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Adelaide Minerva April 5, 2016

The Prosocial Effects of 3,4-methylenedioxymethamphetamine (MDMA) in Squirrel Monkeys

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Abstract

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3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy') is a synthesized drug that shares structural and pharmacological similarities with both hallucinogens and stimulants, yet has unique effects on prosocial and affiliative behavior. Recreational MDMA users often report feelings of increased sociability, friendliness, euphoria, closeness to others, and empathy. In line with these findings, controlled laboratory studies demonstrate that the compound enhances social behaviors and information processing in both humans and rodents. Due to its prosocial effects, MDMA has been proposed as an adjunct to psychotherapy for individuals with treatment resistant social and anxiety conditions, such as post-traumatic stress disorder (PTSD) and autism. This study sought to better understand the neurobiological mechanisms behind the MDMA-induced increase in social behaviors. Four male squirrel monkeys of the black cap subspecies (*Saimiri boliviensis*) served as subjects throughout this experiment. We administered a low dose range (0, 0.01, 0.03, 0.1, 0.3 mg/kg) of MDMA with pretreatment of saline, M100907, a 5-HT_{2A} receptor antagonist, or WAY163909, a 5-HT_{2C} receptor agonist, to subjects twice per week in a laboratory setting. Behavior and vocalizations while on the drug in the lab were analyzed and conditions were compared to each other in order to better understand the mechanism by which MDMA elicits its prosocial effects. MDMA resulted in a dose-dependent increase in prosocial behavior and affiliative calls. Pretreatment with M100907 attenuated these effects, whereas pretreatment with WAY163909 did not have a statistically significant effect. These results suggest that the social effects may be mediated by a system involving the amygdala to reduce social fear rather than a dopaminergic reward system. With the findings of this investigation, we hope to contribute to the future creation of a novel therapeutic with these benefits to social behavior but without the negative side effects and potential neurotoxicity of MDMA.

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Hypothesis: The present study hypothesized that administration of MDMA would lead to an increase in prosocial behaviors and vocalizations observed in group-housed black top squirrel monkeys while on the drug in a laboratory setting. In addition, we hypothesized that the 5-HT_{2A} receptor would be necessary for and activation of the 5-HT_{2C} receptor would enhance this outcome, thus co-administration of M100907 (a 5-HT_{2A} receptor antagonist) and WAY163909 (a 5-HT_{2C} receptor agonist) together with MDMA would attenuate these prosocial effects. If these hypotheses proved correct, we hypothesized that M100907 and WAY163909 would have a synergistic effect, with low doses ineffective on their own building on each other to attenuate the effects of MDMA when administered concurrently. We further hypothesized that the drug-induced prosocial changes would be unique to MDMA, and not occur with the same frequency following administration of methamphetamine, a more traditional psychostimulant.

Purpose and Rationale: This investigation aimed to establish a nonhuman primate model for examining the prosocial affiliative effects of MDMA. Several prior studies have used squirrel monkeys to investigate the pharmacological and neurotoxic effects of MDMA (Ricuarte et al., 1988a,b; Mechan et al., 2006; Mueller et al., 2009), however the

current examination is the first to use the species to model the effects on social behavior and vocalizations. Previous work conducted using human subjects typically involved polydrug users and observational study design, thus allowing for the potential influence of numerous confounding variables (Wu et al., 2009; Danforth et al., 2015). In the present study, the subjects, environment, and method of drug exposure were all controlled. This tightly managed design allowed for greater certainty as to the effects of incremental doses of MDMA in eliciting behavioral and vocal changes in the squirrel monkey cohort in the hour immediately following drug administration. Further, observation of the same behaviors after administration of M100907 and WAY163909 allowed us to better understand the neural mechanisms behind MDMA's prosocial effects. Studies have indicated a role of the 5-HT_{2A} receptor in MDMA-induced effects, with M100907, a 5-HT_{2A} receptor antagonist, blocking locomotor stimulation (Kehne et al., 1996) and striatal dopamine release (Schmidt et al., 1992, 1994), both typically seen following administration of MDMA. Thus, we expected M100907 to similarly attenuate the prosocial effects of the drug. Behavioral and pharmacological research has suggested that activation of the 5-HT_{2A} and 5-HT_{2C} receptors have distinct, even opposite, properties (Cunningham et al., 2012; Robinson et al., 2008; Howell and Cunningham, 2014). In cocaine studies, blockade of the 5-HT_{2A} receptor inhibits some behavioral effects of cocaine, while blockade of the 5-HT_{2C} receptor enhances those same effects (Fletcher et al., 2002). In studies of impulsivity, blockade of the 5-HT_{2A} receptor decreases impulsive responding while blockade of the 5-HT_{2C} receptor increases the same behavior (Robinson et al., 2008). In pharmacological studies,

