT(2A) density is correlated with amygdala reactivity, response habituation, and functional coupling*. Cereb Cortex* 19: 2499-2507.

Fiuzat EC, Rhodes SE, and Murray EA. (2017): The role of orbitofrontal-amygdala interactions in updating action-outcome valuations in macaques*. J Neurosci* 37: 2463-2470.

Franklin TR, Acton PD, Maldjian JA, Gray JD, Croft JR, Dackis CA*, et al.* (2002): Decreased gray matter concentration in the insular, orbitofrontal, cingulate, and temporal cortices of cocaine patients*. Biol Psychiatry* 51: 134-142.

Frau L, Simola N, Plumitallo A, and Morelli M. (2013): Microglial and astroglial activation by 3,4-methylenedioxymethamphetamine (MDMA) in mice depends on S(+) enantiomer and is associated with an increase in body temperature and motility. *J Neurochem* 124: 69-78.

Freudenmann RW, Oxler F, and Bernschneider-Reif S. (2006): The origin of MDMA (ecstasy) revisited: the true story reconstructed from the original documents. *Addiction* 101: 1241-1245.

Fuchs RA, Evans KA, Parker MP, and See RE. (2004): Differential involvement of orbitofrontal cortex subregions in conditioned cue-induced and cocaine-primed reinstatement of cocaine seeking in rats*. J Neurosci* 24: 6600-6610.

Fumagalli F, Caffino L, Racagni G, and Riva MA. (2009): Repeated stress prevents cocaine-induced activation of BDNF signaling in rat prefrontal cortex*. Eur Neuropsychopharmacol* 19: 402-408.

Fumagalli F, Di Pasquale L, Caffino L, Racagni G, and Riva MA. (2007): Repeated exposure to cocaine differently modulates BDNF mRNA and protein levels in rat striatum and prefrontal cortex*. Eur J Neurosci* 26: 2756-2763.

Fumagalli F, Moro F, Caffino L, Orru A, Cassina C, Giannotti G*, et al.* (2013): Region-specific effects on BDNF expression after contingent or non-contingent cocaine i.v. self-administration in rats*. Int J Neuropsychopharmacol* 16: 913-918.

Gabbott PL, Warner TA, and Busby SJ. (2006): Amygdala input monosynaptically innervates parvalbumin immunoreactive local circuit neurons in rat medial prefrontal cortex*. Neuroscience* 139: 1039-1048.

Gabriele A, Setlow B, and Packard MG. (2009): Cocaine self-administration alters the relative effectiveness of multiple memory systems during extinction*. Learn Mem* 16: 296-299.

Gaston TR and Rasmussen GT. (1972): Identification of 3,4 methylenedioxymethamphetamine. *Bureau of Narcotics and Dangerous Drugs* 4: 60-61.

Ghitza UE, Zhai H, Wu P, Airavaara M, Shaham Y, and Lu L. (2010): Role of BDNF and GDNF in drug reward and relapse: a review*. Neurosci Biobehav Rev* 35: 157-171.

Giannotti G, Caffino L, Calabrese F, Racagni G, Riva MA, and Fumagalli F. (2014): Prolonged abstinence from developmental cocaine exposure dysregulates BDNF and its signaling network in the medial prefrontal cortex of adult rats*. Int J Neuropsychopharmacol* 17: 625-634.

Gipson CD, Kupchik YM, Shen H, Reissner KJ, Thomas CA, and Kalivas PW. (2013): Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation*. Neuron* 77: 867-872.

Gourley SL, Kedves AT, Olausson P, and Taylor JR. (2009a): A history of corticosterone exposure regulates fear extinction and cortical NR2B, GluR2/3, and BDNF*. Neuropsychopharmacology* 34: 707-716.

Gourley SL, Koleske AJ, and Taylor JR. (2009b): Loss of dendrite stabilization by the Abl-related gene (Arg) kinase regulates behavioral flexibility and sensitivity to cocaine. *Proc Natl Acad Sci U S A* 106: 16859-16864.

Gourley SL, Lee AS, Howell JL, Pittenger C, and Taylor JR. (2010): Dissociable regulation of instrumental action within mouse prefrontal cortex. *Eur J Neurosci* 32: 1726-1734.

Gourley SL, Olevska A, Warren MS, Taylor JR, and Koleske AJ. (2012a): Arg kinase regulates prefrontal dendritic spine refinement and cocaine-induced plasticity. *J Neurosci* 32: 2314-2323.

Gourley SL, Olevska A, Zimmermann KS, Ressler KJ, Dileone RJ, and Taylor JR. (2013a): The orbitofrontal cortex regulates outcome-based decision-making via the lateral striatum. *Eur J Neurosci* 38: 2382-2388.

Gourley SL, Swanson AM, Jacobs AM, Howell JL, Mo M, Dileone RJ*, et al.* (2012b): Action control is mediated by prefrontal BDNF and glucocorticoid receptor binding. *Proc Natl Acad Sci U S A* 109: 20714-20719.

Gourley SL, Swanson AM, and Koleske AJ. (2013b): Corticosteroid-induced neural remodeling predicts behavioral vulnerability and resilience. *J Neurosci* 33: 3107-3112.

Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, and Self DW. (2007): Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat Neurosci* 10: 1029-1037.

Graybeal C, Feyder M, Schulman E, Saksida LM, Bussey TJ, Brigman JL*, et al.* (2011): Paradoxical reversal learning enhancement by stress or prefrontal cortical damage: rescue with BDNF. *Nat Neurosci* 14: 1507-1509.

Graybiel AM and Grafton ST. (2015): The striatum: where skills and habits meet. *Cold Spring Harb Perspect Biol* 7: a021691.

