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Stereospecificity of the prosocial and neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA) in mice

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By

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B.A., University of Tennessee, 2010

Advisor: Leonard L. Howell, Ph.D.

An abstract of

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Abstract

Stereospecificity of the prosocial and neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA) in mice

By

Daniel Walker Curry

3,4-methylenedioxymethamphetamine (MDMA) is a substituted phenethylamine that became popular as a recreational drug (ecstasy) and therapeutic tool during the late 1970's and early 1980's. Escalating recreational use led to its prohibition, but scientific interest in the drug has persisted due to its unique prosocial effects. Under clinical observation, volunteers report that MDMA increases feelings of closeness towards others, empathy, and sociability. In addition to these acute effects, there is accumulating evidence that MDMA can have powerful and enduring therapeutic benefits. Recent clinical trials have observed that MDMA is effective in treating post-traumatic stress disorder and social anxiety in adults with autism. Large Phase III clinical trials are moving forward despite no clear mechanistic understanding of why MDMA is therapeutically useful. An appropriate animal model with which to evaluate the neurobiological mechanisms of MDMA-induced prosocial behavior and therapeutic-like effects is needed. A more complete understanding of MDMA is especially important because of its widespread illicit use and the risk of serious adverse effects that may accompany its use. MDMA is neurotoxic and can produce potentially lethal hyperthermia even at moderate doses. There is thus significant impetus to isolate the mechanisms of MDMA's prosocial and therapeutic effects so that new therapeutics can be developed that have fewer side effects and lower potential for abuse. To probe the pharmacological mechanisms of MDMA, a mouse model was developed using repeated intermittent drug treatments to elicit robust prosocial behaviors. To determine if these effects are stereospecific, the two enantiomers of MDMA were tested using this paradigm. Although less potent, (–)-MDMA recapitulated the prosocial effects of racemic MDMA, without any locomotor stimulant side effects. (–)-MDMA and racemic MDMA stimulated oxytocinergic neurons; release of this neuropeptide has been suggested as an important factor underlying the unique social effects of MDMA. In contrast, (+)-MDMA, which has previously been considered the active isomer because of its higher potency, had no significant prosocial effects and did not significantly stimulate oxytocinergic neurons. To determine if (–)-MDMA could be a safer therapeutic option than traditional MDMA, markers of neurotoxicity were evaluated postmortem. In comparison to racemic MDMA, (–)-MDMA produced no evidence of neurotoxicity and did not produce hyperthermia, even at very high doses. These results indicate that the prosocial effects of MDMA are separable from the stimulant, neurotoxic, and thermogenic effects of the drug, and suggest that (–)-MDMA could be a more viable therapeutic option than racemic MDMA.

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List of Abbreviations

5-HT	5-hydroxytryptamine; serotonin
aCSF	artificial cerebrospinal fluid
ANOVA	analysis of variance
CAPS	clinician administered PTSD scale
DA	dopamine
DAT	dopamine reuptake transporter
DEA	Drug Enforcement Agency
DOI	1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane
EC_{50}	half maximal effective concentration
GFAP	glial fibrillary acidic protein
GSH	glutathione
HPLC	high pressure liquid chromatography
ICV	intracerebroventricular
i.p.	intraperitoneal
M100	R(+)-MDL100,907
mg/kg	milligrams per kilogram
MDA	3,4-methylenedioxyamphetamine

MDMA	3,4-methylenedioxymethamphetamine
NAC	N-acetylcysteine
NAcc	nucleus accumbens
NE	norepinephrine
NET	norepinephrine reuptake transporter
PFC	prefrontal cortex
PTSD	post-traumatic stress disorder
ROS	reactive oxygen species
SCH	R(+)-SCH23390
SERT	serotonin reuptake transporter
SEM	standard error of the mean
SSRI	serotonin-selective reuptake inhibitor
WAY	WAY163909

I love everything that flows, everything that has time in it and becoming, that brings us back to the beginning where there is never an end . . . that is ecstasy
– Henry Miller, Tropic of Cancer (banned in the US for nearly 30 years…)

Chapter 1. Introduction

A. The History and Current Use of MDMA

The first known synthesis of 3,4-methylenedioxymethamphetamine (MDMA) was performed by the German drug firm Merck in 1912. Merck was attempting to find alternative synthesis pathways for the hemostatic drug Hydrastinine and its derivatives in order to circumvent a patent held by its competitor Bayer Elberfeld (Freudenmann *et al*, 2006). MDMA was an intermediate in one of these syntheses, from Safrole to 3-Methylhydrastinine, and was therefore included in the patent of that synthesis filed on December 24th of that year. There is no evidence that MDMA was tested in any capacity until 1927, the year its patent would expire, when it was briefly investigated for ephedrine-like effects (Bernschneider-Reif *et al*, 2006). Merck had just begun large-scale manufacture and marketing of ephedrine as a decongestant and anti-asthmatic (Dikötter *et al*, 2004) and was likely searching for similar compounds (Figure 1.1). Despite reporting "partly remarkable results" the studies did not continue and MDMA was again forgotten (Freudenmann *et al*, 2006). In that same year, however, another ephedrine-like compound was also rediscovered: amphetamine (Sulzer *et al*, 2005). It would not be forgotten.

Amphetamine was initially studied and commercially released for its decongestant properties, but very quickly its ability to stimulate the central nervous system was discovered and its use rapidly expanded. Under the trade name Benzedrine, amphetamine was first released as an inhaler in 1932 and then as an oral tablet. Over 50 million Benzedrine tablets were sold during the first 3 years of availability (Sulzer *et al*, 2005). Use of amphetamine continued to escalate during the war and post-war years, used to keep soldiers awake and workers productive. During the 1950's there was considerable interest in developing new stimulants from amphetamine derivatives. So due to its structure, MDMA was once again pulled from the archives at Merck and investigated, this time as a stimulant, first in Germany (Bernschneider-Reif *et al*, 2006) and later

by the United States Army, which was also interested in potential hallucinogenic effects of the drug due to its structural similarity with mescaline (Hardman *et al*, 1973).

MDMA studies at the time were performed in animals, from flies to monkeys, and there is no evidence that MDMA was tested in humans (Freudenmann *et al*, 2006). The first human user of MDMA was quite possibly an unwitting recreational drug user who thought they were purchasing 3,4-methylenedioxyamphetamine (MDA), which had become popular as a recreational drug during the 1960s (Pentney, 2001). MDA became illegal after the Controlled Substances Act was passed in 1970 (Public Law 91-513) and an enterprising chemist likely concluded that adding a methyl group would be an easy way to skirt the legal restriction (Passie and Benzenhöfer, 2016). So unsurprisingly, in 1970, MDMA appeared for the first time on the streets of America, detected in drug tablets seized by the police (Gaston and Rasmussen, 1972).

Knowledge of the drug spread by word of mouth and MDMA slowly began to enter the public consciousness (Figure 1.2a). Alexander Shulgin, who had been a pioneer of psychedelic chemistry, was encouraged to try MDMA by a graduate student he was mentoring at UCSF in 1976. Shulgin "developed a great respect and admiration for the material" and felt that MDMA could be useful for psychotherapy (Shulgin and Shulgin, 1991). He gave out samples to friends and clinicians in the San Francisco area and collected comments on its effects. Leo Zeff, who had been a practitioner of LSD-assisted psychotherapy before its prohibition, was especially impressed with the potential of MDMA and went on to travel the country promoting the drug and training therapists in its use (Pentney, 2001; Stolaroff, 2004).

Clinical use of MDMA grew slowly as methods were developed (Greer and Tolbert, 1994) and efficacy was observed for a variety of conditions (Greer and Tolbert, 1986; Pentney, 2001; Shulgin, 1990). Despite the desire of many researchers to keep the drug out of the public

eye, MDMA use began to quickly spread beyond the clinic (Holland, 2001). Michael Clegg, a former priest, saw the commercial potential of MDMA and established a production and distribution network known as the Texas group (Simek, 2015). Clegg is credited with giving MDMA its most enduring street name, “Ecstasy”, supposedly claiming it would let people see God. By 1984, the Texas group was producing one million tablets of MDMA per month, all of it entirely legal. Dallas and Fort Worth were the epicenter; MDMA was sold over the counter at nightclubs and bars. This got the attention of Texas senator Lloyd Bentsen, who urged the Drug Enforcement Administration (DEA) to make the drug illegal (Holland, 2001).

The DEA announced in July of 1984 that they intended to classify MDMA as a Schedule I drug, the most prohibitory classification, reserved for compounds with a high likelihood of abuse and no medical value (Shulgin, 1990). Critically, as a Schedule I drug, clinical use of MDMA would become illegal - prescriptions cannot be written for Schedule I drugs. A group of clinicians and researchers filed a complaint with the DEA and requested hearings on the matter of MDMA’s medical use. Despite a judicial recommendation that MDMA be placed into Schedule III, which would have permitted clinical use and research to continue, the DEA ignored the recommendation and MDMA was officially placed into Schedule I on March 23rd 1988 (Holland, 2001).

Prohibition was effective in halting the clinical use and study of MDMA, but it did not stop the rapid expansion of recreational use (Figure 1.2b). From the US, MDMA spread to Europe where it become associated with large dance parties called “raves”. The rave phenomenon was considered the largest youth movement in Britain’s history (Collin and Godfrey, 2010) and was soon exported back to the United States. Despite Congressional action in 2000 (Public Law 106-310) and 2003 (Public Law 10-821) to crackdown on MDMA use, it has continued to be widely used (Figure 1.2c), primarily by young people. In 2015, 5.9% of American 12th graders

had tried MDMA, making it the most widely used illicit drug besides cannabis (44.7%) among this age group (Johnston *et al*, 2016). However, most of these students were not current users of the drug. Only 1.1% of 12th graders had used MDMA in the past month compared to 21.3% that had used cannabis in that time frame. Therefore, fewer than 1 in 5 students that tried MDMA were current users, compared to 1 in 2 cannabis users. These data suggest that use of MDMA, compared to other illicit drugs, may be less likely to lead to continued use and abuse. Indeed, only 0.1% of American adults over 26 are current MDMA users (Center for Behavioral Health Statistics and Quality, 2015), and most users stop taking the drug of their own volition (Parrott, 2005). But demand for and/or supply of MDMA may be increasing. In 2014, the most recent year for which data are available, MDMA seizures more than doubled (UNODC 2016). New dark web markets such as Silk Road 2, Evolution, and Agora have made acquiring drugs like MDMA far easier than in the past. An analysis by The Economist found that MDMA was the highest selling product across these marketplaces, outselling all other illicit and prescription drugs and non-drug items such as counterfeit money and pirated media (The Economist, 2016). Demand for MDMA is clearly high, and as it becomes easier to acquire, use is likely to increase.

In recent years, MDMA has returned to experimental use in the clinic. Inspired by the brief therapeutic use of the drug during the 1970's and 80's, researchers have begun testing the efficacy of using MDMA to treat post-traumatic stress disorder (PTSD), social anxiety associated with autism, and end of life anxiety. These efforts have been funded and organized by the nonprofit Multidisciplinary Association for Psychedelic Studies (MAPS), which hopes to reschedule MDMA and gain regulatory approval for its prescription use by 2021 (MAPS, 2015). The trials thus far have been small but all report sustained symptom reduction following MDMA treatment. What effect medical use has on recreational use of MDMA remains to be seen, but anecdotal reports suggest that medical use of the drug is decreasing its perceived harmfulness (Parrott, 2014a). Therefore, if licit use of MDMA increases, illicit use may follow suit.

B. Pharmacology of MDMA

MDMA is a substituted phenethylamine, a diverse class of organic compounds comprised of a phenyl ring and an ethylamine sidechain. MDMA contains three additional moieties from this structure: a 3,4-methylenedioxy ring substitution and methyl substitutions at the α-carbon and amine. Other examples of phenethylamines are the catecholamine neurotransmitters, the psychostimulant amphetamine, and the hallucinogen mescaline (Figure 1.1). Its formal IUPAC name is *N*-methyl-1-(3,4-methylenedioxyphenyl)propan-2-amine, but it is more commonly known as 3,4-methylenedioxymethamphetamine. It is a racemic molecule, with one chiral center located at the α-carbon. MDMA is produced and consumed as a 1:1 mixture of its two enantiomers: (+)-MDMA and (–)-MDMA (Pizarro *et al*, 2004).

MDMA is a weak base with a pKa value of 10.14, molar mass of 193.25 g/mol, and plasma protein binding of approximately 34% (Garrett *et al*, 1991). These properties confer easy diffusion across cell membranes and distribution to tissues more acidic than blood such as the brain. It is typically prepared as a hydrochloride salt containing 84% w/w free base MDMA. MDMA is primarily consumed orally, and although bioavailability has not been examined in humans, rodent studies indicate that bioavailability is comparable for oral, intraperitoneal (i.p.), and subcutaneous (s.c.) routes of administration (Finnegan *et al*, 1988). In humans, peak plasma concentrations of MDMA are attained 2 hours after oral administration with an elimination half-life of 8-9 hours (de la Torre *et al*, 2004). In contrast, MDMA is distributed and eliminated much faster in mice. MDMA reaches a peak plasma concentration within 30 minutes following i.p. administration and has an elimination half-life of approximately 30 minutes (Fantegrossi *et al*, 2009; Scheidweiler *et al*, 2011).

MDMA is primarily metabolized in the liver. The major metabolic pathway includes O-demethylenation to 3,4-dihydroxymethamphetamine (HHMA) by cytochrome P450 isoenzymes

CYP2D6 and CYP3A4 followed by O-methylation to 4-hydroxy-3-methoxymethamphetamine (HMMA) by catechol-O-methyltransferase. HHMA and HMMA are subsequently conjugated by sulfotransferase or UDP-glucuronosyltransferase and preferentially excreted. To a lesser extent, MDMA can first be N-demethylated to MDA before proceeding down the previous metabolic pathway to 3,4-dihydroxyamphetamine (HHA) and 4-hydroxy-3-methoxyamphetamine (HMA) (Capela *et al*, 2009). In humans, about 20% of the drug is excreted unaltered in urine at a constant rate. In contrast, non-renal clearance is non-linear due to saturation or inhibition of hepatic metabolism (de la Torre *et al*, 2000).

The pharmacodynamic effects of MDMA are complex and multifaceted but are dominated by its effect on monoamine release. Because of its structural similarity to the endogenous monoamines, MDMA acts as a substrate at the three monoamine transporters with highest affinity for the serotonin (5-HT) transporter (SERT) and lower affinity for the norepinephrine (NE) and dopamine (DA) transporters, NET and DAT, respectively. Ki binding affinity of MDMA is 0.64 μM, 1.74 μM, and 4.87 μM at mouse SERT, NET, and DAT, respectively, with equivalent affinity at human transporters (Han *et al*, 2006). In comparison to amphetamine and methamphetamine, MDMA has 10-fold higher affinity for SERT and 10- to 40-fold lower affinity for NET and DAT (Han *et al*, 2006). MDMA is taken up by the monoamine transporters into the presynaptic terminals where it disrupts vesicular storage of monoamines, possibly by entering vesicles through the vesicular monoamine transporters and depleting monoamine storage by decreasing the pH gradient that enables vesicular storage (Fleckenstein and Hanson, 2003; Partilla *et al*, 2006). This increases the concentration of cytosolic monoamines that can be exported from the cell via reverse action of the monoamine transporters (Fleckenstein *et al*, 2007; Gudelsky and Nash, 1996).

Monoamine release by MDMA has been measured across species using both *in vivo* and *in vitro* techniques. MDMA-stimulated release of radiolabeled neurotransmitters from rat

synaptosomes yields EC_{50} values of 72 nM, 110 nM, and 278 nM for SERT, NET, and DAT respectively (Setola *et al*, 2003). Microdialysis sampling of extracellular release from mouse prefrontal cortex yields similar relative results. A 10 mg/kg i.p. dose of MDMA increased extracellular serotonin 500% above baseline, reaching a peak concentration 40 minutes post-injection. Extracellular 5-HT decreased slowly, remaining 200% of baseline at the cessation of sampling, 200 minutes post-injection. Increases in NE and DA were smaller and returned to baseline more rapidly (Lanteri *et al*, 2013).

In addition to affecting monoamine release, MDMA also has affinity for a variety of transmembrane receptors. An i.p. injection of 10 mg/kg MDMA yields drug concentrations of approximately 5 µM in plasma and 50 µmol/kg in the striatum 30 minutes after administration in mice (Scheidweiler *et al*, 2011). In comparison, 1.6 mg/kg, a common clinical and recreation dose, given orally to human volunteers, yields a lower peak plasma concentration of 1.5 µM but a substantially higher area under the concentration-time curve (Kolbrich *et al*, 2008b). At these concentrations, MDMA has moderate affinity (< 10 µM Ki) for 5-$HT_{2A/C}$ (5 µM), α_2 adrenergic (4 µM), $histamine_1$ (6 µM), and $muscarinic_1$ (6 µM) receptors (Battaglia *et al*, 1988a). More recently it has been discovered that MDMA has high affinity for 5-HT_{2B} (0.5 µM) and heteromeric nicotine (0.76 µM) receptors (Garcia-Ratés *et al*, 2007; Setola *et al*, 2003), which rival the affinity of MDMA for SERT. However, the relative importance of MDMA binding to these receptors is poorly understood.

MDMA also increases plasma concentrations of several pituitary hormones including oxytocin, vasopressin, prolactin, and adrenocorticotropic hormone (Dumont *et al*, 2009; Forsling *et al*, 2001, 2002; Hysek *et al*, 2013; Nash and Meltzer, 1990). Release of these hormones is primarily triggered by MDMA-induced 5-HT release, but direct agonism of MDMA at 5-HT_2 receptors may also play a role (Bagdy, 1996; Jørgensen *et al*, 2003; Zhang *et al*, 2002). The full

extent to which these hormones contribute to the effects of MDMA is still unknown, but they likely influence both the psychological and physiological effects of the drug.

The most frequent psychological effects of MDMA administration are euphoria, increased sense of well-being, happiness, stimulation, increased energy, extroversion, feeling close to others, empathy, sociability, and mild hallucinogen-like effects such as changed perception of colors and sounds, and derealization (Cole and Sumnall, 2003). Common physiological effects include increased blood pressure, tachycardia, bruxism, mydriasis, and hyperthermia (de la Torre *et al*, 2004). Studies with human volunteers have sought to dissect the roles of the different neurotransmitter systems in the psychological and physiological effects of MDMA. Pretreatment with citalopram, which impairs MDMA induced release of 5-HT, attenuated MDMA-induced cardiovascular effects (Liechti and Vollenweider, 2000) and increases in self-confidence, extroversion, positive mood, derealization, and imagination (Liechti *et al*, 2000a). Blockade of NE release by reboxetine had overlapping as well as distinct effects from citalopram. It attenuated the cardiovascular effects and feelings of stimulation, closeness to others, and emotional excitation produced by MDMA (Hysek *et al*, 2011). Haloperidol, a DA D_2 receptor antagonist, attenuated MDMA-induced positive mood but had no other effects (Liechti and Vollenweider, 2001). Lastly, ketanserin, a 5-HT_{2A} antagonist, attenuated several of the perceptual and emotional changes produced by MDMA, effects that were not affected by citalopram, suggesting that direct agonism of MDMA at 5-HT_{2A} receptors may mediate these effects (Liechti *et al*, 2000b). Together, these studies suggest that the effects of MDMA on body, brain, and behavior are complex and involve the interaction of multiple neurotransmitter systems.

C. Prosocial Effects and Therapeutic Use of MDMA

MDMA is structurally similar to amphetamine-like stimulants and mescaline-like hallucinogens. It is therefore not surprising that MDMA shares some effects with both drug

classes. Like stimulants, MDMA produces stimulation and euphoria (Cami *et al*, 2000; Kirkpatrick *et al*, 2012). And like hallucinogens, MDMA induces changes to mood, cognition, and perception (Liechti *et al*, 2000b; Tancer and Johanson, 2003). However, MDMA has many effects that are distinct from these classifications and is considered by some researchers to be the prototypical member of a unique class of substances known as entactogens (Gouzoulis-Mayfrank *et al*, 1999; Nichols, 1986; Sáez-Briones and Hernández, 2013). David Nichols proposed this neologism meaning "to touch within'' to describe the effects of MDMA and related compounds. Entactogens are characterized by their rather unique ability to increase feelings of love and empathy, closeness towards others, and inner peace (Kolbrich *et al*, 2008a; Liechti *et al*, 2000b; Vollenweider *et al*, 1998).

MDMA is frequently referred to as a "love drug" (Holland, 2001) and its prosocial effects are reported to be a major motivator for recreational use (Morgan *et al*, 2013; Sumnall *et al*, 2006). Many blinded placebo-controlled studies have been performed to assess these prosocial effects in a controlled laboratory environment. The dosages tested typically ranged from 75 to 125 mg, with effects generally increasing at higher doses. Relative to placebo, MDMA increases self-reported feelings of love, talkativeness, extroversion, sociability, self-confidence, friendliness, playfulness, openness, trust, emotional concern, and closeness towards others (Kamilar-Britt and Bedi, 2015). Free speech semantic analysis found that while on MDMA, subjects used more words with semantic proximity to concepts like friend, support, and empathy (Bedi *et al*, 2014). Several studies have sought to measure changes in social cognition and perception by measuring subjects' ability to accurately recognize the emotional valence from photos of faces or eyes. MDMA impairs recognition of negative emotions including "fear", "sadness", and "anger" (Bedi *et al*, 2010b; Hysek *et al*, 2014; Kirkpatrick *et al*, 2014b), and may enhance identification of positive emotions like "friendly" (Hysek *et al*, 2012), although other studies have found no effect on recognition of positive emotions (Kamilar-Britt and Bedi, 2015).

An fMRI analysis of subjects performing this task found that MDMA enhanced the ventral striatum response to happy faces, and dampened amygdala activation to angry faces relative to placebo (Bedi *et al*, 2009). Other studies have asked participants to discern the affective state of actors in an emotionally-charged situation. In these studies, MDMA generally failed to affect recognition of emotional states but increased the self-reported emotional response to these situations (Hysek *et al*, 2013; Kuypers *et al*, 2014; Schmid *et al*, 2014). In sum, MDMA appears to have a limited effect on or may impair cognitive empathy, which is the identification of the mental state of another [i.e. "Theory of Mind" (Blair, 2005)], but it robustly increases emotional empathy, which is the internal response to the emotional displays of others (Hysek *et al*, 2013; Kamilar-Britt and Bedi, 2015).

To determine how MDMA affects prosocial decision making, studies have employed behavioral tasks such as the dictator game in which a participant is given a sum of money to divide between themselves and a stranger. MDMA increased preference for a more equal distribution of funds, and on average subjects that were administered MDMA gave away more money than they kept (Stewart *et al*, 2014). Another study asked participants if they would like to interact with a stranger or remain alone. Subjects given MDMA had an increased preference for choosing to socialize (Kirkpatrick *et al*, 2014b). In addition to these simulated social situations, a recent study assessed the effects of MDMA on real social interaction. MDMA increased social interaction (mostly talking) between pairs of participants but not between subjects and research assistants (Kirkpatrick and de Wit, 2014). This suggests that the social setting may be essential for the emergence of certain prosocial effects of MDMA.

Together, the above studies provide insight into the effects of MDMA, and lend validity to the claims of users that MDMA is prosocial. The potential of these effects was recognized early-on by therapists. MDMA was said to facilitate a therapeutic alliance between the patient and therapist. Patients were more at ease and willing to open up and talk honestly about

themselves and their problems when MDMA was administered as an adjunctive during therapy (Greer and Tolbert, 1986; Stolaroff, 2004). While on the drug, fear and anxiety appeared to decrease, and patients were less defensive and more emotionally open, claiming they could access feelings, thoughts, and memories not ordinarily available to them, hence the name "entactogen" (Amoroso, 2015). During the brief stretch from 1978 until its initial prohibition in 1985, it is estimated that thousands of patients were treated with MDMA (Shulgin and Shulgin, 1991). Anecdotal reports from this period suggest that MDMA was useful for treating a wide range of conditions, including PTSD, phobias, psychosomatic disorders, depression, drug and alcohol addiction, relationship difficulties, and end of life anxiety (Adamson and Metzner, 1988; Downing, 1986; Greer and Tolbert, 1986; Grinspoon and Bakalar, 1986; Riedlinger and Riedlinger, 1994). It's supposed effectiveness earned it the nickname "penicillin for the soul" (Shulgin and Shulgin, 1991), but no blinded placebo-controlled studies were performed to rigorously assess any true efficacy. Following prohibition, clinical use and investigation of MDMA was effectively halted, but interest in its therapeutic potential remained. A small dedicated group of MDMA proponents formed the organization MAPS to organize and fund research into the therapeutic use of MDMA and similar drugs. In 2001 MAPS successfully gained FDA approval to begin the first Phase II clinical trials of MDMA (Doblin, 2002).

When given as an adjunctive treatment, reports suggested that MDMA could help patients to access and resolve repressed memories and painful emotional traumas (Greer and Tolbert, 1990; Grinspoon and Bakalar, 1986). In light of these claims there was particular interest among psychiatric researchers to test MDMA treatment in patients with chronic PTSD who are non-responsive to conventional treatments (Danforth *et al*, 2016). PTSD is a severe anxiety disorder that affects 10-30% of individuals who experience a traumatic event (VanElzakker *et al*, 2014). Cues including objects, sounds, and sensations that were present during a trauma, or are generalizable to cues that were, become pathologically linked to the traumatic event. These cues

will then continue to trigger powerful fear responses even though they do not signal a threat (VanElzakker *et al*, 2014). The most effective treatment for PTSD is exposure therapy, in which patients are instructed to focus on and describe the details of a traumatic experience in a safe and controlled environment (Rothbaum and Schwartz, 2002). In theory, repeated re-exposure to the frightening but ultimately safe stimuli will lead to extinction of the fear memory. Although it is more effective than no treatment, exposure therapy has significant limitations. It is time consuming, typically requiring many sessions over months to years with a trained therapist, and symptoms may get worse before any appreciable improvement becomes apparent. It is also emotionally demanding, and some patients, potentially those with the most severe conditions, may resist or incompletely revisit the traumatic experiences (White, 2014). Perhaps because of these limitations, only 6.3% of PTSD-afflicted war veterans are treated with exposure therapy, and dropout rates are as high as 30% (Amoroso and Workman, 2016). MDMA may facilitate therapy by establishing a therapeutic alliance between the patient and therapists (Grinspoon and Bakalar, 1986) and helping patients to lower their defenses and access their traumatic memories while in a state of comfort and inner peace (Amoroso and Workman, 2016).

The first completed placebo-controlled Phase II study of MDMA-assisted psychotherapy for the treatment of PTSD demonstrated promising results in a sample of 20 subjects (Mithoefer *et al*, 2011). The subjects, who were mostly women and victims of sexual trauma, underwent 11 therapy sessions and 2 sessions with adjunctive MDMA (125 mg) or placebo. Improvement of symptoms was assessed using the Clinician-Administered PTSD Scale (CAPS), which is the established gold-standard measure of PTSD symptoms. Subjects that received MDMA had statistically and clinically significant CAPS reductions of 53.7 points, from a mean starting score of 79.2. Participants in the placebo group had a mean starting score of 79.6 and showed an insignificant mean CAPS score reduction of 20.5 points. Improvements were generally maintained upon long-term follow-up an average of 3.8 years later, with 87.5% of MDMA-

treated participants having CAPS scores below 50 and therefore no longer meeting the diagnostic criteria for PTSD (Mithoefer *et al*, 2013). A second double-blind pilot study of MDMA-assisted psychotherapy, using similar methods, was conducted in Switzerland with an equivalent patient population of 12. It compared 125 mg MDMA to 25 mg MDMA as an active placebo and found a clinically but not statistically significant effect of high- versus low-dose MDMA on CAPS scores (Oehen *et al*, 2013). Three weeks following the third and final MDMA treatment session, participants that received the high-dose had CAPS scores 15.6 points lower than at baseline, whereas participants treated with the low-dose scored only 3.2 points lower from baseline. These gains persisted at the 12-month follow-up, with CAPS scores reduced an average of 24 points in the high-dose group relative to baseline.

Although preliminary, these results are extremely promising. But some caution is warranted given the small and rather homogenous patient samples in these studies. Both PTSD trials were almost entirely made up of Caucasian women with a history of sexual abuse or assault, making the generalizability of their results more difficult (White, 2014). Another concern is that MDMA's strong psychoactive effects make it subjectively and objectively difficult to mask. Ineffective blinding of participants and clinicians could present a significant potential bias. In the Mithoefer study, 19 of 20 participants were able to correctly guess their treatment assignment and their therapists guessed correctly in every case (Mithoefer *et al*, 2011). The Oehen study mitigated this problem by using a small dose of MDMA as an active placebo (Oehen *et al*, 2013), but this approach has had limited success in other studies (Mithoefer *et al*, 2016). Using an alternative active placebo such as amphetamine or methylphenidate may be a better option, and will hopefully be explored in future studies. Despite these limitations, the magnitude of the effects observed thus far are truly substantial and exceed the reported efficacy of any existing mainline therapy (Amoroso and Workman, 2016). As a point of comparison, a CAPS reduction of just 10.2 points was sufficient for FDA-approval of the SSRI sertraline for PTSD (Brady *et al*,

2000). And patients must take this medication daily to maintain the benefits. With MDMA, just 2-3 treatment sessions substantially reduced the symptoms of PTSD for years after the cessation of treatment (Mithoefer *et al*, 2013).

Other Phase II trials are ongoing to assess the efficacy of MDMA-assisted therapy for treating other conditions. Early evaluations of MDMA's clinical effects noted that in addition to acute prosocial effects, subjects frequently reported lasting improvements. Grinspoon and Bakalar noted that patients previously treated with MDMA had increased self-esteem, ability to communicate with others, capacity for achieving empathetic rapport, trust, and intimacy (Grinspoon and Bakalar, 1986). In a study of 20 psychiatrists, 50% reported sustained improvement of interpersonal functioning one week after consuming MDMA (Liester *et al*, 1992). These early reports of MDMA's lingering prosocial effects suggest that MDMA may be useful as a treatment for social dysfunction. Autism, which is now diagnosed in 1 in 68 children (Report, 2014), is a spectrum of disorders characterized, in part, by impaired social and communication skills. Social anxiety is pervasive among autistic individuals and is thought to further compound these deficits (White *et al*, 2010). Anecdotal personal accounts posted to online message boards by individuals claiming to have autism purport that MDMA was helpful in reducing their anxieties and helping them to feel more connected with others (Danforth *et al*, 2016). A recently completed clinical trial with 12 autistic adults tested whether MDMA may in fact be beneficial. Using the Leibowitz social anxiety scale as the primary measure, subjects received two MDMA or placebo treatment sessions along with 9 preparatory or integrative sessions. Although preliminary, the results are very promising and indicate that MDMA facilitated a rapid-onset reduction of social anxiety symptoms, with sustained reductions at a 6-month follow up (Yazar-Klosinski *et al*, 2016).

Lastly, a new trial is investigating whether MDMA can alleviate end-of-life anxiety in patients with life-threatening illnesses. Two large double-blind placebo-controlled studies have

recently reported that a single dose of the hallucinogen psilocybin dramatically reduces end-of-life anxiety in advanced stage cancer patients, with enduring effects 6 months later (Griffiths *et al*, 2016; Ross *et al*, 2016). To determine if MDMA has similar efficacy, an ongoing clinical trial is assessing it as an adjunctive treatment alongside psychotherapy (NCT02427568). MDMA has several benefits over classical hallucinogens. It is substantially milder, shorter acting, and enhances introspection without distracting, and sometimes distressing, cognitive distortions (Danforth *et al*, 2016). These features make it much more suitable as an adjunct to psychotherapy, although the relative importance of having a psychotherapy component has not yet been evaluated in any clinical study of MDMA.

Clinical investigations of MDMA are moving forward at a rapidly increasing pace. Between 1970 and 2010 only two Phase II clinical trials of MDMA were performed, of which only one was completed. In 2016 alone, six studies were completed and more are underway (Mithoefer *et al*, 2016). Thus far, all of these trials have evaluated MDMA as a treatment for anxiety-related conditions, but future studies are likely to explore its efficacy for treating other disorders such as substance abuse (Jerome *et al*, 2013). Although preliminary, the results published so far suggest that MDMA may be a powerful new form of therapy for PTSD and other conditions. In November 2016, the FDA-approved larger Phase III clinical trials (Philipps, 2016). If these larger studies, with more diverse populations, bear out similar results, then MDMA may well be on track to gain regulatory approval for clinical use in the near future (MAPS, 2015).

D. Lethality and Neurotoxicity of MDMA

The risk of adverse events from MDMA use is generally considered lower than for other illicit drugs (Nutt *et al*, 2010). Most such effects are mild, including tachycardia, dry mouth, bruxism, confusion, sweating, and hypertension (Hall and Henry, 2006). In 2011 there were approximately 22,498 emergency department (ED) visits due to MDMA compared with 505,224

for cocaine and 258,482 for heroin, in the US. However, these data may not necessarily reflect the harmfulness of a substance as there were 455,668 ED visits due to cannabis (Department Health and Services, 2013). One means of evaluating the safety of a substance is its therapeutic index, i.e. the ratio of its median lethal dose to its median effective dose (Gable, 2006). MDMA has a therapeutic index equivalent to cocaine, which is not exactly a bellwether of safety, and studies in mice suggest that its lethality increases even more when taken in crowded environments, which are exactly the kinds of places the drug is popular (Fantegrossi *et al*, 2003). A prospective study from Israel estimated that the risk of severe morbidity from MDMA was approximately 1 in 400. Particularly alarming was that most ED patients had taken 3 or fewer MDMA tablets and 21% had consumed only 1 or ½ of a tablet. Although the purity and drug concentration was not assayed, and many patients had consumed other intoxicants (principally alcohol), these findings seriously question the image of MDMA as a relatively safe drug (Halpern *et al*, 2011b).

The risk of death from MDMA use has been estimated to be between 1 in 10,000 and 1 in 50,000 (Gore, 1999), and on average, 50 fatalities in the US are attributed to MDMA each year (Rogers *et al*, 2009). Mortality is most often attributed to hyperpyrexia which precedes intravascular coagulation, rhabdomyolysis, and multi-organ failure (Cole and Sumnall, 2003). Doses of MDMA between 1 and 2 mg/kg, administered to volunteers in a controlled clinical setting, increase core body temperature by 0.3 to 1.0°C (Parrott, 2012). Physical exertion and/or high ambient temperature may dramatically exacerbate these increases (Kiyatkin, 2014). What is concerning is that there is not a clear relationship between the amount of MDMA taken and the severity of adverse hyperthermia and death (Henry *et al*, 1992). The first documented fatality from MDMA-related hyperpyrexia was a 16-year-old girl admitted to the hospital after consuming 1 MDMA tablet at a dance club (Chadwick *et al*, 1991). Her body temperature reached 42°C and she died after 36 hours of intensive medical intervention efforts. Toxicological analysis revealed a blood MDMA concentration of just 0.424 mg/L (2.19 μM) upon hospital

admission with no other drugs detected. Other case studies have documented similar cases of hyperthermia and death in patients with even lower blood concentrations, whereas some patients with extremely high MDMA blood levels experienced only mild adverse symptoms and recovered without incident (Henry *et al*, 1992). Cases such as these suggest that there may be a high degree of individual variability in the adverse response to MDMA and/or that the context of use may substantially affect outcomes (Parrott, 2012).

In recent years, as awareness of the dangers of MDMA-induced hyperthermia has increased, MDMA users have been encouraged to drink fluids while using the drug. Nightclubs often provide "chill-out rooms" with easily accessible free water. However, these efforts coupled with drug-induced dry mouth, over-heating, and exertion may lead to excessive fluid intake. Vasopressin, released by MDMA, inhibits the renal response to water load, so hyponatremia can easily develop, which in severe cases can lead to coma and death from cerebral edema (Ghatol and Kazory, 2012; Hall and Henry, 2006). An additional subset of MDMA-related fatalities has been ascribed to sudden cardiac arrest. Individuals with cardiomyopathy, hypertension, or other cardiac abnormalities are susceptible to sudden death from excessive sympathetic stimulation by MDMA (Hall and Henry, 2006). Because many of these heart conditions go undiagnosed in young people, they are unlikely to know that they are at risk.

Therefore, most cases of MDMA-related mortality (hyperthermia, hyponatremia, and cardiac arrest) are not necessarily the result of a traditional overdose, but rather a combination of the drug, environment, behavior, and genetics. Certain individuals are substantially more vulnerable to MDMA-related death than an average user, and engaging in certain behaviors including increased physical exertion and excessive fluid intake can greatly increase the harmfulness of MDMA. First-time users are up to 6 times more likely to die from consuming MDMA than an average user (Gore, 1999). A standard 1.5 mg/kg dose may be relatively safe for most users, but lethal to a small subset. Use of MDMA in a controlled clinical setting should

mitigate most of these risks, but the unpredictability of severe hyperthermic or cardiac responses presents potential limitations for the therapeutic use of MDMA.

While severe adverse acute effects are relatively rare immediately following MDMA use, the long term adverse effects may be much more pervasive. There is substantial evidence that MDMA can lead to long-lasting, potentially permanent brain dysfunction (Biezonski and Meyer, 2011; Capela *et al*, 2009; Parrott, 2013). The severity, consequences, and mechanisms of this dysfunction have been the subject of substantial research for over 25 years. A primary justification for the prohibitive scheduling of MDMA was the observation that its demethylated cousin MDA was neurotoxic (Pentney, 2001; Ricaurte *et al*, 1985). In the years following its initial scheduling, studies in rats and non-human primates confirmed fears that MDMA might have similar toxicity (Insel *et al*, 1989; O'Hearn *et al*, 1988).

In rats, MDMA depletes 5-HT and its major metabolite 5-HIAA, with the most severe loss occurring in the neocortex, striatum, and hippocampus (Capela *et al*, 2009). Evidence of neurotoxicity begins to appear 24 hours to 2 weeks following MDMA administration. The intensity of immuno-labeled serotonergic neurons is lower following MDMA treatment, with axon terminals affected while cell bodies are spared (Fischer *et al*, 1995; O'Hearn *et al*, 1988). SERT and tryptophan hydroxylase, the rate-limiting enzyme of 5-HT synthesis, are also depleted, further suggesting a loss of presynaptic 5-HT terminals (Xie *et al*, 2006). These abnormalities are reported to last for months to years after MDMA administration, and when axonal regrowth occurs it is highly abnormal (Battaglia *et al*, 1988b; Fischer *et al*, 1995). Loss of 5-HT markers is often accompanied by increased astrocytic GFAP immunoreactivity (Ádori *et al*, 2006; Aguirre *et al*, 1999), enhanced expression of which is a marker of reactive astrogliosis (O'CALLAGHAN, 1993) and one of the most widely documented markers of central nervous system damage (Norton *et al*, 1992).

Primates appear to be especially vulnerable to the neurotoxic effects of MDMA (Moratalla *et al*, 2015). Rhesus macaques (*Macaca mulatta*) administered repeated low doses of MDMA have decreased 5-HT and 5-HIAA levels, while higher doses also decrease SERT binding (Insel *et al*, 1989). Squirrel monkeys (*Saimiri sciureus*) treated with similar doses have decreased 5-HT content for at least 7 years following administration of the drug (Hatzidimitriou *et al*, 1999). Long-lasting serotonergic deficits are observed even from a single low oral dose in this species (Mueller *et al*, 2013). The lowest dose tested was equivalent to a 1.6 mg/kg dose in a human, nearly identical to the standard 1.5 mg/kg dose used in many clinical studies. Other studies have found similar results, with 5-HT depletion occurring in animals with MDMA plasma concentrations in-line with those observed in human users (Mechan *et al*, 2006). These findings indicate that MDMA is very likely to be toxic in humans, potentially even at the doses used clinically.