activation of 5-HT_{2A} receptors enhances dopamine release, whereas activation of 5-HT_{2C} receptors inhibits dopamine release (Howell and Cunningham, 2014). Therefore, we hypothesized that the same contrasting relationship would hold true in this study, with WAY163909, a 5-HT_{2C} receptor agonist, inducing the same effect as M100907 in weakening the prosocial behaviors engendered by MDMA. Further, we hypothesized that the opposing properties of these two receptor subtypes would contribute to a synergistic effect between M100907 and WAY163909 when given concurrently at doses ineffective on their own. MDMA has already been used in human psychotherapy (Greer and Tolbert, 1986) and its efficacy as an adjunct to treatment for individuals suffering from PTSD (Mitheofer et al., 2010) and autism (Danforth et al., 2015) is currently being studied. Understanding the detailed neurobiological mechanisms behind MDMA's actions will help to establish a novel therapeutic approach for further clinical use that avoids the liabilities associated with the compound's abuse and potential neurotoxicity.

Introduction

Background and History of MDMA

MDMA has a long history of study covering more than a century as well as a more recent record of illicit use. While working at the Pharmaceutical company Merck, Arthur Koellisch first synthesized MDMA in 1912 (Benzenhofer and Passie, 2010; Bernschneider-Reif et al., 2006; Kalant, 2001). Two years later, the company patented the compound as an appetite suppressant (Cole and Sumnall, 2003; Weir, 2000). However, MDMA remained little known until the 1960s, when Alexander Shulgin

rediscovered the drug's effects and shared his experience with psychologist Leo Zeff (Benzenhofer and Passie, 2010). Soon thereafter, Zeff and others introduced MDMA as an adjunct to psychotherapy, finding positive emotional and subjective effects of the drug in clinical and long-term settings without disrupting an alert state of consciousness (Greer and Tolbert 1986; Greer and Tolbert, 1998; Mithoefer et al., 2010; Cole and Sumnall, 2003). MDMA's use continued to grow and, during the 1970s and 80s, the compound gained popularity as an illicit recreational street drug throughout United States and Europe (Benzenhofer and Passie, 2010; Cole and Sumnall, 2003). In July 1985, the U.S. Drug Enforcement Administration placed MDMA on the list of Schedule I controlled substances (Benzenhofer and Passie, 2010; Greer and Tolbert, 1998; Mithoefer et al., 2010; Vollenweider et al., 1998) due to perceived high risk potential for abuse and lack of significant medical value (Cole and Sumnall, 2003; Green et al., 2003). Most other countries also categorize the compound among their restricted drugs (Bernschneider-Reif et al., 2006; Kalant, 2001). However, emerging literature lists MDMA as among the least dangerous recreational drugs in terms of abuse liability (Nutt et al., 2010), as evidenced by a much lower rate of self-administration of MDMA in comparison to other drugs of abuse in rodents and nonhuman primates (De La Garza et al., 2007; Schenk, 2009).

Commonly referred to as 'ecstasy' in its pill form or 'molly' in its powder form (Kamilar-Britt and Bedi, 2015), MDMA ranks as one of the most recognized recreational drugs (Cole and Sumnall, 2003). The compound is widely known to elicit feelings of euphoria, empathy, closeness to others, self-confidence, and sociability (Mithoefer et al.,

2010; Schwartz and Miller, 1997; Siegel, 1986; Vollenweider et al., 1998; Weir, 2000), distinct from the effects of both stimulants and hallucinogens (Peroutka et al., 1988). Research has quantified and validated these effects through well-controlled observations. One study indicated that MDMA administration attenuated amygdala response to angry faces while simultaneously enhancing ventral striatum reactivity to happy faces (Bedi et al., 2009). Subsequent work further supported these results, revealing that administration of MDMA increased favorable responses to pleasant pictures in a social context but lessened such responses to pleasant pictures absent a social context. These findings imply a "socially-selective" effect of MDMA (Wardle et al., 2014), earning it the designation, "entactogen," which derives from Greek and Latin roots to mean, "producing a touching within" (Bedi et al., 2009; Bernschneider-Reif et al., 2006; Cole and Sumnall, 2003; Kalant, 2001; Ter Bogt and Engels, 2005).