Green AR, King MV, Shortall SE, and Fone KC. (2012): Lost in translation: preclinical studies on 3,4-methylenedioxymethamphetamine provide information on mechanisms of action, but do not allow accurate prediction of adverse events in humans. *Br J Pharmacol* 166: 1523-1536.

Green AR, Mechan AO, Elliott JM, O'Shea E, and Colado MI. (2003): The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacol Rev* 55: 463-508.

Greer G and Tolbert R. (1986): Subjective reports of the effects of MDMA in a clinical setting. *J Psychoactive Drugs* 18: 319-327.

Gremel CM and Costa RM. (2013): Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. *Nat Commun* 4: 2264.

Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, and Shaham Y. (2003): Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. *J Neurosci* 23: 742-747.

Grinspoon L and Bakalar JB. (1986): Can drugs be used to enhance the psychotherapeutic process? *American Journal of Psychotherapy* 40: 393-404.

Gross C, Raj N, Molinaro G, Allen AG, Whyte AJ, Gibson JR, *et al.* (2015): Selective role of the catalytic PI3K subunit p110beta in impaired higher order cognition in fragile X syndrome. *Cell Rep* 11: 681-688.

Harder JA and Ridley RM. (2000): The 5-HT1A antagonist, WAY 100 635, alleviates cognitive impairments induced by dizocilpine (MK-801) in monkeys. *Neuropharmacology* 39: 547-552.

Hardman HF, Haavik CO, and Seevers MH. (1973): Relationship of the structure of mescaline and seven analogs to toxicity and behavior in five species of laboratory animals. *Toxicology and Applied Pharmacology* 25: 299-309.

Hearing MC, Miller SW, See RE, and McGinty JF. (2008): Relapse to cocaine seeking increases activity-regulated gene expression differentially in the prefrontal cortex of abstinent rats. *Psychopharmacology (Berl)* 198: 77-91.

Heidbreder CA and Groenewegen HJ. (2003): The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. *Neurosci Biobehav Rev* 27: 555-579.

Heldt SA, Zimmermann K, Parker K, Gaval M, Weinshenker D, and Ressler KJ. (2014): BDNF deletion or TrkB impairment in amygdala inhibits both appetitive and aversive learning. *J Neurosci* 34: 2444-2450.

Hemmerle AM, Dickerson JW, Herring NR, Schaefer TL, Vorhees CV, Williams MT*, et al.* (2012): (+/-)3,4-methylenedioxymethamphetamine ("ecstasy") treatment modulates expression of neurotrophins and their receptors in multiple regions of adult rat brain. *J Comp Neurol* 520: 2459-2474.

Herin DV, Liu S, Ullrich T, Rice KC, and Cunningham KA. (2005): Role of the serotonin 5-HT2A receptor in the hyperlocomotive and hyperthermic effects of (+)-3,4-methylenedioxymethamphetamine. *Psychopharmacology (Berl)* 178: 505-513.

Hinton EA, Wheeler MG, and Gourley SL. (2014): Early-life cocaine interferes with BDNF-mediated behavioral plasticity. *Learn Mem* 21: 253-257.

Hiramatsu M and Cho AK. (1990): Enantiomeric differences in the effects of 3,4-methylenedioxymethamphetamine on extracellular monoamines and metabolites in the

striatum of freely-moving rats: An in vivo microdialysis study. *Neuropharmacology* 29: 269-275.

Hjorth S. (1993): Serotonin 5-HT1A autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo: a microdialysis study. *J Neurochem* 60: 776-779.

Hofer M, Pagliusi SR, Hohn A, Leibrock J, and Barde YA. (1990): Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *Embo j* 9: 2459-2464.

Homer BD, Solomon TM, Moeller RW, Mascia A, DeRaleau L, and Halkitis PN. (2008): Methamphetamine abuse and impairment of social functioning: a review of the underlying neurophysiological causes and behavioral implications. *Psychol Bull* 134: 301-310.

Hoover WB and Vertes RP. (2011): Projections of the medial orbital and ventral orbital cortex in the rat. *J Comp Neurol* 519: 3766-3801.

Hopf S, Hartmann-Wiesner E, Kuhlmorgen B, and Mayer S. (1974): The behavioral repertoire of the squirrel monkey (Saimiri). *Folia Primatol (Basel)* 21: 225-249.

Horger BA, Iyasere CA, Berhow MT, Messer CJ, Nestler EJ, and Taylor JR. (1999): Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J Neurosci* 19: 4110-4122.

Howell LL and Cunningham KA. (2015): Serotonin 5-HT2 receptor interactions with dopamine function: implications for therapeutics in cocaine use disorder. *Pharmacol Rev* 67: 176-197.

Huang EJ and Reichardt LF. (2003): Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem* 72: 609-642.

Hunt GE, McGregor IS, Cornish JL, and Callaghan PD. (2011): MDMA-induced c-Fos expression in oxytocin-containing neurons is blocked by pretreatment with the 5-HT-1A receptor antagonist WAY 100635. *Brain Res Bull* 86: 65-73.

Hyman SE, Malenka RC, and Nestler EJ. (2006): Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci* 29: 565-598.

Im HI, Hollander JA, Bali P, and Kenny PJ. (2010): MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nat Neurosci* 13: 1120-1127.

Jackson SA, Horst NK, Pears A, Robbins TW, and Roberts AC. (2016): Role of the perigenual anterior cingulate and orbitofrontal cortex in contingency learning in the marmoset. *Cereb Cortex* 26: 3273-3284.

Jang SW, Liu X, Yepes M, Shepherd KR, Miller GW, Liu Y, *et al.* (2010): A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. *Proc Natl Acad Sci U S A* 107: 2687-2692.