Several researchers have questioned the conclusion that MDMA is neurotoxic, suggesting instead that the loss of biochemical 5-HT markers is a neuroadaptive phenomenon rather than evidence of neurodegeneration (Baumann *et al*, 2007; Wang *et al*, 2004). Indeed, low doses of MDMA that deplete 5-HT do not necessarily produce evidence of axotomy. However, following higher doses of MDMA there is clear evidence that neurodegeneration occurs. Argyrophilic deposits are present in the brains of MDMA-treated animals following amino-cupric-silver staining, which selectively marks degenerating neurons (Commins *et al*, 1987; Jensen *et al*, 1993). Fluoro-Jade, another selective marker of degenerating neurons, has also been use to assess the neurotoxicity of MDMA. Following a single 10 mg/kg MDMA treatment in rats, few Fluoro-Jade lesions were detected, but at 20 and 40 mg/kg the number of lesions increased dramatically in a clear dose-response relationship (Schmued, 2003). Yet, even MDMA-treated rats without clear signs of neurodegeneration have long-lasting behavioral abnormalities (Baumann *et al*, 2007). This indicates that clinically relevant deficits may occur even at doses lower than those

that produce obvious signs of degeneration. So regardless of whether 5-HT depletion is always indicative of neurodegeneration, there is clear evidence that MDMA is "neurotoxic" in that it leads to long lasting neural and behavioral dysfunction (Biezonski and Meyer, 2011).

Fewer studies have been performed with humans or human tissue, but they strongly suggest that MDMA has similar long-lasting neurotoxic effects, comparable to the effects observed in animal models. Only one report of direct 5-HT measurement from a human MDMA user exists. A 26 year old poly-drug user with a 9 year history of MDMA use had a 50-80% depletion of striatal 5-HT and 5-HIAA (Kish *et al*, 2010a). CSF samples taken from previous MDMA users have lower concentrations of 5-HIAA than samples taken from control subjects, indicating lower 5-HT turnover (McCann *et al*, 1994, 1999), and these 5-HIAA deficits correlate with the subjects' prior degree of MDMA use (Bolla *et al*, 1998). Neuroimaging studies have found that current and former MDMA users have significantly lower SERT binding than controls (Erritzoe *et al*, 2011a; McCann *et al*, 1998, 2005, 2008; Urban *et al*, 2012). Reduced SERT binding is most evident in heavy MDMA users (Vegting *et al*, 2016), but other brain abnormalities, including decreased white matter volume, have been observed in light MDMA users, indicating that MDMA may be neurotoxic to humans even at low and infrequent doses (de Win *et al*, 2008). There is a strong negative correlation between lifetime use of MDMA and SERT binding, but there is also a more promising positive correlation between SERT binding and the length of abstinence (McCann *et al*, 2005). These findings suggest that with prolonged abstinence, regional recovery of SERT function may occur. However, similar findings from animal studies indicate that 5-HT axonal regrowth is highly disorganized and not representative of normal 5-HT innervation observed in controls (Fischer *et al*, 1995; Hatzidimitriou *et al*, 1999).

5-HT is believed to modulate many behavioral, psychological, and physiological functions (Meneses, 1999; de Win *et al*, 2006), so depletion or dysfunction of 5-HT neural systems would be expected to have wide-ranging deleterious consequences. A variety of studies

have linked MDMA use with such functional deficits. Rats previously treated with multiple doses of MDMA have decreased social interaction (Bull *et al*, 2004; Thompson *et al*, 2008), increased anxiogenic behavior (Baumann *et al*, 2007), and impaired learning and memory (Marston *et al*, 1999; Sprague *et al*, 2003). In humans, past MDMA use is associated with impaired memory and executive function as well as an increased incidence of depression and other psychological problems (Parrott, 2013). Memory impairments are especially severe in heavy users (Reneman *et al*, 2005), and there is a clear correlation between memory impairment, the extent of past MDMA use, and 5-HIAA depletion measured in CSF (Bolla *et al*, 1998). Memory function has been reported to be up to 50% lower in MDMA users than in non-user controls (Morgan 2002, Zakzanis and Campbell 2006). Past users are more likely to report depression (Sumnall and Cole, 2005) and have a higher propensity for aggression (Reid *et al*, 2007). A large study of current and past MDMA users found that they had significantly impaired memory and clinically significant levels of depression, impulsivity, and sleep disturbances compared to non-users and polydrug-user controls (Taurah *et al*, 2014), and despite an average of 5 years of abstinence, past MDMA users showed no improvement compared to current users on most measures.

Not all studies of MDMA users have observed persistent cognitive or psychological impairments. In particular, low doses and limited lifetime usage, is not necessarily associated with functional deficits (Jager *et al*, 2007), or these deficits are relatively minor (Bedi and Redman, 2008; Halpern *et al*, 2011a). Additionally, because the purity of MDMA consumed by recreational users is unknown, functional deficits cannot necessarily be linked conclusively to MDMA. Indeed, ecstasy tablets or powders and crystals ("Molly") contain impurities, contaminants, and in many cases no MDMA at all (Baggott *et al*, 2000; Palhol *et al*, 2002). Furthermore, studies of MDMA users are confounded by polydrug use and are mostly retrospective making the order of causality difficult to establish (Bedi *et al*, 2010a). These findings and limitations have led some researchers to conclude that there is insufficient evidence

to indicate that MDMA is harmful when used infrequently and at moderate doses (Doblin *et al*, 2014; Sessa and Nutt, 2007). However, many studies have already addressed some of these weaknesses. A quantitative meta-analysis of studies assessing the impact of MDMA use on short-term and working memory concluded that MDMA users performed worse across all memory domains compared to both non-users and polydrug-user controls (Nulsen *et al*, 2010). And a prospective study found that even a first low cumulative dose of MDMA is associated with a decline in verbal memory (Schilt *et al*, 2007). With regard to other studies that have reported only modest functional deficits in MDMA users with limited lifetime use, one particularly staunch MDMA critic has argued that rather than "refuting MDMA neurotoxicity [such studies] have empirically confirmed its potential for causing neurobiological damage – even when taken carefully" (Parrott, 2011). The overlapping evidence from animal and human studies clearly indicates that high and repeated doses of MDMA are neurotoxic and produce long-lasting behavioral and psychiatric dysfunctions. To what extent these deficits extend to moderate MDMA users or those that may receive MDMA as part of a therapeutic regimen is unclear, but there is sufficient evidence to at least warrant concern regarding neurotoxicity in these cases.

MDMA neurotoxicity is believed to result from an increased production of free radicals such as reactive oxygen species (ROS). Rats treated with MDMA have elevated free radical formation for 6 hours after treatment (Colado *et al*, 1997), and increased ROS production is evident after MDMA treatment in mouse synaptosomes and human DAT- and SERT-expressing cells (Barbosa *et al*, 2015). Cellular mechanisms exist to cope with normal production of ROS, including antioxidants that donate an electron and three major free radical scavenging enzymes: superoxide dismutase, catalase, and glutathione peroxidase. However, when these defenses are overwhelmed, oxidative stress occurs. This can lead to lipid peroxidation and localized breakdown of the cellular membrane, oxidation of proteins and DNA, and apoptosis (O'Shea *et al*, 1998). There is considerable evidence that the neurotoxicity of MDMA results from excessive

ROS formation that overpowers these endogenous defenses (Capela *et al*, 2009; Moratalla *et al*, 2015). Neurotoxic damage in rats is prevented by administration of the free radical scavenging agent alpha-phenyl-N-tert-butyl (Colado *et al*, 1997) or other antioxidants (Aguirre *et al*, 1999; Gudelsky, 1996) (Gudelsky 1996, Aguirre 1999), and overexpression of superoxide dismutase in mice blocks neurotoxicity (Jayanthi *et al*, 1999), while reduction or inactivation of free radical scavenging enzymes exacerbates MDMA neurotoxicity (Cadet *et al*, 2001).

However, the exact mechanism by which MDMA generates these free radicals is not fully understood and remains an active area of investigation. Early studies with MDMA found that intracerebroventricular (ICV) administration of the drug was not neurotoxic (Esteban *et al*, 2001). This led to the hypothesis that MDMA itself was not toxic, but rather a metabolite formed in the liver was responsible for MDMA-attributed neurotoxicity (Colado *et al*, 1997; Hiramatsu *et al*, 1990). The major metabolites of MDMA, HHMA and HMA, can be further oxidized into their quinone forms, which are highly electrophilic and form adducts with glutathione (GSH) or N-acetylcysteine (NAC). These adducts may be trafficked into the brain via GSH transport mechanisms, and they undergo further metabolism to form new quinol-thioethers that undergo redox cycling and produce ROS (Jones *et al*, 2005). In particular, 5-NAC-HHMA has been suggested as a principle neurotoxic metabolite of MDMA. However, a follow-up study failed to detect significant neurotoxicity when this metabolite was injected directly into the brain (Mueller *et al*, 2009). Furthermore, this study found that there was no correlation between metabolite concentrations and neurotoxicity despite a strong correlation between MDMA concentrations and toxicity. There is thus still no concrete evidence that any metabolite of MDMA is primarily responsible for the neurotoxicity of the drug.

Other authors have suggested that the increased concentration of extracellular dopamine that follows MDMA could be the principle source of ROS responsible for neurotoxicity (Sprague *et al*, 1998). Early studies of amphetamine-derivatives observed that their neurotoxicity is

correlated with their DA-releasing potency (Johnson *et al*, 1986, 1987; Schmidt, 1987a). This theory is supported by the observation that depletion of DA in rats prior to treatment with MDMA significantly reduces its neurotoxic effects (Stone *et al*, 1988), whereas administration of the DA precursor L-DOPA that increases DA synthesis, exacerbates neurotoxicity from MDMA (Schmidt *et al*, 1991). Production of hydroxyl radicals and serotonergic neurotoxicity were prevented in rats by pretreatment with a DAT inhibitor, suggesting that both effects are dopamine-dependent (Shankaran *et al*, 1999).

After MDMA administration, there is a phase of abrupt increase in extravesicular levels of monoamine transmitters inside monoamine nerve terminals, including increased DA taken up by 5-HT neurons (Alves *et al*, 2007; Sprague and Nichols, 1995a). DA is prone to auto-oxidation, and in the presence of GSH it can form a quinone glutathione conjugate capable of redox cycling, promoting the formation of free radicals (Bindoli *et al*, 1992). This property has long been studied as a potential explanation for the neurodegeneration of DA neurons in Parkinson's disease (Spencer *et al*, 1998). Within 5-HT neurons, DA is metabolized by monoamine oxidase B (MAO-B). The deamination of DA by MAO-B produces superoxide and hydrogen peroxide. When hydrogen peroxide removal pathways are overwhelmed, it accumulates and is converted into more reactive hydroxyl radicals. Because of MAO-B's location within the outer mitochondrial wall, generation of ROS during DA catabolism primarily affects the mitochondria (Capela *et al*, 2009). Mitochondrial DNA is not protected by histones or DNA-binding proteins, so it is particularly vulnerable to damage by ROS. MDMA has been demonstrated to damage mitochondrial DNA and impair protein synthesis, leading to incomplete electron transport chain function and further ROS generation as well as a loss of cellular respiration (Alves *et al*, 2007). Oxidative damage to mitochondria can initiate intracellular signaling cascades leading to neural degeneration (Barbosa *et al*, 2015). Pretreatment with an MAO-B inhibitor or an antisense oligonucleotide targeting MAO-B is sufficient to prevent MDMA-induced mitochondrial damage

and serotonergic toxicity in rats (Alves *et al*, 2007; Falk *et al*, 2002; Sprague and Nichols, 1995a, 1995b). Interestingly, the thioether metabolites of MDMA can potentiate uptake of DA into SERT-expressing cells, suggesting that they could play a facilitative role in DA-mediated toxicity (Jones *et al*, 2004).

Hyperthermia also plays a significant role in MDMA neurotoxicity (Capela *et al*, 2009). Studies have shown that elevated body temperature markedly enhances neurotoxicity, while low body temperature is neuroprotective. (Broening *et al*, 1995; Malberg and Seiden, 1998). The formation of hydroxyl radicals in the brain is a temperature-dependent process (Globus *et al*, 1995; Kil *et al*, 1996), and the increased free-radical formation by MDMA is prevented by inhibiting MDMA-induced hyperthermia (Colado *et al*, 1999). Central administration of MDMA may be nontoxic, not because metabolism is necessary, but because it does not produce hyperthermia (Shokry *et al*, 2016). Many drugs that prevent MDMA neurotoxicity, including the NMDA antagonist MK-801 (Farfel *et al*, 1992), the 5-HT_{2A} antagonist ketanserin (Malberg *et al*, 1996), and the D_1 antagonist SCH 23390 (Shioda *et al*, 2008), may do so by blocking hyperthermia. Hyperthermia also disrupts the blood brain barrier, potentially facilitating access of harmful toxicants into the brain (Sharma and Ali, 2008). Silver staining following very high doses of MDMA in rats has indicated that cellular degeneration can occur in non-serotonergic cells. This effect is likely due to hyperthermia and the widespread nonspecific oxidative stress that it produces (Capela *et al*, 2009; Jensen *et al*, 1993). Despite the obviously important role of hyperthermia, there is reason to believe that it is not necessarily essential for MDMA neurotoxicity. Pretreatment with the SSRI fluoxetine is able to prevent neurotoxicity without affecting (at least the initial) MDMA-induced hyperthermia, suggesting that hyperthermia alone is not sufficient for neurotoxic damage (Capela *et al*, 2009; Malberg *et al*, 1996), and although hyperthermia appears to be necessary for neurotoxicity from a single large dose of MDMA, small

repeated doses have been shown to produce toxicity without elevating body temperature, indicating that hyperthermia is not always necessary (O'Shea *et al*, 1998).

Lastly, ROS generated by the above processes likely play a role in triggering an inflammatory response. Evidence of neuroinflammation, including an increased prevalence of microglia and astrocytes, has been observed in the neuronal areas damaged by MDMA (Aguirre *et al*, 1999; Frau *et al*, 2013; Thomas *et al*, 2004). Glial activation may participate in neurodegeneration by releasing cytokines that exert neurotoxic effects on vulnerable neurons (Barcia *et al*, 2011). Cytokine-initiated apoptosis can in turn spur additional free radical formation (Stoian *et al*, 1996). Furthermore, in a pro-oxidant environment microglia release stores of iron that may catalyze hydroxyl radical formation from hydrogen peroxide via the Fenton reaction, and thereby create a self-perpetuating cycle of oxidative stress and neuroinflammation (Yamamoto *et al*, 2010).

In conclusion, there are many potential mediators of MDMA-induced neurotoxicity. Neurotoxic metabolites of MDMA, DA autoxidation and metabolism by MAO-B, hyperthermia, and inflammation likely all play a role. Neurodegeneration may result from the synergistic or additive effect of these, which overwhelm the body's antioxidant defenses. Inhibiting one or more of these mechanisms may be sufficient to lower the oxidative environment enough that neural dysfunction can be avoided. So, in a sense, the mechanism of MDMA-induced neurotoxicity may be the distributed action of multiple independent but interacting drug effects that culminate in the selective, localized degeneration of specific susceptible neuronal populations.

E. Summary of Experimental Aims and Outcomes

From its humble beginning in a Merck laboratory, MDMA has become one of the most widely used illicit drugs. Its fascinating ability to engender feelings of love and closeness towards others has inspired new subcultures and generated significant interest in the drug's therapeutic

potential. Yet, as deaths mount and studies of its neurotoxicity accumulate, the dangers of MDMA have never been clearer. During the 100+ year history of MDMA, a great deal has been learned, but the drug is still ultimately a mystery. The exact mechanisms that underlie its profound prosocial effects are poorly understood, as are the neurotoxic mechanisms. Potentially the greatest question concerns the future of MDMA – whether it will remain classified as a dangerous drug of abuse or become a viable and respectable medication of the future.

The driving motivation for the studies described in this dissertation was to identify the mechanisms of MDMA that induce prosocial behavior, and to develop alternative compounds that could replicate these same effects without the adverse effects of MDMA, particularly the stimulant, neurotoxic, and hyperthermic effects. The first aim of this endeavor was to develop an animal model that recapitulated the social effects of MDMA observed in humans. Due to the wealth of genetic tools available in mice, they were an ideal choice; however, most prior studies had observed no prosocial effects from MDMA in mice. We discovered that prosocial effects only emerge after repeated treatments with MDMA. This previously unreported phenomenon was investigated and found to be dependent on experience and the 5-HT_{2A} receptor. These studies are the focus of Chapter 2.

After our animal model was developed, the next aim was to identify the pharmacological mechanisms of MDMA that increase social interaction. MDMA is a racemic mixture of two pharmacologically distinct enantiomers, so assessing the effects of each enantiomer was a natural starting place to dissect the effects of MDMA. Previous studies had suggested that (+)-MDMA was the dominant enantiomer and likely driver of the social effects. To our surprise, (+)-MDMA had insignificant effects on social interaction, and was primarily a locomotor stimulant. (–)-MDMA, on the other hand, had robust prosocial effects that were equal in magnitude to those elicited by racemic MDMA. Intriguingly, activation of oxytocinergic neurons, which has been

suggested as a possible mechanism of MDMA-induced social behavior, was observed following (+/–)- and (–)-MDMA but not (+)-MDMA. These studies are the focus of Chapter 3.

With the novel finding that (–)-MDMA has prosocial effects, the final aim and the focus of Chapter 4 was to determine whether the prosocial effects of MDMA could be isolated from the neurotoxic and thermogenic effects. Work from other labs had shown that (–)-MDMA was less neurotoxic, but these studies did not account for the lower potency of the enantiomer. Using multiple measures of neurotoxicity, (–)-MDMA was investigated alongside racemic MDMA as a positive control. (–)-MDMA produced no signs of neurotoxicity and did not produce hyperthermia. These findings indicate that (–)-MDMA may be a substantially more viable therapeutic option than traditional racemic MDMA, and suggest that the neurotoxic mechanism of MDMA is largely linked to (+)-MDMA. Preliminary evidence suggests that DA, which (–)-MDMA does not release, is a key differentiator between the enantiomers and is responsible for the hyperthermic effects of racemic MDMA.

Together these studies provide the first indication that (–)-MDMA may be a superior therapeutic agent compared to racemic MDMA. (–)-MDMA produces similar prosocial effects without any of the adverse effects we investigated. Furthermore, the differing pharmacology of (–)-MDMA offers some insight into the key mechanisms of MDMA that are responsible for the prosocial and adverse effects. Future studies will be necessary to confirm these initial findings; toxicological studies in other species are needed, as are clinical studies to determine if (–)-MDMA has similar prosocial effects in humans. Although (–)-MDMA may not prove to be an ideal treatment, these studies arguably have a more significant conclusion: that the prosocial effects of MDMA can be isolated from the stimulant, neurotoxic, and hyperthermic effects. Development of safer and more targeted therapeutics might therefore be possible that would make clinical use of MDMA, in any form, unnecessary.

F. Figures

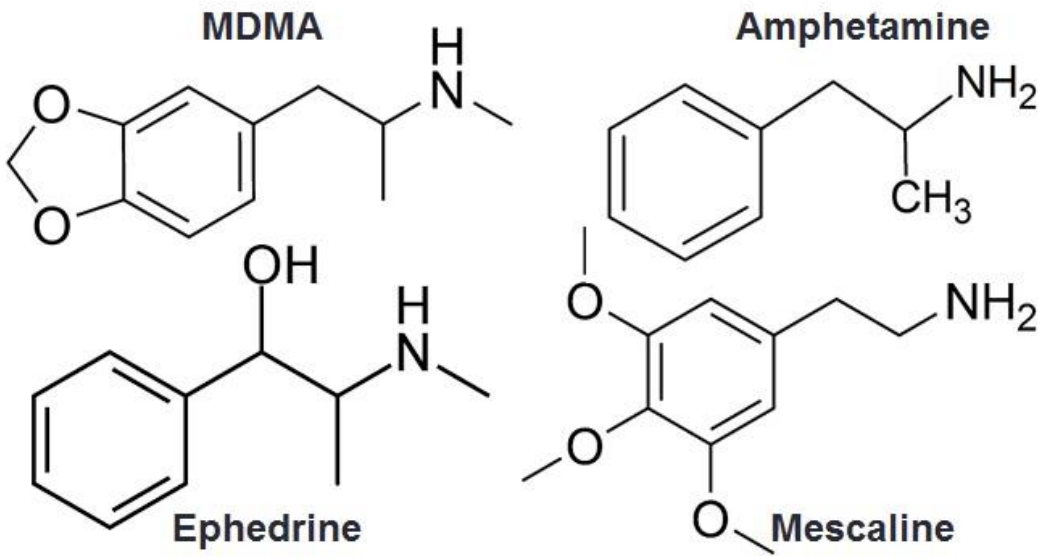

Figure 1.1 Chemical structures of MDMA and related drugs. MDMA is a substituted phenethylamine with structural and functional similarities to other phenethylamines such as amphetamine and mescaline. These similarities played an important role in the history of MDMA, as researchers searched for new drugs with similarities to known compounds.

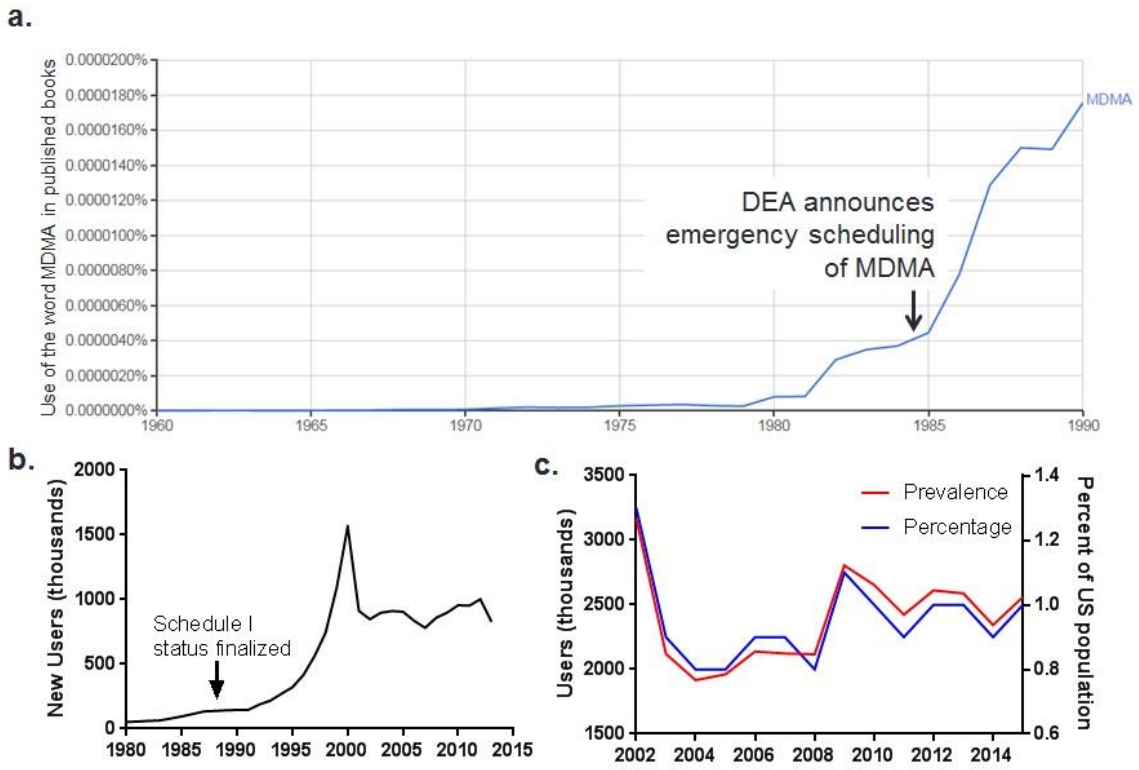

Figure 1.2 Use of MDMA rises rapidly. (a) The word MDMA, essentially unused prior to 1970, rapidly enters the lexicon in the early 1980's. The court battle between the DEA and clinical MDMA researchers brought the drug to the public's attention. These data were obtained using Google's Ngram Viewer, which plots the usage of a word within a sample of published works (Michel *et al*, 2011). **(b)** Classification of MDMA into Schedule I effectively halted clinical use and research of the drug but did not stop the rise of recreational use. Use of MDMA increased dramatically in the US during the 1990's. A series of congressional actions from 2000-2003 that addressed trafficking, distribution, and use of MDMA, may have helped to decrease use. Annual new users are estimated (2001 and prior) from the National Household Survey on Drug Abuse (NHSDA 2001) and (post 2001) from the National Survey on Drug Use and Health (NSDUH 2015). The number of new users per year was calculated from the current age of survey participants and their stated age at first use. For age-brackets (e.g. 30-34), an even distribution of current ages was assumed, and all values were divided across the applicable years. **(c)** Since 2002, MDMA use in America has remained relatively stable, with approximately 1% of US individuals 12 and older using the drug at least once annually (NSDUH 2016).

Chapter 2. Sensitization to the Prosocial Effects of MDMA

A. Introduction

3,4-methylenedioxymethamphetamine (MDMA) is a ring-substituted phenethylamine that became popular as a recreational drug (ecstasy) and therapeutic tool during the late 1970's and early 1980's (Holland, 2001). Escalating use led to its prohibition, but scientific interest in the drug has persisted due to its unique prosocial effects. Under clinical observation, volunteers report that MDMA increases feelings of closeness towards others and sociability (Bedi *et al*, 2010b; Hysek *et al*, 2013). Participants given MDMA are more likely to choose to participate in social situations (Kirkpatrick *et al*, 2014b) and spend more time interacting with one another than those given placebo (Kirkpatrick and de Wit, 2014). Although many recreational drugs are capable of altering social experiences, MDMA is generally regarded as a distinct, prototypically social drug or love drug (Kamilar-Britt and Bedi, 2015). Alcohol and psychostimulants can increase feelings of friendliness and sociability (Bershad *et al*, 2015), but MDMA uniquely also increases trust, generosity, and empathy (Bershad *et al*, 2016a). These effects led early MDMA researchers to propose new drug classifications for MDMA, naming it an "entactogen" (Nichols, 1986) or an "empathogen" (Adamson and Metzner, 1988), and to investigate its therapeutic utility. In addition to acute prosocial effects, a group a psychiatrists testing MDMA reported that it facilitated long-lasting improvements to their interpersonal functioning (Liester *et al*, 1992). More recently, double-blind placebo-controlled clinical trials have been performed to more rigorously assess such effects. Although preliminary, a study of adults with autism found that two MDMA treatments significantly reduced social anxiety symptoms for at least 6 months after treatment (Yazar-Klosinski *et al*, 2016).

There is currently no accepted mechanism for how MDMA produces these unique prosocial and therapeutic effects. Indeed, the underlying biological processes that mediate many

complex social behaviors are poorly defined. Unlike fear, which can be easily induced and investigated in animal models, affiliative social behaviors cannot be easily elicited in the laboratory, making the neurobiology of these behaviors more difficult to study. Select model systems such as the monogamous prairie vole have substantially advanced social neuroscience (McGraw and Young, 2010), but few methods are available to probe these systems in other species. If MDMA produces well defined prosocial behaviors in model organisms it could represent a new and powerful tool to investigate the endogenous biological systems that mediate these social behaviors (Heifets and Malenka, 2016). Understanding the mechanisms by which MDMA modulates these systems may also provide clues as to what has gone awry in disorders characterized by deficits in normal social behavior, and assist the development of new treatments.

Given the wealth of genetic tools available in mice, they are an ideal model organism to investigate the prosocial effects of MDMA, and to eventually probe the endogenous mechanisms of prosocial behavior. Relative to the number of human studies, considerably fewer have assessed the prosocial effects of MDMA in animal models. Most of these studies evaluated the effects of MDMA using a social interaction test, where two conspecifics are paired together and their interactions are scored by an observer blind to the experimental conditions. Perhaps the most reliable behavioral feature of MDMA is that it dramatically reduces aggression across species including rats (Morley and McGregor, 2000), mice (Machalova *et al*, 2012; Navarro and Maldonado, 1999), and fish (Capurro *et al*, 1997). In addition to reducing species typical behavior, several studies have observed that MDMA also increases affiliative-like behaviors in rats and mice (Ando *et al*, 2006; Daza-Losada *et al*, 2009; Morley and McGregor, 2000; Procópio-Souza *et al*, 2011). The prosocial behaviors most commonly elicited by MDMA at doses between 5 - 15 mg/kg are adjacent lying, where subjects lay next to each other in close physical contact, and non-aggressive following. However, a substantial number of other studies have found a conflicting lack of enhanced affiliative behaviors after administering similar doses

of MDMA (Bhattacharya *et al*, 1998; Homberg *et al*, 2007; Maldonado and Navarro, 2001; Navarro *et al*, 2004a). In particular, most studies with mice have observed that MDMA decreases social interaction. In preliminary studies, we also observed that MDMA either did not affect or modestly decreased social interaction in mice. However, when mice were tested with MDMA a second-time social effects became readily apparent. This intriguing effect could explain the discrepancies in the existing literature. Many of the studies that observed robust prosocial effects, had tested their subjects multiple times with multiple doses of MDMA (Ando *et al*, 2006; Morley and McGregor, 2000; Procópio-Souza *et al*, 2011), while many studies that used drug naïve subjects observed no prosocial effects (Bhattacharya *et al*, 1998; Homberg *et al*, 2007; Maldonado and Navarro, 2001; Navarro *et al*, 2004b).

The idea that acute administration of a drug can have lasting effects on the brain and behavior is a major area of ongoing research in the field of drug abuse (Robinson and Berridge, 2008). Subjects given repeated intermittent exposure to drugs of abuse show an enhanced or sensitized behavioral and neurochemical response to subsequent drug exposures. This concept of sensitization has been used to explain the accelerated use of drugs that leads to addiction (Steketee and Kalivas, 2011). We hypothesized that the emergence of prosocial effects from MDMA during subsequent treatment sessions was a form of sensitization. Behavioral sensitization involves the enhancement of multiple discrete and largely dissociable behaviors (Robinson and Becker, 1986). The most commonly studied is locomotor sensitization, typically measured immediately after drug administration by placing the test subject into an open-field chamber and measuring their ambulatory activity. As with other drugs of abuse including stimulants and opioids, repeated intermittent treatment with MDMA produces long-lasting locomotor sensitization (Ball *et al*, 2011; Bradbury *et al*, 2012; Kalivas *et al*, 1998).

Behavioral sensitization is largely believed to result from hyperactivity within the mesolimbic pathway that leads to enhanced drug-induced DA release and a concomitant increase

in locomotor activity and other drug effects. The underlying neural adaptations that produce this sensitization are not fully understood but are dependent upon changes within the monoamine brainstem nuclei (Auclair *et al*, 2004; Cador *et al*, 1995). Several receptors appear to be critical in mediating these neural adaptations. 5-HT_{2A} antagonists and 5-HT_{2C} agonists prevent the development (Auclair *et al*, 2004; Wu *et al*, 2015) and expression (Ramos *et al*, 2005; Zayara *et al*, 2010) of sensitized locomotor and neurochemical responses. Sensitization, independent of the drug that initiates it, is likely mediated by similar neural adaptations. This has been demonstrated with cross-sensitization studies. For example rats sensitized to the locomotor stimulant effect of amphetamine will also display a sensitized locomotor response when treated with other drugs of abuse including other stimulants like cocaine or opioids like morphine (Cador *et al*, 1995). Intriguingly however, amphetamine does not produce cross-sensitization to MDMA, although MDMA produces cross-sensitization with amphetamine (Bradbury *et al*, 2012). This suggests that related but distinct neural adaptations may occur with MDMA treatment compared with other drugs of abuse. Indeed, repeated treatments with MDMA do not appear to produce a sensitized DA response in mice, but rather a sensitization of 5-HT and NE release. This sensitization appears to result from a downregulation of the G protein subunit α_i in the dorsal raphe and the locus coeruleus and is dependent on activation of 5-HT_{2A} receptors (Lanteri *et al*, 2013).

To better understand the sensitization of MDMA-induced social behavior we performed a series of experiments to determine if it is mediated by the same mechanisms that underlie traditional behavioral sensitization. First, we demonstrated that social interaction increased precipitously across 4 treatment sessions. This sensitization persisted for at least 2 weeks but sensitized mice did not have higher baseline social behavior compared to control subjects. We observed only limited evidence of locomotor sensitization in these animals and there was no cross-sensitization to amphetamine. This suggests that social sensitization occurs independently from locomotor sensitization, and could likely have alternative mechanisms. We also observed no

sensitization of MDMA-induced 5-HT release. Because 5-HT_{2A} receptor antagonists prevent the development of locomotor sensitization to MDMA and other drugs of abuse (Ago *et al*, 2008; Auclair *et al*, 2004; Lanteri *et al*, 2013), we pretreated mice with a selective antagonist to determine its effect on social sensitization. Pretreatment prevented the development of social sensitization, but did not attenuate the expression of prosocial behavior when administered to mice that had already been sensitized. And although 5-HT_{2A} activation was necessary for the development of sensitization it was not sufficient. 5-HT_{2C} receptors have been implicated as another mediator of behavioral sensitization (Lanteri *et al*, 2008; Ramos *et al*, 2005; Wu *et al*, 2015). However, we observed that pretreatment with a selective 5-HT_{2C} agonist did not affect the development of social sensitization. Lastly, we observed that social interaction during MDMA treatment was necessary for the development of social sensitization. Together these experiments indicate that social sensitization occurs via independent but potentially overlapping mechanisms from locomotor sensitization. Further investigation is warranted to determine if neural adaptations occur with social sensitization and if the same processes that mediate social sensitization are also involved in the long-lasting therapeutic effects of the drug that have been demonstrated in recent clinical trials.

B. Methods

Subjects

Male Swiss Webster mice (Charles River Laboratories, Wilmington, MA) aged 7-10 weeks served as subjects in all experiments. Mice were housed five per cage in a temperature and humidity controlled colony room at the Yerkes National Primate Research Center with food and water available *ad libitum*. Lights were set to a 14-hour light/dark cycle. All experiments were performed at an ambient temperature of 22±2°C, during the lights-on phase. All studies were carried out in accordance with the Guide for Care and Use of Laboratory Animals as adopted and

promulgated by the National Institutes of Health. Experimental protocols were approved by the Emory University Institutional Animal Care and Use Committee.

Drugs

MDMA and d-amphetamine were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC). The 5-HT_{2A} receptor antagonist R(+)-MDL100,907 (M100) was provided by Dr. Kenner Rice (National Institute on Drug Abuse). The 5-HT_{2A} agonist R(–)-2,5-Dimethoxy-4-iodoamphetamine (DOI) was purchased from Sigma-Aldrich (St. Louis, MO). The 5-HT_{2C} receptor agonist WAY163909 (WAY) was provided by Pfizer (New York City, NY). MDMA, d-amphetamine, and WAY were dissolved in 0.9% sterile saline. M100 was dissolved in 0.9% sterile saline containing HCl. NaOH was added to restore the solution to the pH of saline (5.5). All drugs were administered via intraperitoneal injection (i.p.) at a volume of 10 ml/kg.

A dose-effect study was performed to determine the optimal dose of MDMA that increases social interaction in mice. A large range of doses was tested, from 3 mg/kg to 30 mg/kg. The peak effective dose of MDMA was determined to be 7.8 mg/kg and all experiments presented within this chapter used this dose. D-amphetamine was administered at 2 mg/kg because this dose has previous been shown to robustly increase locomotor activity and is sensitive to cross-sensitization with MDMA (Lanteri *et al*, 2013). Pilot experiments tested doses of M100 (0.3 or 1.0 mg/kg), DOI (0.3 or 1 mg/kg), and WAY (5.6 or 10 mg/kg). Due to better results and/or lack of off-target effects, subsequent experiments used the higher doses (1 mg/kg) of M100 and DOI and the lower dose (5.6 mg/kg) of WAY.

Behavioral Tests

Social Interaction Test:

The social interaction test is a well validated measure of dyadic social behavior in male rodents (File and Seth, 2003) that is sensitive to the behavioral effects of MDMA (Morley and McGregor, 2000). Treatments and pretreatments were administered to subjects prior to temporary isolation in a clean cage. Mice were then paired with a conspecific for a 10 minute test in a clear 35 x 28 cm (unless otherwise specified) Plexiglas arena. While in the testing arena, subjects were free to move around and interact, allowing a diverse range of observable behaviors. Tests were videotaped and scored using JWatcher by an observer blind to the experimental conditions. The durations of 3 behaviors were scored: anogenital investigation (sniffing the conspecific's anogenital area), general investigation (non-anogenital sniffing, grooming, and following the conspecific), and adjacent lying (side-by-side contact or huddling, excluding climbing under/over the conspecific) (Morley *et al*, 2005). These behaviors were averaged for each pair and then summed to produce a total social interaction score, upon which statistical analysis was performed.

Locomotor Activity Test:

Ambulatory activity was tested in a 45 x 39 x 37 cm open field activity monitoring apparatus with 16 x 16 photocells positioned 2.5 cm off the chamber floor (San Diego Instruments, San Diego, CA). Operation of the chambers and data collection was done by an interfaced computer. Accumulative beam breaks of adjacent photocells were recorded as the measure of locomotor activity. The locomotor activity of each subject was monitored for 1 hour immediately after drug administration.

Microdialysis and quantification of 5-HT release

To assess changes in 5-HT release from MDMA, 8 mice were implanted with unilateral guide cannulae directed at the nucleus accumbens (NAcc). Mice were anesthetized with isoflurane and injected i.p. with 1 mg/kg Meloxicam to decrease post-operative pain. After being secured in a Kopf stereotaxic apparatus, cannulae were installed 1.9 mm anterior and 0.9 mm

lateral to bregma, positioned 1 mm above the NAcc. Half of the cannulations were to the right of bregma and half to the left. After surgery, mice were singly housed and given 5 days to recover before testing. Postoperative Meloxicam was administered for 3 days following cannulation. On test days, mice were placed into a clean circular cage with access to food and water. Dialysis probes (CMA 7, 1 mm probe length) were connected via FEP Teflon tubing to a microinfusion syringe mounted on a motor-driven syringe pump. Probes were flushed with artificial cerebrospinal fluid (aCSF; in mM: 142 NaCl, 3 KCl, 1.3 $CaCl_2$, 1 $MgCl_2$, 1 Na_2HPO_4; pH 7.6) for 30 min prior to insertion into the guide cannulae (Thrivikraman *et al*, 2013). The flow of aCSF through the probe was maintained at 1µl/min for the duration of the experiment. After a 5 hour equilibration period, sample collection and experimental manipulations began. The probe outlet was connected to FEP tubing terminating in a refrigerated sample collector. Samples of dialysate were collected into micro-centrifuge tubes every 20 minutes. 3 baseline samples were taken before treatment with MDMA and 6 samples were collected post-treatment. After the final post-treatment sample was collected, a liquid switch was used to change the aCSF flow to a high potassium aCSF (in mM: 96 NaCl, 98 KCl, 1.3 $CaCl_2$, 1 $MgCl_2$, 1 Na_2HPO_4; pH 7.6) for a final sample collection. The increased potassium causes neuronal firing adjacent to the dialysis probe and was used to confirm that the site was active and responsive. The concentration of 5-HT in each sample was quantified using high performance liquid chromatography with electrochemical detection (HPLC-ECD). The HPLC system was composed of a small-bore reverse-phase C_{18} column (3.2 mm x 150 mm x 3 um; 70-0636; Thermo Scientific, Sunnyvale, CA), a Thermo Dionix Ultimate 3000 solvent delivery pump set to a flow rate of 0.6 ml/min, a guard cell (+350 mV; 5020, ESA), and an autosampler (542, ESA, Chelmsford, MA). Detection was carried out with a dual-channel analytical cell (5014B, Thermo Scientific) and an ESA Coulochem III detector. The analytical cell's oxidative channel was set to -150 mV and its reductive channel was set to +200 mV. The mobile phase was prepared with polished water and contained 90 mM Na_2HPO_4, 50 mM citric acid, 1.7 mM octane sulfonic acid, 50µM EDTA, 10% acetonitrile, and

was adjusted with KOH to a pH of 3.0. Data were acquired and analyzed using Chromeleon 6.8 software (Thermo Scientific). 5-HT concentrations were calculated from a standard curve generated with external 5-HT (Sigma) standards.