These subjectively positive social effects, along with its modest hallucinogenic properties, make ecstasy a popular drug of choice at "rave" dance parties. Recreational use of MDMA exploded during the 1980s, at the same time as the rave culture gained worldwide popularity, starting in the United Kingdom (Cole and Sumnall, 2003; Weir, 2000). Today, MDMA usage at dance parties and other similar social events remains widespread in comparison to other drugs and situations (Sumnall et al., 2006**;** Ter Bogt and Engels, 2005; Weir, 2000), and those who regularly attend raves and nightclubs are approximately fourteen times more likely than the general population to have used the drug (Cole and Sumnall, 2003). Empirical evidence suggests that ecstasy not only increases sociability and euphoria, but also the intensity and enjoyment of music and

colors, enhancing the overall positive experience of raves (Green et al., 2003; Sumnall et al., 2006). At the same time, the drug reduces fatigue, allowing users to dance for extremely long periods at a time (Kalant, 2001; Ter Bogt and Engels, 2005). Ecstasy is typically taken as an oral tablet for recreational use, in doses ranging from 50-150mg (Kalant, 2001).

Several problems arise when considering only naturalistic observations of human use when studying MDMA. First, individuals self-administering the drug for recreational use tend to use a considerably higher dose than what would be given safely in a clinical setting (Danforth et al., 2015). Additionally, ecstasy and molly dispensed on the street almost always include other drugs, so the amount of pure MDMA, if any at all, in these uncontrolled forms is extremely variable (Hudson et al., 2014; Cole and Sumnall, 2003; Kalant, 2001; Schwartz and Miller, 1997). Therefore, conclusions about the effects of pure MDMA based on data from recreational use remain suspect at best. Lastly, street users of ecstasy and molly are frequently polydrug users, and thus other drugs confound the correlations one might observe (Wu et al., 2009). Together, these factors imply that research on recreational MDMA use has limited applicability and validity in comparison to controlled clinical studies of pure MDMA.

Animal Laboratory Studies

Existing research notes prosocial effects of MDMA in a variety of laboratory animals. Multiple studies have documented an increase in social behaviors in rodents, usually by use of a social interaction test. Most commonly, MDMA increased the amount of time

rodents engaged in adjacent lying (Morley and McGregor, 2000; Morley et al., 2005; Thompson et al., 2007; Ando et al., 2006; Ramos et al., 2013), defined as when animals lie next to each other in immediate proximity (Kamilar-Britt and Bedi, 2015). Other prosocial behaviors such as peaceful following and social investigation are also increased in rodents following administration of MDMA (Morley and McGregor, 2000; Morley et al., 2005; Procopio-Souza et al., 2011). MDMA also induced decreased rearing (Morley et al., 2005; Thompson et al., 2007; Ando et al., 2006) and anogenital sniffing (Morley et al., 2005; Thompson et al., 2007; Procopio-Souza et al., 2011). These behaviors are used to identify novel conspecifics (Ramos et al., 2013) and in assessment of risk (Kamilar-Britt and Bedi, 2015), respectively. Thus a reduction in the frequency of either behavior suggests an increase in social comfort with unfamiliar individuals due to MDMA, consistent with the compound's facilitation of prosocial behaviors.

Corresponding with the above results, MDMA has been found to reduce aggression in both rodents and fish (Morley and McGregor, 2000; Capurro et al., 1997). In Wistar rats encountering a conspecific for the first time, the compound reduced aggressive behaviors in comparison to placebo (Morley and McGregor, 2000). MDMA also resulted in a dose-dependent decrease in the typical aggressive behaviors *Gymnotus carapo* fish display upon first meeting another conspecific (Westby, 1975), often replacing such behaviors with parallel swimming, which has been interpreted as "friendly" (Capurro et al., 1997). In all but one (Ando et al., 2006) of the several studies noted above, the effects induced by low doses of MDMA were not accompanied by a

decrease in locomotion, signifying that the observed changes were in fact behavioral and not due to drowsiness. MDMA has decreased aggression in other studies, however the effects noted in these studies are less straightforward. For instance, MDMA in comparison to placebo decreased the amount of threatening and attack behaviors in mice that underwent prolonged social isolation (Navarro and Maldonado, 1999; Maldonado and Navarro, 2001). Yet, at the same time, these mice displayed more avoidance, defense, and submission postures in addition to reduced social investigation, suggesting that the decrease in aggression may be a result of increased social anxiety rather than an increase in prosocial behavior. Additional research is needed to confirm the effect of MDMA on aggressive behavior in laboratory animals.