Jay TM and Witter MP. (1991): Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. *J Comp Neurol* 313: 574-586.

Jentsch JD and Taylor JR. (1999): Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl)* 146: 373-390.

Jerome L, Schuster S, Yazar-Klosinski BB, Shira Schuster MA, and Yazar-Klosinski BB. (2013): Can MDMA Play a Role in the Treatment of Substance Abuse? *Curr Drug Abuse Rev* 6: 54-62.

Jiang DG, Jin SL, Li GY, Li QQ, Li ZR, Ma HX*, et al.* (2016): Serotonin regulates brain-derived neurotrophic factor expression in select brain regions during acute psychological stress*. Neural Regen Res* 11: 1471-1479.

Johansen PO and Krebs TS. (2009): How could MDMA (ecstasy) help anxiety disorders? A neurobiological rationale*. J Psychopharmacol* 23: 389-391.

Johnson M, Letter AA, Merchant K, Hanson GR, and Gibb JW. (1988): Effects of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine isomers on central serotonergic, dopaminergic and nigral neurotensin systems of the rat. *J Pharmacol Exp Ther* 244: 977-982.

Jurgens U. (1979): Vocalization as an emotional indicator. A neuroethological study in the squirrel monkey*. Behaviour* 69: 88-117.

Kabir ZD, Katzman AC, and Kosofsky BE. (2013): Molecular mechanisms mediating a deficit in recall of fear extinction in adult mice exposed to cocaine in utero*. PLoS One* 8: e84165.

Kalivas PW. (2009): The glutamate homeostasis hypothesis of addiction*. Nat Rev Neurosci* 10: 561-572.

Kamilar-Britt P and Bedi G. (2015): The prosocial effects of 3,4-methylenedioxymethamphetamine (MDMA): Controlled studies in humans and laboratory animals*. Neurosci Biobehav Rev* 57: 433-446.

Kessler RC, Aguilar-Gaxiola S, Berglund PA, Caraveo-Anduaga JJ, DeWit DJ, Greenfield SF*, et al.* (2001): Patterns and predictors of treatment seeking after onset of a substance use disorder*. Arch Gen Psychiatry* 58: 1065-1071.

Kolb B and Muhammad A. (2014): Harnessing the power of neuroplasticity for intervention*. Front Hum Neurosci* 8: 377.

Kuypers KP, de la Torre R, Farre M, Yubero-Lahoz S, Dziobek I, Van den Bos W*, et al.* (2014): No evidence that MDMA-induced enhancement of emotional empathy is related to peripheral oxytocin levels or 5-HT1a receptor activation*. PLoS One* 9: e100719.

Kuznetsova A, Brockhoff PB, and Christensen RHB. (2015): Package 'lmerTest'*. R package version* 2.0-29.

Lawn J. (1986): Schedules of controlled substances: Extension of temporary control of 3,4-methylenedioxymethamphetamine (MDMA) in Schedule 1*. Federal Register* 51: 21911-21912.

Le Foll B, Diaz J, and Sokoloff P. (2005): A single cocaine exposure increases BDNF and D3 receptor expression: implications for drug-conditioning*. Neuroreport* 16: 175-178.

LeBlanc KH, Maidment NT, and Ostlund SB. (2013): Repeated cocaine exposure facilitates the expression of incentive motivation and induces habitual control in rats*. PLoS One* 8: e61355.

Lee MR, Rohn MC, Tanda G, and Leggio L. (2016): Targeting the oxytocin system to treat addictive disorders: Rationale and progress to date*. CNS Drugs* 30: 109-123.

Li X, DeJoseph MR, Urban JH, Bahi A, Dreyer JL, Meredith GE*, et al.* (2013): Different roles of BDNF in nucleus accumbens core versus shell during the incubation of cue-induced cocaine craving and its long-term maintenance*. J Neurosci* 33: 1130-1142.

Li X and Wolf ME. (2015): Multiple faces of BDNF in cocaine addiction*. Behav Brain Res* 279: 240-254.

Liechti ME, Baumann C, Gamma A, and Vollenweider FX. (2000a): Acute psychological effects of 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') are attenuated by the serotonin uptake inhibitor citalopram*. Neuropsychopharmacology* 22: 513-521.

Liechti ME, Saur MR, Gamma A, Hell D, and Vollenweider FX. (2000b): Psychological and physiological effects of MDMA ('Ecstasy') after pretreatment with the 5-HT2 antagonist ketanserin in healthy humans. *Neuropsychopharmacology* 23: 396-404.

Liu QR, Lu L, Zhu XG, Gong JP, Shaham Y, and Uhl GR. (2006): Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. *Brain Res* 1067: 1-12.

Logrip ML, Barak S, Warnault V, and Ron D. (2015): Corticostriatal BDNF and alcohol addiction. *Brain Res*.

Lu B, Nagappan G, and Lu Y. (2014): BDNF and synaptic plasticity, cognitive function, and dysfunction. *Handb Exp Pharmacol* 220: 223-250.

Lu H, Cheng PL, Lim BK, Khoshnevisrad N, and Poo MM. (2010): Elevated BDNF after cocaine withdrawal facilitates LTP in medial prefrontal cortex by suppressing GABA inhibition. *Neuron* 67: 821-833.

Lu Y, Christian K, and Lu B. (2008): BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? *Neurobiol Learn Mem* 89: 312-323.

Lucantonio F, Stalnaker TA, Shaham Y, Niv Y, and Schoenbaum G. (2012): The impact of orbitofrontal dysfunction on cocaine addiction. *Nat Neurosci* 15: 358-366.