Experimental Procedures

Preliminary experiments indicated that MDMA did not affect social interaction when administered to mice for the first time, but after a second treatment administered 48 hours later social interaction increased. To determine if the amount of social interaction would continue to increase with subsequent treatments of MDMA, mice were given treatments of MDMA (7.8 mg/kg i.p.) or saline (10 mice per treatment group) 4 times, once every 48 hours. After each treatment, mice were isolated in a clean cage for 25 minutes and then paired with an unfamiliar conspecific that had received the same treatment for a 10-minute social interaction test. Due to high aggression within one saline treated pair, the two mice had to be separated during the first test day and were removed from the study and not tested on subsequent days. To determine if increased social interaction following subsequent treatments with MDMA was purely due to experience with the testing procedure, a second cohort of 10 mice were treated with saline during the first 3 social interaction tests but received MDMA on the 4th test day (day 7). To determine the persistence of MDMA-induced social sensitization and its effect on baseline social behavior, a new cohort of 24 mice (12 per treatment group) underwent the same sensitization procedure as above, receiving either saline or MDMA on each test day. On day 11, 4 days after the last treatment session, mice were paired with an unfamiliar conspecific but did not receive any drug treatments. On day 21, 14 days after the last treatment session, a challenge dose of MDMA or saline was given.

Sensitization to the locomotor stimulant effect of MDMA was tested in a new group of mice (6 per treatment group). Mice received 4 injections of saline or MDMA, each 48-hours apart, and were immediately placed into activity monitoring chambers. To measure cross-

sensitization, all mice were given an injection of 2 mg/kg d-amphetamine 2 weeks after the last MDMA or saline test session. To determine if social sensitization involved neurochemical sensitization, 8 mice had intracranial cannulae implanted and CSF dialysate was collected following treatment with MDMA. Mice underwent the same treatment protocol described above, receiving MDMA every 48 hours over 7 days for a total of 4 treatment sessions. Microdialysis was performed during the first and forth treatment sessions. Due to poor dialysis probe placement or loss of the head cap during the course of the experiment, 3 mice were removed from analysis.

The role that 5-HT_{2A} receptors play in mediating social sensitization was assessed with several related experiments. First, to determine if activation of 5-HT_{2A} receptors is necessary for the development of sensitization, mice (8 per treatment group) received pretreatments of 1 mg/kg M100 or saline 15 minutes before treatment with MDMA. Mice were then isolated for 25 minutes before being paired for social interaction testing. This procedure was repeated during a total of 3 treatment session each 48-hours apart. On day 7, 48 hours after the last treatment session, mice were all given MDMA without a pretreatment, and then underwent social interaction testing. Next, to determine if activation of 5-HT_{2A} receptors is sufficient to induce the development of sensitization, 32 mice (8 per treatment group) were administered DOI (1mg/kg), MDMA, or saline for three treatment sessions as described above. On day 7, sensitization was tested by administering a challenge dose of MDMA or saline. One MDMA treated subject from the MDMA sensitization group escaped the testing arena during testing and was therefore excluded from analysis. To determine if 5-HT_{2A} activation is necessary for the expression of MDMA-induced social behavior, 16 additional mice were given MDMA across 3 treatment sessions as above. On day 7 they were given a pretreatment of saline or M100 (8 per treatment group) 15 minutes before MDMA. Social interaction was then tested as described above.

Given the opposing function of 5-HT_{2A} and 5-HT_{2C} receptors we tested whether the 5-HT_{2C} agonist WAY could also prevent the development of sensitization. As with M100, mice (8 per treatment group) were pretreated with WAY or saline 15 minutes before treatment with

MDMA, followed by a 25-minute isolation period, and a 10-minute social interaction test. This procedure was repeated on days 3 and 5, with each treatment session separated by 48 hours. On day 7 all mice received MDMA with no pretreatment and were tested for social interaction. To assess off target effects of WAY and M100 that may interfere with behavior, locomotor activity was monitored in mice (5 per treatment group) for 1 hour after treatment with these drugs.

Finally, to determine if social sensitization was dependent on social interaction during the treatment sessions or if it would occur regardless of the social context, we administered MDMA to mice (8 per treatment group) for 3 sensitization sessions, once every 48 hours. Mice were given MDMA and then either isolated for 2 hours or paired for 2 hours, before being returned to their home cages. On day 7, mice were given MDMA, isolated for 25 minutes, and then paired in a novel 30 x 18 cm testing arena. Their social interaction was compared to a separate group of 8 MDMA treated mice that underwent normal social interaction testing on all treatment days.

Data Analysis

Data were analyzed with Prism 7 (Graphpad, La Jolla, CA). Sensitization experiments covering multiple days were analyzed with two-way ANOVAs. When possible, a repeated measures analysis was used, but because social interaction testing was always averaged for each unique pairing, within-subjects analyses were not possible for these experiments. Unless otherwise specified, Sidak's multiple comparisons test was used to determine group differences. When one-way ANOVAs were performed on data sets spanning just one or two treatment days, Tukey's or Dunnett's post-hoc tests were performed. Alpha for all experiments was set at 5%. Error bars represent the standard error of the mean (SEM).

C. Results

Sensitization to the prosocial effects of MDMA

Treatment with 7.8 mg/kg MDMA every 48 hours significantly increased murine social interaction (Figure 2.1a). A two-way ANOVA indicated a significant effect of treatment $F(3,28) = 50.1$ $p < 0.0001$, effect of day $F(1,28)=115.2$ $p < 0.0001$, and interaction $F(3,28) = 29.32$ $p < 0.0001$. Sidak's multiple comparisons test revealed that social interaction following MDMA treatment was significantly greater than following saline on days 5 and 7 ($p < 0.0001$). Additionally, social interaction following MDMA was greater on these days than on the first or third days of MDMA treatment ($p < 0.0001$). And social interaction was higher on the seventh day than on the fifth day ($p = 0.0003$). The increased social interaction produced by MDMA following each successive treatment, is a clear indication of a sensitization-like phenomena. To analyze the individual social behaviors that were sensitized following MDMA treatment, a two-way, within-subjects ANOVA was performed comparing three individual behaviors. There was a significant effect of day $F(3, 16) = 82.75$ $p < 0.0001$, behavior $F(2,32)=122.8$ $p < 0.0001$, and an interaction $F(6,32)=12.44$ $p < 0.0001$. Sidak's multiple comparisons test indicated that general investigation (days 1v5, 1v7, 3v5, 3v7 $p < 0.0001$) and adjacent lying (1v7 $p = 0.0008$, 3v7 $p = 0.001$; 5v7 $p = 0.214$) behaviors sensitized, significantly increasing across subsequent MDMA treatments, but anogenital investigation did not (Figure 2.1b).

Social sensitization was not an artifact of familiarity with the testing procedure. A one-way ANOVA comparing the social interaction of mice on day 7 indicated that there were significant group differences $F(2,11) = 60.04$ $p < 0.0001$ based on treatment history (Figure 2.1c). Mice that had received prior MDMA treatments had significantly increased social interaction upon treatment with MDMA compared to mice receiving MDMA or Saline that had received prior saline treatments (Tukey's post-hoc test, $p < 0.0001$).

Sensitization was long-lasting but did not affect baseline social behavior (Figure 1.2d). Social interaction testing on days 11 and 21 were analyzed by a one-way ANOVA to permit additional post-hoc testing. There were significant group differences $F(3,20) = 6.746$ $p = 0.0025$.

Orthogonal comparisons were made within each test day and were adjusted for multiplicity using Sidak's method (two independent t-tests yielded equivalent significance). There was no increase in baseline social behavior between animals with a treatment history of saline or MDMA ($p = 0.994$). 14 days after the last treatment session, subjects were given a challenge dose of MDMA or saline. MDMA treated mice engaged in significantly more social interaction than saline treated controls $p = 0.0062$.

Locomotor sensitization and cross-sensitization

To determine if social sensitization was accompanied by locomotor sensitization, the locomotor stimulating effect of MDMA was tested across 4 treatments, each 48 apart (Figure 2.2). A two-way repeated measures ANOVA indicated that there was a significant effect of treatment day $F(3,30) = 4.688$ $p = 0.0084$ but not a significant effect of treatment, $F(1,10) = 4.627$ $p = 0.0570$ or interaction $F(3,30) = 2.451$ $p = 0.0828$, although both were nearly significant. Within the saline treatment group a Sidak's multiple comparisons test revealed that there was no effect of sensitization, i.e. locomotor activity did not significantly increase across the treatment days. Within the MDMA treatment group there was evidence of sensitization, but only on the 4^{th} treatment day (Day 1 vs Day 7 $p = 0.0007$; Day 5 vs Day 7 $p = 0.0309$). A second Sidak's multiple comparisons test indicated that the saline and MDMA treatment groups were only different on day 7 ($p = 0.0296$). To determine if this subtle locomotor sensitization would lead to a sensitized amphetamine response, both treatment groups were administered amphetamine 2 weeks later. A Student's t-test indicated that there was no cross sensitization ($p = 0.9142$).

Neurochemical sensitization

5-HT overflow was measured in the nucleus accumbens during the first and forth (day 7) treatment sessions (Figure 2.3). A two-way repeated measures ANOVA found that there was an effect of time $F(11,44) = 2.474$ $p = 0.0165$, indicating that MDMA affected 5-HT overflow, but

no effect of day F(1,4) = 0.04376 p = 0.8445 and no interaction F(11,44)= 1.854 = p = 0.0733 indicating that there was no neurochemical sensitization on day 7.

Role of 5-HT_{2A} receptors in social sensitization to MDMA

Pretreatment with the 5-HT_{2A} antagonist M100 prevented the development of social sensitization (Figure 2.4a). A two-way ANOVA indicated there was a significant effect of pretreatment F(1,24)=9.257 p = 0.0056, no effect of day F(3,24)=1.406; p=0.2653, and a significant interaction F(3,24)=3.129 p = 0.0444. Sidak's multiple comparisons test was used to assess relevant differences within and between the treatment groups. Within the saline pretreatment group, mice had significantly higher social interaction on day 7 compared to day 1 (p = 0.0242) and to animals that had received M100 pretreatments (p = 0.0358).

Mice administered the 5-HT_{2A} agonist DOI instead of MDMA did not show a sensitized social response when administered MDMA on day 7 (Figure 2.4b). Comparison of total social interaction of day 7 in mice given MDMA or saline with a treatment history of DOI, MDMA, or saline indicated a significant effect of treatment (ANOVA, F(3,11)=12.43; p = 0.0007). Tukey's post-hoc test assessed group differences and indicated that all treatment groups differed significantly from MDMA treated animals that had received prior MDMA treatments (Saline/Saline p = 0.001; Saline/MDMA p = 0.0021; DOI/MDMA p = 0.0018).

To test if 5-HT_{2A} receptor activation was necessary for the expression of MDMA-induced social behavior (Figure 2.2c), mice sensitized with 3 prior MDMA treatments were given a pretreatment of saline or M100 on day 7 before MDMA treatment. A Student's t-test indicated that there was no effect of M100 on the expression of social interaction t(6)=1.31 p = 0.2380.

Role of 5-HT_{2C} receptors in social sensitization to MDMA and locomotor activity

Pretreatment with the 5-HT_{2C} agonist WAY during the first three MDMA treatment session did not affect the development of social sensitization when mice were treated with

MDMA on day 7 (Figure 2.5a). A Student's t-test found no difference between mice that had been pretreated with WAY compared to mice that had been pretreated with saline $t(6)=0.1922$ $p= 0.8539$.

We observed that animals pretreated with WAY appeared more subdued than mice pretreated with saline. Given the similarity of 5-HT_{2A} antagonists and 5-HT_{2C} agonists we assessed the effects of both drugs on normal locomotor activity in an open field (Figure 2.5b). A one-way ANOVA indicated that there was a significant effect of treatment $F(3,16) = 11.06$ $p = 0.0004$. A Dunnett's post-hoc test comparing each drug to saline revealed that WAY 5.6 mg/kg decreased locomotor behavior ($p = 0.0037$). A higher dose of WAY (10 mg/kg) had an even greater effect on locomotor activity ($p = 0.0004$). M100, which completely prevented the development of social sensitization, did not reduce baseline locomotor activity.

Role of social interaction in social sensitization to MDMA

To determine if social sensitization was purely a product of MDMA or if it involved an interaction between the drug and the social environment it was given in, we tested for the development of social sensitization in mice that were either isolated or paired during each MDMA treatment. On day 7 all test subjects were given MDMA and paired as normal. A one-way ANOVA indicated that there was a significant between groups difference $F(3,12) = 12.68$ $p = 0.0005$. A Tukey's post-hoc test indicated that isolated mice given MDMA 4 times displayed significantly less social interaction compared to mice that had received MDMA while paired ($p = 0.0076$). Isolated mice were not significantly different from mice receiving MDMA for the first time ($p = 0.9815$), indicating that social pairing was necessary for the development of social sensitization (Figure 2.6).

D. Discussion

The initial impetus for this set of experiments was to develop a mouse model for investigating the prosocial effects of MDMA. These effects are well documented in humans but studies with rodents have presented conflicting results. The development of a reliable murine protocol would facilitate mechanistic inquiry into the pharmacological and neurobiological mechanisms that drive MDMA-induced prosocial behaviors. Here we demonstrated a 7 day, 4 MDMA treatment protocol that produces robust and reliable increases in murine social interaction. We observed that the prosocial effects produced by MDMA only emerge after repeated treatments, and continue to increase in a sensitization-like manner upon subsequent treatments. This previously unreported phenomenon was investigated and found to be dependent on 5-HT_{2A} receptor activation and a social context.

Pairs of rodents that are placed into an area where neither has established territorial control will engage in a variety of social behaviors. These include active social behaviors such as sniffing, following, and allogrooming as well as passive social behaviors such as lying in close contact (de Angelis and File, 1979; File and Hyde, 1978). We observed that a single dose of MDMA, administered 25 minutes prior to testing, did not affect the duration of social interaction between unfamiliar male mice. However, subsequent doses of MDMA, given every 48 hours, steadily increased the duration of social interaction by these mice. By the third and fourth MDMA treatments, mice were engaging in significantly more social interaction than saline treated conspecifics. Both active and passive social behaviors were increased by MDMA across treatment sessions. In comparison, mice treated with saline interacted at a relatively stable amount upon each pairing. This progressive enhancement of MDMA-induced social interaction appeared strikingly similar to the locomotor sensitization produced by other drugs of abuse such as amphetamine and cocaine. Like locomotor sensitization, it developed rapidly with an amplified response to the drug after just one or two treatments, it did not affect off-drug behavior, and was long-lasting (Vanderschuren *et al*, 1999). The similarity between locomotor sensitization and the

social sensitization we observed could indicate that the two phenomena are related and/or share the same underlying neurobiological mechanism. Indeed like other amphetamine derivatives, MDMA can also produce locomotor sensitization, but this is typically only observed from higher doses and daily treatment regimens (Hamida *et al*, 2008; Kalivas *et al*, 1998; Lanteri *et al*, 2013). In the present study, we observed only limited evidence that our subjects sensitized to the locomotor stimulant effects of MDMA. The magnitude of increased ambulatory activity across treatment sessions was very small and there was no cross-sensitization with amphetamine.

Sensitization of drug-induced behaviors such as locomotor activity is widely thought to emerge from a disinhibition of the mesolimbic dopamine system (Cador *et al*, 1995) and increased synaptic potentiation of striatal D_1 receptor expressing medium spiny neurons (Pascoli *et al*, 2012). DA neurotransmission is therefore amplified upon subsequent drug treatments leading to increased DA related behavioral output. Although sensitized dopamine responses to MDMA have been reported in rats (Kalivas *et al*, 1998), this effect has not been observed in mice. Rather, behavioral sensitization to MDMA appears to be driven by a disinhibition of 5-HT and NE release rather than DA release in this species (Bradbury *et al*, 2012; Lanteri *et al*, 2013). This distinction potentially stems from the species-specific neurotoxicity of MDMA. DA sensitization in rats may be a direct consequence of 5-HT depletion (Schenk, 2011). 5-HT normally provides an inhibitory check on DA neurotransmission (Howell and Cunningham, 2015), a loss of 5-HT following repeated large doses of MDMA could therefore lead to augmented DA release. But regardless of the species, neurochemical sensitization to MDMA has only been observed following daily treatments that also produced robust locomotor sensitization. Our subjects that were only treated every-other day did not have sensitized 5-HT responses to MDMA. Future studies will be necessary to determine if NE or DA release are sensitized, but is unlikely that either would be affected differently from 5-HT given that their sensitization is intricately linked (Auclair *et al*, 2004; Lanteri *et al*, 2008). These findings all suggest that social

sensitization is distinct and perhaps entirely unrelated to traditional locomotor and neurochemical sensitization.

Several monoamine receptors have been implicated as key modulators of drug sensitization. 5-HT_{2A} receptors, which are expressed in all monoaminergic brainstem nuclei as well as the hippocampus, amygdala, striatum, and many cortical regions (Guiard and Di Giovanni, 2015), have been extensively studied. 5-HT_{2A} antagonists can prevent the development as well as expression of locomotor sensitization to psychostimulants, opiates, and even MDMA (Ago *et al*, 2008; Auclair *et al*, 2004; Filip *et al*, 2001; Lanteri *et al*, 2013). We found that pretreatment with the selective 5-HT_{2A} antagonist M100, completely blocked the development of social sensitization in our mice. However, M100 did not attenuate social interaction in mice that were already sensitized, indicating that it does not affect the expression of social sensitization. Repeated prior treatments with the 5-HT_{2A} agonist DOI have previously been demonstrated to enhance the locomotor stimulant effects of MDMA (Ross *et al*, 2006), indicating that 5-HT_{2A} activation is sufficient for the development of locomotor sensitization to MDMA. Here we demonstrated that such activation does not induce social sensitization, as mice previously treated with DOI did not have increased social behavior when treated with MDMA. Thus, while 5-HT_{2A} activation may be sufficient to induce locomotor sensitization, it is not sufficient for social sensitization, suggesting that other MDMA effects are also necessary.

We also investigated the role that 5-HT_{2C} receptors might play in mediating social sensitization. These receptors are widely distributed throughout the brain, with high expression in the PFC, VTA, amygdala, and striatum (Nocjar *et al*, 2015; Pompeiano *et al*, 1994). And like 5-HT_{2A} receptors, they play a critical role in mediating locomotor sensitization. Both receptors likely regulate sensitization through the same mechanism but in an opposing manner. Whereas 5-HT_{2A} receptors stimulate DA neurotransmission, 5-HT_{2C} receptors suppress DA neurotransmission. This opposing functionality is likely due to the relative cellular localizations

of the two receptors (Howell and Cunningham, 2015). 5-HT_{2A} receptors are expressed more predominantly on glutamatergic projection neurons and DA VTA neurons, so agonists like DOI increase DA cell firing and release, and antagonist like M100 suppress firing (Bortolozzi *et al*, 2005). 5-HT_{2C} receptors, though, are expressed preferentially on inhibitory interneurons rather than on DA neurons in the substantia nigra or VTA (Eberle-Wang *et al*, 1997), and thus their activation by an agonist like WAY reduces DA neuronal firing (Gobert *et al*, 2000). Given that augmented DA release is the causative factor believed to drive behavioral sensitization to most drugs (Cador *et al*, 1995), 5-HT_{2A} antagonists and 5-HT_{2C} agonists both likely block locomotor sensitization by suppressing DA neurotransmission. Indeed much like 5-HT_{2A} antagonists, 5-HT_{2C} agonists prevent the development of locomotor sensitization to drugs of abuse such as amphetamine and heroin (Lanteri *et al*, 2008; Wu *et al*, 2015). Yet in the present study, we observed that a 5-HT_{2C} agonist had no effect on the development of social sensitization. This additional discrepancy from the canonical behavioral sensitization literature, further suggests that social sensitization emerges via distinct mechanisms and is not a product of enhanced DA release, thus raising the question of why a 5-HT_{2A} antagonist prevented its development.

One possible explanation is that social sensitization requires associative learning. 5-HT_{2A} agonists facilitate associative learning, whereas inverse agonists like M100 impair learning (Boulougouris *et al*, 2008; Harvey, 2003a; Vanover *et al*, 2006). An initial hypothesis to explain the lack of prosocial effects elicited by MDMA on day 1 was that mice may need to gain familiarity with either the testing procedure or the drug itself. Non-contingently administered MDMA is known to be anxiogenic in mice (Navarro and Maldonado, 2002), which could conceivably obscure the social effects of the drug initially, but progressively less as mice gained experience with the procedure and treatment. However, while this might play a role, familiarity with the task and/or MDMA was not sufficient to increase social behavior. Mice given saline injections and paired repeatedly for social interaction testing, displayed no more social behavior

when given MDMA than did mice that were entirely naïve. And mice given MDMA multiple times without pairing, similarly showed no more social behavior when eventually paired than did naïve mice. Thus, the critical factor in social sensitization was the repeated social pairing of MDMA treated mice. While contextual associations between where a drug is first administered and where it is later tested is important in mediating the magnitude of behavioral sensitization (Ball *et al*, 2011; Varela *et al*, 2011), the complete lack of sensitization in these animals suggests that social pairing is more than just a context clue. It seems more likely that social interaction itself is necessary for the development of social sensitization to MDMA.

Social interaction is a natural reward that stimulates the mesolimbic system, and social bonds have been described in similar terms to drug addiction (Insel, 2003). Oxytocin has been proposed as the key neural signal than links social stimuli to the mesolimbic dopamine system and increases their salience (Ross and Young, 2009). MDMA appears to affect social behavior, at least in part, by enhancing social reward (Ramos *et al*, 2015). Interestingly, MDMA increases activation of many brain areas involved in reward (e.g. NAcc and VTA) and social behavior (e.g. medial preoptic area and central amygdala), but only when administered in a social context (Thompson *et al*, 2009). Mice will preferentially investigate social stimuli over non-social stimuli and this preference can be entirely blocked with administration of an oxytocin receptor antagonist (Lukas *et al*, 2011). And much like MDMA, exogenously administered oxytocin increases social interaction in rats (Ramos *et al*, 2013). Unsurprisingly, oxytocin has been proposed as a critical mediator of MDMA's prosocial effects (Thompson *et al*, 2007). MDMA increases oxytocin release (Dumont *et al*, 2009), likely via 5-HT stimulation of the paraventricular nucleus of the hypothalamus (PVN) (Jørgensen *et al*, 2003; Van de Kar *et al*, 1995).

Intriguingly, oxytocin mediates social reward and produces long-lasting synaptic plasticity in the NAcc in a 5-HT dependent manner (Dölen *et al*, 2013) that is analogous to the DA dependent plasticity that produces behavioral sensitization to drugs of abuse like cocaine

(Brebner *et al*, 2005; Thomas *et al*, 2001). Both phenomena may produce incentive sensitization, or an increased motivation for cues associated with the progenitor of the plasticity (Robinson and Berridge, 2008). In the case of MDMA, this means an increased motivation for social interaction. Therefore, social sensitization may be a process akin to reinforcement learning. MDMA makes social interaction more reinforcing, and just as mice will learn to press a lever for cocaine, they "learn" to engage in more social behavior. To what extent this learning might be associative versus non-associative is unclear, but this would be an interesting question to assess in future studies given the observed role of 5-HT_{2A} receptors in this effect.

Alterations in synaptic plasticity and other neurobiological changes are likely to underlie the development and long-term persistence of social sensitization to MDMA. It is possible that these same mechanisms also explain why MDMA has been demonstrated to have extremely long-lasting therapeutic effects (Mithoefer *et al*, 2013). MDMA administration not only produces acute increases in 5-HT and oxytocin, but may also produce long term changes in the regulation of these systems. MDMA increases transcription of 5-HT_{1B} receptor and oxytocin mRNA (Kindlundh-Högberg *et al*, 2006; van Nieuwenhuijzen *et al*, 2010). Increased expression of these could have profound effects on social behavior. The role of oxytocin in social behavior has been discussed above, but 5-HT_{1B} receptors are also critical mediators of social interaction and reward and their dysfunction may be involved in autism (Dölen *et al*, 2013; Orabona *et al*, 2009; Saudou *et al*, 1994). Future studies should investigate what kinds of long term neurobiological changes occur following MDMA and what role a social context plays in these effects. Acutely in humans, MDMA clearly increases the incentive and salience for social stimuli and interaction (Kamilar-Britt and Bedi, 2015). How these effects change overtime with repeated MDMA treatments and whether these effects influence off-drug behavior is still to be determined.

Lastly, a discussion of sensitization would not be complete without also discussing tolerance. With repeated administration of many drugs, sensitization develops to some effects and

tolerance develops to others. The species, dose, and frequency all influence which of the two occurs. Clearly in the present case of MDMA-induced social behavior, sensitization is occurring. However, if continued or larger treatments had been given there is evidence to suggest that tolerance would instead develop. Rats given repeated binge doses of MDMA have decreased baseline social interaction, and a blunted prosocial response to subsequent MDMA treatments (Thompson *et al*, 2008). Even 12 weeks later, a higher dose of MDMA was required to produce the drug's normal prosocial effects in these subjects. This kind of "chronic tolerance" has been extensively described in recreational MDMA users (Parrott, 2005, 2013). After initial MDMA use, tolerance to the desired effects of the drug develops, which may be one reason why many users eventually stop taking the drug. But there are indications that a subset of users not only escalate the dose they consume, but also increase their frequency of use. Some of these users even meet the DSM criteria for substance abuse and/or dependence (Cottler *et al*, 2001; Leung and Cottler, 2008). Tolerance to MDMA likely develops due to the chronic depletion of 5-HT associated with MDMA neurotoxicity (Baumann *et al*, 2008; Jones *et al*, 2010). Not only does this likely reduce many of the desired effects of MDMA, including the social effects, but it also may increase the addictive potential of MDMA (Ball and Slane, 2014). It is hypothesized that a loss of 5-HT produces a disinhibition of MDMA-induced DA release, making it a more efficacious reinforcer (Schenk, 2009).

So, while sensitization may indicate something about the therapeutic efficacy of MDMA, neurotoxicity and concomitant tolerance should also be taken into consideration when considering therapeutic use of the drug. Although profound and exciting gains may be achieved with the first treatment, it is not yet clear how often these treatments will need to be repeated. It is conceivable that the magnitude of the therapeutic effects could diminish with subsequent uses, requiring progressively more frequent treatments at higher and higher doses (Parrott, 2013, 2014b). This is one more compelling reason to develop new treatments that have the same

prosocial and therapeutic effects as MDMA but without its neurotoxicity and other adverse effects.

E. Figures.

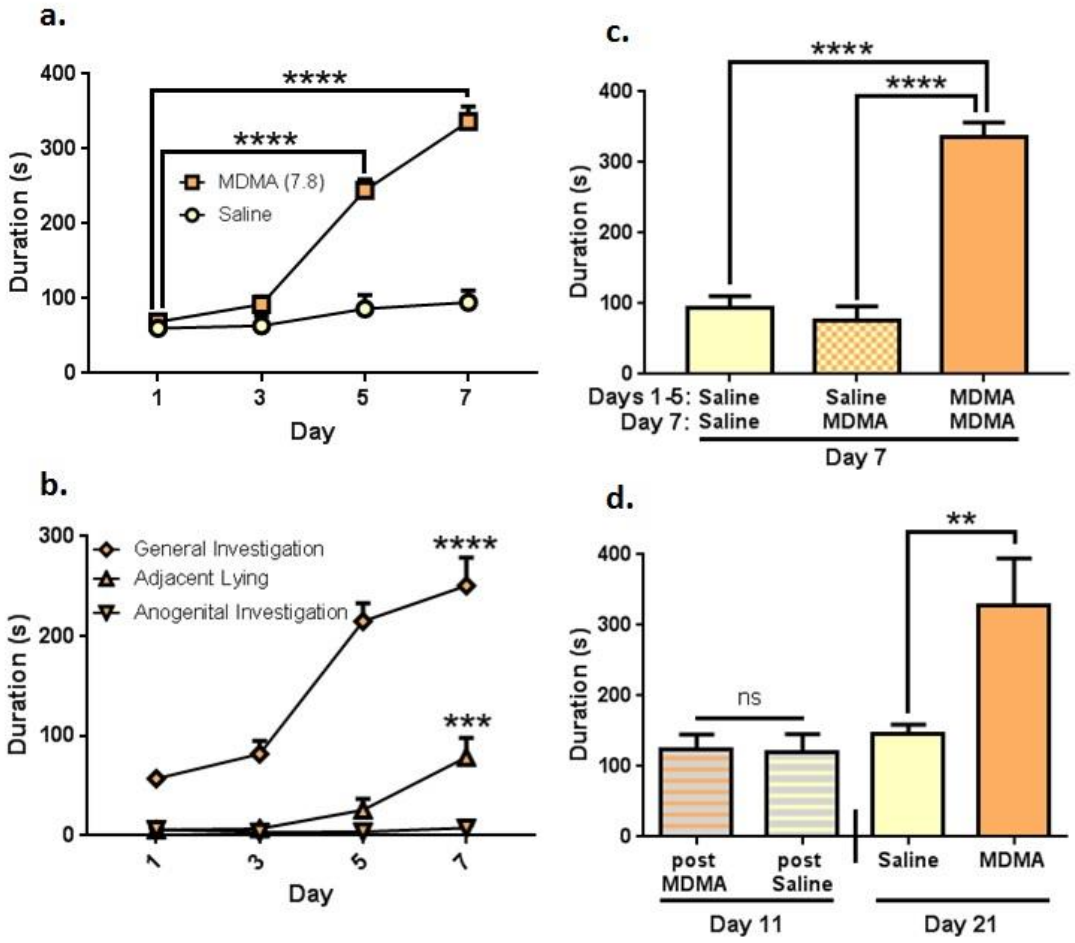

Figure 2.1 Mice sensitize to the prosocial effects of MDMA. Mice were treated every-other day with MDMA or saline and paired with a novel conspecific for a 10-minute social interaction test. The duration of total social interaction is displayed unless otherwise specified. **(a)** When administered on day 1, MDMA did not increase social interaction compared to saline. However, across subsequent treatments the duration of social interaction increased in MDMA treated mice, but not in saline treated mice. On days 5 and 7, MDMA treated mice interacted significantly more than they had on day 1 (****$p < 0.0001$). **(b)** Total social interaction is the accumulated duration of three specific social behaviors: general investigation (mostly nose-to-nose sniffing, allogrooming, and close following), adjacent lying, and anogenital investigation. The amount of general investigation and adjacent lying increased across subsequent treatments of MDMA. On day 7 MDMA treated mice spent more time engaging in general investigation (****$p < 0.0001$)

and adjacent lying (***p = 0.0008) than they did on day 1. **(c)** The increased social interaction produced by MDMA enhanced across subsequent treatments due to familiarity with the testing procedure. Mice given MDMA for the 4th time engaged in significantly more social interaction than mice treated with saline for the 4th time or mice with prior saline treatments that were given MDMA for the first time (****p < 0.0001), indicating that the development of this sensitization was dependent on MDMA. **(d)** Sensitization of MDMA-induced social interaction did not affect baseline social interaction when animals were paired with a novel conspecific without drug treatment, but it was long-lasting and when treated again with MDMA 2 weeks later mice displayed significantly more social interaction that saline treated mice (**p = 0.0062).

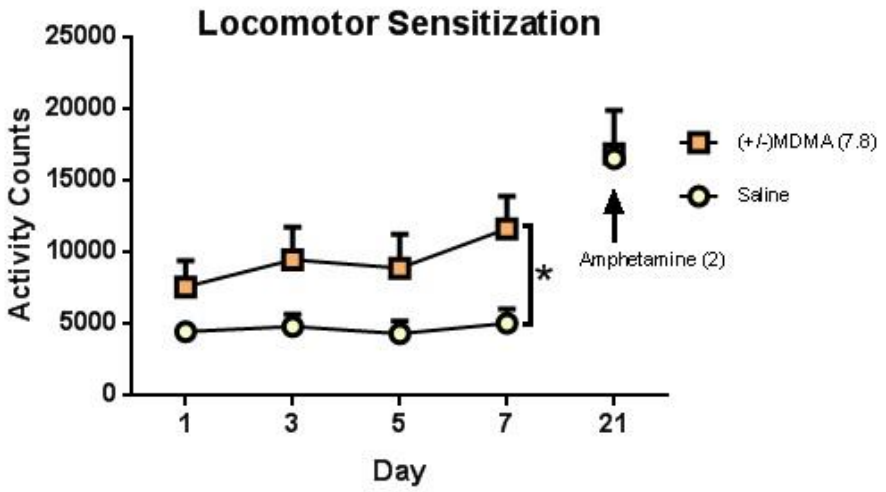

Figure 2.2 Limited locomotor sensitization and no cross-sensitization. This MDMA treatment regimen produced only limited evidence of locomotor sensitization and did not produce cross-tolerence with the locomotor stimulating effect of amphetamine. MDMA increased locomotor activity in mice compared to saline, but this effect was only significant after the 4th MDMA treatment (*p = 0.0296).

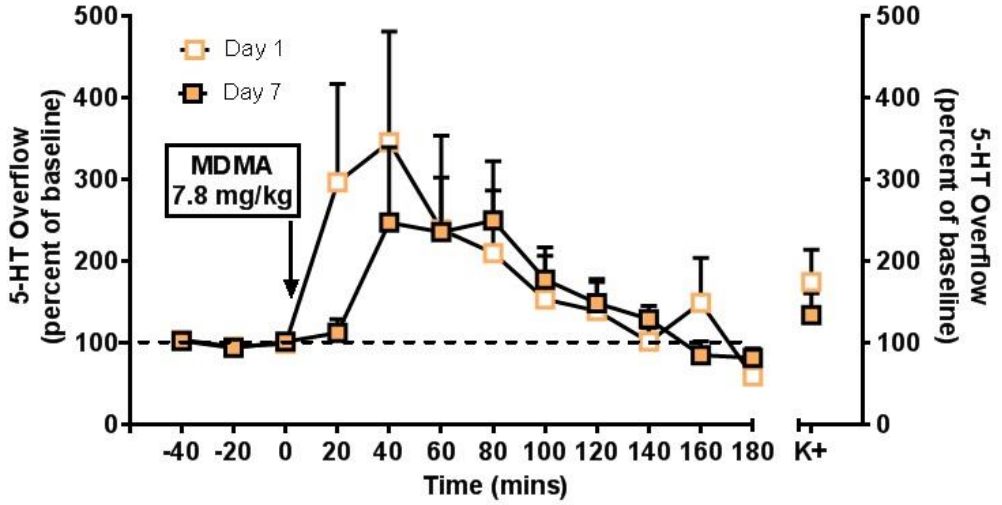

Figure 2.3 MDMA-induced 5-HT release did not sensitize. 5-HT release was measured in the nucleus accumbens of mice recieving MDMA for the first time and then again during their 4th treatment on day 7. MDMA-induced release of 5-HT was not significantly higher or lower on day 7 compared to day 1.

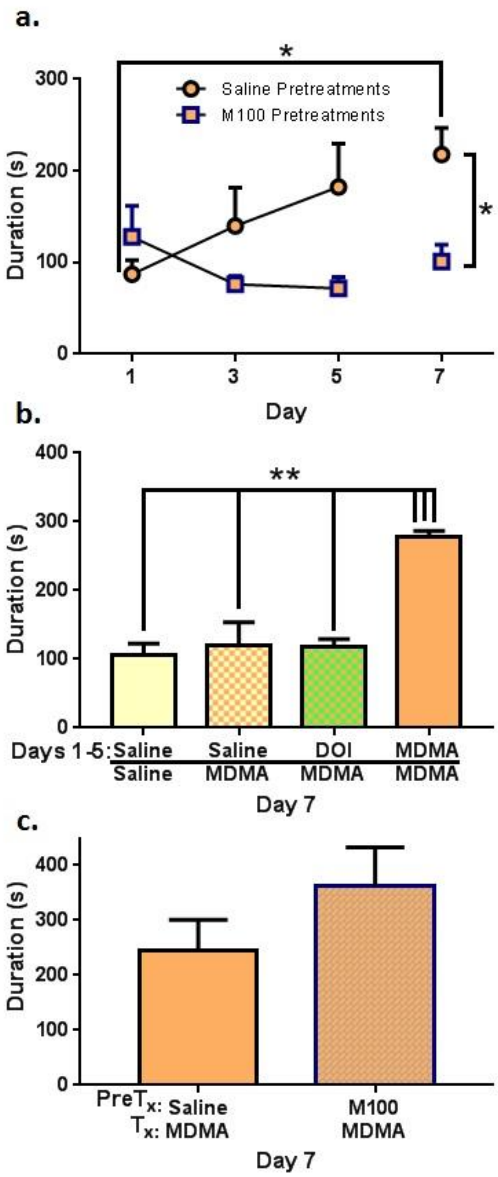

Figure 2.4 5-HT$_{2A}$ receptor activity is necessary but not sufficient for the development of social sensitization. (a) Duration of total social interaction in mice administered MDMA with pretreatments of saline or the 5-HT$_{2A}$ antagonist M100. On day 7 MDMA was given alone with no pretreatments. Mice that had receieved saline pretreatments displayed significantly more social interaction than they had on day 1(*p = 0.0242) and significantly more than mice that had received M100 pretreatments (*p = 0.0358). **(b)** Treatment with the 5-HT$_{2A}$ agonist DOI every other day over 5 days did not sesntize mice to MDMA-induced social ineraction when it was administered on day 7. Mice with prior MDMA treatments enganged in significatnly more social interaction that mice with prior DOI (**p = 0.0018) or saline (**p = 0.0021) treatments, and significantly more than mice receiving a 4th saline treatment (**p = 0.001). **(c)** Pretreatment with M100 did not attenuate the expression of sensitization or decrease MDMA-induced social interaction when administered to Mice with 3 prior MDMA treatments.

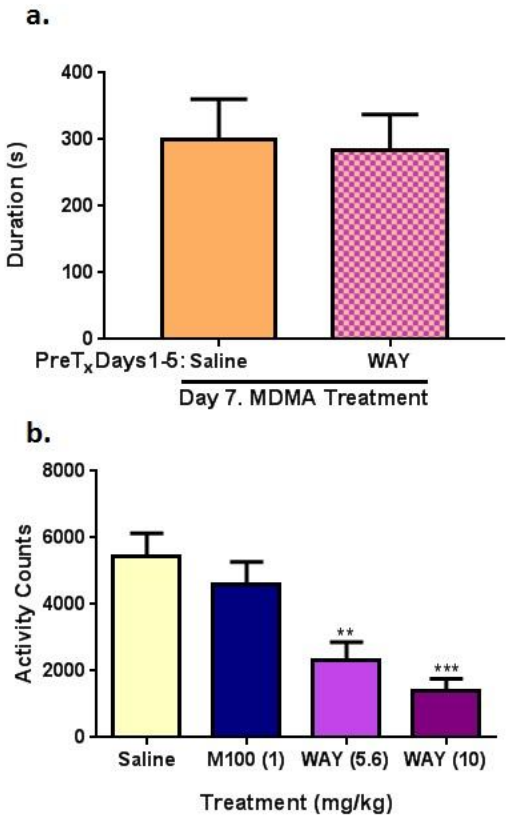

Figure 2.5 A 5-HT_{2C} agonist does not prevent social sensitization. (a) Pretreatment with the 5-HT_{2C} agonist WAY before MDMA treatment every other day did not diminish MDMA-induced social interaction when it was given alone on day 7. (b) WAY, at 5.6 mg/kg and 10 mg/kg, decreased locomotor activity compared to saline treated mice (**p = 0.0037 and ***p = 0.0004, respectively). M100, at 1 mg/kg, did not affect locomotor activity.