Human Studies

In recent years, a number of controlled laboratory studies involving the administration of MDMA to human subjects have been conducted. Results show that MDMA increased self-reporting of many positive social mood states including 'loving' (Bedi et al., 2010; Kirkpatrick et al., 2014a,b; Wardle and de Wit, 2014; Wardle et al., 2014), 'talkative' (Tancer and Johanson, 2007), 'extroversion' (Lietchti et al., 2000b; Hysek et al., 2014a), 'social/sociable' (Bedi et al., 2009; Kirkpatrick et al., 2014a,b; Tancer and Johanson, 2003), 'friendly' (Johanson et al., 2006; Kirkpatrick et al., 2014a,b; Tancer and Johanson 2003, 2007), 'open' (Hysek et al., 2012), and 'close to other people' (Hysek et al., 2012, 2014a; Kolbrich et al., 2008). The results from these studies are consistent with the subjective reports of recreational MDMA users, who

report similar increases in prosocial emotions and mental states. Despite these encouraging findings, recent evidence suggests that prior exposure to MDMA may alter the prosocial effects observed in laboratory studies (Ando et al., 2006; Thompson et al., 2008), and thus it is important to use drug-naïve individuals or subjects that serve as their own controls.

While the above-mentioned human studies assessed mood state and emotion using subjective self-report measures, others employed a more quantitative objective approach, examining the effect of MDMA on the semantic structure of free speech. One method used Latent Semantic Analyses (LSA) to measure mood changes following MDMA administration (Bedi et al., 2014). These researchers found that MDMA induced speech with greater LSA proximity to socially relevant concepts such as 'friend', 'support', and 'empathy' compared to placebo. Two additional studies measuring semantic speech content (Wardle and de Wit, 2014; Baggot et al., 2015) used the Interpersonal Perception Task (IPT), during which subjects describe to an interviewer an important individual in their life and a specialized software application subsequently analyzes the speech for changes in emotional content (Kamilar-Britt and Bedi, 2015). In one IPT study, MDMA increased the use of positive, but not negative, emotional words in comparison to placebo (Wardle and de Wit, 2014). In the second, MDMA relative to placebo resulted in an increased use of words with social content (Baggot et al., 2015). In this study, an increase in self-reporting of positive social and emotional mood states correlated with the level of increase of social words, supporting the idea that MDMA targets internal cognitive and mood changes that then have an effect on behavior.

Together, results from both subjective self-report and more objective quantitative studies suggest that MDMA induces prosocial feelings and mental states in humans.

In controlled laboratory studies, MDMA not only increases social feelings, but also affects the social processing underlying interpersonal behavior (Bedi et al., 2009; Hysek et al., 2012; Wardle et al., 2014). These abilities to interpret others' emotional and mental states from external cues and to generate an appropriate affective response to others' emotions are known as cognitive empathy and emotional empathy, respectively (Blair, 2005). Cognitive empathy is important when considering social interaction, as deficits in cognitive empathy are associated with interpersonal difficulties and deficits in social skills (Kornreich et al, 2002; Phillips et al., 2003; Carton et al., 1999). A common way to assess cognitive empathy is through facial emotion recognition (FER). Using FER, Bedi et al. (2010) found that MDMA in comparison to placebo impaired recognition of fearful faces. In another study, MDMA reduced recognition of fearful, sad, angry, disgusted, and surprised faces relative to placebo, but did not significantly affect recognition of happy faces (Hysek et al., 2014a). Two subsequent studies also found significantly decreased accuracy in decoding of negative emotions, such as fear, anger, and sadness, on the FER task (Hysek et al., 2014b; Kirkpatrick et al., 2014b). However, these studies found a slight reduction in overall FER recognition trending towards significance (Hysek et al., 2014a; Kirkpatrick et al., 2014b), suggesting that MDMA impairs the overall ability to recognize facial emotions with a stronger effect on negative, threat-related emotions.