Lyon RA, Glennon RA, and Titeler M. (1986): 3,4-Methylenedioxymethamphetamine (MDMA): Stereoselective interactions at brain 5-HT1 and 5-HT2 receptors. *Psychopharmacology (Berl)* 88: 525-526.

Macenski MJ and Meisch RA. (1998): Ratio size and cocaine concentration effects on oral cocaine-reinforced behavior. *J Exp Anal Behav* 70: 185-201.

MAPS, *Clinical Research with Psychedelics* 2015.

Marchant NJ, Li X, and Shaham Y. (2013): Recent developments in animal models of drug relapse. *Curr Opin Neurobiol* 23: 675-683.

Martínez-Turrillas R, Moyano S, Del Rio J, and Frechilla D. (2006): Differential effects of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") on BDNF mRNA expression in rat frontal cortex and hippocampus. *Neurosci Lett* 402: 126-130.

Matyas F, Lee J, Shin HS, and Acsady L. (2014): The fear circuit of the mouse forebrain: connections between the mediodorsal thalamus, frontal cortices and basolateral amygdala. *Eur J Neurosci* 39: 1810-1823.

McClung J, Fantegrossi W, and Howell LL. (2010): Reinstatement of extinguished amphetamine self-administration by 3,4-methylenedioxymethamphetamine (MDMA) and its enantiomers in rhesus monkeys. *Psychopharmacology (Berl)* 210: 75-83.

McDonald AJ. (1991): Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. *Neuroscience* 44: 1-14.

McDonald AJ, Mascagni F, and Guo L. (1996): Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience* 71: 55-75.

McGinty JF, Whitfield TW, Jr., and Berglind WJ. (2010): Brain-derived neurotrophic factor and cocaine addiction. *Brain Res* 1314: 183-193.

McGregor IS and Bowen MT. (2012): Breaking the loop: oxytocin as a potential treatment for drug addiction. *Horm Behav* 61: 331-339.

Meller R, Babity JM, and Grahame-Smith DG. (2002): 5-HT2A receptor activation leads to increased BDNF mRNA expression in C6 glioma cells. *Neuromolecular Med* 1: 197-205.

Merighi A, Salio C, Ghirri A, Lossi L, Ferrini F, Betelli C, *et al.* (2008): BDNF as a pain modulator. *Prog Neurobiol* 85: 297-317.

Miczek KA, Woolley J, Schlisserman S, and Yoshimura H. (1981): Analysis of amphetamine effects on agonistic and affiliative behavior in squirrel monkeys (Saimiri sciureus). *Pharmacol Biochem Behav* 14 Suppl 1: 103-107.

Miczek KA and Yoshimura H. (1982): Disruption of primate social behavior by d-amphetamine and cocaine: differential antagonism by antipsychotics. *Psychopharmacology (Berl)* 76: 163-171.

Miles FJ, Everitt BJ, and Dickinson A. (2003): Oral cocaine seeking by rats: action or habit? *Behav Neurosci* 117: 927-938.

Miller T and Hendrie D. *Substance abuse prevention dollars and cents: A cost-benefit analysis*. 2008, Center for Substance Abuse Prevention, Substance Abuse and Mental Health Services Administraiton: Rockville, MD.

Mithoefer MC, Grob CS, and Brewerton TD. *Novel psychopharmacological therapies for psychiatric disorders: Psilocybin and MDMA*, in *The Lancet Psychiatry*. 2016. p. 481-488.

Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, and Doblin R. (2011): The safety and efficacy of {+/-}3,4-methylenedioxymethamphetamine-assisted psychotherapy in subjects with chronic, treatment-resistant posttraumatic stress disorder: the first randomized controlled pilot study. *J Psychopharmacol* 25: 439-452.

Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, Martin SF, Yazar-Klosinski B*, et al.* (2013): Durability of improvement in post-traumatic stress disorder symptoms and absence of harmful effects or drug dependency after 3,4-methylenedioxymethamphetamine-assisted psychotherapy: a prospective long-term follow-up study. *J Psychopharmacol* 27: 28-39.

Moorman DE, James MH, McGlinchey EM, and Aston-Jones G. (2014): Differential roles of medial prefrontal subregions in the regulation of drug seeking. *Brain Res*.

Morecraft RJ, Geula C, and Mesulam MM. (1992): Cytoarchitecture and neural afferents of orbitofrontal cortex in the brain of the monkey. *J Comp Neurol* 323: 341-358.

Morrow JD, Saunders BT, Maren S, and Robinson TE. (2015): Sign-tracking to an appetitive cue predicts incubation of conditioned fear in rats. *Behav Brain Res* 276: 59-66.

Mouri A, Noda Y, Niwa M, Matsumoto Y, Mamiya T, Nitta A, *et al.* (2017): The involvement of brain-derived neurotrophic factor in 3,4-methylenedioxymethamphetamine-induced place preference and behavioral sensitization. *Behav Brain Res* 329: 157-165.

Mueller M, Peters FT, Huestis MA, Ricaurte GA, and Maurer HH. (2009): Simultaneous liquid chromatographic-electrospray ionization mass spectrometric quantification of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and its metabolites 3,4-dihydroxymethamphetamine, 4-hydroxy-3-methoxymethamphetamine and 3,4-methylenedioxyamphetamine in squirrel monkey and human plasma after acidic conjugate cleavage. *Forensic Sci Int* 184: 64-68.

Murnane KS, Fantegrossi WE, Godfrey JR, Banks ML, and Howell LL. (2010): Endocrine and neurochemical effects of 3,4-methylenedioxymethamphetamine and its stereoisomers in rhesus monkeys. *J Pharmacol Exp Ther* 334: 642-650.