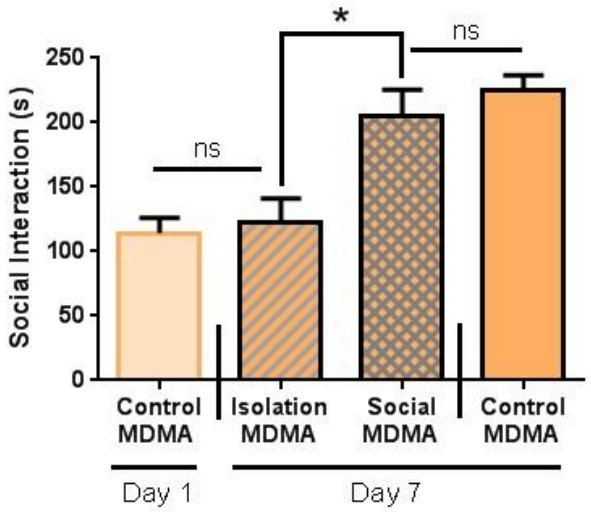

Figure 2.6 Social interaction is necessary for the development of social sensitization. Control mice treated with MDMA and paired for a social interaction test every other day sensitize to the prosocial effects of MDMA. Mice treated with the same regimen of MDMA but isolated during treatments 1-3 did not display sensitized social interaction when social interaction was tested on day 7. Mice that were paired for 2 hours after treatment displayed normal social interaction in the novel social interaction test and interacted significantly more than mice that had been isolated (*p = 0.0076).

Chapter 3. Stereoselectivity of MDMA-induced prosocial behavior

A. Introduction

The U.S. is facing a growing epidemic of mental health disorders. For autism alone, the CDC recently announced that 1 in 68 children in the U.S. are identified as autistic (Report, 2014). Yet, there are relatively few treatment options available for patients with such disorders. While behavioral therapy can often be helpful, it is expensive and often takes many months or years to be effective. Anecdotal reports from the 1980's suggest that using MDMA as an adjunct to traditional therapy can both quicken and significantly increase its effectiveness. MDMA is known to increase feelings of closeness towards others, empathy, and sociability (Bedi *et al*, 2010b; Hysek *et al*, 2013) and was reported to have often been a "breakthrough" for patients that had previously been resistant to treatment (Greer and Tolbert, 1986). These reports and its unique prosocial effects have led to a resurgence of interest in the drug's therapeutic potential and several double-blind placebo controlled studies have recently been completed and more are underway.

The first Phase II clinical trial of MDMA investigated its efficacy as an adjunctive treatment with psychotherapy for patients suffering from post-traumatic stress disorder (PTSD) (Mithoefer *et al*, 2011). Patients were randomized to receive MDMA or placebo during 2 sessions in addition to 11 non-drug treatment sessions. The primary outcome measure was the Clinician-Administered PTSD Scale (CAPS), which was given to patients 4 days after each drug treatment and during a 2-month follow-up. After the first MDMA treatment, patients' CAPS scores improved by an average of 41.4 points compared to placebo treated patients who improved by only 5.5 points. At the two month follow up, 80% of MDMA treated patients no longer met the diagnostic criteria for PTSD. At a later follow-up, given 17-74 months later, the gains achieved

had generally persisted with 87.5% of MDMA treated patients no longer meeting the diagnostic criteria for PTSD (Mithoefer *et al*, 2013).

These huge therapeutic gains ignited significant interest in MDMA (Amoroso and Workman, 2016) and led to the initiation of many additional clinical trials for PTSD as well as for other conditions, including autism spectrum disorder (Yazar-Klosinski *et al*, 2016). But despite the significant efficacy that MDMA may have as a treatment for certain mental health conditions, it is unlikely that it will see widespread clinical use because of the significant adverse effects associated with its use including neurotoxicity and associated psychiatric problems (Parrott, 2014b). There is thus significant impetus to better understand the pharmacological mechanisms that are responsible for MDMA's therapeutic benefits so that these mechanisms can be isolated and new therapeutics can be developed that have similar prosocial and therapeutic effects with fewer dangerous adverse effects.

MDMA's primary mechanism of action is the reversal of monoamine reuptake at presynaptic terminals that leads to substantial increases in the extracellular concentrations of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) (Green *et al*, 2003). The role of these monoamine neurotransmitters in mediating the psychological and physiological effects of MDMA has been investigated in human studies using various transport inhibitors or receptor antagonists. These studies have highlighted a significant role for 5-HT and supporting roles for DA, NE, and 5-HT_{2A} receptors. MDMA, however, has many additional targets and downstream effects that have just begun to be investigated. MDMA releases a variety of hormones that may be key mediators of the drug's effects. Cortisol and associated HPA hormones may mediate the feelings of increased energy and stimulation produced by MDMA (Parrott, 2009). And prolactin, vasopressin, and oxytocin, which are all modulators of social behavior, have been suggested as effectors of the drug's prosocial effects (Emanuele *et al*, 2006; Passie *et al*, 2005). Oxytocin, in particular, has generated interest because it has some prosocial effects in humans (Kirkpatrick *et*

al, 2014c) and increases social interaction in rats (Ramos *et al*, 2013). MDMA increases oxytocin release (Dumont *et al*, 2009; Kirkpatrick *et al*, 2014a) and oxytocin antagonists attenuate some of the social effects of MDMA in rats and mice (Kuteykin-teplyakov and Maldonado, 2014; Thompson *et al*, 2007).

It is likely that many of MDMA's pharmacological effects interact to create the rather unique prosocial effects of the drug, and potentially also mediate its therapeutic effects. To better understand which pharmacological mechanisms are important, we investigated the stereoisomers of MDMA. MDMA is a racemic mixture of two functionally distinct enantiomers. Both enantiomers act as substrate-type monoamine releasers and increase the concentration of extracellular monoamines (Johnson *et al*, 1986; Murnane *et al*, 2010; Setola *et al*, 2003) via a carrier-mediated exchange mechanism (Rothman and Baumann, 2002). But there are significant differences in their potency and selectivity. The right-handed enantiomer, (+)-MDMA, is a fairly nonselective monoamine releaser that increases synaptic 5-HT, NE, and DA with similar potency (74 nM, 136 nM, and 142 nM, respectively; EC_{50} of tritiated monoamine release from pre-loaded rat synaptosomes) (Setola *et al*, 2003). The left-handed enantiomer, (–)-MDMA, is substantially less potent as a monoamine releaser and is more selective, increasing 5-HT, NE, and DA at EC_{50} values of 340 nM, 560 nM, and 3700 nM, respectively. Due to its high potency as a monoamine releaser, (+)-MDMA has long been considered the "active isomer" of MDMA (Anderson *et al*, 1978). However, (–)-MDMA has higher affinity as a direct agonist at many transmembrane receptors including 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2B} receptors (Huot *et al*, 2011; Lyon *et al*, 1986; Nash *et al*, 1994; Setola *et al*, 2003). Racemic MDMA, which herein is denoted as (+/–)-MDMA or simply MDMA, is a 50/50 mixture of these two enantiomers.

The relative contribution of each enantiomer to the prosocial effects of MDMA has not been previously investigated. To determine if the enantiomers have comparable effects to racemic MDMA, we utilized an abbreviated version of the social interaction procedure demonstrated in

Chapter 2 and a locomotor activity assessment. Given that the neuropeptide oxytocin has been proposed as an important mediator of MDMA's prosocial effects, we investigated the activation of oxytocinergic neurons by MDMA and its enantiomers. To further clarify the role of oxytocin in MDMA-induced social interaction, a group of mice was pretreated with a selective oxytocin receptor antagonist prior to MDMA and social interaction testing. Together these studies demonstrate the stereoselectivity of certain MDMA-induced behaviors, the substantial therapeutic potential of (–)-MDMA, and a partial role for oxytocin.

B. Methods

Subjects

Male Swiss Webster mice (Charles River Laboratories, Wilmington, MA) aged 7-10 weeks served as subjects in all experiments. Mice were housed five per cage in a temperature and humidity controlled colony room at the Yerkes National Primate Research Center with food and water available *ad libitum*. Lights were set to a 14-hour light/dark cycle. All experiments were performed at an ambient temperature of 22±2°C, during the lights-on phase. All studies were carried out in accordance with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Experimental protocols were approved by the Emory University Institutional Animal Care and Use Committee.

Drugs

(+/–)-MDMA, (–)-MDMA, and (+)-MDMA were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC). L-368,899 (OTA) was acquired from Tocris. Doses were calculated and are expressed as HCl salts. All drugs were dissolved in 0.9% sterile saline and administered via intraperitoneal injection at a volume of 10 ml/kg.

Social Interaction Dose-Effect Curves

Dose-effect studies were performed to determine the prosocial effects of MDMA and its enantiomers and the optimal dosages to increase the duration of social interaction in mice. Preliminary experiments indicated that MDMA did not affect social interaction when administered to mice for the first time. Mice were therefore treated twice. During the first session mice (10 per treatment group) were injected with (+/–)-MDMA, (–)-MDMA, (+)-MDMA, or saline and isolated for 30 minutes before being paired with an unfamiliar weight-matched conspecific that received the same treatment. Pairing took place in a 30 x 18 cm clear Plexiglas testing chamber for 10 minutes. 48 hours later, these procedures were repeated with the same mice, pairs, and treatments. While in the testing arena, subjects were free to move around and interact, allowing a diverse range of observable behaviors. Several saline treated pairs had to be separated and removed from evaluation because of sustained fighting. No fighting was observed in the other treatment groups. Tests were videotaped and the experimental sessions were scored using JWatcher or BORIS (Friard and Gamba, 2016) by an observer blind to the experimental conditions. The durations of 3 behaviors were scored: anogenital investigation (sniffing the conspecific's anogenital area), general investigation (non-anogenital sniffing, grooming, and following the conspecific), and adjacent lying (side-by-side contact or huddling, excluding climbing under/over the conspecific) (Morley *et al*, 2005). These behaviors were averaged for each pair and then summed to produce a total social interaction score, upon which statistical analysis was performed.

Locomotor Behavior

Drug effects on locomotor activity were tested in 45 x 39 x 37 cm open field chambers with 16 x 16 photocells positioned 2.5 cm off the chamber floor (San Diego Instruments, San Diego, CA). Operation of the chambers and data collection was done by an interfaced computer. Mice (13 per treatment group) were treated with (–/+)-MDMA, (–)-MDMA, (+)-MDMA, or saline immediately before being placed into the chambers for 1 hour. Testing was performed in a dark, enclosed space. Accumulative beam breaks of adjacent photocells were recorded as the

measure of locomotor activity. Activity plots were generated in MATLAB (MathWorks, Natick, MA) using the 32 photocells to produce a 256 pixel heatmap of beam breaks by each subject.

Immunofluorescence

Subjects were administered an i.p. injection of (–/+)-MDMA, (–)-MDMA, (+)-MDMA, or saline (4-5 mice per treatment) 85 minutes before transcardial perfusion with 4% formaldehyde. Prior to perfusion, subjects were deeply anesthetized with 150 mg/kg sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI). Their brains were removed and post fixed for 24 hours in the same formaldehyde solution. Brains were immersed in 15% and 30% sucrose solutions for two consecutive days and then frozen in chilled methyl butane, sectioned at 35 µm, and stored at -20°C. Tissue sections of the PVN from each subject were washed in PBS and endogenous peroxidase activity was blocked with 3% H_2O_2 in a 10% methanol PBS solution for 15 minutes and then blocked with a solution of 2% normal donkey serum, 5% bovine serum albumin, and 0.3% Triton X in PBS for 90 minutes. The sections were then incubated with a primary antibody against oxytocin (ab2078, Abcam, Cambridge, UK; 1:3000) diluted in blocking buffer for 72 hours at 4°C. They were then rinsed with PBS and incubated with a fluorescent secondary antibody (Alexa Fluor 488 (A21206), ThermoFisher; 1:1000) at room temperature for 1 hour while protected from light. Sections were again washed with PBS and then blocked in the same blocking solution for 90 minutes before a second primary incubation with an anti-cFos antibody (sc-52-G, Santa Cruz Biotechnology, Dallas, TX; 1:400) overnight at 4°C. Finally, the sections were rinsed with PBS and incubated with a fluorescent secondary antibody (Alexa Fluor 594 (A11058), Life Technologies; 1:100) at room temperature for 1 hour and then mounted to slides with SlowFade mountant (ThermoFisher). 4-6 sections from each subject were photographed at 20x magnification and fluorescent-labeled oxytocin (OT+) and double-labeled (OT+cFos) neurons were counted manually by an observer blind to the experimental conditions. The percent activation was calculated by dividing the number of

OT+cFos cells by the number of OT+ cells. Tissue sections from one subject treated with (+/–)-MDMA were damaged during processing and were excluded from analysis.

Oxytocin Antagonist Test

To determine if oxytocin receptor activation is necessary for the prosocial effects of MDMA, mice were sensitized to MDMA with four 7.8 mg/kg treatments, each separated by 48 hours. After each treatment mice were paired with the same initially unfamiliar conspecific. One week later, on day 14, mice (6-8 per treatment group) were administered a pretreatment of 10 mg/kg OTA or saline 15 minutes prior to saline or MDMA. 25 minutes later mice were paired and social interaction was tested as described above.

Data Analysis

Data were analyzed with Prism 7 (Graphpad, La Jolla, CA). All experiments were analyzed using one-way between subjects ANOVAs with Dunnett's or Tukey's post-hoc tests. Alpha for all experiments was set at 5%. Error bars represent the standard error of the mean (SEM).

C. Results

Increased social interaction by MDMA and its enantiomers

The effects of MDMA and its enantiomers on murine social interaction were tested across a range of doses. (+/–)-MDMA treatment increased total social interaction, $F(3, 16) = 3.749$, $p = 0.0326$ (Figure 3.1a). A Dunnett's post-hoc test revealed that 7.8 mg/kg differed significantly from saline, 95% CI [15.01, 243.7], $p = 0.0255$. (–)-MDMA increased total social interaction with similar efficacy, $F(3, 16) = 3.317$, $p = 0.0468$ (Figure 3.1b), but was less potent. A Dunnett's post-hoc test revealed that 17 mg/kg differed significantly from saline, 95% CI [15.98, 274], $p = 0.0265$. (+)-MDMA did not significantly alter total social interaction, $F(4, 20) = 1.194$, $p = 0.344$, but there was a trend towards significance at the 7.8 mg/kg dose (Figure 3.1c).

To increase statistical power, a Student's t-test was performed comparing this dose to saline, but there was still not a significant difference, $t(8)=2.116$, $p = 0.0672$.

Locomotor activity following treatment with MDMA or its enantiomers

The locomotor stimulant effects of (+/–)-MDMA, (–)-MDMA, and (+)-MDMA were tested at the doses found to produce peak prosocial effects (Figure 3.2a). There was an effect of treatment on the quantity of horizontal beam breaks, $F(3, 48) = 15.56$, $p < 0.0001$ (Figure 3.2b). A Dunnett's post-hoc test revealed that (+/–)-MDMA and (+)-MDMA significantly increased locomotor activity relative to saline, 95% CIs [58.37, 8121], [5283, 13345] and $p = 0.0461$, $p < 0.0001$, respectively). Activity in both treatment groups was concentrated around the periphery of the open field. (–)-MDMA did not affect locomotor activity relative to saline, $p = 0.9299$.

Activation of oxytocinergic neurons

Activation of oxytocinergic neurons was assessed in the PVN after treatment with (+/–)-MDMA, (–)-MDMA, (+)-MDMA or saline by measuring the percent of oxytocinergic neurons that co-expressed cFos (Figure 3.3a). There was a significant effect of treatment on the percent of $cFos^+$ oxytocin cells, $F(3, 15) = 4.577$, $p = 0.0182$ (Figure 3.3b). A Dunnett's post-hoc test indicated that the (+/–)-MDMA and (–)-MDMA treated groups differed significantly from saline, 95% CIs [2.285, 42.18], [5.466, 43.08] and $p = 0.028$, $p = 0.0112$, respectively, while the (+)-MDMA treated group was not significantly different from saline, $p = 0.2203$.

Effect of an oxytocin receptor antagonist on MDMA-induced social interaction

To determine if oxytocin receptor activation was necessary for the prosocial effects of MDMA, mice were pretreated with 10 mg/kg OTA or saline (Figure 3.4). There was a significant effect of treatment on the duration of total social interaction, $F(3,16)=5.934$, $p = 0.0064$. Tukey's post-hoc test revealed that, as expected, MDMA increased social interaction relative to saline,

95% CI [15.69, 300.7], p = 0.0270. OTA pretreatment did not decrease baseline social interaction in saline treated mice, p = 0.9933. OTA pretreatment did not significantly attenuate the duration of social interaction in MDMA treated mice, p = 0.4932, but OTA+MDMA mice also did not have significantly higher social interaction than saline treated mice, p = 0.2157. Social interaction in these subjects therefore appears to be intermediary to saline treated and MDMA treated mice.

D. Discussion

The prosocial effects of MDMA have been extensively studied in humans. It increases feelings of closeness towards others, empathy, and gregariousness (Bedi *et al*, 2010b; Dumont *et al*, 2009; Hysek *et al*, 2013). Recently, behavioral and cognitive tests have been used to probe these effects and have confirmed that these self-reported feelings do generally reflect quantifiable increases in emotional empathy, trust, and other prosocial measures (Kamilar-Britt and Bedi, 2015). MDMA appears to have similar prosocial effects in non-human animals. MDMA increases allogrooming in long-tailed macaques (Ballesta *et al*, 2016), social interaction in rats (Morley and McGregor, 2000), and a preference for social contexts in mice (Kuteykin-teplyakov and Maldonado, 2014). However, the prosocial effects of MDMA's individual enantiomers have not been previously investigated. While the only structural distinction between the two is the orientation of the methyl group affixed to the alpha-carbon (see Figure 1.1), the two are functionally very different.

As described in Chapter 2, we developed an MDMA treatment protocol that produces robust increases in murine social interaction. Using an abbreviated version of this protocol we investigated the dose-effect relationships of MDMA and its enantiomers on social interaction behaviors in mice. The peak effective dose of (+/–)-MDMA was 7.8 mg/kg, which corresponds to approximately a 75-mg dose in an average person after accounting for body size using the interspecies scaling equation, $D_{human} = D_{animal} (W_{human}/W_{animal})^{0.7}$ where D is dose and W is weight. Although the accuracy of such scaling has been questioned (Vollenweider *et al*, 2001), a 75 mg

dose is within the range commonly used recreationally and in clinical studies, suggesting that the prosocial effects of MDMA occur at an equivalent dose in both humans and mice. The dose-effect curve generated for (+/–)-MDMA formed a pronounced inverted-V shape, with no other dose significantly altering social interaction. The narrowness of this curve, could explain why some previous studies with mice did not observe prosocial effects (Daza-Losada *et al*, 2009; Navarro and Maldonado, 1999). 3, 5, and 10 mg/kg are frequently tested MDMA doses in mice, and all would have missed the effective dose range.

A similar dose-effect curve was observed with (–)-MDMA. Its peak effective dose was 17 mg/kg, with no other dose producing significant effects. Although less potent, this dose increased total social interaction with equivalent efficacy to racemic MDMA. In contrast, (+)-MDMA had a much flatter dose-effect curve and no dose had a statistically significant effect on social interaction. Although (+)-MDMA treatment did not meet the predefined level of significance (α) to reject the null hypothesis, subjectively it appears likely that this enantiomer also increased social interaction in a dose-dependent manner. Although no previous study has examined the prosocial effects of (+)-MDMA, it is unlikely that it would have no effect on social interaction given that racemic MDMA has such robust effects. Rather than outright acceptance of the null hypothesis, the more appropriate conclusion may be that (+)-MDMA increases social interaction but to a lesser degree or with greater variance than racemic MDMA and (–)-MDMA. A comparison of the mean difference and standard error of their peak effective doses relative to saline is informative. (+/–)-MDMA and (–)-MDMA increased the duration of social interaction by 129.4 ± 44.11 and 145 ± 49.76 seconds respectively, whereas the peak "effective" dose of (+)-MDMA, 7.8 mg/kg, increased the duration of social interaction by 94.73 ± 61.84 seconds. The only way to clarify the effect of (+)-MDMA on social interaction and its effect relative to (+/–)- and (–)-MDMA is with additional study and a larger sample size, but the current data suggests

that (+)-MDMA may also produce behaviorally relevant increases in social interaction, but to a lesser extent than (+/–)-MDMA or (–)-MDMA.

The specific social behaviors increased by each treatment differed considerably. Both (+)-MDMA and (+/–)-MDMA rather selectively increased general investigation behaviors, which were predominantly nose-to-nose sniffing and non-aggressive following, while (–)-MDMA preferentially increased adjacent lying. Adjacent lying is one of the primary behavioral effects of MDMA in rats (Morley *et al*, 2005) and similar MDMA-induced behaviors are observed in other species including humans who may form a "cuddle puddle", i.e. a group of people cuddling while under the influence of MDMA (Leneghan, 2013). One potential explanation for the different behavioral effects observed is that locomotor activity was significantly higher in (+/–)-MDMA treated and especially in (+)-MDMA treated mice compared to saline, whereas (–)-MDMA had no locomotor stimulant effects. Hyperactivity in (+/–)-MDMA treated mice might mask the more sedentary social behaviors, and perhaps all social behavior in (+)-MDMA treated mice. Indeed, MDMA taken by humans promotes all night dance parties and has clear stimulant effects, but can also produce the aforementioned cuddling (Adamson and Metzner, 1988). Mice may have less behavioral control over these competing effects, and the stimulant effects of the drug may dominate. Interestingly though, in Chapter 2 we observed that adjacent lying behavior increased significantly after additional MDMA treatments. Therefore, although the behavioral effects of (+/–)-MDMA and (–)-MDMA are not identical, the social interaction increased by (–)-MDMA is still very MDMA-like, and quite likely indicates that this enantiomer will have similar prosocial effects in humans.

To my knowledge, there are only two published works that describe the effects of the enantiomers in humans (Anderson *et al*, 1978; Shulgin and Shulgin, 1991). Both focused on the intoxicating properties of the drugs and did not mention social effects. (+/–)-MDMA and (+)-MDMA both produced significant intoxication and physical side effects such as bruxism and

mydriasis, however (–)-MDMA had none of these effects. The authors concluded that with the exception of enhanced color perception reported by 2 participants, (–)-MDMA was "otherwise ineffective" even at the highest dose tested, 200mg (Anderson *et al*, 1978). This raises the potentially very significant possibility that, as we have observed, (–)-MDMA produces robust prosocial effects without any locomotor stimulant effects, intoxication, or other unwanted side effects associated with (+/–)-MDMA.

This is especially relevant given the current interest in using MDMA as a therapeutic adjunct to facilitate psychotherapy and help to treat a variety of mental health conditions. Given the strong prosocial effects of MDMA, it has unsurprisingly drawn interest as a treatment for social dysfunction and social anxiety. One of the earliest clinical studies of MDMA found that it increased self-reported interpersonal functioning for at least a week after treatment (Liester *et al*, 1992). A double-blind placebo controlled clinical trial, which has just concluded, found that MDMA paired with therapy significantly decreased social anxiety in adults with autism for at least 6 months after treatment (Danforth *et al*, 2016; Yazar-Klosinski *et al*, 2016). Another major area of clinical interest is using MDMA to treat PTSD. When paired with psychotherapy, just two or three MDMA treatment sessions significantly reduced PTSD symptoms for at least 17 months (Mithoefer *et al*, 2011, 2013). Several additional clinical trials are ongoing and the FDA recently approved larger Phase III clinical trials of MDMA (Philipps, 2016). While these clinical effects are very promising, the adverse side effects of MDMA will likely limit or preclude widespread clinical use of the drug. FDA approval is based not only on therapeutic efficacy but also on safety. MDMA is neurotoxic and can be potentially lethal even at low doses due to hyperthermia or cardiac dysrhythmia (Capela *et al*, 2009; Hall and Henry, 2006). If the prosocial and therapeutic effects of MDMA could be separated from these negative adverse effects, the result would be a substantially more viable therapeutic.

It is not presently clear why MDMA has proven to be such an effective therapeutic, but several possible explanations have been proposed (Mithoefer *et al*, 2016). One is that the prosocial effects of MDMA improve the "therapeutic alliance" between the patient and therapist. With increased trust, patients are less defensive and more willing to revisit and reflect openly on their traumatic memories (Grinspoon and Bakalar, 1986). Mithoefer, who conducted the first Phase II clinical trial of MDMA, observed that it made it possible for patients, who had often struggled with this for months or years, to finally revisit their traumas effectively. Another explanation is that MDMA promotes memory retrieval and reconsolidation of previously traumatic memories with new associations of safety. This second proposal is especially interesting given the use of MDMA for PTSD. PTSD is often conceptualized as a deficit in the extinction of fear conditioning, whereby cues associated with a traumatic memory continue to trigger a powerful fear response even when those cues no longer signal an actual threat (VanElzakker *et al*, 2014). If MDMA promotes extinction of these fear memories that would be a powerful mechanism of action. And indeed, a mouse model designed to test this found that treatment of fear-conditioned mice with (+/–)-MDMA prior to extinction training facilitated long-lasting extinction of conditioned fear (Young *et al*, 2015). A companion experiment to the present study was performed to test the effects of both (–)-MDMA and (+)-MDMA on this same test and is presented in Appendix A. (+)-MDMA had no effect, but (–)-MDMA significantly facilitated the extinction of conditioned fear at the same 17 mg/kg dose that increases social interaction. Thus, it seems that (–)-MDMA could have the same therapeutic efficacy as (+/–)-MDMA. The prosocial effects demonstrated herein indicate that (–)-MDMA would have similar benefits towards strengthening a therapeutic alliance, and this fear-extinction paradigm indicates comparable effects on learning and memory. Although other factors are likely to also be involved, these data strongly suggest that (–)-MDMA could have similar therapeutic capabilities without many of the adverse side effects of (+/–)-MDMA.

There are several key pharmacological differences between (+)-MDMA and (–)-MDMA that may explain the profound behavioral differences they produce. One of the major differences is their selectivity as monoamine releasers. (+)-MDMA increases levels of 5-HT, DA, and NE all with similar potency (Setola *et al*, 2003). In contrast, (–)-MDMA is much more selective as a 5-HT releaser, and does not increase DA levels at all (Hiramatsu and Cho, 1990; Murnane *et al*, 2010; Setola *et al*, 2003). Clinical studies have found that the majority of MDMA's subjective effects, including prosocial effects, can be blocked by pretreatment with an SSRI, which prevents the transporter-mediated release of serotonin (Liechti *et al*, 1998). Conversely, blocking dopamine signaling only decreased the euphoric and stimulatory effects, with no effect on other subjective measures (Liechti and Vollenweider, 2001). This suggests that MDMA's release of dopamine is of limited importance to the prosocial effects of MDMA, which appear to be driven to a large extent by 5-HT release. This likely explains why (–)-MDMA retained the prosocial effects of MDMA without the stimulant effects; since DA is generally necessary for the locomotor stimulant effects of drugs (French, 1986). A second key difference may explain the stereospecificity observed in these studies and in Appendix A. (–)-MDMA has much higher binding affinity than (+)-MDMA at several neuronal receptors. Of particular importance may be its affinity for 5-HT_{2A} receptors. These receptors are important mediators of associative learning and other 5-HT_{2A} agonists are known to enhance learning and facilitate fear extinction (Harvey, 2003b; Zhang and Stackman, 2015). Thus, activation of these receptors by (+/–)-MDMA and (–)-MDMA, but not (+)-MDMA, may explain their facilitative effect on fear extinction learning. Although 5-HT released by MDMA would seemingly also bind to these receptors, there is reason to believe that (–)-MDMA may activate and affect downstream signaling in ways distinct from endogenous serotonin. 5-HT_{2A} receptors display a high degree of functional selectivity whereby different agonists can have distinct effects at the receptor (González-Maeso *et al*, 2007). Functional selectivity at 5-HT_{2A} receptors has been proposed as the key to the entactogenic effects of MDMA (Ray, 2016).

Both monoamine release and 5-HT_{2A} binding are likely important mediators of the prosocial and therapeutic effects of MDMA, and may explain the different behavioral effects of (+)-MDMA and (–)-MDMA. Another key mediator may be oxytocin. Oxytocin is a major regulator of social behavior in mammals, with well-defined roles in maternal behavior, pair bonding, social learning, and consoling behavior (Burkett *et al*, 2016; Lee *et al*, 2009; Ross and Young, 2009). MDMA increases oxytocin release in humans as well as in rodents, and this has been suggested as a mechanism underlying the prosocial effects of MDMA (Dumont *et al*, 2009; Emanuele *et al*, 2006; Thompson *et al*, 2007). Oxytocin release is likely driven by MDMA-induced 5-HT release binding at 5-HT receptors, but direct agonism by MDMA at these receptors may also play a role (Bagdy, 1996; Jørgensen *et al*, 2003; Saydoff *et al*, 1991). Given its potential importance, we investigated the relative activation of oxytocineric neurons by MDMA and its enantiomers. Activation of oxytocinergic neurons was correlated with the duration of social interaction produced by MDMA and the enantiomers. Both (+/–)-MDMA and (–)-MDMA significantly increased oxytocin activation, but (+)-MDMA did not. These findings demonstrate again that (–)-MDMA has similar efficacy to racemic MDMA despite its lower potency. As with the social interaction test results, oxytocin activation is inconclusive in (+)-MDMA treated subjects, with the mean effect located intermediate to saline treated controls and (+/–)- or (–)-MDMA treated subjects. These findings further suggest that (+)-MDMA has sub-optimal social effects, and suggest that oxytocin may indeed have an important role in the prosocial effects of MDMA.

To clarify such a role for oxytocin, we evaluated whether a selective oxytocin receptor antagonist would block the prosocial effects of MDMA in our social interaction test. OTA decreased social interaction in MDMA treated mice but this effect was not statistically significant. Previous studies examining the role of oxytocin in MDMA-induced social behavior have yielded conflicting results. The first study to administer an oxytocin antagonist prior to

MDMA found that it blocked MDMA-induced social behavior in rats (Thompson *et al*, 2007). However, the antagonist used, tocinoic acid, is not highly selective. A follow up study from the same group found that vasopressin V1a receptors, rather than oxytocin receptors, appeared to mediate the prosocial effects of MDMA (Ramos *et al*, 2013). Another study that utilized the same antagonist, at the same dose, as the present study, found that it prevented MDMA-induced increases in murine social investigation (Kuteykin-teplyakov and Maldonado, 2014). This suggests that other behavioral measures may be more sensitive to the role of oxytocin. Unlike MDMA, the effects of oxytocin are quite subtle and recipients are generally unable to discern treatment from placebo (MacDonald *et al*, 2011; Macdonald and Macdonald, 2010). Two recent clinical studies have directly compared the effects of intranasal oxytocin with MDMA. Although oxytocin produces some overlapping effects with MDMA such as increased feelings of friendliness (Kirkpatrick *et al*, 2014c), these effects were much milder than for MDMA, and unlike MDMA, oxytocin did not affect measures of empathy or social interaction (Kuypers *et al*, 2014). And other drugs that increase oxytocin, such as fenfluramine, are not prosocial (File and Guardiola-Lemaitre, 1988; Lee *et al*, 2003; Saydoff *et al*, 1991). But interestingly, homozygous carriers of an oxytocin receptor gene SNP have a blunted response to MDMA-induced sociability (Bershad *et al*, 2016b). Together, these studies suggest a supporting role for oxytocin in MDMA-induced social behavior, but it is unlikely that it is sufficient or necessary for all of MDMA's prosocial effects.

In conclusion, the present study examined the stereoselectivity of MDMA-induced social interaction, locomotor activity, and oxytocinergic neuronal activation. (–)-MDMA significantly increased social interaction, had no locomotor stimulant effects, and significantly increased oxytocin activity. In stark contrast, (+)-MDMA had the opposite effects, not significantly increasing social interaction or oxytocin activity, but robustly increasing locomotor activity. The racemic mixture produced effects intermediary to the enantiomers, though at a lower dose than

would be expected based on the potencies of the individual enantiomers. This apparent synergy of the enantiomers when administered together has been observed previously (Anderson *et al*, 1978; Murnane *et al*, 2010) but remains poorly understood. What is clear is that (–)-MDMA appears to have prosocial and therapeutic-like effects that are similar to (+/–)-MDMA without the stimulant effects and potentially other side effects. If (–)-MDMA also lacks the neurotoxic effects of MDMA it could be a far superior therapeutic option that should be investigated in future clinical trials. It is still unclear what the exact neural mechanisms of MDMA-induced prosocial behavior are. Neuronal oxytocin activation paralleled the social interaction produced by MDMA and its enantiomers, but it is unlikely that oxytocin is the sole mediator of MDMA-induced social interaction. More likely, it is just one of several different factors involved. Future studies should continue to investigate the role of vasopressin as well as 5-HT receptors, given the high affinity of (–)-MDMA at these receptors.

It is quite possible that no human has consumed (–)-MDMA since the 1970's. At the time, the researchers involved in its study were more interested in the psychedelic effects of MDMA and related drugs and appeared to have little interest in (–)-MDMA, which at least at the doses tested had no such effects (Anderson *et al*, 1978; Shulgin and Shulgin, 1991). Since then, most pre-clinical researchers have not bothered to evaluate (–)-MDMA, perhaps assuming that it wouldn't have interesting behavioral effects given its weak psychotropic effects. Indeed, prior to these experiments I did not expect it would have such pronounced effects on social interaction, but the results herein strongly suggest that (–)-MDMA is prosocial in much the same way that racemic MDMA is. With the medical use of psychedelics there has been a compelling narrative that their therapeutic benefits are intimately tied to their mind altering effects (Nichols *et al*, 2016; Sessa, 2005). But (–)-MDMA appears as a challenge to that assumption. It seems to lack the strong psychotropic effects of racemic MDMA, yet it has very similar prosocial and therapeutic-like effects, suggesting that at least in the case of MDMA, these effects may be

dissociable. Therefore, not only could (–)-MDMA represent a new form of MDMA with therapeutic utility and fewer side effects, but it could also change our understanding of why MDMA is useful.