Emotional empathy is equally significant in assessing proper social interaction. One study indicated that MDMA in comparison to placebo attenuated amygdala response to angry faces versus neutral faces and also enhanced ventral striatum reactivity to happy faces versus neutral faces (Bedi et al., 2009). These data imply that MDMA may reduce emotional responding to social threats while simultaneously heightening reactivity to social rewards. Other investigations utilized the multifaceted empathy test (MET), which has participants infer others' affective states and also rate their own emotional states (Hysek et al., 2012, 2014a). In line with previous research, Hysek et al. (2012) found that MDMA increased subjective self-report of feelings of "closeness," "open," and "talkative," and improved ability to recognize positively valenced emotions while impairing ability to recognize negatively valenced emotions. In addition, MDMA enhanced subjective emotional empathy ratings, specifically increasing responses to positive rather than negative emotions (Hysek et al., 2014a). A similar study found comparable results, with MDMA relative to placebo increasing affective responses to positive but not negative emotional stimuli (Schmid et al., 2014).

Together, these data suggest that MDMA reduces overall cognitive empathy, specifically the ability to recognize the negative emotions of others, and enhances emotional empathy, boosting emotional responses to the positive emotions of others, and feelings of mood and extroversion. In this way, the increased sociability seen after administration of MDMA may be attributed to: 1) increased responses to positive socioemotional signals; 2) decreased responses to—and recognition of—negative or threatening socioemotional signals; and 3) increases in subjective ratings of happiness

and closeness to others. These effects may benefit an individual in therapy by helping him to form a closer bond with his therapist while at the same time reducing a fear response to traumatizing memories that may be perpetuating his condition.

MDMA was first used as an adjunct to psychotherapy in the 1970s and 1980s for its prosocial and positive emotional effects, which led scientists and doctors to believe the compound would enhance the therapy's effectiveness (Greer and Tolbert, 1986; Greer and Tolbert, 1998). The drug appeared to enhance positive feelings of closeness and euphoria, helping patients speak more openly (Greer and Tolbert, 1986; Danforth et al., 2015). More recently, clinical investigations have focused on the ability of MDMA to reduce symptoms of social disorders such as post-traumatic stress disorder (PTSD) (Mithoefer et al., 2010; Oehen et al., 2013) and autism (Danforth et al., 2016) in treatment resistant individuals. Mithoefer et al. (2010) proposed two mechanisms by which MDMA may enhance psychotherapy. Generally, successful psychotherapy occurs within the "optimal arousal zone" or "window of tolerance" during which there is significant emotional engagement (Mithoefer et al., 2010; Oehen et al., 2013). Patients with PTSD often suffer from emotional numbing and extensive anxiety, which leads to a narrow "window of tolerance" (Mithoefer et al., 2010). Mithoefer et al. (2010) proposed that MDMA might improve the efficacy of therapy sessions by promoting emotional engagement and reducing anxiety, thus widening the window during which therapy can have an effect. In addition, patients suffering from PTSD often have difficulty revisiting the trauma that led to their condition, which is a frequent component of therapy for PTSD. Mitheofer et al. (2010) proposed that MDMA might help control the fear response

to this exposure, first, through its ability to help individuals confront emotionally intense memories (Greer and Tolbert, 1998; Danforth et al., 2015) and, second, by increasing trust between the patient and therapist through its ability to induce feelings of empathy, compassion, and closeness to others (Vollenweider et al., 1998; Danforth et al., 2015), further enhancing the therapy. This study found a significant reduction in CAPS (Clinician Administered PTSD Scale) scores, a measure of PTSD symptoms, in those patients who received MDMA administration concurrent with their psychotherapy sessions relative to those who received a placebo. A subsequent study by Oehen et al. (2013) confirmed that no serious adverse effects resulted from the drug treatment, suggesting its safety for clinical use.

A more recent study launched in 2014 is investigating the effectiveness of MDMA for reduction of social anxiety in autistic individuals (Danforth et al., 2015). While the results of this work have not yet been published, the authors propose multiple reasons why MDMA-assisted therapy helps treat autism. Most notably, MDMA has been found to relieve symptoms of social anxiety and increase feelings of connectedness to others. Specifically, a large survey involving subjects from 13 countries found that, as a result of the drug, 72% of participants with prior use of MDMA reported “more comfort in social settings,” 78% reported “feeling at ease in my own body,” 77% reported that it was “easier than usual to talk to others,” and 22% reported “increased insight into own thought processes” (Danforth et al., 2015). Each of these findings point to the relevance of MDMA in treating social anxiety and establishing a positive relationship between patient and therapist that may further enhance the efficacy of therapy. Despite these

positive effects in recent clinical trials, there are concerns about using MDMA in psychotherapy due to its neurotoxic effects and potential for abuse. The current investigation aims to understand the neurobiological mechanisms behind the prosocial and potential therapeutic effects of MDMA in hopes of developing a novel drug with fewer risks.