Mutschler J, Eifler S, Dirican G, Grosshans M, Kiefer F, Rossler W, *et al.* (2013): Functional social support within a medical supervised outpatient treatment program. *Am J Drug Alcohol Abuse* 39: 44-49.

Nelson A and Killcross S. (2006): Amphetamine exposure enhances habit formation. *J Neurosci* 26: 3805-3812.

Nelson AJ and Killcross S. (2013): Accelerated habit formation following amphetamine exposure is reversed by D1, but enhanced by D2, receptor antagonists. *Front Neurosci* 7: 76.

Nichols DE. (1986): Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: Entactogens. *J Psychoactive Drugs* 18: 305-313.

Nordquist RE, Voorn P, de Mooij-van Malsen JG, Joosten RN, Pennartz CM, and Vanderschuren LJ. (2007): Augmented reinforcer value and accelerated habit formation after repeated amphetamine treatment. *Eur Neuropsychopharmacol* 17: 532-540.

Oehen P, Traber R, Widmer V, and Schnyder U. (2013): A randomized, controlled pilot study of MDMA (± 3,4-Methylenedioxymethamphetamine)-assisted psychotherapy for treatment of resistant, chronic Post-Traumatic Stress Disorder (PTSD). *J Psychopharmacol* 27: 40-52.

Ongur D and Price JL. (2000): The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cereb Cortex* 10: 206-219.

Ostlund SB and Balleine BW. (2007): Orbitofrontal cortex mediates outcome encoding in Pavlovian but not instrumental conditioning. *J Neurosci* 27: 4819-4825.

Otis JM, Fitzgerald MK, and Mueller D. (2014): Infralimbic BDNF/TrkB enhancement of GluN2B currents facilitates extinction of a cocaine-conditioned place preference. *J Neurosci* 34: 6057-6064.

Park H and Poo MM. (2013): Neurotrophin regulation of neural circuit development and function. *Nat Rev Neurosci* 14: 7-23.

Park H, Popescu A, and Poo MM. (2014): Essential role of presynaptic NMDA receptors in activity-dependent BDNF secretion and corticostriatal LTP. *Neuron* 84: 1009-1022.

Parkes SL and Balleine BW. (2013): Incentive memory: evidence the basolateral amygdala encodes and the insular cortex retrieves outcome values to guide choice between goal-directed actions. *J Neurosci* 33: 8753-8763.

Parrott AC. (2013): Human psychobiology of MDMA or 'Ecstasy': an overview of 25 years of empirical research. *Hum Psychopharmacol* 28: 289-307.

Parrott AC. (2014): MDMA is certainly damaging after 25 years of empirical research: a reply and refutation of Doblin et al. (2014). *Hum Psychopharmacol* 29: 109-119.

Patel R and Titheradge D. (2015): MDMA for the treatment of mood disorder: all talk no substance? *Ther Adv Psychopharmacol* 5: 179-188.

Pentney AR. (2001): An exploration of the history and controversies surrounding MDMA and MDA. *J Psychoactive Drugs* 33: 213-221.

Peters J, Kalivas PW, and Quirk GJ. (2009): Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learn Mem* 16: 279-288.

Peterson AB, Abel JM, and Lynch WJ. (2014): Dose-dependent effects of wheel running on cocaine-seeking and prefrontal cortex Bdnf exon IV expression in rats. *Psychopharmacology (Berl)* 231: 1305-1314.

Phillips D. *F.D.A. agrees to new trial for ecstasy as relief for PTSD patients*, in *The New York Times*. 2016.

Pierce RC and Vanderschuren LJ. (2010): Kicking the habit: the neural basis of ingrained behaviors in cocaine addiction. *Neurosci Biobehav Rev* 35: 212-219.

Pitts EG, Taylor JR, and Gourley SL. (2016): Prefrontal cortical BDNF: A regulatory key in cocaine- and food-reinforced behaviors. *Neurobiol Dis* 91: 326-335.

Preller KH, Herdener M, Schilbach L, Stampfli P, Hulka LM, Vonmoos M*, et al.* (2014): Functional changes of the reward system underlie blunted response to social gaze in cocaine users. *Proc Natl Acad Sci U S A* 111: 2842-2847.

Preller KH, Hulka LM, Vonmoos M, Jenni D, Baumgartner MR, Seifritz E*, et al.* (2014): Impaired emotional empathy and related social network deficits in cocaine users*. Addict Biol* 19: 452-466.

Quinn JJ, Hitchcott PK, Umeda EA, Arnold AP, and Taylor JR. (2007): Sex chromosome complement regulates habit formation*. Nat Neurosci* 10: 1398-1400.

R Core Team. *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/*. 2014.

Radley JJ, Anderson RM, Cosme CV, Glanz RM, Miller MC, Romig-Martin SA*, et al.* (2015): The contingency of cocaine administration accounts for structural and functional medial prefrontal deficits and increased adrenocortical activation*. J Neurosci* 35: 11897-11910.

Rapanelli M, Lew SE, Frick LR, and Zanutto BS. (2010): Plasticity in the rat prefrontal cortex: linking gene expression and an operant learning with a computational theory*. PLoS One* 5: e8656.

Rattiner LM, Davis M, French CT, and Ressler KJ. (2004): Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning*. J Neurosci* 24: 4796-4806.

Reichardt LF. (2006): Neurotrophin-regulated signalling pathways*. Philos Trans R Soc Lond B Biol Sci* 361: 1545-1564.

Ren Q, Zhang JC, Fujita Y, Ma M, Wu J, and Hashimoto K. (2013): Effects of TrkB agonist 7,8-dihydroxyflavone on sensory gating deficits in mice after administration of methamphetamine*. Pharmacol Biochem Behav* 106: 124-127.