E. Figures

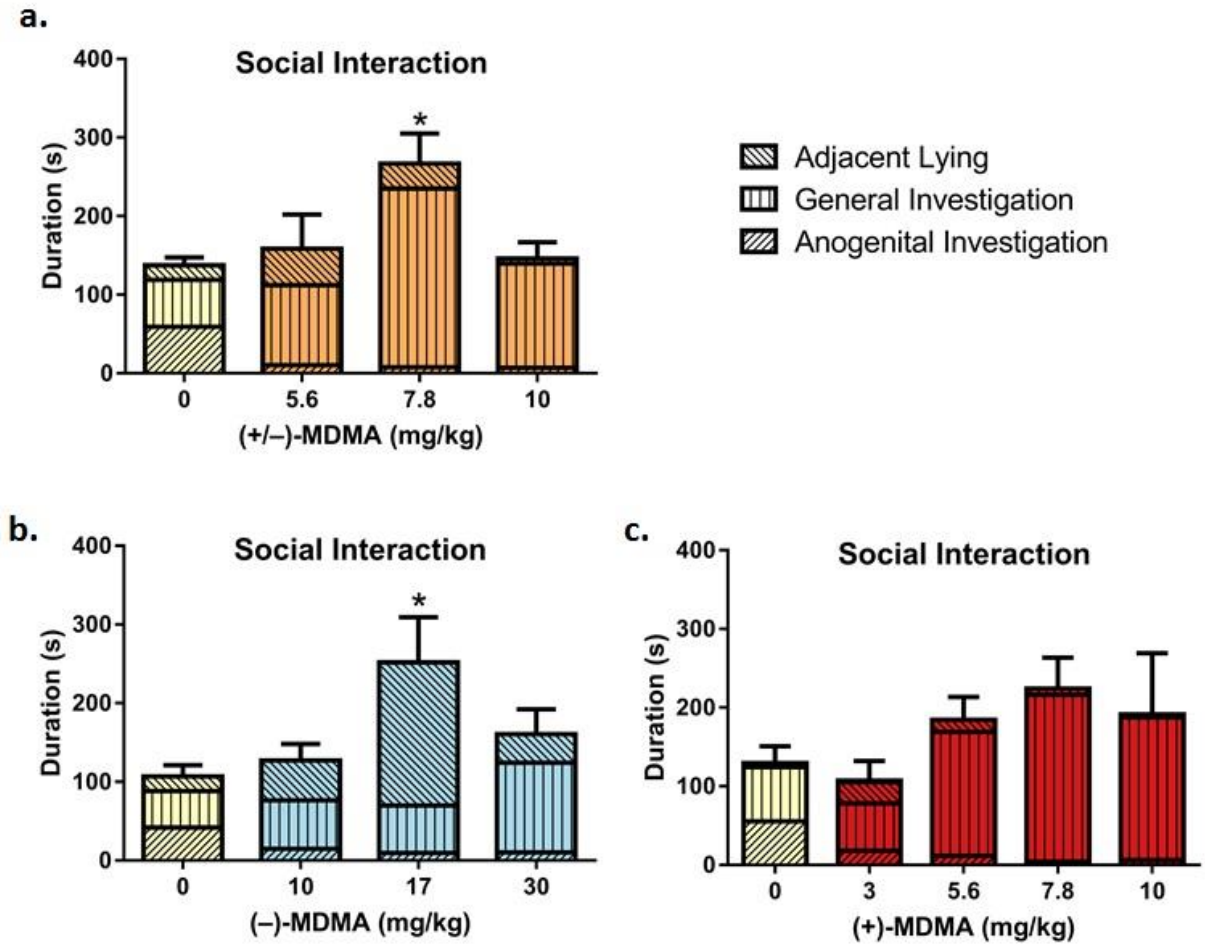

Figure 3.1 Social interaction after treatment with (+/–)-MDMA, (–)-MDMA, or (+)-MDMA. The durations of 3 social behaviors during a 10-minute social interaction test are shown stacked to produce mean ± SEM total social interaction. **(a)** (+/–)-MDMA increased total social interaction with a peak effective doses of 7.8 mg/kg, *p = 0.0255. (+/–)-MDMA preferentially increased general investigation behaviors, mostly nose-to-nose sniffing and non-aggressive following. **(b)** (–)-MDMA increased total social interaction with a peak effective doses of 17 mg/kg, *p = 0.0265. (–)-MDMA preferentially increased adjacent lying behavior. **(c)** (+)-MDMA did not significantly alter total social interaction but had peak effects at 7.8 mg/kg. *N = 5 pairs per treatment dose.*

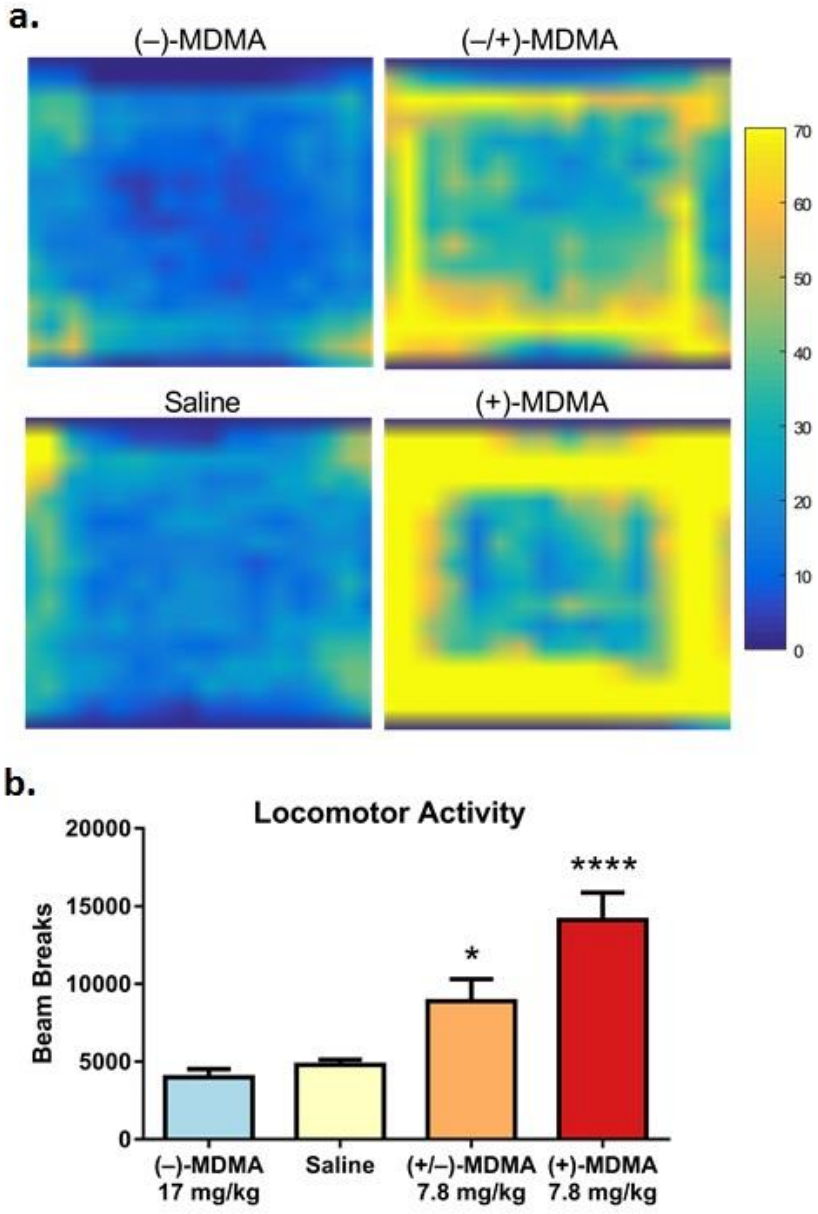

Figure 3.2 Effects of (–)-MDMA, (+/–)-MDMA, and (+)-MDMA on locomotor activity. (a) Activity plots indicate the location of beam breaks by a representative subject in each treatment group. Colors correspond to the number of beam breaks, with lighter colors indicating increased activity. Locomotor activity was concentrated in the periphery of the chambers. **(b)** (+/–)-MDMA and (+)-MDMA significantly increased locomotor activity compared to saline, *$p = 0.0461$ and ****$p < 0.0001$, respectively. (–)-MDMA had no locomotor-stimulant effects compared to saline, $p = 0.9299$. *N = 13 mice per treatment group.*

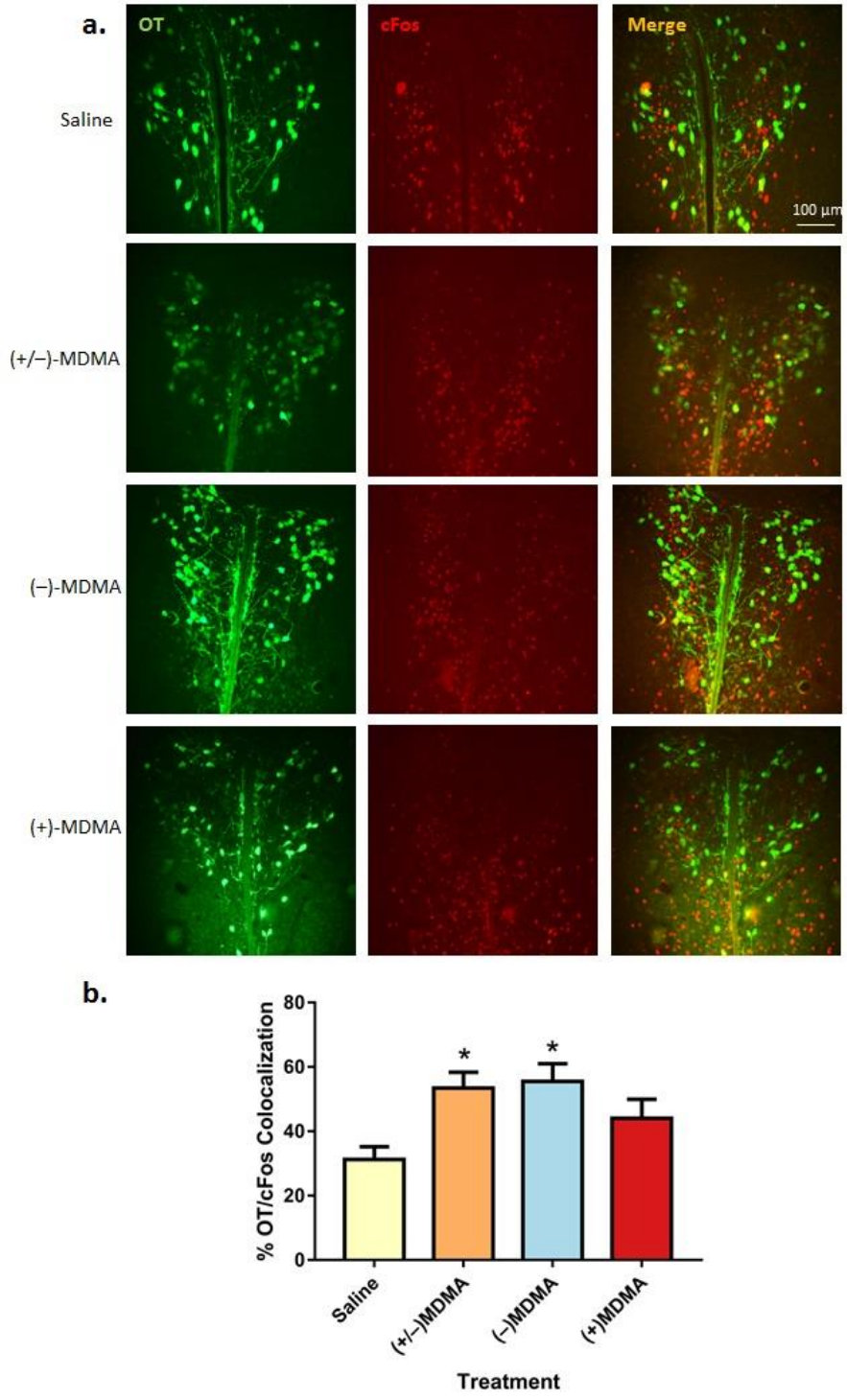

Figure 3.3 Activation of oxytocinergic neurons. (a) The expression of cFos in immunolabeled oxytocin cells was assessed in the PVN after treatment with (+/–)-MDMA, (–)-MDMA, (+)-MDMA or saline. **(b)** The percent of oxytocinergic neurons that were co-labeled with c-Fos was significantly higher in 7.8 mg/kg (+/–)-MDMA and 17 mg/kg (–)-MDMA treated mice compared to saline, *p = 0.028 and *p = 0.0112, respectively, while the 7.8 mg/kg (+)-MDMA treated group was not significantly different. *N = 4-5 mice per treatment group.*

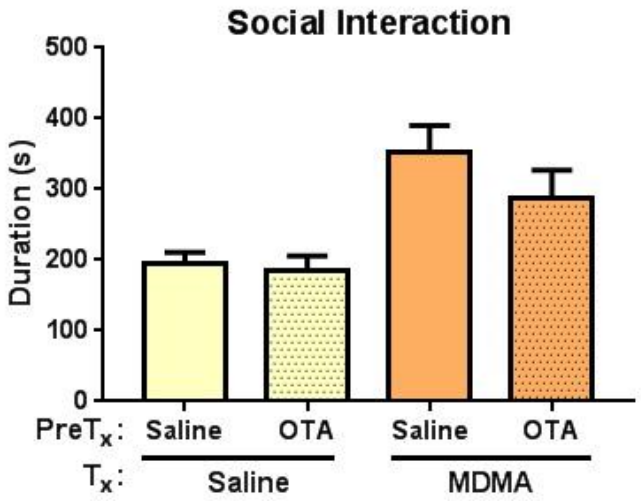

Figure 3.4 Effect of oxytocin antagonist on murine social interaction. Mice treated with saline + 7.8 mg/kg MDMA had increased social interaction compared to saline + saline treated subjects, p = 0.0270. Pretreatment with the oxytocin receptor antagonist OTA (10 mg/kg) did not significantly decrease social interaction in saline or MDMA treated mice. But OTA + MDMA treated mice were also not significantly different from saline + saline treated mice. The duration of social interaction was on average 92.64 seconds higher than baseline in this group, compared to 158.2 seconds higher in the saline + MDMA treated group. *N = 3-4 pairs per treatment group*.

Chapter 4. Separating the Agony from Ecstasy

A. Introduction

There has long been interest in the therapeutic potential of MDMA. Before it became popular as a recreational drug, it was used by a small group of therapists to facilitate and augment psychotherapy. The largely anecdotal accounts of its therapeutic potential from this period have been substantially bolstered by recent clinical trials that appear to confirm the drug's efficacy. But despite these promising results, serious limitations remain to wider clinical use. Most concerning is the accumulated evidence that MDMA is neurotoxic (Moratalla *et al*, 2015; Parrott, 2013), and that it can be potentially fatal even at relatively low doses due to severe hyperthermia (Green *et al*, 2003; Henry *et al*, 1992). Current and abstinent MDMA users have neurocognitive deficits and higher rates of depression than nonusers or users of other drugs (Rogers *et al*, 2009; Taurah *et al*, 2014). And even the low doses used clinically may pose neurotoxic risk (Mueller *et al*, 2013). There is thus significant impetus to develop a safer medication that isolates the unique prosocial and therapeutic effects of MDMA from these adverse effects.

The enantiomers of MDMA have distinct pharmacological profiles (Fantegrossi, 2008), that when combined have synergistic effects to produce prosocial and therapeutic effects at doses lower than would be expected from the effects of either enantiomer alone. As presented in Chapter 3 and Appendix A, (+)-MDMA has insignificant prosocial effects in mice and no effect on fear extinction, respectively. Conversely, (–)-MDMA has robust prosocial and therapeutic-like effects that are comparable to racemic MDMA but only at a substantially higher dose. Intriguingly though, even at such a high dose (–)-MDMA had no detectable locomotor stimulant side effects, suggesting that some of the adverse effects of (+/–)-MDMA may stem exclusively from the (+) enantiomer. Indeed, several previous studies have suggested that (+)-MDMA may be responsible for the neurotoxicity of the racemic mixture (Frau *et al*, 2013; Schmidt *et al*, 1987).

These studies found that (–)-MDMA had little to no neurotoxicity in rats or mice, but the doses used did not account for its lower potency. Few studies have evaluated the effects of (–)-MDMA because it has long been considered the inactive isomer, with relatively weak psychotropic effects (Anderson *et al*, 1978). However, our findings that it has both profound prosocial and therapeutic-like effects suggest that further investigation into the clinical viability of this enantiomer is needed. To this end, we investigated the neurotoxicity and thermogenic effect of (–)-MDMA using a dosing regimen based on its potency relative to racemic MDMA.

In most species, (+/–)-MDMA is a selective neurotoxin that produces widespread serotonergic neuronal terminal pruning that spares cell bodies (Capela *et al*, 2009). Within hours of administration to rats, brain levels of 5-HT plummet to less than 20% of control values. This is followed by an abrupt full recovery within 24 hours. However, a gradual dying back of 5-HT terminals begins, accompanied by reactive astrogliosis, which can occur as a result of neuronal damage and lead to enhanced expression of GFAP within astrocytes (O'Callaghan and Miller, 1994). Within a week following treatment, 5-HT levels are approximately 75% of control values (Schmidt, 1987b). The magnitude of neurochemical loss varies by the dosing regimen but follows this same pattern, with reductions in central 5-HT persisting for months to years after treatment (Battaglia *et al*, 1988b). Loss of 5-HT is accompanied by similar decreases in 5-HT transporter (SERT) and tryptophan hydroxylase expression (Xie *et al*, 2006). Studies of human MDMA users have observed similar deficits. Former users have decreased levels of the 5-HT metabolite 5-HIAA (McCann *et al*, 1994, 1999) and decreased SERT binding (Erritzoe *et al*, 2011b; Kish *et al*, 2010b; McCann *et al*, 2008), suggesting that similar processes occur at the doses consumed by humans. Multiple studies have tried to link human use of MDMA with functional deficits. Most such studies have been retrospective making causality difficult to assess, but the accumulated data makes it clear that current and former MDMA users have impaired prospective and retrospective memory (Parrott, 2013). A meta-analysis of over 100 studies concluded that memory deficits in

former MDMA users were significant compared to non-users and poly-drug users (Rogers *et al*, 2009).

MDMA has similar neurotoxic effects in mice, but instead of damaging 5-HT neurons, it is selective for dopamine neurons (Granado *et al*, 2008b). The reason for this stark difference is not fully understood but may stem from the species specific distribution of free radical scavenging enzymes (Cadet *et al*, 1995; Granado *et al*, 2008a). Despite the difference in neuronal vulnerability, the mechanisms of neurotoxicity appear to be consistent across species and involve excessive production of reactive oxygen species that coupled with hyperpyrexia and other pro-oxidant factors overwhelm the neurons' anti-oxidant defenses (Cadet and Brannock, 1997). Therefore, despite the distinct pattern of neurotoxic dysfunction in mice, findings regarding the neurotoxicity of (–)-MDMA should be translatable to other species.

To determine if (–)-MDMA is neurotoxic at high doses, we assessed reactive astrogliosis, a reliable and universal marker of CNS damage (Norton *et al*, 1992; O'Callaghan and Miller, 1994), as well as dopamine (DA) content and DA transporter (DAT) expression as markers of DA terminal pruning. Drug-induced changes to body temperature were also measured. Hyperthermia is a major contributing factor to neurotoxicity and is the most common cause of MDMA-related mortality. The mechanism by which MDMA produces hyperthermia, and how to reverse it, has been a popular area of research. Many recent studies have implicated NE and 5-HT as dominant factors (Dao *et al*, 2014; Docherty and Green, 2010a), however this does not make sense in the case of (–)-MDMA, which has been reported to have no hyperthermic effects (Fantegrossi *et al*, 2003), but has relatively similar effects upon NE and 5-HT relative to (+/–)-MDMA. One of the key pharmacological differences between (–)-MDMA and racemic MDMA is that (–)-MDMA does not release DA (Hiramatsu and Cho, 1990; Murnane *et al*, 2010; Setola *et al*, 2003). To determine if DA signaling is necessary for MDMA-induced hyperthermia in mice we administered a selective D_1 receptor antagonist prior to MDMA. Together these studies provide

multiple measures to compare the toxicity of (–)-MDMA and (+/–)-MDMA and provide mechanistic insight into why their effects are so different.

B. Methods

Subjects

Male Swiss Webster mice (Charles River Laboratories, Wilmington, MA) aged 7-10 weeks served as subjects in all experiments. Mice were housed five per cage in a temperature and humidity controlled colony room at the Yerkes National Primate Research Center with food and water available *ad libitum*. Lights were set to a 14-hour light/dark cycle. All experiments were performed at an ambient temperature of 22±2°C, during the lights-on phase. All studies were carried out in accordance with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. Experimental protocols were approved by the Emory University Institutional Animal Care and Use Committee.

Drugs

(+/–)-MDMA and (–)-MDMA were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC). R(+)-SCH23390 was acquired from Research Biochemicals International (Natick, MA). Doses were calculated and are expressed as HCl salts. All drugs were dissolved in 0.9% sterile saline and administered via intraperitoneal injection at a volume of 10 ml/kg.

Neurotoxic Dosing and Tissue Collection

Subjects received a total of four injections of either (+/–)-MDMA (20 mg/kg), (–)-MDMA (50 mg/kg), or saline given twice, two hours apart on two consecutive days. Previous studies have demonstrated that this dose of racemic MDMA is neurotoxic in mice (Capela *et al*, 2009). The (–)-MDMA dose was chosen based on the lower potency of this enantiomer relative to

racemic MDMA as determined during behavioral testing. Subjects were isolated during treatment and returned to their home cages 2 hours after the second daily dose. Survival was 75% for (+/–)-MDMA and 100% for (–)-MDMA treated mice. Following treatment, subjects were divided into two groups. 48 hours after the final injection, subjects from group 1 (n = 7 per treatment group) were deeply anesthetized with 150 mg/kg sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI) and transcardially perfused with 4% formaldehyde. Their brains were removed and post fixed for 24 hours in the same formaldehyde solution. Brains were immersed in 15% sucrose for 48 hours, frozen in chilled methyl butane, sectioned at 35 µm, and stored at -20°C until analysis by immunohistochemistry. Subjects in group 2 (n = 13-15 per treatment group) were euthanized by cervical dislocation 14 days after the last injection. Their brains were removed and prefrontal cortex, striatum, and hippocampus were rapidly dissected and frozen for subsequent analysis by HPLC or Western blot. These regions of interest were selected because previous studies have consistently observed monoamine and/or protein depletion these brain areas following MDMA administration in rats and mice (Capela *et al,* 2009).

Immunohistochemistry (IHC)

Reactive astrogliosis was assessed in the dorsal striatum by quantification of glial fibrillary acidic protein (GFAP). This brain area was chosen because of the quantity of dopaminergic axon terminals and prior reports of extensive MDMA-induced reactive gliosis in this region (Fra*u et al,* 2013). Tissue sections were washed in PBS and endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 10 minutes. Sections were then blocked with a solution of 2% goat serum and 0.2% Tween 20 in PBS for one hour and then incubated in blocking buffer overnight at 4°C with a primary antibody against GFAP (ab4674, Abcam, Cambridge, UK; 1:8000). They were then rinsed with PBS and incubated with a biotinylated secondary antibody (Vector Labs, Burlingame, CA; 1:200; 1 h). Immunolabeling was visualized with VECTASTAIN ABC and Impact DAB (Vector Labs). 4-6 sections from each subject were

photographed at 10x magnification, and the percent area of GFAP immunoreactivity in the dorsal striatum was quantified using ImageJ.

High Pressure Liquid Chromatography (HPLC)

Brain tissue was homogenized by sonication in 0.1 M perchloric acid and centrifuged for 25 minutes at 14000 x g. The pellet was dissolved in 0.3 M NaOH and total protein content was determined via BCA assay (Pierce Biotechnology, Rockford, IL). HPLC with electrochemical detection was used to determine the quantity of DA and serotonin (5-HT) in the supernatant. The HPLC system was composed of a small-bore column (3.2 mm x 150 mm x 3 um; 70-0636; Thermo Scientific, Sunnyvale, CA), a Thermo Dionix Ultimate 3000 solvent delivery pump set to a flow rate of 0.6 ml/min, a guard cell (350 mV; 5020, ESA), and an autosampler (542, ESA, Chelmsford, MA). Detection was carried out with a dual-channel analytical cell (5014B, Thermo Scientific) and an ESA Coulochem III detector. The analytical cell's oxidative channel was set to -150 mV and its reductive channel was set to 220 mV. The mobile phase was commercially prepared MDTM (Thermo Scientific). Data were acquired and analyzed using Chromeleon 6.8 software (Thermo Scientific). DA and 5-HT content are presented as a percent of their concentration in saline treated controls. Of 129 possible samples, 10 were not included in analysis. Of these, 2 were lost during tissue collection, 7 had unquantifiable peaks, and 1 was an extreme outlier (saline, hippocampus; $Z > 2.507$, $p < 0.05$). All subjects had at least one tissue sample analyzed.

Western Blot

Striatal tissue (n = 8-9 per treatment group) was homogenized by sonication in Tris/HCl buffer and centrifuged for 20 minutes at 18,000 x g. The pellet was suspended in RIPA buffer (Sigma-Aldrich, St. Louis, MO) and shaken on ice for 2 hours. Samples were centrifuged for 20 minutes at 14,000 x g and analysis was performed on the supernatant. Total protein content was

determined with a BCA assay (Pierce Biotechnology). Samples were diluted in Running Buffer (Life Technologies, Carlsbad, CA), heated to 70°C for 10 minutes, and then separated on 8% Bis-Tris gels (Life Technologies). Proteins separated by electrophoresis were transferred to PVDF membranes (Bio-Rad, Hercules, CA). Membranes were blocked for 60 minutes at room temperature in TBS containing 5% nonfat dry milk and 0.05% Tween 20. Membranes were incubated overnight in blocking buffer at 4°C with primary antibodies against DAT (AB2231, Millipore, Darmstadt, Germany; 1:20000) and Na^+/K^+-ATPase (ab76020,1:500000, Abcam). Membranes were washed with TBS containing 0.1% Tween 20 and then incubated for 1 hour at room temperature with an HRP-labeled secondary antibody (1:200000, Jackson Immuno, West Grove, PA) in blocking buffer. After washing, the antibody complex was visualized by chemiluminescence (Amersham ECL Prime, GE Healthcare, Buckinghamshire, UK). Bands were quantified using ImageJ and the relative expression of DAT was calculated as a percent of the Na^+/K^+-ATPase loading control. DAT expression following each treatment is presented as a percent relative to saline treated controls.

Body Temperature Monitoring

The effect of (–)-MDMA (50 mg/kg) or (+/–)-MDMA (20 mg/kg) on body temperature, given twice at a two-hour interval, was monitored using a rectal thermometer (n = 5 per treatment group). Measurements were taken every 30 minutes. To determine if dopamine D1 receptor activity was necessary for (+/–)-MDMA-induced hyperthermia, a second group of mice (n = 9-13 per treatment group) received pretreatments of the selective D1 antagonist (+)-SCH23390 (0.5 mg/kg) 30 minutes before a single (+/–)-MDMA treatment.

Data Analysis

Data were analyzed with Prism 7 (Graphpad, La Jolla, CA). Immunohistochemistry and western blot results were analyzed using one-way between subjects ANOVAs with Dunnett's

post-hoc comparisons of each treatment to saline. Neurochemistry results were analyzed using a two-way repeated measures ANOVA with a Dunnett's post-hoc comparison of each treatment to saline. Body temperature results were analyzed using a repeated measures two-way ANOVA with Dunnet's or Tukey's post-hoc comparisons. Alpha for all experiments was set at 5%. Error bars represent the standard error of the mean (SEM).

C. Results

Reactive astrogliosis 48 hours after treatment

Reactive astrogliosis was assessed in the dorsal striatum 48 hours after treatment with (+/–)-MDMA, (–)-MDMA, or saline (Figure 4.1a). There was a significant effect of treatment on the percent area of GFAP immunoreactivity $F(2, 18) = 6.85$, $p = 0.0061$(Figure 4.1b). Mice treated with a neurotoxic regimen of (+/–)-MDMA had significantly increased GFAP immunoreactivity relative to saline treated controls, 95% CI [1.159, 10.26], $p = 0.0141$. An equivalent dosing regimen of (–)-MDMA did not increase GFAP immunoreactivity, $p = 0.9102$.

Neurochemistry and DAT expression 14 days after treatment

Long-lasting changes to DA and 5-HT content and DAT expression were assessed two weeks after treatment with (+/–)-MDMA, (–)-MDMA, or saline. There was a significant effect of treatment on DA tissue content assayed in three brain regions $F(2, 110) = 4.023$, $p = 0.0206$ (Figure 4.2a). Mice treated with (+/–)-MDMA had lower DA content in all regions relative to saline treated controls, 95% CI [-3.516, -42.66], $p = 0.0178$, whereas (–)-MDMA treated mice were not significantly different from controls, $p = 0.9366$. 5-HT concentrations in the same brain regions were not affected by either treatment, $F(2, 110) = 1.112$, $p = 0.3326$ (Figure 4.2b), indicating that neurotoxicity was selective to DA neurons. DAT expression in the striatum was quantified by Western blot as an additional marker of DA neuronal pruning. DAT expression relative to Na^+/K^+-ATPase loading controls was normalized to expression as a percentage of

saline treated controls. There was a significant effect of treatment on DAT expression, F (2, 22) = 4.807, p = 0.0185 (Figure 4.2c). Post-hoc analysis revealed that (+/–)-MDMA, but not (–)-MDMA, significantly reduced striatal DAT expression relative to saline treated controls, 95% CI [-0.427, -34.2], p = 0.0441, and p = 0.8842, respectively.

Changes in body temperature

The effects of (+/–)-MDMA and (–)-MDMA on body temperature were assessed by taking rectal thermal measurements at 30-minute intervals (Figure 4.3). There was a significant effect of treatment, F(2, 12) = 26.85, p < 0.0001; time, F(7,84) = 4.646, p = 0.0002; and interaction, F(14,84) = 6.586, p < 0.0001. Dunnett's post-hoc analysis revealed that (+/–)-MDMA significantly increased body temperature relative to saline treated controls, 95% CI [0.887, 3.003], p = 0.0012. Conversely, (–)-MDMA significantly decreased body temperature, 95% CI [-0.057, -2.173], p= 0.0392. (+/–)-MDMA-induced hyperpyrexia exceeded 42°C in one subject and was fatal.

Role of D1R activity in (+/–)-MDMA-induced hyperthermia

To investigate the role of DA in the hyperthermic effect of (+/–)-MDMA, subjects were pretreated with the D1 receptor antagonist SCH or saline 30 minutes before treatment with (+/–)-MDMA or saline (Figure 4.4). There was a significant effect of treatment on body temperatures measured post-treatment, F(3, 40) = 6.923, p = 0.0007; time F(3, 120) = 10.02, p < 0.0001; and interaction, F(9, 120) = 1.397, p = 0.1967. Tukey's post-hoc analysis revealed that (+/–)-MDMA increased body temperature relative to saline (Saline/MDMA x Saline/Saline, 95% CI [0.026, 2.247], p = 0.0431). Pretreatment with SCH attenuated this effect (SCH/MDMA x Saline/MDMA, 95% CI [-0.426, -2.647], p = 0.0034), but did not significantly reduce baseline body temperature (SCH/Saline x Saline/Saline, p = 0.811).

D. Discussion

MDMA has profound prosocial effects and may have significant therapeutic utility when given as an adjunct to psychotherapy. But despite promising results from recent clinical trials, significant debate remains over the wisdom of using it as a therapeutic (Doblin *et al*, 2014; Parrott, 2014a). MDMA is a widely used illicit drug, particularly among young people (Center for Behavioral Health Statistics and Quality, 2015), and medical use may send the message that MDMA is safe for recreational use (Parrott, 2013). Even modest doses of MDMA can be fatal due to hyperthermia (Henry *et al*, 1992), especially when consumed in hot and crowded environments like dance clubs where use of the drug is popular (Halpern *et al*, 2011b). Furthermore, MDMA use may have long-term deleterious consequences. There is substantial evidence that it is neurotoxic and its use is associated with an increased incidence of psychiatric problems, which are presumably manifestations of underlying neural dysfunction (Moratalla *et al*, 2015; Parrott, 2013; Rogers *et al*, 2009; Taurah *et al*, 2014). These factors are all likely to limit the therapeutic viability of MDMA even if it has proven medical efficacy. And it is currently unclear how often therapy will need to be repeated. The risks from MDMA increase with repeated use, and may come to outweigh the therapeutic gains. In particular, the risk of neurotoxicity might limit clinical use to only a few specific debilitating or terminal conditions. But if the prosocial and therapeutic effects could be isolated from these adverse effects, the result would be a much more viable therapeutic.

As discussed in Chapter 2, (–)-MDMA increases social interaction and has therapeutic-like effects in mice that are equivalent to racemic MDMA. To determine if (–)-MDMA has fewer adverse effects we evaluated the neurotoxic and hyperthermic effects of (–)-MDMA relative to racemic MDMA, using a dosing regimen that accounted for the significant potency difference between the two. (+/–)-MDMA produced severe hyperthermia and several subjects died after treatment. In contrast, (–)-MDMA produced no hyperthermia and there were no fatalities following its administration. Neurotoxicity was assessed using multiple measures. Reactive

astrogliosis, which is a reliable marker of neuronal damage (O'Callaghan and Miller, 1994), was assessed 48 hours after dosing by measuring striatal GFAP immunoreactivity. (+/–)-MDMA treated mice had significantly elevated GFAP expression relative to saline treated controls, but there was no evidence of reactive astrogliosis in (–)-MDMA treated mice. MDMA is largely considered to be a selective dopaminergic neurotoxin in mice, although in this strain, Swiss Webster, there is evidence that it may also be a serotonergic toxin (Itzhak and Achat-Mendes, 2004). To evaluate loss of dopamine neuronal terminals we quantified DA tissue content and striatal DAT expression. Potential serotonergic toxicity was assessed by measuring 5-HT tissue content. (+/–)-MDMA treated mice, had significant depletions of DA and lower DAT than saline treated controls. However, there was no depletion of 5-HT indicating that, at this dose, toxicity was likely limited to DA neurons. Across all measures (–)-MDMA treatment produced no evidence of neurotoxicity. DA and 5-HT tissue concentrations and DAT expression were equivalent to the levels in saline treated controls. The data obtained indicate that (–)-MDMA has no discernible neurotoxic or hyperthermic effects in mice, and suggest that (–)-MDMA may be a substantially more viable therapeutic option than racemic MDMA.

One of the key differences between (–)-MDMA and (+/–)-MDMA is that (–)-MDMA does not release DA (Acquas *et al*, 2007; Hiramatsu and Cho, 1990; Murnane *et al*, 2010). Given that DA release is necessary for the locomotor stimulant effects of amphetamine and similar drugs (French, 1986), this difference likely explains why (–)-MDMA does not increase locomotor activity. It may also be the reason that it is not neurotoxic and does not produce hyperthermia. Neurotoxic damage by MDMA is correlated with the degree of hyperthermia and in most cases can be attenuated or eliminated by preventing hyperthermia (Green *et al*, 2003). Hyperthermia occurs when heat production exceeds heat dissipation. In mammals, metabolic processes, which are required for basal function, generate heat that maintains a constant body temperature (Rusyniak and Sprague, 2005). In response to changing external temperatures or physical

exertion, adaptive processes work to regulate and maintain this constant temperature. To decrease body temperature, heat is primarily dissipated through peripheral vasodilation as well as perspiration in some species. MDMA impairs both of these processes; it delays the onset of sweating (Gordon, 2007), and increased 5-HT release constricts cutaneous blood vessels (Pedersen and Blessing, 2001). To increase body temperature, the hypothalamus acts to regulate the sympathetic nervous system and increase mitochondrial oxidative phosphorylation as well as stimulate shivering (Lowell and Spiegelman, 2000). Serotonin, dopamine, and norepinephrine have all been suggested to play major roles in regulating hypothalamic control of thermogenesis, and given that MDMA increases release of each of these, it is not surprising that it can have a profound effect on core body temperature. More attention has been given to the potential roles of 5-HT and NE in mediating MDMA-induced thermogenesis (Dao *et al*, 2014; Docherty and Green, 2010b), but because (–)-MDMA also increases release of these neurotransmitters, this does not explain why it would have no hyperthermic effects. Several previous studies in rats have suggested that DA may play a significant role in mediating MDMA thermogenesis (Mechan *et al*, 2002; Shioda *et al*, 2008). Given that (–)-MDMA does not release DA and does not produce hyperthermia, we hypothesized that DA activity might be necessary for MDMA-induced hyperthermia. And indeed, we observed that pretreatment with a selective D1 receptor antagonist fully prevented hyperthermia.

In addition to playing a thermogenic role, DA release may also have a critical role mediating MDMA neurotoxicity (Sprague *et al*, 1998). Following release by MDMA, DA can be taken up by monoamine transporters into DA and 5-HT terminals. There the deamination of DA by monoamine oxidase produces reactive oxygen species (ROS) that can lead to cellular degeneration (Cadet and Brannock, 1997). Depletion of DA or inhibition of MAO-B activity is sufficient to prevent neurotoxicity in rats (Falk *et al*, 2002; Sprague and Nichols, 1995a; Stone *et al*, 1988). The neuronal distribution and capacity of ROS scavenging enzymes may account for

the different neurotoxic profiles of MDMA between species (Granado *et al*, 2008a). Expression of human superoxide dismutase eliminates neurotoxicity in mice (Cadet *et al*, 1995). So, although MDMA produces a distinct pattern of toxicity in mice compared to other species, the underlying mechanisms of hyperthermia and neurotoxicity are likely universal, with DA release being a necessary component of both. Therefore, our finding that (–)-MDMA is not neurotoxic in mice, should translate to other species. However, future studies will be necessary to confirm this.

Despite the apparently favorable profile of (–)-MDMA, some additional safety concerns remain. The dosing regimen used in this study was high and was scaled to account for the lower relative potency of (–)-MDMA. However, dangerous effects including neurotoxicity and lethality might emerge if higher doses were tested. A stark survival difference was observed between (–)-MDMA and (+/–)-MDMA treated mice, but a previous study found that their median lethal doses were fairly similar (Fantegrossi *et al*, 2003). Non-neural toxicity may also be a concern. (–)-MDMA has high affinity for 5-HT_{2B} receptors and can stimulate mitogenesis in primary human heart valve interstitial cells in a fenfluramine-like manner (Setola *et al*, 2003). Fenfluramine, an appetite suppressant, and pergolide, an anti-Parkinson's drug, were both removed from the US market after it was discovered that they could cause heart valve damage. Their activation of 5-HT_{2B} receptors expressed on heart valve leaflets leads to a proliferation of myofibroblasts in an abundant extracellular matrix, thickening and reducing flexibility of the cardiac muscle (Elangbam, 2010). This can lead to valvular dysfunction and heart failure. The prevalence of cardiac toxicity in fenfluramine-treated patients ranges from 6% to 30% depending on the duration and frequency of use. A small clinical study found that 28% of heavy MDMA users who took approximately 3.6 ecstasy tablets per week for 6 years had an increased incidence of mild to moderate heart valve disease (Droogmans *et al*, 2007). But the risk of cardiotoxicity may only be significant with consistent and frequent use of 5-HT_{2B} activating drugs over an extended period of time (Hopkins and Polukoff, 2003). Hepatotoxicity may also be a risk, as metabolism of (–)-

MDMA in the liver produces redox-active quinone metabolites that can generate ROS and damage liver cells (Lourenço *et al*, 2013). In both of these cases, the risks are likely negligible with responsible and infrequent clinical use, but future studies should test whether periodic low doses of (–)-MDMA pose a significant danger.

Despite these potential limitations, the present study demonstrates that, in mice, (–)-MDMA lacks the two most worrying effects of MDMA: hyperthermia and neurotoxicity. Chapter III described a set of experiments establishing that (–)-MDMA has prosocial and potentially therapeutic effects that are equivalent to (+/–)-MDMA, which has recently been approved for Phase III clinical trials. Based on these findings, clinical study of (–)-MDMA is warranted as it may be a far safer alternative to racemic MDMA. It does not increase extracellular DA and has no locomotor stimulant effects, both of which are features of nearly every drug of abuse. This could potentially mean that (–)-MDMA has a lower likelihood for abuse, although self-administration studies have not been conclusive in this regard (Fantegrossi *et al*, 2002; Wang and Woolverton, 2007). At the very least, using (–)-MDMA in the clinic rather than racemic MDMA will limit the mixed message that MDMA is both a dangerous illegal drug and a medicine. Although these results are preliminary and further studies will be necessary to confirm these findings in other species, the prospect that the beneficial effects of MDMA can be produced without the severe negative side effects is extremely promising. Indeed, the agony can be separated from ecstasy.