Structure and Pharmacological Mechanism of Action

MDMA has both hallucinogenic and psychostimulant properties. Hallucinogens are known to cause hallucinations and 'psychedelic' effects, including perceptual distortions, visual illusions, and synaesthesia (Stolerman, 2010). Psychostimulants elevate mood and increase energy, as well as psychomotor activity (Miller-Keane and O'Toole, 2003). MDMA causes perceptual distortions and intensification of visual, tactile, and acoustic stimuli (Liechti et al., 2000a; Green et al., 2003) similar to the mild hallucinations associated with hallucinogenic drugs, while also increasing mood-state and psychomotor activity (de la Torre et al., 2004). The latter effects are most likely due to MDMA's molecular structure. MDMA is an amphetamine derivative, and thus many of its properties would be predicted to be similar to those of amphetamine. Despite these similarities with both hallucinogenic and psychostimulant drugs, MDMA's actions are distinct from both, as exhibited by its "socially-selective" effects (Dumont et al., 2009; Wardle et al., 2014) that have earned it the classification of "entactogen" (Bedi et al., 2009; Bernschneider-Reif et al., 2006; Cole and Sumnall, 2003; Kalant, 2001; Ter Bogt and Engels, 2005), separate from other hallucinogens and stimulants.

Once administered, MDMA stimulates the release of serotonin, dopamine, and norepinephrine (de la Torre et al., 2004; Fitzgerald and Reid, 1990; Koch and Galloway, 1997; Rothman et al., 2001; Green et al., 2003). While much study has suggested that the psychoactive effects of the drug in humans are due to release of serotonin, norepinephrine, and dopamine (Hysek et al., 2012; Liechti et al., 2000a,b; Liechti and Vollenweider, 2000), the primary subjective socioemotional effects of the drug may result specifically from increased extracellular serotonin (5-hydroxytryptamine, 5-HT) concentrations (Mithoefer et al., 2010; Bernschneider-Reif et al., 2006). MDMA acts as an indirect serotonin agonist. The compound binds with the serotonin transporter and gets taken back up into the presynaptic terminal, disrupting neurotransmitter storage and promoting serotonin release (de la Torre et al., 2004; Rudnick and Wall, 1992; Danforth et al., 2015). At the same time, this binding prevents synaptic serotonin from binding to the transporter, thus preserving its extracellular concentration (Nichols, 1986). Together, these actions enhance the likelihood that a serotonin receptor will be activated. Previous findings on the molecular activity of MDMA support the role of the serotonin system, specifically the 5-HT_2 receptor, in generating the positive prosocial effects associated with the compound. Morley et al. (2005) found that MDMA increased social interaction in newly acquainted rats, but co-administration of SB206553, a 5-$HT_{2B/2C}$ receptor antagonist, attenuated this result. In humans, pretreatment with citalopram, a 5-HT reuptake inhibitor, lowered MDMA-induced ratings of “extroversion” and “self-confidence” (Liechti et al., 2000a; Liechti and Vollenweider, 2001) and pretreatment with ketanserin, a 5-HT_{2A} receptor antagonist, decreased MDMA-induced

ratings of “friendliness” and “positive mood” (van Wel et al., 2012). However, Liechti et al. (2000b) did not find a significant effect of ketanserin on MDMA-induced feelings associated with well-being or positive mood. Further, binding of medial prefrontal cortex (mPFC) 5-HT_{2A} receptors was inversely correlated with reactivity to angry and fearful faces in humans (Fisher et al., 2009), suggesting a potential mechanism for the finding that MDMA decreases reactivity to angry faces (Bedi et al., 2009). These data, while not unequivocal, suggest that the prosocial effects of MDMA could operate, in part, through a mechanism involving the serotonin system.