Ren Q, Zhang JC, Ma M, Fujita Y, Wu J, and Hashimoto K. (2014): 7,8-Dihydroxyflavone, a TrkB agonist, attenuates behavioral abnormalities and neurotoxicity in mice after administration of methamphetamine*. Psychopharmacology (Berl)* 231: 159-166.

Rhodes SE and Murray EA. (2013): Differential effects of amygdala, orbital prefrontal cortex, and prelimbic cortex lesions on goal-directed behavior in rhesus macaques. *J Neurosci* 33: 3380-3389.

Riedlinger TJ and Riedlinger JE. (1994): Psychedelic and entactogenic drugs in the treatment of depression. *J Psychoactive Drugs* 26: 41-55.

Rietjens SJ, Hondebrink L, Westerink RH, and Meulenbelt J. (2012): Pharmacokinetics and pharmacodynamics of 3,4-methylenedioxymethamphetamine (MDMA): interindividual differences due to polymorphisms and drug-drug interactions. *Crit Rev Toxicol* 42: 854-876.

Robbins TW, Ersche KD, and Everitt BJ. (2008): Drug addiction and the memory systems of the brain. *Ann N Y Acad Sci* 1141: 1-21.

Rodriguez-Romaguera J, Do-Monte FH, Tanimura Y, Quirk GJ, and Haber SN. (2015): Enhancement of fear extinction with deep brain stimulation: evidence for medial orbitofrontal involvement. *Neuropsychopharmacology* 40: 1726-1733.

Rogers G, Elston J, Garside R, Roome C, Taylor R, Younger P*, et al.* (2009): The harmful health effects of recreational ecstasy: a systematic review of observational evidence. *Health technology assessment* 13: 1-315.

Rosen GD, Williams AG, Capra JA, Connolly MT, Cruz B, Lu L*, et al.* (2000): The Mouse Brain Library @ www.mbl.org. . *Int Mouse Genome Conference* 14: 166.

Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI*, et al.* (2001): Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* 39: 32-41.

Russo SJ, Mazei-Robison MS, Ables JL, and Nestler EJ. (2009): Neurotrophic factors and structural plasticity in addiction. *Neuropharmacology* 56 Suppl 1: 73-82.

Sadri-Vakili G, Kumaresan V, Schmidt HD, Famous KR, Chawla P, Vassoler FM*, et al.* (2010): Cocaine-induced chromatin remodeling increases brain-derived neurotrophic factor transcription in the rat medial prefrontal cortex, which alters the reinforcing efficacy of cocaine*. J Neurosci* 30: 11735-11744.

Saunders BT and Robinson TE. (2010): A cocaine cue acts as an incentive stimulus in some but not others: implications for addiction*. Biol Psychiatry* 67: 730-736.

Saunders BT and Robinson TE. (2011): Individual variation in the motivational properties of cocaine*. Neuropsychopharmacology* 36: 1668-1676.

Schilman EA, Uylings HB, Galis-de Graaf Y, Joel D, and Groenewegen HJ. (2008): The orbital cortex in rats topographically projects to central parts of the caudate-putamen complex*. Neurosci Lett* 432: 40-45.

Schmidt CJ, Levin JA, and Lovenberg W. (1987): In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain*. Biochem Pharmacol* 36: 747-755.

Schmidt CJ, Sullivan CK, and Fadayel GM. (1994): Blockade of striatal 5-hydroxytryptamine2 receptors reduces the increase in extracellular concentrations of dopamine produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine*. J Neurochem* 62: 1382-1389.

Schmidt HD, McGinty JF, West AE, and Sadri-Vakili G. (2013): Epigenetics and psychostimulant addiction*. Cold Spring Harb Perspect Med* 3: a012047.

Schmitzer-Torbert N, Apostolidis S, Amoa R, O'Rear C, Kaster M, Stowers J*, et al.* (2015): Post-training cocaine administration facilitates habit learning and requires the infralimbic cortex and dorsolateral striatum*. Neurobiol Learn Mem* 118: 105-112.

Schoenbaum G and Setlow B. (2005): Cocaine makes actions insensitive to outcomes but not extinction: implications for altered orbitofrontal-amygdalar function. *Cereb Cortex* 15: 1162-1169.

Schoenbaum G, Setlow B, Saddoris MP, and Gallagher M. (2003): Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. *Neuron* 39: 855-867.

Schwabe L. (2013): Stress and the engagement of multiple memory systems: integration of animal and human studies. *Hippocampus* 23: 1035-1043.

Schwabe L, Dickinson A, and Wolf OT. (2011): Stress, habits, and drug addiction: a psychoneuroendocrinological perspective. *Exp Clin Psychopharmacol* 19: 53-63.

Sesack SR, Deutch AY, Roth RH, and Bunney BS. (1989): Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J Comp Neurol* 290: 213-242.

Seu E, Groman SM, Arnold AP, and Jentsch JD. (2014): Sex chromosome complement influences operant responding for a palatable food in mice. *Genes Brain Behav* 13: 527-534.

Shively CA, Register TC, Higley JD, and Willard SL. (2014): Sertraline effects on cerebrospinal fluid monoamines and species-typical socioemotional behavior of female cynomolgus monkeys. *Psychopharmacology (Berl)* 231: 1409-1416.

Shulgin A and Shulgin A. *Pihkal: A chemical love story*. 1991, Berkeley, CA: Transform Press.

Simchon Tenenbaum Y, Weizman A, and Rehavi M. (2015): The impact of chronic early administration of psychostimulants on brain expression of BDNF and other neuroplasticity-relevant proteins. *J Mol Neurosci*.