E. Figures

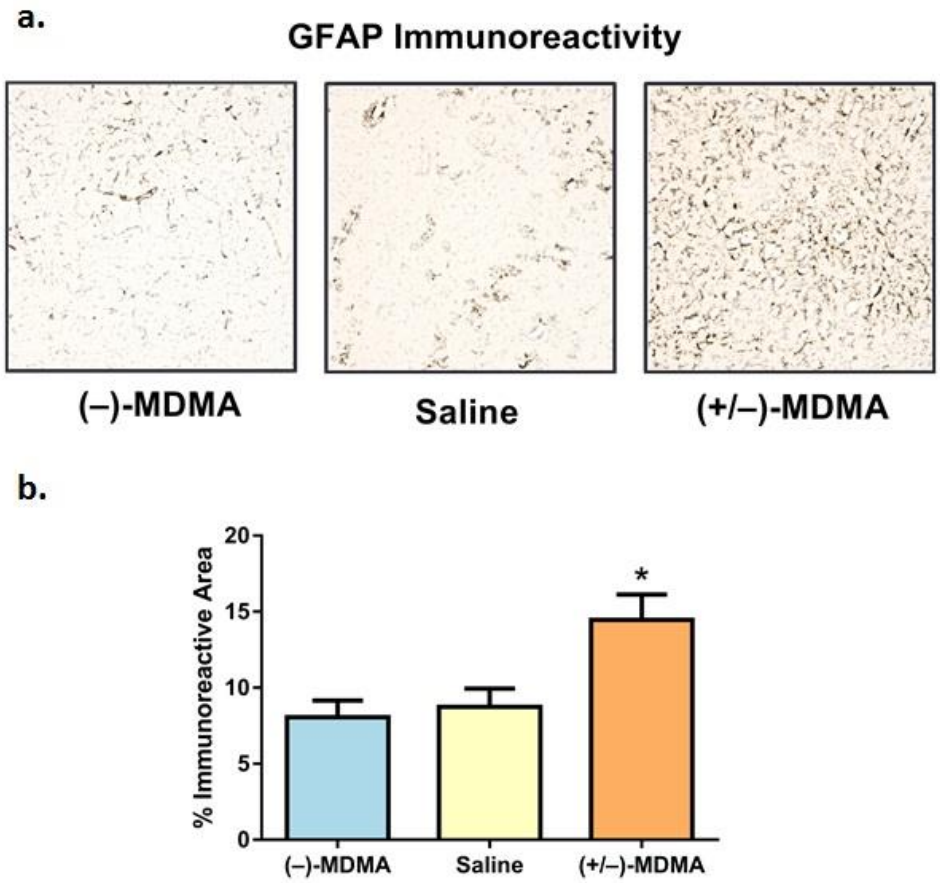

Figure 4.1 Astrogliosis 48 hours after treatment with (+/–)-MDMA or (–)-MDMA (a) Reactive astrogliosis, a marker of CNS damage, was assessed by quantification of GFAP immunoreactivity in the dorsal striatum 48 hours after a neurotoxic dosing regimen of (+/–)-MDMA or an equivalent regimen of (–)-MDMA. **(b)** (+/–)-MDMA significantly increased GFAP immunoreactivity in the striatum relative to saline, *p = 0.0141. (–)-MDMA did not affect GFAP immunoreactivity, p = 0.9102. *N = 7 per treatment group.*

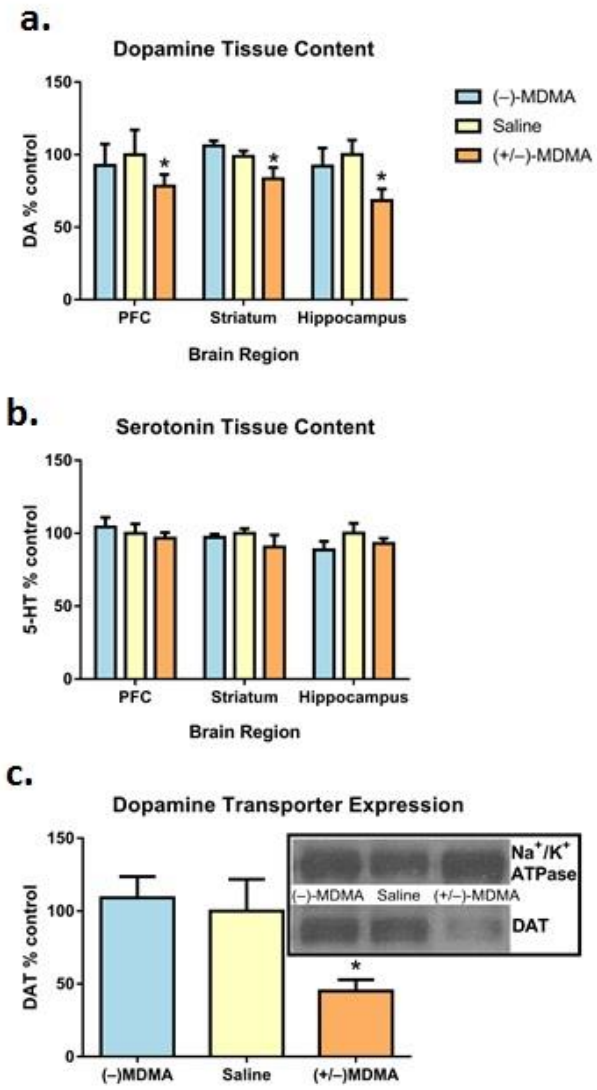

Figure 4.2 Markers of neuronal terminal pruning. 2 weeks after binge dose treatments with (+/–)-MDMA or (–)-MDM, markers of neuronal terminal pruning were assayed. **(a)** DA content was assessed in 3 brain regions as a marker of DA terminal pruning. (+/–)-MDMA treatment significantly decreased DA content, *p = 0.0178. (–)-MDMA had no effect on DA concentrations relative to saline treated controls, p = 0.9366. **(b)** There was no effect of treatment on 5-HT content in the same brain regions, indicating that pruning was isolated to DA neurons. **(c)** DAT expression in the striatum was quantified as an additional marker of DA neuronal pruning. (+/–)-MDMA, but not (–)-MDMA, significantly reduced striatal DAT expression, *p = 0.0441 and p = 0.8842, respectively. *a,b: n = 13-15 per treatment group, c: n = 8-9 per treatment group.*

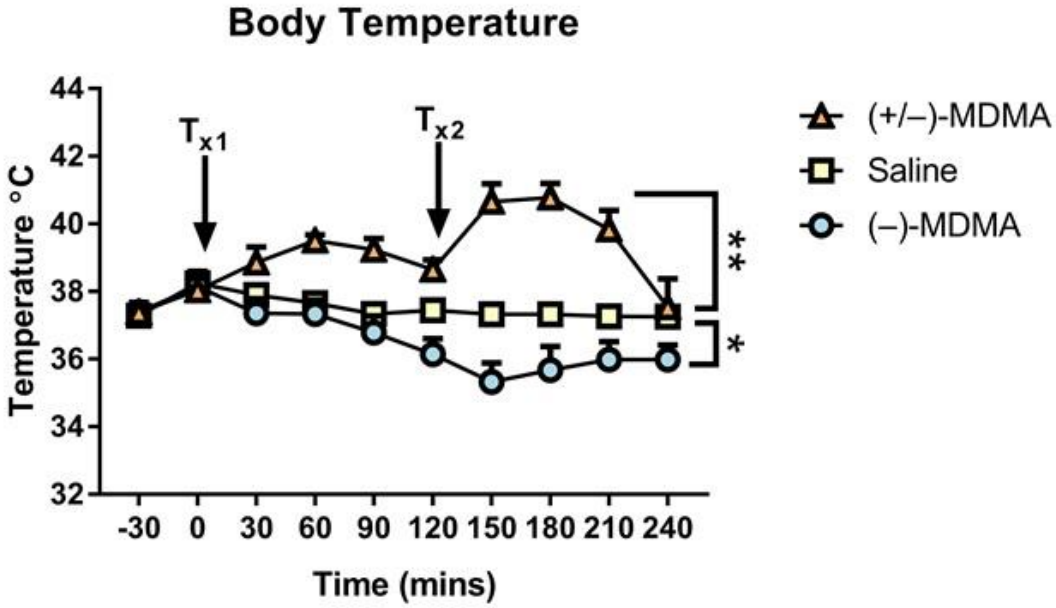

Figure 4.3 Effects of (+/–)-MDMA and (–)-MDMA on murine body temperature. Subjects were treated with (+/–)-MDMA (20 mg/kg), (–)-MDMA (50 mg/kg), or saline at times T_{x1} and T_{x2}. Relative to saline treated controls, (+/–)-MDMA significantly increased body temperature, **p = 0.0012, and (–)-MDMA significantly decreased body temperature, *p= 0.0392. *N = 5 per treatment group.*

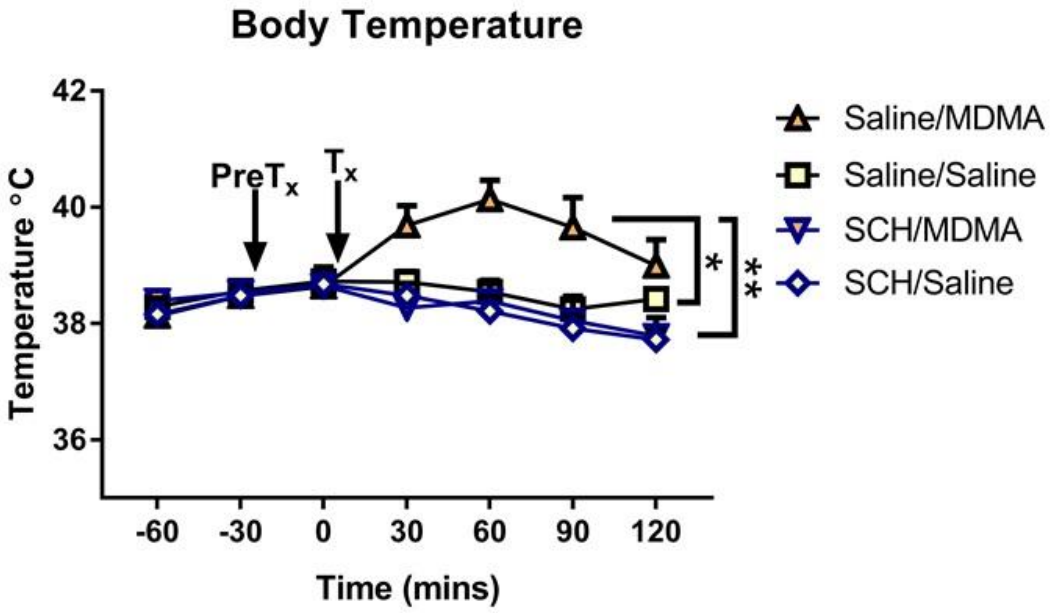

Figure 4.4 D1 antagonist prevents MDMA-induced hyperthermia. To investigate the role of DA in the hyperthermic effect of (+/–)-MDMA, subjects were pretreated ($PreT_x$) with the D1 receptor antagonist (+)-SCH23390 (SCH; 0.5 mg/kg) or saline 30 minutes before treatment (T_x) with (+/–)-MDMA (20 mg/kg) or saline. (+/–)-MDMA increased body temperature compared to saline treated controls, *p = 0.0431. Pretreatment with SCH attenuated this effect, **p = 0.0034, but did not significantly reduce baseline body temperature, p = 0.811. *N = 9-13 per treatment group*.

Chapter 5. Conclusions

A. Summary of Key Findings

The primary findings of these studies are three-fold. First, we observed that MDMA increases social interaction in mice in a sensitization-like manner, with repeated intermittent treatments producing subsequently larger effects. Second, we discovered that the effects of MDMA on murine social behavior are stereospecific, with (–)-MDMA increasing social interaction with similar efficacy to racemic MDMA, while (+)-MDMA had no significant effects. Conversely, the locomotor stimulant effects of MDMA were exclusive to (+)-MDMA. And third, we observed that unlike racemic MDMA, (–)-MDMA did not produce hyperthermia or any evidence of neurotoxicity, even when administered at very high doses. Together these studies advance our understanding of MDMA and provide a new paradigm to study its social effects. Furthermore, this work reveals that (–)-MDMA may be a substantially more viable therapeutic option than racemic MDMA.

The unique prosocial effects of MDMA have been a subject of sustained interest since the early days of its recreational and clinical use. These effects have been extensively studied and characterized in humans, but little is known regarding their mechanistic underpinnings (Kamilar-Britt and Bedi, 2015). Several well-designed animal studies have been conducted to probe these effects (Ramos *et al*, 2013; Thompson *et al*, 2007), but increases in social behavior by MDMA have been inconsistent across studies. In particular, studies using mice have mostly failed to observe any enhancement of social interaction following treatment with MDMA (Maldonado and Navarro, 2001; Navarro *et al*, 2004a). Given the large number of useful genetic tools available in mice, we set out to develop an MDMA treatment protocol with this species that would reliably produce prosocial effects. We observed that when drug-naïve mice were treated with MDMA for the first time the drug had no discernible social effects. However, if treated again 48 hours later, MDMA reliably increased social interaction among conspecifics. Subsequent treatments

continued to elicit increasing amounts of social interaction in a sensitization-like manner. The relevance of this phenomenon to other species is unknown, but may explain why some previous studies did not observe prosocial effects from MDMA (Bhattacharya *et al*, 1998; Homberg *et al*, 2007).

This effect, which we termed "social sensitization", occurred independently from locomotor or neurochemical sensitization, and unlike traditional sensitization it was not affected by pretreatment with a 5-HT_{2C} receptor agonist. Pretreatment with a 5-HT_{2A} receptor antagonist fully prevented the development of social sensitization, despite having no effect on the expression of MDMA-induced prosocial behavior in already sensitized mice. Intriguingly, mice that were given repeated intermittent treatments of MDMA while isolated did not display a sensitized response to the drug when tested later for social interaction. MDMA had to be given in a social setting for sensitization to occur. This suggests that there is a complex interaction between the drug and the environment in which it is administered.

With a reliable paradigm to investigate the social effects of MDMA established, we next evaluated whether these effects were stereospecific. MDMA is a racemic 50/50 mixture of two functionally distinct enantiomers (Steele *et al*, 1987). (+)-MDMA is the more potent of the two and has long been considered the "active isomer" (Anderson *et al*, 1978). It is primarily a monoamine releaser that increases synaptic concentrations of serotonin (5-HT), norepinephrine (NE), and dopamine (DA). (–)-MDMA is less potent as a monoamine releaser and has no appreciable effect on dopamine release (Acquas *et al*, 2007). However, it has higher potency as a direct agonist at several 5-HT receptors (Lyon *et al*, 1986). In the social interaction test, (–)-MDMA robustly increased murine social behavior with similar efficacy to racemic MDMA. Specifically, it increased adjacent lying behavior which is a hallmark of MDMA in rats and comparable to huddling behavior produced by MDMA in non-human primates. It also had no locomotor stimulant side effects. In stark contrast, (+)-MDMA substantially elevated locomotor

activity but did not significantly increase social interaction. The neuropeptide oxytocin has been suggested as a key mediator of MDMA's prosocial effects (Bershad *et al*, 2016b; Thompson *et al*, 2007). Both (+/–)-MDMA and (–)-MDMA significantly increased activation of oxytocinergic neurons, while (+)-MDMA had negligible effects. This correlation between prosocial effects and oxytocin activation supports the hypothesis that oxytocin may be involved in mediating these effects. But a follow up experiment utilizing a selective oxytocin antagonist (OTA), demonstrated that oxytocin receptor signaling is not necessary for MDMA-induced social behavior, suggesting that oxytocin is likely just one of several factors involved in mediating these drug effects.

A number of Phase II clinical trials are underway or have recently completed, testing the efficacy of MDMA as an adjunctive treatment with psychotherapy for patients suffering from chronic treatment-resistant post-traumatic stress disorder (PTSD). Two recently completed trials both observed substantial and enduring improvements following treatment with MDMA (Mithoefer *et al*, 2011; Oehen *et al*, 2013). PTSD is often conceptualized as a deficit of fear extinction learning (VanElzakker *et al*, 2014). A companion experiment by Dr. Matthew Young (Appendix A) assessed the effects of MDMA's enantiomers in a fear-conditioning and extinction paradigm. Previous work had demonstrated that MDMA facilitates extinction of cued fear in mice (Young *et al*, 2015). As with prosocial behavior, this effect was found to be stereospecific to (–)-MDMA, with (+)-MDMA having no effect. Facilitation of extinction learning by MDMA during therapy may explain why MDMA was helpful in treating these patients. The stereospecificity of this effect is particularly relevant, and indicates that (–)-MDMA may have the same therapeutic efficacy.

Although this apparent efficacy is extremely promising, the clinical viability of MDMA is limited by its potentially severe adverse effects and widespread recreational use. MDMA is neurotoxic and produces long-lasting neuronal dysfunction in humans and animals (Capela *et al*, 2009). It can also produce potentially lethal elevations in body temperature, even at modest doses

(Chadwick *et al*, 1991). If the therapeutic effects of MDMA could be isolated from these adverse effects, the result would be a much safer and more viable therapeutic with a greater likelihood of receiving regulatory approval. Given that (–)-MDMA produces prosocial and therapeutic-like effects without locomotor stimulant effects, we tested whether it might also lack neurotoxic and hyperthermic effects. High, binge doses of (–)-MDMA were tested in comparison with racemic MDMA, with concurrent body temperature monitoring and post-mortem examination of neurotoxic markers. (+/–)-MDMA increased expression of glial fibrillary acidic protein (GFAP), an indication of reactive astrogliosis and marker of CNS damage, and decreased brain tissue content of DA and DA transporter (DAT) expression, both markers of DA neuronal terminal pruning. (+/–)-MDMA also produced severe hyperthermia that was fatal to some experimental subjects. (–)-MDMA had none of these effects. It produced no evidence of neurotoxicity and slightly lowered body temperature. Even the high doses tested were well-tolerated, and the treated mice displayed no signs of acute toxicity.

(–)-MDMA therefore appears to produce prosocial and therapeutic-like effects equivalent to racemic MDMA without the stimulant, neurotoxic, and hyperthermic side effects. This discovery suggests that (–)-MDMA may be a much more suitable therapeutic than racemic MDMA. But perhaps more significant is the finding that these effects are separable. This means that new drugs could be developed that also specifically produce only the desired therapeutic or prosocial effects of MDMA without the adverse side effects. Conceivably these drugs could also be engineered to reduce other side effects associated with (–)-MDMA, as long as those effects are not directly related to the therapeutic effects. For now though, these findings highlight the currently untapped potential of (–)-MDMA. It is not a perfect medication, but few drugs are; if these findings are translatable to humans, (–)-MDMA is likely to have therapeutic efficacy similar to traditional MDMA, but with substantially fewer adverse effects and less potential for abuse.

B. Future Directions

A primary goal of this work was to develop a reliable murine model to study the prosocial effects of MDMA so that the mechanisms underlying these effects could be investigated with new sophisticated genetic tools. A better understanding of how MDMA increases social behaviors may elucidate the endogenous neurobiological systems that drive these behaviors as well as facilitate the development of new therapeutics that have similar prosocial effects. Several previous attempts have been made to dissect the pharmacological underpinnings of these effects, but they have been incomplete or mostly conjectural (Liechti and Vollenweider, 2001; Ramos *et al*, 2013; Ray, 2016; Sáez-Briones and Hernández, 2013; Thompson *et al*, 2007). It seems likely that 5-HT release is one critical factor, which leads to additional downstream effects such as increased oxytocin and vasopressin release that may also be critical modulators. Increased norepinephrine release may also be important, and direct agonism by MDMA at certain 5-HT receptors is likely necessary. We evaluated what roles 5-HT_{2A} and oxytocin receptors might play in mediating the prosocial effects of MDMA through several preliminary experiments, but further studies will be needed to clarify the importance of these and other pharmacological factors.

Manipulating central oxytocin signaling is complicated by the limited brain permeability of exogenously administered oxytocin and oxytocin receptor antagonists. To overcome this obstacle we developed a DREADDs (designer receptors exclusively activated by designer drugs) model so that oxytocinergic neurons could be turned on or off by convenient systemic administration of an otherwise inert agonist (clozapine-N-oxide, (CNO)) (Urban and Roth, 2015). DREADDs are G protein-coupled receptors that are quiescent unless activated by CNO. Depending on the specific subtype, they can transiently stimulate (hM3Dq) or inhibit (hM4Di) DREADD-expressing neurons in awake and behaving animals without any need for guide cannulae or other head-mounted equipment. Recently available transgenic mice that express Cre recombinase only in OT-producing neurons (Wu *et al*, 2012) were bred with floxed DREADD

mice to produce mice expressing stimulatory or inhibitor DREADDs exclusively in oxytocin-producing neurons. This non-invasive model system will allow future experiments to examine the behavioral effects of stimulating or inhibiting oxytocin and help to clarify the role of oxytocin in MDMA-induced prosocial behaviors. Specifically, we will test whether administration of CNO blunts the prosocial effects of MDMA in hM4Di-expressing mice, and if CNO increases social interaction in hM3Dq-expressing mice. Additionally, we will test the role of oxytocin in the development of MDMA-induced social sensitization. We observed that 5-HT_{2A} receptor activity was necessary for the development of sensitization but not the expression of MDMA-induced prosocial behavior. Oxytocin could function similarly.

Another target that warrants further investigation is 5-HT_{2B} receptors. MDMA has very high affinity for these receptors, higher than for any other G protein-coupled receptor yet tested, but little is known about their importance in mediating the effects of MDMA (Setola *et al*, 2003). 5-HT_{2B} receptors are well known for their role in drug-induced valvulopathy that led to the withdrawal of fenfluramine and pergolide from the US market (Elangbam, 2010). They are also expressed in multiple brain regions including the amygdala, hypothalamus, lateral septum, and dorsal raphe nucleus. They are presynaptic and are thought to regulate basal 5-HT concentrations through phosphorylation of the serotonin transporter (SERT) (Diaz *et al*, 2012). Their expression is required for the antidepressant actions of SSRIs, and 5-HT_{2B} receptor agonists have anxiolytic and antidepressant effects (Duxon *et al*, 1997). A gene sequencing study of Finnish violent offenders found a high incidence of a 5-HT_{2B} stop codon mutation that led to nonsense RNA (Bevilacqua *et al*, 2010). The antisocial and impulsive behaviors reported in this cohort of criminals suggests that 5-HT_{2B} receptors are important mediators of personality and behavior (Tikkanen *et al*, 2015). Understanding what role these receptors might play in the prosocial effect of MDMA would be an intriguing area of future research. Previous research has demonstrated that functional 5-HT_{2B} receptors are necessary for MDMA-induced 5-HT release *in vivo* and *in*

vitro (Dol*y et al*, 2008). To what extent MDMA agonism facilitates 5-HT release remains to be determined, and agonism may have additional behavioral effects unrelated to 5-HT release.

The most relevant and immediately translatable finding from these experiments is that (–)-MDMA has prosocial and therapeutic-like effects that are equivalent to racemic MDMA without producing hyperthermia or evidence of neurotoxicity in mice. Clinical trials of MDMA are moving forward rapidly, but the drug's adverse effects may significantly limit its therapeutic viability. Our findings indicate that (–)-MDMA may have similar therapeutic benefits without these safety limitations. To confirm these assumptions, additional studies will be required. We predict that the increased social interaction observed in mice following treatment with MDMA is indicative of the prosocial effects reported by human users. Since (–)-MDMA produces similar effects in our model, we predict that it will have similar prosocial effects in humans. However, there have been no published accounts regarding the social effects of (–)-MDMA in humans. Clinical studies are needed to verify if (–)-MDMA indeed has similar effects to racemic MDMA. Additional studies are also needed to extend our neurotoxicity findings. MDMA is a selective dopaminergic neurotoxin in mice, but a 5-HT neurotoxin in seemingly all other species including humans (Capela *et al*, 2009). Similar mechanisms are likely involved in both forms of toxicity, but the toxicity of (–)-MDMA should still be evaluated in a model system more homologous to humans, ideally a non-human primates model. If our findings derived in mice are confirmed with these additional studies, then clinical trials should be performed to test the therapeutic efficacy of (–)-MDMA for conditions that racemic MDMA has proven effective for.

C. Overcoming Prohibition

For the past three decades, MDMA has been primarily viewed as a public health concern. It is consumed by large numbers of young people, and its role in the premature deaths of even a small number of its users is a tragedy. Perhaps most concerning, though, is the prospect that

millions of young people who use MDMA may be inadvertently damaging their brains. The neurotoxic effects of MDMA are well documented in animal models (Capela *et al*, 2009), and studies of human users present significant cause for concern (Parrott, 2013). Former users have cognitive impairments and a higher rate of psychiatric illness compared to non-users, both potential indications of the neurological dysfunction precipitated by MDMA. More worrying is that these effects do not dissipate with abstinence, suggesting that damage from acute MDMA use is long-lasting or potentially permanent.

However, in recent years another perspective has emerged that not only is MDMA not particularly dangerous (Nutt *et al*, 2010), but that it also may be an incredibly effective medicine (Mithoefer *et al*, 2011, 2013). The disparity of these two views is confounding, and reconciling the apparent duality of MDMA will be a fascinating public health story. As MDMA moves into Phase III clinical trials, the US may be on the verge of a regulatory first, the rescheduling of a Schedule I drug. Proponents of MDMA's therapeutic use hope to have MDMA FDA-approved and rescheduled by 2021 (MAPS, 2015). The plausibility of this goal is hard to estimate. Cannabis, another Schedule I drug with purported medical benefits, remains illegal at the federal level even as many states have sanctioned its medical and even recreational use. In the case of both drugs, early fears of their adverse effects were likely overblown, and proponents of their continued prohibition likely over-estimate their threat to users and society (Parrott, 2014a). Conversely, advocates for their legalization and medical use may be underestimating their potential for harm (Bostwick, 2012; Sessa and Nutt, 2007).

Comparison of these two drugs provides intriguing parallels, and the fate of cannabis may portend that of MDMA. Cannabis has been cultivated for at least 6,000 years, making it one of the oldest agricultural crops (Zuardi, 2006). Despite religious, medical, and recreational use throughout Asia, its psychoactive effects were not appreciated in the west until the mid-19th century. The first report on its effects was published in 1839, and in 1870 it was added to the US

pharmacopoeia as a medicine. As a medication, typically in the form of an oil based extract, cannabis had a quiet, apparently useful role for the treatment of menstrual cramps and several other conditions. However, cannabis as "marijuana", smoked predominantly by Mexican migrants and popular in African-American jazz clubs, ignited fears within white America (Bostwick, 2012). Its prohibition is intricately tied into the country's racial transformations, prejudices, and cultural anxieties. Much as anti-Chinese sentiment in San Francisco had fueled the nation's first drug prohibition, so too was the case with cannabis. Newspapers and magazines circulated explicitly racist and sensationalist stories about marijuana. Harry Anslinger, director of the newly created Federal Bureau of Narcotics, successfully pushed for prohibition in 1937 (Chasin, 2016). Testifying before congress he called "Marijuana the most violence causing drug known to man". Anslinger remained the head of federal narcotics efforts until 1962 before moving on to shape global drug policy as an American representative to the U.N.

Since 1972, efforts have been underway to reschedule cannabis to allow medical use of the drug to resume at the national level. There is interest in using cannabis to treat a variety of conditions including glaucoma, AIDS wasting syndrome, neuropathic pain, cancer, multiple sclerosis, chemotherapy-induced nausea, and certain seizure disorders (Bostwick, 2012). Although data from controlled human studies is limited, there is some compelling evidence that cannabis is helpful for many of these conditions (Alexander, 2016; Crippa *et al*, 2016; Shelef *et al*, 2016; Tzadok *et al*, 2016). Given its potential, 28 states have made cannabis available for medical use. Yet at the federal level, cannabis and all its constituent cannabinoids are illegal at the most restrictive level, Schedule I (Drug Enforcement Administration, 2016). In August 2016, the DEA again rejected calls to reschedule marijuana, hypocritically citing that it has no proven medical value, even though two pharmaceutical products of synthetically produced cannabinoids, including one that is pure THC (the primary psychoactive component of cannabis) have been FDA-approved and are legal for medical use (Baron, 2015).

A primary roadblock for medical approval of cannabis is that it contains 483 known chemicals. FDA approval could ultimately hinge on isolation and clinical testing of the individual cannabinoids. Meanwhile, eight states and the District of Columbia have legalized or are in the process of legalizing recreational cannabis. Thus, the medical use and legalization of cannabis may diverge. It appears increasingly likely that cannabis will become fully legalized and regulated like alcohol or tobacco products, but that it may never be FDA-approved for any medical condition. Instead, pharmaceutical products using synthetic versions of various cannabinoids will be patented, FDA-approved, and ultimately take the place of cannabis for medical use.

Much like cannabis, MDMA had a relatively quiet existence before its prohibition. "Rediscovered" in 1976, MDMA quickly gained a following within the drug-assisted psychotherapeutic community (Holland, 2001). Anecdotal case reports from this period suggest that it was effective in helping to treat a variety of psychiatric conditions (Greer and Tolbert, 1990). But as clinical investigation of MDMA was just beginning, public concern about drug use was also building. In 1981, Nancy Reagan launched a highly-publicized anti-drug campaign, "Just Say No." As crack cocaine use began to escalate, primarily in minority communities, new laws were passed in Congress, and state legislatures enacted zero-tolerance policies and draconian sentencing guidelines for drug possession (Reinarman and Levine, 1997). Into this environment emerged "ecstasy", which became popular at gay and straight nightclubs in conservative Texas (Simek, 2015). And so, as with cannabis and so many drugs before it, cultural anxieties mixed with legitimate safety concerns and well-intentioned reform impulses led to its swift prohibition and placement into Schedule I.

Years of lawsuits from the medical and scientific community failed to change this placement, but recent clinical trials have begun to confirm previous anecdotal evidence of MDMA's therapeutic utility. Large Phase III clinical trials are underway that, if successful, could

pave the way for FDA approval. Presumably, if FDA approval were given, the DEA would be forced to reschedule MDMA to allow legal medical use. However, such a situation has never occurred, and the procedures for such a rescheduling are not well-defined. Rescheduling MDMA would also bring the US into conflict with international law (U.N. Convention on Psychotropic Substances), further complicating matters. The FDA and DEA may be in no hurry to wade into such a quagmire.

The Kefauver-Harris amendment, which established the modern FDA approval process, requires that drugs not only be proven effective but also safe. As discussed previously, there are significant safety concerns associated with MDMA use. It is neurotoxic and can produce severe and potentially life-threatening hyperthermia or cardiac dysrhythmias in vulnerable individuals. Therefore, even if MDMA is demonstrated to have clinical efficacy, the FDA could still deny approval due to a lack of safety. Alternatively, approval could be given but the viability of widespread medical use could be crippled with onerous licensure requirements and other restrictions designed to limit its therapeutic availability. Although MDMA may be useful for treating a variety of conditions including PTSD, phobias, psychosomatic disorders, depression, drug addiction, relationship difficulties, social anxiety, and the psychological distress of a life-threatening illness, clinical use might be limited to just the narrowest spectrum of one or two severe chronic conditions (Adamson and Metzner, 1988; Danforth *et al*, 2016; Downing, 1986; Greer and Tolbert, 1986; Grinspoon and Bakalar, 1986; Mithoefer *et al*, 2016; Riedlinger and Riedlinger, 1994).

These legitimate safety concerns are further complicated by the widespread illicit use of MDMA. If medical use of MDMA moves forward, this could affect the perceived harmfulness of MDMA and increase recreational use and abuse of the drug. Clearly in the case of cannabis, medical use preceded and arguably permitted the eventually successful efforts to legalize state-wide recreational use of the drug. The director of NORML a national cannabis advocacy

organization, even called medical marijuana a “red-herring to give marijuana a good name” (Emory Wheel, 1979). By legitimizing cannabis, reshaping perceptions about its harmfulness, and increasing supply, legalized use has significantly increased the number of people using the drug. Use of cannabis, including illegal use by minors, is significantly higher in states that allow medical use (RMHIDTA, 2016), and higher still in states that allow recreational use. After Colorado legalized cannabis, drug-related school suspensions and expulsions increased by 40%, cannabis-related emergency room visits increased by 77%, and traffic accidents involving the drug increased 154% (Wong and Clarke, 2015). Without a sea change in federal drug policy, there is no reason to suspect that legalization of MDMA for recreational use will ever occur, but federal regulators will no doubt be cognizant of the effect sanctioned medical use could have on illicit use. People might erroneously believe that because it is used as a medicine it must be safe, potentially increasing irresponsible use and abuse of the drug. There is evidence that this is already occurring without regard to the real and present dangers associated with MDMA (Parrott, 2014a).

Whether by precluding FDA approval or limiting it, the therapeutic use of MDMA will almost certainly be curtailed by these concerns. The value of (–)-MDMA could therefore be multifold. If it produces therapeutic benefits similar to racemic MDMA with fewer safety concerns, it may shift the risk/benefit ratio such that many more conditions might be considered worth treating with (–)-MDMA that would not warrant the risks associated with racemic MDMA. Chemotherapy, for example, has awful side effects, but it is worthwhile because cancer is worse. The same argument can be made for MDMA use in some situations, but (–)-MDMA could significantly expand use to less severe psychiatric conditions. Second, because (–)-MDMA is chemically distinct from the compound “ecstasy”, it does not have the same issues that (+/–)-MDMA would have juggling the dual image as both a dangerous street drug that the government prohibits and a beneficial medication. Much as THC is dual-scheduled, with plant-derived THC

being Schedule I while synthetic THC is Schedule III, MDMA could remain Schedule I while (–)-MDMA is moved to Schedule III, allowing widespread medical use.

D. The Hope for MDMA

MDMA may represent a new form of pharmacotherapy. Traditional psychiatric drugs like SSRIs or antipsychotics are thought to work by adjusting the concentration or signaling of one or more neurochemicals within the brain, treating psychopathology by normalizing or counteracting a neurological imbalance. These drugs usually have acute therapeutic effects that wear off as they are metabolized or excreted. As a result, they typically must be taken every day in order to maintain their medical benefits. MDMA does not appear to work this way. Although the exact mechanisms by which MDMA is therapeutically efficacious are not fully understood, its usefulness is clearly not limited to the half-life of the drug. The therapeutic gains achieved during MDMA-assisted therapy appear to last for months to years after treatment, and potentially may be permanent.

A shift may be occurring in psychiatry away from drugs that are taken daily, to drugs that are taken only periodically but have long-lasting therapeutic gains. Psychiatric use of hallucinogens, which (like MDMA use) also was once in vogue, has returned to popularity with several well designed clinical trials finding significant and long-lasting therapeutic gains for treating depression, anxiety, and addiction (Mithoefer *et al*, 2016; Nichols *et al*, 2016; Sessa, 2005). The dissociative drug ketamine has also emerged as a powerful new tool to treat depression, with rapid onset and effects that last a week or more (DeWilde *et al*, 2015). These drugs may all work by disrupting ingrained functional networks within the brain (Nichols *et al*, 2016). It is possible, indeed likely, that many psychiatric conditions, including depression, addiction, and PTSD, stem from aberrant Hebbian circuits that drive inveterate pathology. Drugs

like psilocybin, ketamine, and MDMA might acutely disrupt these circuits and allow ingrained and ineradicable behaviors, thoughts, and feelings to be extinguished.

Unlike hallucinogens or dissociatives, though, MDMA does not produce cognitive and perceptual distortions or alterations in one's sense of self. And rather than disrupting, it may actually enhance concurrent psychotherapy (Danforth *et al*, 2016). Beginning in the late 1970s, therapists recognized that MDMA might be uniquely suited as an adjunct to therapy, and began using it in their practices. It was reported to be effective in helping to treat conditions as benign as marital strife or as severe as PTSD and alcoholism. Its wide-ranging utility earned it the nickname "penicillin for the soul" (Shulgin and Shulgin, 1991). It reportedly facilitated heightened states of introspection, and patients were more emotionally open with less defensive anxieties, allowing them to access and evaluate feelings and thoughts not ordinarily available to them (Danforth *et al*, 2016; Mithoefer *et al*, 2016). Although thousands of patients were likely treated with MDMA at the time, no placebo-controlled clinical trials were performed; the only evidence of any actual efficacy comes from anecdotal reports. But as modern, well-controlled clinical trials have begun to validate these past claims of therapeutic utility, the question arises as to how far this clinical efficacy might extend. If MDMA is effective for treating PTSD, what about generalized anxiety disorder or phobias? If it is effective for treating social anxiety in autistic patients, what about social anxiety in typically developing individuals? Perhaps it can facilitate and improve psychotherapy in general, without regard to the psychiatric condition for which it is used. While skepticism is certainly warranted – we should not forget that cocaine too was once proposed as a cure-all (Jay, 2015) – ignoring the clinical data on MDMA is equally misguided. The trials so far have been very small with homogenous patient populations, and blinding is certainly an issue, but the solution to these weaknesses is not rebuttal but more and larger trials. The potential for MDMA-assisted therapy is considerable, and the best hope for it being realized may be through (–)-MDMA.

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Appendix A

Companion study performed by Matthew Young, PhD

Introduction

Post-traumatic stress disorder (PTSD) is a chronic and often debilitating condition that may develop following a traumatic event. Approximately 6.8% of the general population will develop PTSD over their lifetimes, but the incidence is substantially higher in at risk populations such as war veterans, where the rate has been estimated to be as high as 24.5% (Amoroso and Workman, 2016). Few treatment options currently exist, but recent clinical trials have found that MDMA may be an effective emerging treatment option (Mithoefer *et al*, 2011; Oehen *et al*, 2013). When paired with psychotherapy, just two or three MDMA treatment sessions significantly reduced PTSD symptoms for at least 17 months (Mithoefer *et al*, 2013). PTSD is often conceptualized as a deficit in the extinction of fear conditioning, whereby cues associated with a traumatic memory continue to trigger a powerful fear response even when those cues do not signal an actual threat (VanElzakker *et al*, 2014). A prior study with fear-conditioned mice demonstrated that treatment with MDMA facilitates long-lasting extinction (Young *et al*, 2015). The same fear conditioning and extinction paradigm was used to assess the therapeutic-like effects of the enantiomers.

Methods

Male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) aged 10-16 weeks served as subjects. The effect of (–)-MDMA and (+)-MDMA on fear extinction was evaluated using established protocols (Young *et al*, 2015). Briefly, mice were exposed to cued fear conditioning on day 1, fear extinction training on day 3, and extinction testing on day 4. Cued fear conditioning consisted of a single pairing of a conditioned stimulus (CS) tone (80 dB, 4.5 kHz, 30 s) and an unconditioned stimulus (US) foot shock (1 mA, 2 s) and was carried out by placing the

subject in the conditioning apparatus for 2 minutes before the CS-tone turned on and co-terminated with the US-footshock. Extinction training was carried out 48 hours later in a new context from conditioning. (–)-MDMA, (+)-MDMA, or saline were administered on day 3, 30 minutes before training. Extinction training began 2 minutes after placing the subject into the extinction apparatus, and consisted of a sub-optimal regimen of 4 CS-tone re-exposures separated by 45 seconds. Extinction testing was performed 24 hours later to determine the lasting effect of treatment on fear extinction. Extinction testing was performed in the same context as training and followed the same procedure. Throughout these experiments, %freezing was estimated by scoring the presence or absence of non-respiratory movement every 5 seconds. Changes to conditioned freezing during extinction training and extinction testing by doses of (–)-MDMA or (+)-MDMA were analyzed with one-way ANOVAs and Dunnett's post-hot tests comparing each treatment to saline treated controls.

Results

The timeline of fear conditioning and extinction is shown in Figure A.1a. Tests evaluated whether treatment with (–)-MDMA or (+)-MDMA, given 30 minutes prior to extinction training, would facilitate lasting extinction. Extinction was measured as the %freezing in response to a CS tone. Acute administration of (–)-MDMA (N = 7/dose) had no effect during extinction training ($F(3, 24) = 1.002$, $p = 0.4089$; Figure A.1b), but significantly reduced freezing during extinction testing ($F(3, 24) = 3.64$, $p = 0.027$; Figure A.1c). A Dunnett's post-hoc analysis revealed that 17 mg/kg (–)-MDMA significantly reduced freezing during extinction testing compared to saline ($p = 0.0124$). (+)-MDMA (N = 6/dose) reduced conditioned freezing during training ($F(3, 20) = 5.581$, $p = 0.006$; Figure A.1d), with a Dunnett's post hoc test indicating that 5.6 mg/kg and 7.8 mg/kg (+)-MDMA were significantly different from saline ($p = 0.007$ and $p = 0.0387$, respectively). However, this effect did not persist the following day during extinction testing ($F(3, 20) = 1.142$, $p = 0.3564$; Figure A.1e).

Discussion

There is significant interest in the adjunctive use of MDMA to treat PTSD. Two small clinical trials recently completed and found that when MDMA was given during psychotherapy it helped to substantially reduce the symptoms of PTSD in patients that had been resistant to other treatments (Mithoefer *et al*, 2011; Oehen *et al*, 2013). Larger Phase III clinical trials ae underway and MDMA may one day become an FDA-approved medication for PTSD (Philipps, 2016). However little is known about why MDMA might therapeutically efficacious. One explanation is that it facilitates the extinction of conditioned fears that have become linked to a past traumatic event. To test this theory in mice, a recent experiment observed that treatment with MDMA during extinction training decreased conditioned freezing not only during the training, but also the next day during extinction testing (Young *et al*, 2015). Even though no drug was administered during testing, mice formerly treated with MDMA displayed significantly less freezing compared to saline treated controls. This suggests that MDMA is not functioning as a palliative treatment, but rather is increasing the effectiveness of exposure training to extinguish conditioned fear.

In the present study, we tested the enantiomers of MDMA using the same procedure. As with racemic MDMA, (–)-MDMA facilitated lasting fear extinction when tested 24 hours after treatment and training. (+)-MDMA reduced freezing during training but had no lasting effect on fear extinction. Reduced freezing during training may have been due to the locomotor stimulant effect of the drug, or could indicate an effect similar to that of benzodiazepines which can acutely decrease fear responses but do not facilitate lasting extinction (Bouton *et al*, 1990). The stereospecificity of this effect is surprising and suggests that the pharmacodynamic distinctions between (–)-MDMA and (+)-MDMA may be essential to the therapeutic effects of MDMA. One of the key differences between the two enantiomers is the significantly higher potency of (–)-MDMA at 5-HT_{2A} receptors (Huot *et al*, 2011). These receptors are known to be involved in associative learning, and other 5-HT_{2A} agonists can enhance learning (Harvey, 2003b). Whether

activation of 5-HT_{2A} receptors is necessary for the facilitative effect of MDMA and (–)-MDMA on fear extinction learning will be an important question to assess in future experiments.

In addition to the promising therapeutic effects of MDMA, it can also have severe adverse effects that may significantly limit its therapeutic viability. In particular, it has been demonstrated to be highly neurotoxic in animal studies (Capela *et al*, 2009), and evidence from human users suggests that toxicity may occur at the doses used recreationally as well as clinically (Mueller *et al*, 2013; Parrott, 2013). It is also known to induce precipitous increases in body temperature that can be potentially fatal (Halpern *et al*, 2011b; Landry, 2002). Our findings indicate that (–)-MDMA does not have these same adverse effects, at least in mice. If (–)-MDMA has therapeutic effects similar to (+/–)-MDMA, as our model indicates, then it may be a substantially more viable clinical option that racemic MDMA. Clinical study of (–)-MDMA will be the only way to confirm these results. We sincerely hope that clinical researchers will pursue it.