Smith HJ, Newman JD, and Symmes D, *Vocal concomitants of affiliative behavior*, in *Primate communication*, Brown, Petersen, and Snowden, Editors. 1982, Cambridge University Press: Cambridge, UK.

Sober SJ and Brainard MS. (2009): Adult birdsong is actively maintained by error correction. *Nat Neurosci* 12: 927-931.

Stalnaker TA, Cooch NK, and Schoenbaum G. (2015): What the orbitofrontal cortex does not do. *Nat Neurosci* 18: 620-627.

Su H, Tao J, Zhang J, Xie Y, Sun Y, Li L*, et al.* (2014): An association between BDNF Val66Met polymorphism and impulsivity in methamphetamine abusers. *Neurosci Lett* 582: 16-20.

Substance Abuse and Mental Health Services Administration. *The DAWN Report: Highlights of the 2010 Drug Abuse Warning Network (DAWN) findings on drug-related emergency deparment visits*, S.A.a.M.H.S. Administration, Editor. 2012: Rockville, MD.

Substance Abuse and Mental Health Services Administration. *Results from the 2013 National Survey on Drug Use and Health: Mental health findings*, S.A.a.M.H.S. Administration, Editor. 2014: Rockville, MD.

Sumnall HR, Cole JC, and Jerome L. (2006): The varieties of ecstatic experience: an exploration of the subjective experiences of ecstasy. *J Psychopharmacol* 20: 670-682.

Sun H, Cocker PJ, Zeeb FD, and Winstanley CA. (2012): Chronic atomoxetine treatment during adolescence decreases impulsive choice, but not impulsive action, in adult rats and alters markers of synaptic plasticity in the orbitofrontal cortex. *Psychopharmacology (Berl)* 219: 285-301.

Sun WL, Eisenstein SA, Zelek-Molik A, and McGinty JF. (2014): A single brain-derived neurotrophic factor infusion into the dorsomedial prefrontal cortex attenuates cocaine self-

administration-induced phosphorylation of synapsin in the nucleus accumbens during early withdrawal. *Int J Neuropsychopharmacol* 18.

Swanson AM, Allen AG, Shapiro LP, and Gourley SL. (2015): GABAAalpha1-mediated plasticity in the orbitofrontal cortex regulates context-dependent action selection. *Neuropsychopharmacology* 40: 1027-1036.

Thompson MR, Callaghan PD, Hunt GE, Cornish JL, and McGregor IS. (2007): A role for oxytocin and 5-HT(1A) receptors in the prosocial effects of 3,4 methylenedioxymethamphetamine ("ecstasy"). *Neuroscience* 146: 509-514.

Tian M, Zeng Y, Hu Y, Yuan X, Liu S, Li J*, et al.* (2015): 7, 8-Dihydroxyflavone induces synapse expression of AMPA GluA1 and ameliorates cognitive and spine abnormalities in a mouse model of fragile X syndrome. *Neuropharmacology* 89: 43-53.

Torregrossa MM, Corlett PR, and Taylor JR. (2011): Aberrant learning and memory in addiction. *Neurobiol Learn Mem* 96: 609-623.

Torregrossa MM, Quinn JJ, and Taylor JR. (2008): Impulsivity, compulsivity, and habit: the role of orbitofrontal cortex revisited. *Biol Psychiatry* 63: 253-255.

Tzeng WY, Chuang JY, Lin LC, Cherng CG, Lin KY, Chen LH*, et al.* (2013): Companions reverse stressor-induced decreases in neurogenesis and cocaine conditioning possibly by restoring BDNF and NGF levels in dentate gyrus. *Psychoneuroendocrinology* 38: 425-437.

Vaidya VA, Marek GJ, Aghajanian GK, and Duman RS. (1997): 5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J Neurosci* 17: 2785-2795.

van Wel JH, Kuypers KP, Theunissen EL, Bosker WM, Bakker K, and Ramaekers JG. (2012): Effects of acute MDMA intoxication on mood and impulsivity: role of the 5-HT2 and 5-HT1 receptors. *PLoS One* 7: e40187.

Vassoler FM, White SL, Schmidt HD, Sadri-Vakili G, and Pierce RC. (2013): Epigenetic inheritance of a cocaine-resistance phenotype. *Nat Neurosci* 16: 42-47.

Verheij MM, Vendruscolo LF, Caffino L, Giannotti G, Cazorla M, Fumagalli F*, et al.* (2016): Systemic delivery of a brain-penetrant trkB antagonist reduces cocaine self-administration and normalizes trkB signaling in the nucleus accumbens and prefrontal cortex. *J Neurosci* 36: 8149-8159.

Viola TW, Tractenberg SG, Levandowski ML, Pezzi JC, Bauer ME, Teixeira AL*, et al.* (2014): Neurotrophic factors in women with crack cocaine dependence during early abstinence: the role of early life stress. *Journal of Psychiatry & Neuroscience : JPN* 39: 206-214.

Volkow ND, Baler RD, and Goldstein RZ. (2011a): Addiction: pulling at the neural threads of social behaviors. *Neuron* 69: 599-602.

Volkow ND and Fowler JS. (2000): Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. *Cereb Cortex* 10: 318-325.

Volkow ND, Hitzemann R, Wang GJ, Fowler JS, Wolf AP, Dewey SL*, et al.* (1992): Long-term frontal brain metabolic changes in cocaine abusers. *Synapse* 11: 184-190.

Volkow ND, Wang GJ, Fowler JS, Tomasi D, and Telang F. (2011b): Addiction: beyond dopamine reward circuitry. *Proc Natl Acad Sci U S A* 108: 15037-15042.

Vollenweider FX, Gamma A, Liechti M, and Huber T. (1998): Psychological and cardiovascular effects and short-term sequelae of MDMA ('Ecstasy') in MDMA-naive healthy volunteers. *Neuropsychopharmacology* 19: 241-251.

von Diemen L, Kapczinski F, Sordi AO, de Magalhães Narvaez JC, Guimarães LSP, Kessler FHP*, et al.* (2014): Increase in brain-derived neurotrophic factor expression in early crack cocaine withdrawal*. The International Journal of Neuropsychopharmacology* 17: 33-40.

Wang Z and Woolverton WL. (2007): Estimating the relative reinforcing strength of (+/-)-3,4-methylenedioxymethamphetamine (MDMA) and its isomers in rhesus monkeys: comparison to (+)-methamphetamine*. Psychopharmacology (Berl)* 189: 483-488.

Warnault V, Darcq E, Morisot N, Phamluong K, Wilbrecht L, Massa SM*, et al.* (2015): The BDNF valine 68 to methionine polymorphism increases compulsive alcohol drinking in mice that is reversed by tropomyosin receptor kinase B activation*. Biol Psychiatry*.

Warren MS, Bradley WD, Gourley SL, Lin YC, Simpson MA, Reichardt LF*, et al.* (2012): Integrin beta1 signals through Arg to regulate postnatal dendritic arborization, synapse density, and behavior*. J Neurosci* 32: 2824-2834.

Warton DI, Lyons M, Stoklosa J, and Ives AR. (2016): Three points to consider when choosing a LM or GLM test for count data*. Methods in Ecology and Evolution*.

Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP*, et al.* (2006): Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice*. Science* 313: 536-540.

Wellman LL, Gale K, and Malkova L. (2005): GABAA-mediated inhibition of basolateral amygdala blocks reward devaluation in macaques*. J Neurosci* 25: 4577-4586.

West EA, Forcelli PA, Murnen AT, McCue DL, Gale K, and Malkova L. (2012): Transient inactivation of basolateral amygdala during selective satiation disrupts reinforcer devaluation in rats*. Behav Neurosci* 126: 563-574.

Whitfield TW, Jr., Shi X, Sun WL, and McGinty JF. (2011): The suppressive effect of an intra-prefrontal cortical infusion of BDNF on cocaine-seeking is Trk receptor and extracellular

signal-regulated protein kinase mitogen-activated protein kinase dependent. *J Neurosci* 31: 834-842.

Winter P, Ploog D, and Latta J. (1966): Vocal repertoire of the squirrel monkey (Saimiri sciureus), its analysis and significance. *Exp Brain Res* 1: 359-384.

Wong WC, Ford KA, Pagels NE, McCutcheon JE, and Marinelli M. (2013): Adolescents are more vulnerable to cocaine addiction: behavioral and electrophysiological evidence. *J Neurosci* 33: 4913-4922.

Yager LM and Robinson TE. (2013): A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue. *Psychopharmacology (Berl)* 226: 217-228.

Yamada K and Nabeshima T. (2003): Brain-derived neurotrophic factor/TrkB signaling in memory processes. *J Pharmacol Sci* 91: 267-270.

Yan QS, Feng MJ, and Yan SE. (2007): RNA interference-mediated inhibition of brain-derived neurotrophic factor expression increases cocaine's cytotoxicity in cultured cells. *Neurosci Lett* 414: 165-169.

Yin HH, Mulcare SP, Hilario MR, Clouse E, Holloway T, Davis MI, *et al.* (2009): Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat Neurosci* 12: 333-341.

Yin HH, Ostlund SB, and Balleine BW. (2008): Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. *Eur J Neurosci* 28: 1437-1448.

Young MB, Andero R, Ressler KJ, and Howell LL. (2015): 3,4-Methylenedioxymethamphetamine facilitates fear extinction learning. *Transl Psychiatry* 5: e634.

Zapata A, Minney VL, and Shippenberg TS. (2010): Shift from goal-directed to habitual cocaine seeking after prolonged experience in rats. *J Neurosci* 30: 15457-15463.

Zeng Y, Lv F, Li L, Yu H, Dong M, and Fu Q. (2012): 7,8-dihydroxyflavone rescues spatial memory and synaptic plasticity in cognitively impaired aged rats. *J Neurochem* 122: 800-811.

Zhang JC, Wu J, Fujita Y, Yao W, Ren Q, Yang C*, et al.* (2014a): Antidepressant effects of TrkB ligands on depression-like behavior and dendritic changes in mice after inflammation. *Int J Neuropsychopharmacol* 18.

Zhang Y, Zhu X, Huang C, and Zhang X. (2015): Molecular changes in the medial prefrontal cortex and nucleus accumbens are associated with blocking the behavioral sensitization to cocaine. *Scientific Reports* 5.

Zhang Z, Liu X, Schroeder JP, Chan CB, Song M, Yu SP*, et al.* (2014b): 7,8-dihydroxyflavone prevents synaptic loss and memory deficits in a mouse model of Alzheimer's disease. *Neuropsychopharmacology* 39: 638-650.

Zimmermann KS, Li C, Rainnie DG, Ressler KJ, and Gourley SL. (2017a): Memory retention involves the ventrolateral orbitofrontal cortex: Comparison with the basolateral amygdala. *Neuropsychopharmacology*. In press.

Zimmermann KS, Yamin JA, Rainnie DG, Ressler KJ, and Gourley SL. (2017b): Connections of the mouse orbitofrontal cortex and regulation of goal-directed action selection by brain-derived neurotrophic factor. *Biol Psychiatry* 81: 366-377.

Zingg B, Hintiryan H, Gou L, Song MY, Bay M, Bienkowski MS*, et al.* (2014): Neural networks of the mouse neocortex. *Cell* 156: 1096-1111.