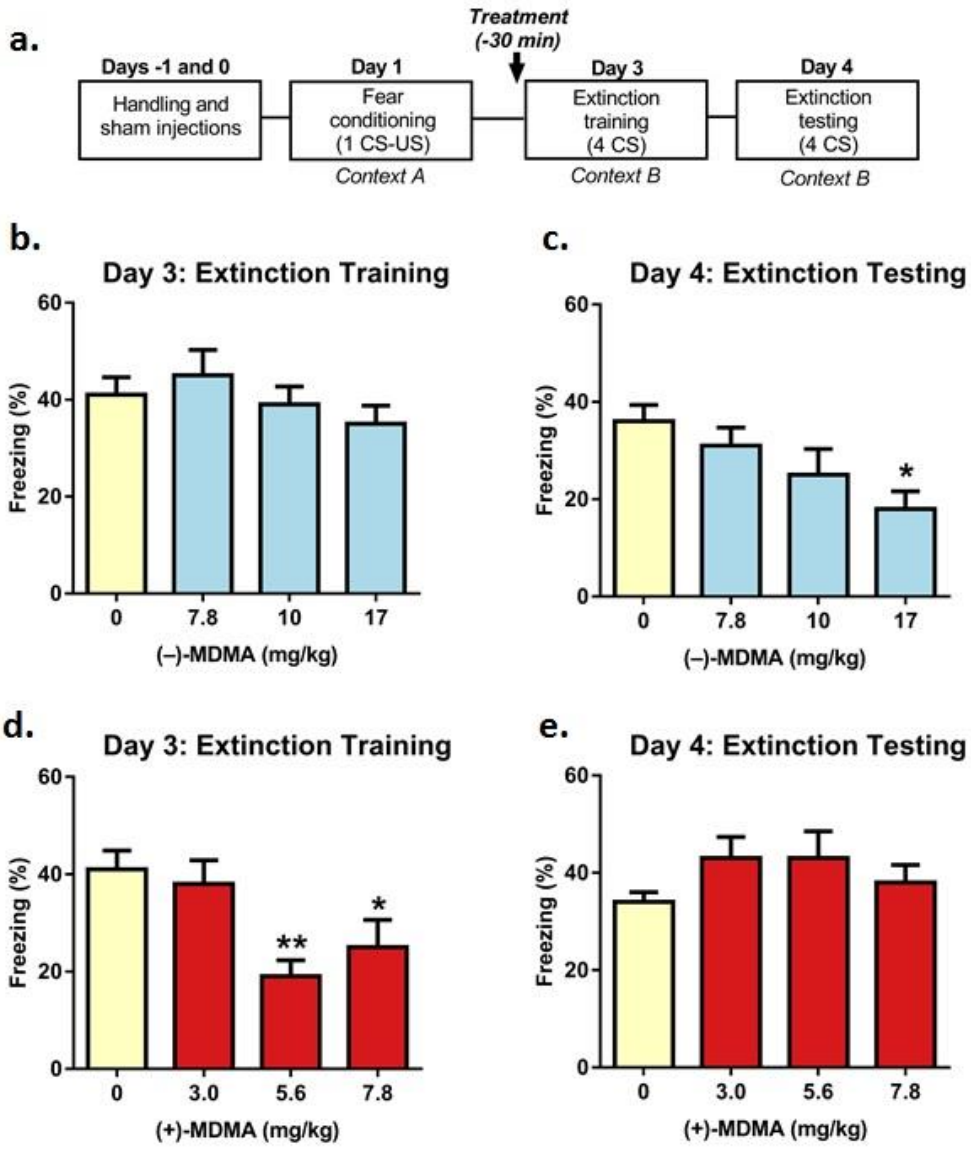

Figure A.1 Dose-dependent effects of (–)-MDMA and (+)-MDMA on fear extinction. **(a)** Timeline of fear conditioning and extinction experiment. **(b)** (–)-MDMA did not affect freezing during extinction training. **(c)** But this prior (–)-MDMA treatment reduced freezing during extinction testing 24 hours later. A treatment dose of 17 mg/kg significantly reduced freezing during extinction testing compared to saline (*p = 0.0124) **(d)** (+)-MDMA decreased freezing during extinction training with doses of 5.6 mg/kg and 7.8 mg/kg significantly decreasing freezing compared to saline (**p = 0.007 and *p = 0.0387, respectively). **(e)** However, (+)-MDMA treatment did not facilitate lasting extinction; there was no effect of treatment on freezing during extinction testing. *Bars represent mean ± SEM of %freezing across 4 CS tones. CS, conditioned stimulus; US, unconditioned stimulus.*

References

Acquas E, Pisanu A, Spiga S, Plumitallo A, Zernig G, Chiara G Di (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**: 121–32.

Adamson S, Metzner R (1988). The Nature of the MDMA Experience and Its Role in Healing, Psychotherapy, and Spiritual Practice. *ReVision* **10**: .

Ádori C, Andó RD, Kovács GG, Bagdy G (2006). Damage of serotonergic axons and immunolocalization of Hsp27, Hsp72, and Hsp90 molecular chaperones after a single dose of MDMA administration in Dark Agouti rat: Temporal, spatial, and cellular patterns. *J Comp Neurol* **497**: 251–269.

Ago Y, Nakamura S, Baba A, Matsuda T (2008). Neuropsychotoxicity of Abused Drugs: Effects of Serotonin Receptor Ligands on Methamphetamine- and Cocaine-Induced Behavioral Sensitization in Mice. *J Pharmacol Sci J Pharmacol Sci* **106**: 15–211.

Aguirre N, Barrionuevo M, Ramírez MJ, Río J Del, Lasheras B (1999). Alpha-lipoic acid prevents 3,4-methylenedioxy-methamphetamine (MDMA)-induced neurotoxicity. *Neuroreport* **10**: 3675–80.

Alexander SPH (2016). Therapeutic potential of cannabis-related drugs. *Prog Neuro-Psychopharmacology Biol Psychiatry* **64**: 157–166.

Alves E, Summavielle T, Juliana Alves C, Gomes-da-Silva J, Custódio Barata J, Fernandes E, *et al* (2007). Monoamine Oxidase-B Mediates Ecstasy-Induced Neurotoxic Effects to Adolescent Rat Brain Mitochondria. doi:10.1523/JNEUROSCI.2645-07.2007.

Amoroso T (2015). The Psychopharmacology of ±3,4 Methylenedioxymethamphetamine and its Role in the Treatment of Posttraumatic Stress Disorder. *J Psychoactive Drugs* **0**: 1–8.

Amoroso T, Workman M (2016). Treating posttraumatic stress disorder with MDMA-assisted psychotherapy: A preliminary meta-analysis and comparison to prolonged exposure therapy. *J Psychopharmacol* doi:10.1177/0269881116642542.

Anderson GM, Braun G, Braun U, Nichols DE, Shulgin a T (1978). Absolute configuration and psychotomimetic activity. *NIDA Res Monogr* 8–15at <http://www.ncbi.nlm.nih.gov/pubmed/101890>.

Ando RD, Benko A, Ferrington L, Kirilly E, Kelly PAT, Bagdy G (2006). Partial lesion of the serotonergic system by a single dose of MDMA results in behavioural disinhibition and enhances acute MDMA-induced social behaviour on the social interaction test. *Neuropharmacology* **50**: 884–96.

Angelis L de, File SE (1979). Acute and chronic effects of three benzodiazepines in the social interaction anxiety test in mice. *Psychopharmacology (Berl)* **64**: 127–9.

Auclair A, Drouin C, Cotecchia S, Glowinski J, Tassin J-P (2004). 5-HT2A and alpha1b-adrenergic receptors entirely mediate dopamine release, locomotor response and

behavioural sensitization to opiates and psychostimulants. *Eur J Neurosci* **20**: 3073–84.

Bagdy G (1996). Role of the hypothalamic paraventricular nucleus in 5-HT1A, 5-HT2A and 5-HT2C receptor-mediated oxytocin, prolactin and ACTH/corticosterone responses. *Behav Brain Res* **73**: 277–80.

Baggott M, Heifets B, Jones RT, Mendelson J, Sferios E, Zehnder J (2000). Chemical analysis of ecstasy pills. *JAMA* **284**: 2190.

Ball KT, Klein JE, Plocinski J a, Slack R (2011). Behavioral sensitization to 3,4-methylenedioxymethamphetamine is long-lasting and modulated by the context of drug administration. *Behav Pharmacol* **22**: 847–50.

Ball KT, Slane M (2014). *Tolerance to the locomotor-activating effects of 3,4-methylenedioxymethamphetamine (MDMA) predicts escalation of MDMA self-administration and cue-induced reinstatement of MDMA seeking in rats*. *Behav Brain Res* **274**: .

Ballesta S, Reymond G, Pozzobon M, Duhamel JR (2016). Effects of MDMA Injections on the Behavior of Socially-Housed Long-Tailed Macaques (Macaca fascicularis). *PLoS One* **11**: 1–10.

Barbosa DJ, Capela JP, Feio-Azevedo R, Teixeira-Gomes A, Bastos M de L, Carvalho F (2015). Mitochondria: key players in the neurotoxic effects of amphetamines. *Arch Toxicol* **89**: 1695–1725.

Barcia C, Ros CM, Annese V, Gómez A, Ros-Bernal F, Aguado-Yera D, *et al* (2011). IFN-γ signaling, with the synergistic contribution of TNF-α, mediates cell specific microglial and astroglial activation in experimental models of Parkinson's disease. *Cell Death Dis* **2**: e142.

Baron EP (2015). Comprehensive Review of Medicinal Marijuana, Cannabinoids, and Therapeutic Implications in Medicine and Headache: What a Long Strange Trip It's Been …. *Headache J Head Face Pain* **55**: 885–916.

Battaglia G, Brooks BP, Kulsakdinun C, Souza EB De (1988a). Pharmacologic profile of MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites. *Eur J Pharmacol* **149**: 159–63.

Battaglia G, Yeh SY, Souza EB De (1988b). MDMA-induced neurotoxicity: Parameters of degeneration and recovery of brain serotonin neurons. *Pharmacol Biochem Behav* **29**: 269–274.

Baumann MH, Clark RD, Franken FH, Rutter JJ, Rothman RB (2008). Tolerance to 3,4-methylenedioxymethamphetamine in rats exposed to single high-dose binges. *Neuroscience* **152**: 773–84.

Baumann MH, Wang X, Rothman RB (2007). 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. *Psychopharmacology (Berl)* **189**: 407–24.

Bedi G, Cecchi G a, Slezak DF, Carrillo F, Sigman M, Wit H de (2014). A window into the intoxicated mind? Speech as an index of psychoactive drug effects. *Neuropsychopharmacology* **39**: 2340–8.

Bedi G, Dam N Van, Redman J (2010a). Ecstasy (MDMA) and high prevalence psychiatric symptomatology: somatic anxiety symptoms are associated with polydrug, not ecstasy, use. *J Psychopharmacol* **24**: 233–240.

Bedi G, Hyman D, Wit H de (2010b). Is ecstasy an "empathogen"? Effects of ±3,4-methylenedioxymethamphetamine on prosocial feelings and identification of emotional states in others. *Biol Psychiatry* **68**: 1134–40.

Bedi G, Phan KL, Angstadt M, Wit H de, Manuscript A (2009). Effects of MDMA on sociability and neural response to social threat and social reward. *Psychopharmacology (Berl)* **207**: 73–83.

Bedi G, Redman J (2008). Ecstasy use and higher-level cognitive functions: weak effects of ecstasy after control for potential confounds. *Psychol Med* **38**: 1319–30.

Bernschneider-Reif S, Oxler F, Freudenmann RW (2006). The origin of MDMA ("ecstasy")--separating the facts from the myth. *Pharmazie* **61**: 966–72.

Bershad AK, Kirkpatrick MG, Seiden JA, Wit H de (2015). Effects of Acute Doses of Prosocial Drugs Methamphetamine and Alcohol on Plasma Oxytocin Levels. *J Clin Psychopharmacol* **35**: 308–312.

Bershad AK, Miller MA, Baggott MJ, Wit H de (2016a). The effects of MDMA on socio-emotional processing: Does MDMA differ from other stimulants? *J Psychopharmacol* **30**: 1248–1258.

Bershad AK, Weafer JJ, Kirkpatrick MG, Wardle MC, Miller MA, Wit H de (2016b). Oxytocin receptor gene variation predicts subjective responses to MDMA. *Soc Neurosci* **919**: 17470919.2016.1143026.

Bevilacqua L, Doly S, Kaprio J, Yuan Q, Tikkanen R, Paunio T, *et al* (2010). A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature* **468**: 1061–6.

Bhattacharya SK, Bhattacharya A, Ghosala S (1998). Anxiogenic activity of methylenedioxymethamphetamine (Ecstasy): an experimental study. *Biog Amin* **14**: .

Biezonski DK, Meyer JS (2011). The Nature of 3, 4-Methylenedioxymethamphetamine (MDMA)-Induced Serotonergic Dysfunction: Evidence for and Against the Neurodegeneration Hypothesis. *Curr Neuropharmacol* **9**: 84–90.

Bindoli A, Rigobello MP, Deeble DJ (1992). Biochemical and toxicological properties of the oxidation products of catecholamines. *Free Radic Biol Med* **13**: 391–405.

Blair RJR (2005). Responding to the emotions of others: Dissociating forms of empathy through the study of typical and psychiatric populations. *Conscious Cogn* **14**: 698–718.

Bolla KI, McCann UD, Ricaurte GA (1998). Memory impairment in abstinent MDMA ("Ecstasy") users. *Neurology* **51**: 1532–7.

Bortolozzi A, Díaz-Mataix L, Scorza MC, Celada P, Artigas F (2005). The activation of 5-HT receptors in prefrontal cortex enhances dopaminergic activity. *J Neurochem* **95**: 1597–607.

Bostwick JM (2012). Blurred Boundaries: The Therapeutics and Politics of Medical Marijuana. *Mayo Clin Proc* **87**: 172–186.

Boulougouris V, Glennon JC, Robbins TW (2008). Dissociable Effects of Selective 5-HT 2A and 5-HT 2C Receptor Antagonists on Serial Spatial Reversal Learning in Rats. *Neuropsychopharmacology* **33**: .

Bouton ME, Kenney F a, Rosengard C (1990). State-dependent fear extinction with two benzodiazepine tranquilizers. *Behav Neurosci* **104**: 44–55.

Bradbury S, Gittings D, Schenk S (2012). Repeated exposure to MDMA and amphetamine: sensitization, cross-sensitization, and response to dopamine D(1)- and D (2)-like agonists. *Psychopharmacol* doi:10.1007/s00213-012-2726-9.

Brady K, Pearlstein T, Asnis GM, Baker D, Rothbaum B, Sikes CR, *et al* (2000). Efficacy and Safety of Sertraline Treatment of Posttraumatic Stress Disorder. *JAMA* **283**: 1837.

Brebner K, Wong TP, Liu L, Liu Y, Campsall P, Gray S, *et al* (2005). Nucleus accumbens long-term depression and the expression of behavioral sensitization. *Science* **310**: 1340–3.

Broening HW, Bowyer JF, Slikker W (1995). Age-dependent sensitivity of rats to the long-term effects of the serotonergic neurotoxicant (+/-)-3,4-methylenedioxymethamphetamine (MDMA) correlates with the magnitude of the MDMA-induced thermal response. *J Pharmacol Exp Ther* **275**: 325–33.

Bull EJ, Hutson PH, Fone KC. F (2004). Decreased social behaviour following 3,4-methylenedioxymethamphetamine (MDMA) is accompanied by changes in 5-HT2A receptor responsivity. *Neuropharmacology* **46**: 202–10.

Burkett JP, Andari E, Johnson Z V, Curry DC, Waal FBM De, Young LJ (2016). Supplementary Material for Oxytocin-dependent consolation behavior in rodents. **375**: .

Cadet JL, Brannock C (1997). Free radicals and the pathobiology of brain dopamine systems. *Neurochem Int* **32**: 117–131.

Cadet JL, Ladenheim B, Hirata H, Rothman RB, Ali S, Carlson E, *et al* (1995). Superoxide radicals mediate the biochemical effects of methylenedioxymethamphetamine (MDMA): evidence from using CuZn-superoxide dismutase transgenic mice. *Synapse* **21**: 169–76.

Cadet JL, Thiriet N, Jayanthi S (2001). Involvement of free radicals in MDMA-induced neurotoxicity in mice. *Ann Med Interne (Paris)* IS57-9at <http://www.ncbi.nlm.nih.gov/pubmed/11435997>.

Cador M, Bjijou Y, Stinus L (1995). Evidence of a complete independence of the neurobiological substrates for the induction and expression of behavioral sensitization to amphetamine. *Neuroscience* **65**: 385–395.

Cami J, Farré M, Mas M, Roset PN, Poudevida S, Mas A, *et al* (2000). Human pharmacology of 3,4-methylenedioxymethamphetamine ("ecstasy"): psychomotor performance and subjective effects. *J Clin Psychopharmacol* **20**: 455–66.

Capela JP, Carmo H, Remião F, Bastos ML, Meisel A, Carvalho F (2009). Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: an overview. *Mol Neurobiol* **39**: 210–71.

Capurro A, Reyes-Parada M, Olazabal D, Perrone R, Silveira R, Macadar O (1997). Aggressive behavior and jamming avoidance response in the weakly electric fish Gymnotus carapo: Effects of 3,4-methylenedioxymethamphetamine (MDMA). *Comp Biochem Physiol Part A*

Physiol **118**: 831–840.

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 Pulication No SMA 15-4927, NSDUH Ser H-50* 64at <http://www.samhsa.gov/data/sites/default/files/NSDUH-FRR1-2014/NSDUH-FRR1-2014.pdf%5Cnhttp://www.samhsa.gov/data/>.

Chadwick IS, Curry PD, Linsley A, Freemont AJ, Doran B (1991). Ecstasy, 3-4 methylenedioxymethamphetamine (MDMA), a fatality associated with coagulopathy and hyperthermia. *J R Soc Med* **84**: 371.

Chasin A (University of Chicago Press: Chicago, 2016). *Assassin of youth : a kaleidoscopic history of Harry J. Anslinger's war on drugs*. .

Colado MI, O'Shea E, Esteban B, Granados R, Green AR (1999). In vivo evidence against clomethiazole being neuroprotective against MDMA ('ecstasy')-induced degeneration of rat brain 5-HT nerve terminals by a free radical scavenging mechanism. *Neuropharmacology* **38**: 307–14.

Colado MI, O'Shea E, Granados R, Murray TK, Green AR (1997). *In vivo* evidence for free radical involvement in the degeneration of rat brain 5-HT following administration of MDMA ("ecstasy") and *p* -chloroamphetamine but not the degeneration following fenfluramine. *Br J Pharmacol* **121**: 889–900.

Cole JC, Sumnall HR (2003). Altered states: the clinical effects of Ecstasy. *Pharmacol Ther* **98**: 35–58.

Collin M, Godfrey J (Serpent's Tail: London, 2010). *Altered State: The story of Ecstasy culture and acid house*. .

Commins DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS (1987). Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. *J Pharmacol Exp Ther* **241**: 338–45.

Cottler LB, Womack SB, Compton WM, Ben-Abdallah A (2001). Ecstasy abuse and dependence among adolescents and young adults: Applicability and reliability of DSM-IV criteria. *Hum Psychopharmacol* **16**: 599–606.

Crippa JAS, Crippa ACS, Hallak JEC, Martín-Santos R, Zuardi AW (2016). Δ9-THC Intoxication by Cannabidiol-Enriched Cannabis Extract in Two Children with Refractory Epilepsy: Full Remission after Switching to Purified Cannabidiol. *Front Pharmacol* **7**: 359.

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

Dao CK, Nowinski SM, Mills EM (2014). The heat is on: Molecular mechanisms of drug-induced hyperthermia. *Temp (Austin, Tex)* **1**: 183–91.

Daza-Losada M, Rodríguez-Arias M, Maldonado C, Aguilar MA, Guerri C, Miñarro J (2009). Acute behavioural and neurotoxic effects of MDMA plus cocaine in adolescent mice. *Neurotoxicol Teratol* **31**: 49–59.

Department Health UO, Services H (2013). Drug Abuse Warning Network, 2011: National Estimates of Drug-Related Emergency Department Visits. .

DeWilde KE, Levitch CF, Murrough JW, Mathew SJ, Iosifescu D V. (2015). The promise of ketamine for treatment-resistant depression: current evidence and future directions. *Ann N Y Acad Sci* **1345**: 47–58.

Diaz SL, Doly S, Narboux-Nême N, Fernández S, Mazot P, Banas SM, *et al* (2012). 5-HT(2B) receptors are required for serotonin-selective antidepressant actions. *Mol Psychiatry* **17**: 154–63.

Dikötter F, Laamann LP, Zhou X (Hurst & Company: London, 2004). *Narcotic Culture: A History of Drugs in China*. .

Doblin R (2002). A Clinical Plan for MDMA (Ecstasy) in the Treatment of Post-Traumatic Stress Disorder (PTSD): Partnering with the FDA. *Multidiscip Assoc Psychedelic Stud* **12**: .

Doblin R, Greer G, Holland J, Jerome L, Mithoefer MC, 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**: 105–8.

Docherty JR, Green AR (2010a). The role of monoamines in the changes in body temperature induced by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and its derivatives. *Br J Pharmacol* **160**: 1029–44.

Docherty JR, Green AR (2010b). The role of monoamines in the changes in body temperature induced by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and its derivatives. *Br J Pharmacol* **160**: 1029–1044.

Dölen G, Darvishzadeh A, Huang KW, Malenka RC (2013). Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature* **501**: 179–84.

Doly S, Valjent E, Setola V, Callebert J, Hervé D, Launay J-M, *et al* (2008). Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. *J Neurosci* **28**: 2933–40.

Downing J (1986). The psychological and physiological effects of MDMA on normal volunteers. *J Psychoactive Drugs* **18**: 335–40.

Droogmans S, Cosyns B, D'haenen H, Creeten E, Weytjens C, Franken PR, *et al* (2007). Possible association between 3,4-methylenedioxymethamphetamine abuse and valvular heart disease. *Am J Cardiol* **100**: 1442–5.

Drug Enforcement Administration (2016). *Establishment of a New Drug Code for Marihuana Extract*. *Fed Regist* **81**: .

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

Duxon MS, Kennett G a, Lightowler S, Blackburn TP, Fone KC (1997). Activation of 5-HT2B receptors in the medial amygdala causes anxiolysis in the social interaction test in the rat. *Neuropharmacology* **36**: 601–8.

Eberle-Wang K, Mikeladze Z, Uryu K, Chesselet MF (1997). Pattern of expression of the serotonin2C receptor messenger RNA in the basal ganglia of adult rats. *J Comp Neurol* **384**: 233–47.

Elangbam CS (2010). Drug-induced valvulopathy: an update. *Toxicol Pathol* **38**: 837–48.

Emanuele E, Arra M, Pesenti S (2006). Vasopressin and oxytocin as neurohormonal mediators of MDMA (ecstasy) sociosexual behavioural effects. *Med Hypotheses* **67**: 1250–1.

Erritzoe D, Frokjaer VG, Holst KK, Christoffersen M, Johansen SS, Svarer C, *et al* (2011a). In vivo imaging of cerebral serotonin transporter and serotonin(2A) receptor binding in 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") and hallucinogen users. *Arch Gen Psychiatry* **68**: 562–576.

Erritzoe D, Frokjaer VG, Holst KK, Christoffersen M, Johansen SS, Svarer C, *et al* (2011b). In vivo imaging of cerebral serotonin transporter and serotonin(2A) receptor binding in 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") and hallucinogen users. *Arch Gen Psychiatry* **68**: 562–76.

Esteban B, O'Shea E, Camarero J, Sanchez V, Green AR, Colado MI (2001). 3,4-Methylenedioxymethamphetamine induces monoamine release, but not toxicity, when administered centrally at a concentration occurring following a peripherally injected neurotoxic dose. *Psychopharmacology (Berl)* **154**: 251–260.

Falk EM, Cook VJ, Nichols DE, Sprague JE (2002). An antisense oligonucleotide targeted at MAO-B attenuates rat striatal serotonergic neurotoxicity induced by MDMA. *Pharmacol Biochem Behav* **72**: 617–622.

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

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**: 202–11.

Fantegrossi WE, Murai N, Brian O, Torre R De, Mathúna BO, Pizarro N, *et al* (2009). Discriminative stimulus effects of 3,4-methylenedioxymethamphetamine and its enantiomers in mice: pharmacokinetic considerations. *J Pharmacol Exp Ther* **329**: 1006–15.

Fantegrossi WE, Ullrich T, Rice KC, Woods JH, Winger G (2002). 3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") and its stereoisomers as reinforcers in rhesus monkeys: serotonergic involvement. *Psychopharmacology (Berl)* **161**: 356–64.

Farfel GM, Vosmer GL, Seiden LS (1992). The N-methyl-D-aspartate antagonist MK-801 protects against serotonin depletions induced by methamphetamine, 3,4-methylenedioxymethamphetamine and p-chloroamphetamine. *Brain Res* **595**: 121–7.

File SE, Guardiola-Lemaitre BJ (1988). l-fenfluramine in tests of dominance and anxiety in the rat. *Neuropsychobiology* **20**: 205–11.

File SE, Hyde JRG (1978). Can Social Interaction Be Used To Measure Anxiety? *Br J Pharmacol* **62**: 19–24.

File SE, Seth P (2003). A review of 25 years of the social interaction test. *Eur J Pharmacol* **463**: 35–53.

Filip M, Nowak E, Papla I (2001). On the role of serotonin2A/2C receptors in the sensitization to cocaine. *J Physiol Pharmacol* **52**: 471–81.

Finnegan KT, Ricaurte GA, Ritchie LD, Irwin I, Peroutka SJ, Langston JW (1988). Orally administered MDMA causes a long-term depletion of serotonin in rat brain. *Brain Res* **447**: 141–144.

Fischer C, Hatzidimitriou G, Wios J, Katz J, Ricaurtel G (1995). Reorganization of Ascending 5HT Axon Projections in Animals Previously Exposed to the Recreational Drug (+)3,4-Methylenedioxymethamphetamine (MDMA, " Ecstasy "). *J Neurosci* **15**: 5478–5485.

Fleckenstein AE, Hanson GR (2003). Impact of psychostimulants on vesicular monoamine transporter function. *Eur J Pharmacol* **479**: 283–289.

Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR (2007). New insights into the mechanism of action of amphetamines. *Annu Rev Pharmacol Toxicol* **47**: 681–98.

Forsling M, Fallon JK, Kicman AT, Hutt AJ, Cowan DA, Henry JA (2001). Arginine vasopressin release in response to the administration of 3,4-methylenedioxymethamphetamine ("ecstasy"): is metabolism a contributory factor? *J Pharm Pharmacol* **53**: 1357–63.

Forsling ML, Fallon JK, Shah D, Tilbrook GS, Cowan D a, Kicman AT, *et al* (2002). The effect of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") and its metabolites on neurohypophysial hormone release from the isolated rat hypothalamus. *Br J Pharmacol* **135**: 649–656.

Frau L, Simola N, Plumitallo A, 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.

French ED (1986). Preliminary Notes. *Neuropharmacology* **25**: 447–450.

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

Friard O, Gamba M (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol Evol* doi:10.1111/2041-210X.12584doi:10.1111/2041-210X.12584.

Gable RS (2006). The Toxicity of Recreational Drugs. *Am Sci* .

Garcia-Ratés S, Camarasa J, Escubedo E, Pubill D (2007). Methamphetamine and 3,4-methylenedioxymethamphetamine interact with central nicotinic receptors and induce their up-regulation. *Toxicol Appl Pharmacol* **223**: 195–205.

Garrett ER, Seyda K, Marroum P (1991). High performance liquid chromatographic assays of the illicit designer drug "Ecstasy", a modified amphetamine, with applications to stability, partitioning and plasma protein binding. *Acta Pharm Nord* **3**: 9–14.

Gaston TR, Rasmussen GT (Chicago, 1972). *IDENTIFICATION OF 3,4 METHYLENEDIOXYMETHAMPHETAMINE*. **15**: .

Ghatol A, Kazory A (2012). Ecstasy-associated acute severe hyponatremia and cerebral edema: a role for osmotic diuresis? *J Emerg Med* **42**: e137-40.

Globus MY-T, Busto R, Lin B, Schnippering H, Ginsberg MD (1995). Detection of free radical activity during transient global ischemia and recirculation: effects of intraischemic brain temperature modulation. *J Neurochem* **65**: 1250–6.

Gobert A, Rivet J-M, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas J-P, *et al* (2000). Serotonin2C receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: A combined dialysis and electrophysiological analysis in the rat. *Synapse* **36**: 205–221.

González-Maeso J, Weisstaub N V, Zhou M, Chan P, Ivic L, Ang R, *et al* (2007). Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron* **53**: 439–52.

Gordon CJ (2007). Thermophysiological responses to hyperthermic drugs: extrapolating from rodent to human. *Prog Brain Res* **162**: 63–79.

Gore SM (1999). Fatal uncertainty: death-rate from use of ecstasy or heroin. *Lancet* **354**: 1265–1266.

Gouzoulis-Mayfrank E, Thelen B, Habermeyer E, Kunert HJ, Kovar K-A, Lindenblatt H, *et al* (1999). Psychopathological, neuroendocrine and autonomic effects of 3,4-methylenedioxyethylamphetamine (MDE), psilocybin and d -methamphetamine in healthy volunteers. *Psychopharmacology (Berl)* **142**: 41–50.

Granado N, Escobedo I, O'Shea E, Colado I, Moratalla R (2008a). Early loss of dopaminergic terminals in striosomes after MDMA administration to mice. *Synapse* **62**: 80–4.

Granado N, O'Shea E, Bove J, Vila M, Colado MI, Moratalla R (2008b). Persistent MDMA-induced dopaminergic neurotoxicity in the striatum and substantia nigra of mice. *J Neurochem* **107**: 1102–1112.

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

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

Greer GR, Tolbert R (1990). The Therapeutic Use of MDMA. 21–35doi:10.1007/978-1-4613-1485-1_2.

Greer GR, Tolbert R (1994). A method of conducting therapeutic sessions with MDMA. *J Psychoactive Drugs* **30**: 371–9.

Griffiths RR, Johnson MW, Carducci MA, Umbricht A, Richards WA, Richards BD, *et al* (2016). Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. *J Psychopharmacol* **30**: 1181–1197.

Grinspoon L, Bakalar JB (1986). Can Drugs Be Used to Enhance the Psychotherapeutic Process? *Am J Psychother* **40**: .

Gudelsky GA (1996). Effect of ascorbate and cysteine on the 3,4-methylenedioxymethamphetamine-induced depletion of brain serotonin. *J Neural Transm* **103**: 1397–1404.

Gudelsky GA, Nash JF (1996). Carrier-mediated release of serotonin by 3,4-methylenedioxymethamphetamine: implications for serotonin-dopamine interactions. *J Neurochem* **66**: 243–9.

Guiard BP, Giovanni G Di (2015). Central serotonin-2A (5-HT2A) receptor dysfunction in depression and epilepsy: The missing link? *Front Pharmacol* **6**: 1–17.

Hall AP, Henry JA (2006). Acute toxic effects of "Ecstasy" (MDMA) and related compounds: overview of pathophysiology and clinical management. *Br J Anaesth* **96**: 678–85.

Halpern JH, Sherwood AR, Hudson JI, Gruber S, Kozin D, Pope HG, *et al* (2011a). Residual neurocognitive features of long-term ecstasy users with minimal exposure to other drugs. *Addiction* **106**: 777–86.

Halpern P, Moskovich J, Avrahami B, Bentur Y, Soffer D, Peleg K (2011b). Morbidity associated with MDMA (ecstasy) abuse: a survey of emergency department admissions. *Hum Exp Toxicol* **30**: 259–66.

Hamida S Ben, Plute E, Cosquer B, Kelche C, Jones BC, Cassel J-C (2008). Interactions between ethanol and cocaine, amphetamine, or MDMA in the rat: thermoregulatory and locomotor effects. *Psychopharmacology (Berl)* **197**: 67–82.

Han DD, Gu HH, Harwood H, Fountain D, Fountain G, Iversen L, *et al* (2006). Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacol* **6**: 6.

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

Harvey JA (2003a). Role of the serotonin 5-HT(2A) receptor in learning. *Learn Mem* **10**: 355–62.

Harvey J a (2003b). Role of the serotonin 5-HT(2A) receptor in learning. *Learn Mem* **10**: 355–62.

Hatzidimitriou G, McCann UD, Ricaurte GA (1999). Altered Serotonin Innervation Patterns in the Forebrain of Monkeys Treated with (±)3,4-Methylenedioxymethamphetamine Seven Years Previously: Factors Influencing Abnormal Recovery. *J Neurosci* **19**: .

Heifets BD, Malenka RC (2016). MDMA as a Probe and Treatment for Social Behaviors. *Cell* **166**: 1–4.

Henry JA, Jeffreys KJ, Dawling S (1992). Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("ecstasy"). *Lancet* **340**: 384–387.

Hiramatsu M, 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**:

269–75.

Hiramatsu M, Kumagai Y, Unger SE, Cho AK (1990). Metabolism of methylenedioxymethamphetamine: formation of dihydroxymethamphetamine and a quinone identified as its glutathione adduct. *J Pharmacol Exp Ther* **254**: .

Holland J (Park Street Press: Rochester, Vermont, 2001). *Ecstasy: The Complete Guide*. .

Homberg JR, Schiepers OJG, Schoffelmeer ANM, Cuppen E, Vanderschuren LJMJ (2007). Acute and constitutive increases in central serotonin levels reduce social play behaviour in peri-adolescent rats. *Psychopharmacology (Berl)* **195**: 175–82.

Hopkins PN, Polukoff GI (2003). Risk of valvular heart disease associated with use of fenfluramine. *BMC Cardiovasc Disord* **3**: 5.

Howell LL, Cunningham KA (2015). Serotonin 5-HT 2 Receptor Interactions with Dopamine Function: Implications for Therapeutics in Cocaine Use Disorder. *Pharmacol Rev Pharmacol Rev* **67**: 176–197.

Huot P, Johnston TH, Lewis KD, Koprich JB, Reyes MG, Fox SH, *et al* (2011). Characterization of 3,4-methylenedioxymethamphetamine (MDMA) enantiomers in vitro and in the MPTP-lesioned primate: R-MDMA reduces severity of dyskinesia, whereas S-MDMA extends duration of ON-time. *J Neurosci* **31**: 7190–8.

Hysek CM, Domes G, Liechti ME (2012). MDMA enhances “mind reading” of positive emotions and impairs “mind reading” of negative emotions. *Psychopharmacol* doi:10.1007/s00213-012-2645-9.

Hysek CM, Schmid Y, Simmler LD, Domes G, Heinrichs M, Eisenegger C, *et al* (2013). MDMA enhances emotional empathy and prosocial behavior. *Soc Cogn Affect Neurosci* **222**: 293–302.

Hysek CM, Simmler LD, Ineichen M, Grouzmann E, Hoener MC, Brenneisen R, *et al* (2011). The norepinephrine transporter inhibitor reboxetine reduces stimulant effects of MDMA (“ecstasy”) in humans. *Clin Pharmacol Ther* **90**: 246–255.

Hysek CM, Simmler LD, Schillinger N, Meyer N, Schmid Y, Donzelli M, *et al* (2014). Pharmacokinetic and pharmacodynamic effects of methylphenidate and MDMA administered alone or in combination. *Int J Neuropsychopharmacol* **17**: 371–81.

Insel TR (2003). Is social attachment an addictive disorder? *Physiol Behav* **79**: 351–7.

Insel TR, Battaglia G, Johannessen JN, Marra S, Souza EB De (1989). 3,4-Methylenedioxymethamphetamine (“ecstasy”) selectively destroys brain serotonin terminals in rhesus monkeys. *J Pharmacol Exp Ther* **249**: 713–20.

Itzhak Y, Achat-Mendes C (2004). Methamphetamine and MDMA (ecstasy) neurotoxicity: “of mice and men”. *IUBMB Life* **56**: 249–55.

Jager G, Win MM de, Vervaeke HK, Schilt T, Kahn RS, Brink W van den, *et al* (2007). Incidental use of ecstasy: no evidence for harmful effects on cognitive brain function in a prospective fMRI study. *Psychopharmacology (Berl)* **193**: 403–414.

Jay M (2015). Chapter Three – Miracle or Menace?: The Arrival of Cocaine 1860–1900. *Int Rev*

Neurobiol **120**: 27–39.

Jayanthi S, Ladenheim B, Andrews AM, Cadet JL (1999). Overexpression of human copper/zinc superoxide dismutase in transgenic mice attenuates oxidative stress caused by methylenedioxymethamphetamine (Ecstasy). *Neuroscience* **91**: 1379–1387.

Jensen KF, Olin J, Haykal-Coates N, O'Callaghan J, Miller DB, Olmos JS de (1993). Mapping toxicant-induced nervous system damage with a cupric silver stain: a quantitative analysis of neural degeneration induced by 3,4-methylenedioxymethamphetamine. *NIDA Res Monogr* **136**: 133-49–4.

Jerome L, Shira Schuster MA, Yazar-Klosinski BB (2013). Can MDMA Play a Role in the Treatment of Substance Abuse? *Curr Drug Abuse Rev* at <http://www.ncbi.nlm.nih.gov/pubmed/23627786>.

Johnson M, Hanson GR, Gibb JW (1987). Effects of N-ethyl-3,4-methylenedioxyamphetamine (MDE) on central serotonergic and dopaminergic systems of the rat. *Biochem Pharmacol* **36**: 4085–4093.

Johnson MP, Hoffman a J, Nichols DE (1986). Effects of the enantiomers of MDA, MDMA and related analogues on [3H]serotonin and [3H]dopamine release from superfused rat brain slices. *Eur J Pharmacol* **132**: 269–276.

Johnston LD, O 'malley PM, Miech RA, Bachman JG, Schulenberg JE (Ann Arbor, 2016). *Monitoring the Future national survey results on drug use, 1975-2015*. .

Jones DC, Duvauchelle C, Ikegami A, Olsen CM, Lau SS, la Torre R de, *et al* (2005). Serotonergic Neurotoxic Metabolites of Ecstasy Identified in Rat Brain. *J Pharmacol Exp Ther* **313**: .

Jones DC, Lau SS, Monks TJ (2004). Thioether metabolites of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine inhibit human serotonin transporter (hSERT) function and simultaneously stimulate dopamine uptake into hSERT-expressing SK-N-MC cells. *J Pharmacol Exp Ther* **311**: 298–306.

Jones K, Brennan KA, Colussi-Mas J, Schenk S (2010). Tolerance to 3,4-methylenedioxymethamphetamine is associated with impaired serotonin release. *Addict Biol* **15**: 289–298.

Jørgensen H, Riis M, Knigge U, Kjaer a, Warberg J (2003). Serotonin receptors involved in vasopressin and oxytocin secretion. *J Neuroendocrinol* **15**: 242–9.

Kalivas PW, Duffy P, White SR (1998). MDMA elicits behavioral and neurochemical sensitization in rats. *Neuropsychopharmacology* **18**: 469–79.

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

Kar LD Van de, Rittenhouse P a, Li Q, Levy a D, Brownfield MS (1995). Hypothalamic paraventricular, but not supraoptic neurons, mediate the serotonergic stimulation of oxytocin secretion. *Brain Res Bull* **36**: 45–50.

Kil HY, Zhang J, Piantadosi CA (1996). Brain temperature alters hydroxyl radical production

during cerebral ischemia/reperfusion in rats. *J Cereb Blood Flow Metab* **16**: 100–6.

Kindlundh-Högberg AMS, Svenningsson P, Schiöth HB (2006). Quantitative mapping shows that serotonin rather than dopamine receptor mRNA expressions are affected after repeated intermittent administration of MDMA in rat brain. *Neuropharmacology* **51**: 838–47.

Kirkpatrick MG, Francis SM, Lee R, Wit H de, Jacob S (2014a). Plasma oxytocin concentrations following MDMA or intranasal oxytocin in humans. *Psychoneuroendocrinology* **46**: 23–31.

Kirkpatrick MG, Gunderson EW, Perez AY, Haney M, Foltin RW, Hart CL (2012). A direct comparison of the behavioral and physiological effects of methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacol* **219**: 109–122.

Kirkpatrick MG, Lee R, Wardle MC, Jacob S, Wit H de (2014b). Effects of MDMA and Intranasal Oxytocin on Social and Emotional Processing. *Neuropsychopharmacology* **39**: 1–37.

Kirkpatrick MG, Lee R, Wardle MC, Jacob S, Wit H de (2014c). Effects of MDMA and Intranasal oxytocin on social and emotional processing. *Neuropsychopharmacology* **39**: 1654–63.

Kirkpatrick MG, Wit H de (2014). MDMA: a social drug in a social context. *Psychopharmacology (Berl)* **232**: 1155–1163.

Kish SJ, Fitzmaurice PS, Chang LJ, Furukawa Y, Tong J (2010a). Low striatal serotonin transporter protein in a human polydrug MDMA (ecstasy) user: a case study. *J Psychopharmacol* **24**: 281–4.

Kish SJ, Lerch J, Furukawa Y, Tong J, McCluskey T, Wilkins D, *et al* (2010b). Decreased cerebral cortical serotonin transporter binding in ecstasy users: a positron emission tomography/[(11)C]DASB and structural brain imaging study. *Brain* **133**: 1779–97.

Kiyatkin EA (2014). State-dependent and environmental modulation of brain hyperthermic effects of psychoactive drugs of abuse. *Temp (Austin, Tex)* **1**: 201–13.

Kolbrich E a, Goodwin RS, Gorelick D a, Hayes RJ, Stein E a, Huestis M a (2008a). Physiological and subjective responses to controlled oral 3,4-methylenedioxymethamphetamine administration. *J Clin Psychopharmacol* **28**: 432–40.

Kolbrich EA, Goodwin RS, Gorelick DA, Hayes RJ, Stein EA, Huestis MA (2008b). Plasma pharmacokinetics of 3,4-methylenedioxymethamphetamine after controlled oral administration to young adults. *Ther Drug Monit* **30**: 320–32.

Kuteykin-teplyakov K, Maldonado R (2014). Looking for prosocial genes : ITRAQ analysis of proteins involved in MDMA-induced sociability in mice. *Eur Neuropsychopharmacol* **24**: 1773–1783.

Kuypers KPC, la Torre R de, Farre M, Yubero-Lahoz S, Dziobek I, Bos W Van den, *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.

la Torre R de, Farré M, Ortuño J, Mas M, Brenneisen R, Roset PN, *et al* (2000). Non-linear pharmacokinetics of MDMA (’ecstasy’) in humans. *Br J Clin Pharmacol* **49**: 104–9.

la Torre R de, Farré M, Roset PN, Pizarro N, Abanades S, Segura M, *et al* (2004). Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition. *Ther Drug Monit* **26**: 137–44.

Landry MJ (2002). MDMA: a review of epidemiologic data. *J Psychoactive Drugs* **34**: 163–9.

Lanteri C, Doucet EL, Hernández Vallejo SJ, Godeheu G, Bobadilla a-C, Salomon L, *et al* (2013). Repeated exposure to MDMA triggers long-term plasticity of noradrenergic and serotonergic neurons. *Mol Psychiatry* 1–11doi:10.1038/mp.2013.97.

Lanteri C, Salomon L, Torrens Y, Glowinski J, Tassin J-P (2008). Drugs of Abuse Specifically Sensitize Noradrenergic and Serotonergic Neurons Via a Non-Dopaminergic Mechanism. *Neuropsychopharmacology* **33**: 1724–1734.

Lee H-J, Macbeth AH, Pagani JH, Young WS (2009). Oxytocin: the great facilitator of life. *Prog Neurobiol* **88**: 127–51.

Lee R, Garcia F, Kar LD Van De, Hauger RD, Coccaro EF (2003). Plasma oxytocin in response to pharmaco-challenge to D-fenfluramine and placebo in healthy men. *Psychiatry Res* **118**: 129–136.

Leneghan S (2013). The varieties of ecstasy experience: a phenomenological ethnography. *J Psychoactive Drugs* **45**: 347–54.

Leung KS, Cottler LB (2008). Ecstasy and other club drugs: a review of recent epidemiologic studies. *Curr Opin Psychiatry* **21**: 234–41.

Liechti ME, Baumann C, Gamma A, Vollenweider FX (1998). Acute Psychological Effects of (MDMA , " Ecstasy ") are Attenuated by the Serotonin Uptake Inhibitor Citalopram. .

Liechti ME, Baumann C, Gamma A, Vollenweider FX (2000a). Acute psychological effects of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") are attenuated by the serotonin uptake inhibitor citalopram. *Neuropsychopharmacology* **22**: 513–21.

Liechti ME, Saur MR, Gamma a, Hell D, Vollenweider FX (2000b). Psychological and physiological effects of MDMA ("Ecstasy") after pretreatment with the 5-HT(2) antagonist ketanserin in healthy humans. *Neuropsychopharmacology* **23**: 396–404.

Liechti ME, Vollenweider FX (2000). The serotonin uptake inhibitor citalopram reduces acute cardiovascular and vegetative effects of 3,4-methylenedioxymethamphetamine ('Ecstasy') in healthy volunteers. *J Psychopharmacol* **14**: 269–74.

Liechti ME, Vollenweider FX (2001). Which neuroreceptors mediate the subjective effects of MDMA in humans? A summary of mechanistic studies. *Hum Psychopharmacol* **16**: 589–598.

Liester MB, Grob CS, Bravo GL, Walsh RN (1992). Phenomenology and sequelae of 3,4-methylenedioxymethamphetamine use. *J Nerv Ment Dis* **180**: 345-52–4.

Lourenço TC, Bósio GC, Cassiano NM, Cass QB, Moreau RLM, Lourenco TC, *et al* (2013). Chiral separation of 3,4-methylenedioxymethamphetamine (MDMA) enantiomers using batch chromatography with peak shaving recycling and its effects on oxidative stress status in rat liver. *J Pharm Biomed Anal* **73**: 13–17.

Lowell BB, Spiegelman BM (2000). Towards a molecular understanding of adaptive thermogenesis. *Nature* **404**: 652–60.

Lukas M, Toth I, Reber SO, Slattery D a, Veenema AH, Neumann ID (2011). The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. *Neuropsychopharmacology* **36**: 2159–68.

Lyon RA, Glennon RA, Titeler M (1986). 3,4-Methylenedioxymethamphetamine (MDMA): stereoselective interactions at brain 5-HT1 and 5-HT2 receptors. *Psychopharmacology (Berl)* **88**: 525–6.

MacDonald E, Dadds MR, Brennan JL, Williams K, Levy F, Cauchi AJ (2011). A review of safety, side-effects and subjective reactions to intranasal oxytocin in human research. *Psychoneuroendocrinology* **36**: 1114–26.

Macdonald K, Macdonald TM (2010). The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. *Harv Rev Psychiatry* **18**: 1–21.

Machalova A, Slais K, Vrskova D, Sulcova A (2012). Differential effects of modafinil, methamphetamine, and MDMA on agonistic behavior in male mice. *Pharmacol Biochem Behav* **102**: 215–223.

Malberg JE, Sabol KE, Seiden LS (1996). Co-administration of MDMA with drugs that protect against MDMA neurotoxicity produces different effects on body temperature in the rat. *J Pharmacol Exp Ther* **278**: 258–67.

Malberg JE, Seiden LS (1998). Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. *J Neurosci* **18**: 5086–94.

Maldonado E, Navarro JF (2001). MDMA ("ecstasy") exhibits an anxiogenic-like activity in social encounters between male mice. *Pharmacol Res* **44**: 27–31.

MAPS (2015). Clinical Research with Psychedelics. .

Marston HM, Reid ME, Lawrence J a., Olverman HJ, Butcher SP (1999). Behavioural analysis of the acute and chronic effects of MDMA treatment in the rat. *Psychopharmacology (Berl)* **144**: 67–76.

McCann UD, Mertl M, Eligulashvili V, Ricaurte GA (1999). Cognitive performance in (±) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") users: a controlled study. *Psychopharmacology (Berl)* **143**: 417–425.

McCann UD, Ridenour A, Shaham Y, Ricaurte GA (1994). Serotonin Neurotoxicity after (±)3,4-Methylenedioxymethamphetamine (MDMA; "Ecstasy"): A Controlled Study in Humans. *Neuropsychopharmacology* **10**: 129–138.

McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA (1998). Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet (London, England)* **352**: 1433–7.

McCann UD, Szabo Z, Seckin E, Rosenblatt P, Mathews WB, Ravert HT, *et al* (2005). Quantitative PET Studies of the Serotonin Transporter in MDMA Users and Controls Using [11C]McN5652 and [11C]DASB. *Neuropsychopharmacology* **30**: 1741–1750.

McCann UD, Szabo Z, Vranesic M, Palermo M, Mathews WB, Ravert HT, *et al* (2008). Positron emission tomographic studies of brain dopamine and serotonin transporters in abstinent (±)3,4-methylenedioxymethamphetamine ("ecstasy") users: relationship to cognitive performance. *Psychopharmacology (Berl)* **200**: 439–450.

McGraw L a, Young LJ (2010). The prairie vole: an emerging model organism for understanding the social brain. *Trends Neurosci* **33**: 103–9.

Mechan A, Yuan J, Hatzidimitriou G, Irvine RJ, McCann UD, Ricaurte G a (2006). Pharmacokinetic profile of single and repeated oral doses of MDMA in squirrel monkeys: relationship to lasting effects on brain serotonin neurons. *Neuropsychopharmacology* **31**: 339–50.

Mechan AO, Esteban B, O'Shea E, Elliott JM, Colado MI, Green AR (2002). The pharmacology of the acute hyperthermic response that follows administration of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") to rats. *Br J Pharmacol* **135**: 170–80.

Meneses A (1999). 5-HT system and cognition. *Neurosci Biobehav Rev* **23**: 1111–1125.

Michel J-B, Shen YK, Aiden AP, Veres A, Gray MK, Google Books Team, *et al* (2011). Quantitative analysis of culture using millions of digitized books. *Science* **331**: 176–82.

Mithoefer MC, Grob CS, Brewerton TD (2016). Novel psychopharmacological therapies for psychiatric disorders: psilocybin and MDMA. *The Lancet Psychiatry* **3**: 481–488.

Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, 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**: 439–52.

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.

Moratalla R, Khairnar A, Simola N, Granado N, García-Montes JR, Porceddu PF, *et al* (2015). Amphetamine-related drugs neurotoxicity in humans and in experimental animals: Main mechanisms. *Prog Neurobiol* **In Press**: 10.1016/j.pneurobio.2015.09.011.

Morgan CJA, Noronha LA, Muetzelfeldt M, Feilding A, Fielding A, Curran HV (2013). Harms and benefits associated with psychoactive drugs: findings of an international survey of active drug users. *J Psychopharmacol* **27**: 497–506.

Morley KC, Arnold JC, McGregor IS (2005). Serotonin (1A) receptor involvement in acute 3,4-methylenedioxymethamphetamine (MDMA) facilitation of social interaction in the rat. *Prog Neuropsychopharmacol Biol Psychiatry* **29**: 648–57.

Morley KC, McGregor IS (2000). (+/-)-3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") increases social interaction in rats. *Eur J Pharmacol* **408**: 41–9.

Mueller M, Yuan J, Felim A, Neudörffer A, Peters FT, Maurer HH, *et al* (2009). Further studies on the role of metabolites in (+/-)-3,4-methylenedioxymethamphetamine-induced

serotonergic neurotoxicity. *Drug Metab Dispos* **37**: 2079–86.

Mueller M, Yuan J, McCann UD, Hatzidimitriou G, Ricaurte G a. (2013). Single oral doses of (±) 3,4-methylenedioxymethamphetamine ('Ecstasy') produce lasting serotonergic deficits in non-human primates: relationship to plasma drug and metabolite concentrations. *Int J Neuropsychopharmacol* **16**: 791–801.

Murnane KS, Fantegrossi WE, Godfrey JR, Banks ML, Howell LL (2010). Endocrine and neurochemical effects of 3,4-methylenedioxymethamphetamine and its stereoisomers in rhesus monkeys. *J Pharmacol Exp Ther* **334**: 642–50.

Nash JF, Meltzer HY (1990). Neuroendocrinological Effects of MDMA in the Rat. *Ecstasy Clin Pharmacol Neurotoxicological Eff drug MDMA* 225–241at <https://books.google.com/books?id=e87cBwAAQBAJ&pg=PA230&lpg=PA230&dq=mdma+acth&source=bl&ots=NGk9tM3tjM&sig=u2KxjYlYUbAAK-QoGeJe3j0SUTk&hl=en&sa=X&ved=0ahUKEwiCvffd1tHQAhUBZyYKHShfDXsQ6AEILDAD#v=onepage&q=mdma acth&f=false>.

Nash JF, Roth BL, Brodkin JD, Nichols DE, Gudelsky GA (1994). Effect of the R(-) and S(+) isomers of MDA and MDMA on phosphotidyl inositol turnover in cultured cells expressing 5-HTzA or 5-HTzc receptors. **177**: 111–115.

Navarro JF, Maldonado E (1999). Behavioral profile of 3,4-methylenedioxy-methamphetamine (MDMA) in agonistic encounters between male mice. *Prog Neuropsychopharmacol Biol Psychiatry* **23**: 327–34.

Navarro JF, Maldonado E (2002). Acute and subchronic effects of MDMA ("ecstasy") on anxiety in male mice tested in the elevated plus-maze. *Prog Neuropsychopharmacol Biol Psychiatry* **26**: 1151–1154.

Navarro JF, Rivera A, Maldonado E, Cavas M, la Calle A de (2004a). Anxiogenic-like activity of 3,4-methylenedioxy-methamphetamine ("Ecstasy") in the social interaction test is accompanied by an increase of c-fos expression in mice amygdala. *Prog Neuropsychopharmacol Biol Psychiatry* **28**: 249–254.

Navarro JF, Rivera A, Maldonado E, Cavas M, La Calle A De (2004b). Anxiogenic-like activity of 3,4-methylenedioxy-methamphetamine ("Ecstasy") in the social interaction test is accompanied by an increase of c-fos expression in mice amygdala. *Prog Neuro-Psychopharmacology Biol Psychiatry* **28**: 249–254.

Nichols D, Johnson M, Nichols C (2016). Psychedelics as Medicines: An Emerging New Paradigm. *Clin Pharmacol Ther* doi:10.1002/cpt.557.

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.

Nieuwenhuijzen PS van, Long LE, Hunt GE, Arnold JC, McGregor IS (2010). Residual social, memory and oxytocin-related changes in rats following repeated exposure to gamma-hydroxybutyrate (GHB), 3,4-methylenedioxymethamphetamine (MDMA) or their combination. *Psychopharmacol* **212**: 663–674.

Nocjar C, Alex KD, Sonneborn A, Abbas AI, Roth BL, Pehek EA (2015). SEROTONIN-2C

AND -2A RECEPTOR CO-EXPRESSION ON CELLS IN THE RAT MEDIAL PREFRONTAL CORTEX. *Neuroscience* **297**: 22–37.

Norton WT, Aquino DA, Hozumi I, Chiu FC, Brosnan CF (1992). Quantitative aspects of reactive gliosis: a review. *Neurochem Res* **17**: 877–85.

Nulsen CE, Fox AM, Hammond GR (2010). Differential effects of ecstasy on short-term and working memory: a meta-analysis. *Neuropsychol Rev* **20**: 21–32.

Nutt DJ, King LA, Phillips LD (2010). Drug harms in the UK: a multicriteria decision analysis. *Lancet* **376**: 1558–1565.

O'CALLAGHAN JP (1993). Quantitative Features of Reactive Gliosis following Toxicant-induced Damage of the CNS. *Ann N Y Acad Sci* **679**: 195–210.

O'Callaghan JP, Miller DB (1994). Neurotoxicity profiles of substituted amphetamines in the C57BL/6J mouse. *J Pharmacol Exp Ther* **270**: 741–51.

O'Hearn E, Battaglia G, Souza EB De, Kuhar MJ, Molliver ME (1988). Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. *J Neurosci* **8**: 2788–803.

O'Shea E, Granados R, Esteban B, Colado M., Green A. (1998). The relationship between the degree of neurodegeneration of rat brain 5-HT nerve terminals and the dose and frequency of administration of MDMA (`ecstasy'). *Neuropharmacology* **37**: 919–926.

Oehen P, Traber R, Widmer V, 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.

Orabona GM, Griesi-Oliveira K, Vadasz E, Bulcão VLS, Takahashi VNVO, Moreira ES, *et al* (2009). HTR1B and HTR2C in autism spectrum disorders in Brazilian families. *Brain Res* **1250**: 14–9.

Palhol F, Boyer S, Naulet N, Chabrillat M (2002). Impurity profiling of seized MDMA tablets by capillary gas chromatography. *Anal Bioanal Chem* **374**: 274–281.

Parrott a C (2005). Chronic tolerance to recreational MDMA (3,4-methylenedioxymethamphetamine) or Ecstasy. *J Psychopharmacol* **19**: 71–83.

Parrott AC (2009). Cortisol and 3,4-Methylenedioxymethamphetamine: Neurohormonal Aspects of Bioenergetic Stress in Ecstasy Users. *Neuropsychobiology* **60**: 148–158.

Parrott AC (2011). Residual neurocognitive features of ecstasy use: a re-interpretation of Halpern et al. (2011) consistent with serotonergic neurotoxicity. *Addiction* **106**: 1365-8–2.

Parrott AC (2012). MDMA and temperature: a review of the thermal effects of "Ecstasy" in humans. *Drug Alcohol Depend* **121**: 1–9.

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

Parrott AC (2014a). MDMA is certainly damaging after 25 years of empirical research: a reply

and refutation of Doblin et al. (2014). *Hum Psychopharmacol* **29**: 109–19.

Parrott AC (2014b). The Potential Dangers of Using MDMA for Psychotherapy. *J Psychoactive Drugs* **46**: 37–43.

Partilla JS, Dempsey AG, Nagpal AS, Blough BE, Baumann MH, Rothman RB (2006). Interaction of Amphetamines and Related Compounds at the Vesicular Monoamine Transporter. **319**: 237–246.

Pascoli V, Turiault M, Lüscher C (2012). Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour. *Nature* **481**: 71–5.

Passie T, Benzenhöfer U (2016). The History of MDMA as an Underground Drug in the United States, 1960–1979. *J Psychoactive Drugs* **48**: 67–75.

Passie T, Hartmann U, Schneider U, Emrich HM, Krüger THC (2005). Ecstasy (MDMA) mimics the post-orgasmic state: impairment of sexual drive and function during acute MDMA-effects may be due to increased prolactin secretion. *Med Hypotheses* **64**: 899–903.

Pedersen NP, Blessing WW (2001). Cutaneous vasoconstriction contributes to hyperthermia induced by 3,4-methylenedioxymethamphetamine (ecstasy) in conscious rabbits. *J Neurosci* **21**: 8648–54.

Pentney AR (2001). An exploration of the history and controversies surrounding MDMA and MDA. *J Psychoactive Drugs* **33**: 213–21.

Philipps D (2016). F.D.A. Agrees to New Trials for Ecstasy as Relief for PTSD Patients. *New York Times* A11at <http://www.nytimes.com/2016/11/29/us/ptsd-mdma-ecstasy.html?_r=0>.

Pizarro N, Farré M, Pujadas M, Peiró AM, Roset PN, Joglar J, *et al* (2004). Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites in human samples including the catechol-type metabolite (3,4-dihydroxymethamphetamine). *Drug Metab Dispos* **32**: 1001–7.

Pompeiano M, Palacios JM, Mengod G (1994). Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. *Brain Res Mol Brain Res* **23**: 163–78.

Procópio-Souza R, Fukushiro DF, Trombin TF, Wuo-Silva R, Zanlorenci LHF, Lima AJO, *et al* (2011). Effects of group exposure on single injection-induced behavioral sensitization to drugs of abuse in mice. *Drug Alcohol Depend* **118**: 349–359.

Ramos L, Hicks C, Caminer A, Goodwin J, McGregor IS (2015). Oxytocin and MDMA (’Ecstasy’) enhance social reward in rats. *Psychopharmacology (Berl)* **232**: 2631–2641.

Ramos L, Hicks C, Kevin R, Caminer A, Narlawar R, Kassiou M, *et al* (2013). Acute Prosocial Effects of Oxytocin and Vasopressin When Given Alone or in Combination with 3,4-Methylenedioxymethamphetamine in Rats: Involvement of the V1A Receptor. *Neuropsychopharmacology* **38**: 2249–59.

Ramos M, Goñ i-Allo B, Aguirre N (2005). Administration of SCH 23390 into the Medial Prefrontal Cortex Blocks the Expression of MDMA-Induced Behavioral Sensitization in Rats: An Effect Mediated by 5-HT 2C Receptor Stimulation and not by D 1 Receptor

Blockade. doi:10.1038/sj.npp.1300735.

Ray TS (2016). Constructing the ecstasy of MDMA from its component mental organs: Proposing the primer/probe method. *Med Hypotheses* **87**: 48–60.

Reid LW, Elifson KW, Sterk CE (2007). Hug drug or thug drug? Ecstasy use and aggressive behavior. *Violence Vict* **22**: 104–19.

Reinarman C, Levine HG (1997). The Crack Attack Politics and Media in the Crack Scare. .

Reneman L, Schilt T, Win MM de, Booij J, Schmand B, Brink W van den, *et al* (2005). Memory function and serotonin transporter promoter gene polymorphism in ecstasy (MDMA) users. *J Psychopharmacol* **20**: 389–399.

Report MW (2014). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *MMWR Surveill Summ* **63 Suppl 2**: 1–21.

Ricaurte G, Bryan G, Strauss L, Seiden L, Schuster C (1985). Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. *Science* **229**: 986–8.

Riedlinger TJ, Riedlinger JE (1994). Psychedelic and Entactogenic Drugs in the Treatment of Depression. *J Psychoactive Drugs* **26**: 41–55.

RMHIDTA (2016). *Latest Results for Colorado Youth and Adult Marijuana Use*. .

Robinson TE, Becker JB (1986). Enduring Changes in Brain and Behavior Produced by Chronic Amphetamine Administration: A Review and Evaluation of Anima1 Models of Amphetamine Psychosis. *Brain Res Rev* **11**: 157–198.

Robinson TE, Berridge KC (2008). Review. The incentive sensitization theory of addiction: some current issues. *Philos Trans R Soc Lond B Biol Sci* **363**: 3137–46.

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 Technol Assess (Rockv)* **13**: 1–315.

Ross HE, Young LJ (2009). Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front Neuroendocrinol* **30**: 534–47.

Ross JD, Herin D V, Frankel PS, Thomas ML, Cunningham KA (2006). Chronic treatment with a serotonin(2) receptor (5-HT(2)R) agonist modulates the behavioral and cellular response to (+)-3,4-methylenedioxymethamphetamine [(+)-MDMA]. *Drug Alcohol Depend* **81**: 117–27.

Ross S, Bossis A, Guss J, Agin-Liebes G, Malone T, Cohen B, *et al* (2016). Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. *J Psychopharmacol* **30**: 1165–1180.

Rothbaum BO, Schwartz a C (2002). Exposure therapy for posttraumatic stress disorder. *Am J Psychother* **56**: 59–75.

Rothman RB, Baumann MH (2002). Therapeutic and adverse actions of serotonin transporter substrates. *Pharmacol Ther* **95**: 73–88.

Rusyniak DE, Sprague JE (2005). Toxin-Induced Hyperthermic Syndromes. *Med Clin N Am* **89**: 1277–1296.

Sáez-Briones P, Hernández A (2013). MDMA (3,4-Methylenedioxymethamphetamine) Analogues as Tools to Characterize MDMA-Like Effects: An Approach to Understand Entactogen Pharmacology. *Curr Neuropharmacol* **11**: 521–34.

Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L, *et al* (1994). Enhanced aggressive behavior in mice lacking 5-HT1B receptor. *Science* **265**: 1875–8.

Saydoff JA, Rittenhouse PA, Kar LD van de, Brownfield MS (1991). Enhanced serotonergic transmission stimulates oxytocin secretion in conscious male rats. *J Pharmacol Exp Ther* **257**: 95–9.

Scheidweiler KB, Ladenheim B, Barnes AJ, Cadet JL, Huestis MA (2011). Metabolite Disposition in Plasma and Striatum of Wild-Type and Multidrug Resistance Protein 1a Knock-Out Mice. **35**: 470–480.

Schenk S (2009). MDMA Self-Administration in Laboratory Animals: A Summary of the Literature and Proposal for Future Research. *Neuropsychobiology* **60**: 130–136.

Schenk S (2011). MDMA ("ecstasy") abuse as an example of dopamine neuroplasticity. *Neurosci Biobehav Rev* **35**: 1203–1218.

Schilt T, Win MML de, Koeter M, Jager G, Korf DJ, Brink W van den, *et al* (2007). Cognition in Novice Ecstasy Users With Minimal Exposure to Other Drugs. *Arch Gen Psychiatry* **64**: 728.

Schmid Y, Hysek CM, Simmler LD, Crockett MJ, Quednow BB, Liechti ME (2014). Differential effects of MDMA and methylphenidate on social cognition. *J Psychopharmacol* **28**: 847–56.

Schmidt CJ (1987a). Acute administration of methylenedioxymethamphetamine: comparison with the neurochemical effects of its N-desmethyl and N-ethyl analogs. *Eur J Pharmacol* **136**: 81–88.

Schmidt CJ, Black CK, Taylor VL (1991). L-DOPA potentiation of the serotonergic deficits due to a single administration of 3,4-methylenedioxymethamphetamine, p-chloroamphetamine or methamphetamine to rats. *Eur J Pharmacol* **203**: 41–49.

Schmidt CJ, Levin JA, Lovenberg W (1987). In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochem Pharmacol* **36**: 747–55.

Schmidt J (1987b). Neurotoxicity of the Psychedelic I lIIU IHIHII llID. **24**: 1–7.

Schmued LC (2003). Demonstration and localization of neuronal degeneration in the rat forebrain following a single exposure to MDMA. *Brain Res* **974**: 127–33.

Sessa B (2005). Can psychedelics have a role in psychiatry once again? *Br J Psychiatry* **186**: 457–8.

Sessa B, Nutt DJ (2007). MDMA, politics and medical research: have we thrown the baby out with the bathwater? *J Psychopharmacol* **21**: 787–91.

Setola V, Hufeisen SJ, Grande-Allen KJ, Vesely I, Glennon R a, Blough B, *et al* (2003). 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. *Mol Pharmacol* **63**: 1223–1229.

Shankaran M, Yamamoto BK, Gudelsky GA (1999). Mazindol attenuates the 3,4-methylenedioxymethamphetamine-induced formation of hydroxyl radicals and long-term depletion of serotonin in the striatum. *J Neurochem* **72**: 2516–2522.

Sharma HS, Ali SF (2008). Acute administration of 3,4-methylenedioxymethamphetamine induces profound hyperthermia, blood-brain barrier disruption, brain edema formation, and cell injury. *Ann N Y Acad Sci* **1139**: 242–58.

Shelef A, Barak Y, Berger U, Paleacu D, Tadger S, Plopsky I, *et al* (2016). Safety and Efficacy of Medical Cannabis Oil for Behavioral and Psychological Symptoms of Dementia: An-Open Label, Add-On, Pilot Study. *J Alzheimer's Dis* **51**: 15–19.

Shioda K, Nisijima K, Yoshino T, Kuboshima K, Iwamura T, Yui K, *et al* (2008). Risperidone attenuates and reverses hyperthermia induced by 3,4-methylenedioxymethamphetamine (MDMA) in rats. *Neurotoxicology* **29**: 1030–6.

Shokry IM, Callanan JJ, Sousa J, Tao R, Baumann M, Clark R, *et al* (2016). New Insights on Different Response of MDMA-Elicited Serotonin Syndrome to Systemic and Intracranial Administrations in the Rat Brain. *PLoS One* **11**: e0155551.

Shulgin a T (1990). History of MDMA. *Ecstasy Clin Pharmacol Toxicol Eff Drug MDMA* 1–20at <http://137.187.144.24/endnote_pdfs/rm-010625.pdf>.

Shulgin A, Shulgin A (Transform Press: Berkeley, 1991). *PIHKAL: A Chemical Love Story*. .

Simek P (2015). Ecstasy Was Legal in 1984, and It Was Glorious. *Playboy* .

Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC, Halliwell B (1998). Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. *J Neurochem* **71**: 2112–22.

Sprague JE, Everman SL, Nichols DE (1998). An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine. *Neurotoxicology* **19**: 427–41.

Sprague JE, Nichols DE (1995a). Inhibition of MAO-B protects against MDMA-induced neurotoxicity in the striatum. *Psychopharmacology (Berl)* **118**: 357–9.

Sprague JE, Nichols DE (1995b). The monoamine oxidase-B inhibitor L-deprenyl protects against 3,4-methylenedioxymethamphetamine-induced lipid peroxidation and long-term serotonergic deficits. *J Pharmacol Exp Ther* **273**: 667–673.

Sprague JE, Preston AS, Leifheit M, Woodside B (2003). Hippocampal serotonergic damage induced by MDMA (ecstasy): effects on spatial learning. *Physiol Behav* **79**: 281–287.

Steele TD, Nichols DE, Yim GK (1987). Stereochemical effects of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [3H]monoamines into synaptosomes from different regions of rat brain. *Biochem Pharmacol* **36**: 2297–303.

Steketee JD, Kalivas PW (2011). Drug Wanting : Behavioral Sensitization and Relapse to Drug-Seeking Behavior. **63**: 348–365.

Stewart L, Ferguson B, Morgan C, Swaboda N, Jones L, Fenton R, *et al* (2014). Effects of ecstasy on cooperative behaviour and perception of trustworthiness: A naturalistic study. *J Psychopharmacol* doi:10.1177/0269881114544775.

Stoian I, Oros A, Moldoveanu E (1996). Apoptosis and free radicals. *Biochem Mol Med* **59**: 93–7.

Stolaroff MJ (Multidisciplinary Association for Psychedelic Studies: Sarasota, FL, 2004). *The Secret Chief Revealed Conversations with a pioneer of the underground psychedelic therapy movement*. .

Stone DM, Johnson M, Hanson GR, Gibb JW (1988). Role of endogenous dopamine in the central serotonergic deficits induced by 3,4-methylenedioxymethamphetamine. *J Pharmacol Exp Ther* **247**: 79–87.

Sulzer D, Sonders MS, Poulsen NW, Galli A (2005). Mechanisms of neurotransmitter release by amphetamines: A review. *Prog Neurobiol* **75**: 406–433.

Sumnall HR, Cole JC (2005). Self-reported depressive symptomatology in community samples of polysubstance misusers who report Ecstasy use: a meta-analysis. *J Psychopharmacol* **19**: 84–92.

Sumnall HR, Cole JC, Jerome L (2006). The varieties of ecstatic experience: an exploration of the subjective experiences of ecstasy. *J Psychopharmacol* **20**: 670–82.

Tancer M, Johanson C-E (2003). Reinforcing, subjective, and physiological effects of MDMA in humans: a comparison with d-amphetamine and mCPP. *Drug Alcohol Depend* **72**: 33–44.

Taurah L, Chandler C, Sanders G (2014). Depression, impulsiveness, sleep, and memory in past and present polydrug users of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). *Psychopharmacology (Berl)* **231**: 737–51.

The Economist (2016). Buying drugs online: Shedding light on the dark web. *Econ* at <http://www.economist.com/news/international/21702176-drug-trade-moving-street-online-cryptomarkets-forced-compete>.

Thomas DM, Dowgiert J, Geddes TJ, Francescutti-Verbeem D, Liu X, Kuhn DM (2004). Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. *Neurosci Lett* **367**: 349–54.

Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001). Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. doi:10.1038/nn757.

Thompson MR, Callaghan PD, Hunt GE, Cornish JL, 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.

Thompson MR, Callaghan PD, Hunt GE, McGregor IS (2008). Reduced sensitivity to MDMA-induced facilitation of social behaviour in MDMA pre-exposed rats. *Prog Neuropsychopharmacol Biol Psychiatry* **32**: 1013–21.

Thompson MR, Hunt GE, McGregor IS (2009). Neural correlates of MDMA ("Ecstasy")-induced

social interaction in rats. *Soc Neurosci* **4**: 60–72.

Thrivikraman K V, Kinkead B, Murray KE, Owens MJ (2013). In vivo dialysis setup with a loop injection valve facilitates retrodialysis studies. *J Pharmacol Toxicol Methods* **68**: 217–24.

Tikkanen R, Tiihonen J, Rautiainen MR, Paunio T, Bevilacqua L, Panarsky R, *et al* (2015). Impulsive alcohol-related risk-behavior and emotional dysregulation among individuals with a serotonin 2B receptor stop codon. *Transl Psychiatry* **5**: e681.

Tzadok M, Uliel-Siboni S, Linder I, Kramer U, Epstein O, Menascu S, *et al* (2016). CBD-enriched medical cannabis for intractable pediatric epilepsy. *Seizure* **35**: 41–44.

UNODC (New York, 2016). *World Drug Report*. .

Urban DJ, Roth BL (2015). DREADDs (Designer Receptors Exclusively Activated by Designer Drugs): Chemogenetic Tools with Therapeutic Utility. *Annu Rev Pharmacol Toxicol* **55**: 399–417.

Urban NB, Girgis RR, Talbot PS, Kegeles LS, Xu X, Frankle WG, *et al* (2012). Sustained recreational use of ecstasy is associated with altered pre and postsynaptic markers of serotonin transmission in neocortical areas: a PET study with [(1)(1)C]DASB and [(1)(1)C]MDL 100907. *Neuropsychopharmacology* **37**: 1465–1473.

Vanderschuren LJMJ, Schmidt ED, Vries TJ De, Moorsel CAP Van, Tilders FJH, Schoffelmeer ANM (1999). A Single Exposure to Amphetamine Is Sufficient to Induce Long- Term Behavioral, Neuroendocrine, and Neurochemical Sensitization in Rats. .

VanElzakker MB, Kathryn Dahlgren M, Caroline Davis F, Dubois S, Shin LM (2014). From Pavlov to PTSD: The extinction of conditioned fear in rodents, humans, and anxiety disorders. *Neurobiol Learn Mem* **113**: 3–18.

Vanover KE, Weiner DM, Makhay M, Veinbergs I, Gardell LR, Lameh J, *et al* (2006). Pharmacological and behavioral profile of N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N'-(4-(2-methylpropyloxy)phenylmethyl) carbamide (2R,3R)-dihydroxybutanedioate (2:1) (ACP-103), a novel 5-hydroxytryptamine(2A) receptor inverse agonist. *J Pharmacol Exp Ther* **317**: 910–8.

Varela MJ, Brea J, Loza MI, Maldonado R, Robledo P (2011). Sensitization to MDMA locomotor effects and changes in the functionality of 5-HT(2A) and D_2 receptors in mice. *Behav Pharmacol* **22**: 362–9.

Vegting Y, Reneman L, Booij J (2016). The effects of ecstasy on neurotransmitter systems: a review on the findings of molecular imaging studies. *Psychopharmacology (Berl)* **233**: 3473–3501.

Vollenweider FX, Gamma A, Liechti M, Huber T (1998). Psychological and cardiovascular effects and short-term sequelae of MDMA ("ecstasy") in MDMA-naïve healthy volunteers. *Neuropsychopharmacology* **19**: 241–51.

Vollenweider FX, Jones RT, Baggott MJ (2001). Caveat emptor: editors beware. *Neuropsychopharmacology* **24**: 461–3.

Wang X, Baumann MH, Xu H, Rothman RB (2004). 3,4-methylenedioxymethamphetamine (MDMA) administration to rats decreases brain tissue serotonin but not serotonin

transporter protein and glial fibrillary acidic protein. *Synapse* **53**: 240–8.

Wang Z, 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**: 483–8.

White CM (2014). 3,4-Methylenedioxymethamphetamine's (MDMA's) Impact on Posttraumatic Stress Disorder. *Ann Pharmacother* **48**: 908–915.

White SW, Albano AM, Johnson CR, Kasari C, Ollendick T, Klin A, *et al* (2010). Development of a cognitive-behavioral intervention program to treat anxiety and social deficits in teens with high-functioning autism. *Clin Child Fam Psychol Rev* **13**: 77–90.

Win MML de, Jager G, Booij J, Reneman L, Schilt T, Lavini C, *et al* (2008). Sustained effects of ecstasy on the human brain: a prospective neuroimaging study in novel users. *Brain* **131**: 2936–45.

Win MML de, Schilt T, Reneman L, Vervaeke H, Jager G, Dijkink S, *et al* (2006). Ecstasy use and self-reported depression, impulsivity, and sensation seeking: a prospective cohort study. *J Psychopharmacol* **20**: 226–235.

Wong K, Clarke C (2015). *The Legalization of Marijuana in Colorado: The Impact*. .

Wu X, Pang G, Zhang Y-M, Li G, Xu S, Dong L, *et al* (2015). Activation of serotonin 5-HT2C receptor suppresses behavioral sensitization and naloxone-precipitated withdrawal symptoms in heroin-treated mice. *Neurosci Lett* **607**: 23–28.

Wu Z, Xu Y, Zhu Y, Sutton AK, Zhao R, Lowell BB, *et al* (2012). An obligate role of oxytocin neurons in diet induced energy expenditure. *PLoS One* **7**: e45167.

Xie T, Tong L, McLane MW, Hatzidimitriou G, Yuan J, McCann U, *et al* (2006). Loss of serotonin transporter protein after MDMA and other ring-substituted amphetamines. *Neuropsychopharmacology* **31**: 2639–51.

Yamamoto BK, Moszczynska A, Gudelsky GA (2010). Amphetamine toxicities. *Ann N Y Acad Sci* **1187**: 101–121.

Yazar-Klosinski BB, Grob CS, Danforth AL (2016). Safety of mdma therapy for social anxiety in autistic adults. *Soc Neurosci* .

Young MB, Andero R, Ressler KJ, Howell LL (2015). 3,4-Methylenedioxymethamphetamine facilitates fear extinction learning. *Transl Psychiatry* **5**: 1–8.

Zayara AE, McIver G, Valdivia PN, Lominac KD, McCreary AC, Szumlinski KK (2010). Blockade of nucleus accumbens 5-HT2A and 5-HT2C receptors prevents the expression of cocaine-induced behavioral and neurochemical sensitization in rats. *Psychopharmacology (Berl)* **213**: 321–335.

Zhang G, Stackman RW (2015). The role of serotonin 5-HT 2A receptors in memory and cognition. doi:10.3389/fphar.2015.00225.

Zhang Y, Damjanoska KJ, Carrasco GA, Dudas B, D'Souza DN, Tetzlaff J, *et al* (2002). Evidence that 5-HT2A receptors in the hypothalamic paraventricular nucleus mediate neuroendocrine responses to (-)DOI. *J Neurosci* **22**: 9635–42.

Zuardi AW (2006). History of cannabis as a medicine: a review. *Rev Bras Psiquiatr* **28**: .