Research also suggests a possible oxytocin-mediated mechanism for the prosocial effects of MDMA. Oxytocin is a neuropeptide that plays an important role in mammalian pair bonding and other social behaviors (Bos et al., 2012). Kirkpatrick et al. (2014b) found that intranasal oxytocin administration increased subjective ratings of “friendly,” “elated,” “insightful,” and “social,” similar to those elicited by MDMA. In one study, MDMA-induced prosocial feelings were positively correlated with plasma oxytocin concentrations in humans (Dumont et al., 2009). However, subsequent investigations did not find any correlation between prosocial feelings and blood oxytocin levels (Hysek et al., 2012, 2014a; Kirkpatrick et al., 2014a). In male Wistar rats, MDMA-induced increases in adjacent laying, a measure of prosocial behavior, was positively correlated with plasma levels of oxytocin (Thompson et al., 2007). In this same study, pretreatment with tocinoic acid, an oxytocin receptor antagonist, blocked the increase in adjacent laying. Further, pretreatment with WAY100635, a 5-HT_{1A} antagonist, attenuated both the levels of oxytocin in the blood and prosocial behaviors seen following MDMA

administration. These results suggest that MDMA-induced prosocial behaviors may be due to increased oxytocin release mediated by interactions with 5-HT_{1A} receptors. Despite these findings, there is significant evidence supporting the role of serotonin in inducing the prosocial effects of MDMA, and thus additional research is needed to confirm the involvement of oxytocin.

Undesirable Effects and Neurotoxicity

MDMA has significant adverse effects and a high degree of neurotoxicity in both experimental animals and humans. Studies have described high doses of MDMA resulting in acute symptoms such as elevated heart rate and blood pressure, dry mouth, nausea, tremor, palpitations, excessive sweating, jaw clenching, teeth grinding, restlessness, ataxia, and anxiety (de la Torre et al., 2004; Green et al., 2003; Peroutka et al., 1988). Fatigue, loss of appetite, irritability, difficulty concentrating, dry mouth, and headache can be present for up to 24 hours (de la Torre et al., 2004; Green et al., 2003). Other residual physical symptoms that can last for days or weeks post-MDMA use are anxiety, fatigue, depressed mood, insomnia, muscle tension, and paranoid delusions (de la Torre et al., 2004; Green et al., 2003). These side effects can be divided into mild, moderate, and severe categories of MDMA toxicity (de la Torre et al., 2004). Mild symptoms include nausea and vomiting, pupil dilation, dry mouth, restlessness, and irritability. Moderate toxic effects involve hyperactivity, confusion, muscle tension, and an increase in body temperature. Side effects classified as severe include delirium, coma, seizures, hyperthermia, and renal failure. Hyperthermia has been noted as a

major acute symptom of MDMA in both rats and humans (Green et al., 1995), and may be associated with other side effects such as acute renal failure (Green et al., 2003). Hyperthermia appears to be aggravated by high ambient temperatures, crowding, poor hydration, and physical activity (Green et al., 1995; Green et al., 2003), suggesting that recreational use of the drug at “rave” parties that promote all of these conditions would exacerbate the toxic effects. It may be hypothesized, then, that the neurotoxic effects could be attenuated within a controlled laboratory setting. Studies also showed an MDMA-induced serotonin syndrome in experimental animals, characterized by muscle rigidity, hyperthermia, ataxia, convulsions, hyperactivity, head weaving, salivation, and defecation (de la Torre et al., 2004; Green et al., 1995; Green et al., 2003).

MDMA also produces significant neurotoxic effects. Specifically, the compound is associated with neurodegeneration of the serotonin neurotransmitter system, damaging serotonergic nerve fibers in many animal species (de la Torre et al., 2004; Green et al., 1995; Ricuarte et al., 1988a). In one study, multiple administrations of 0.5mg/kg of MDMA, either subcutaneously or orally, for 4 consecutive days significantly depleted serotonin levels in the somatosensory cortex, frontal cortex, caudate nucleus, putamen, thalamus, hippocampus, and hypothalamus of male squirrel monkeys two weeks after the final administration (Ricuarte et al., 1988a). Additional research supports these findings (Aguirre et al., 1995; Battaglia et al., 1987). The depletion in serotonin concentration may be partially due to suppressed serotonergic transmission related to MDMA’s sustained inhibition of tryptophan hydroxylase, the rate-limiting enzyme crucial for serotonin synthesis (Green et al., 1995; Green et al., 2003). Alternatively, MDMA

may stimulate direct neurodegenerative damage to serotonergic terminals. Agguire et al. (1995) showed a significant reduction in presynaptic 5-HT transporter density in the brains of rats following MDMA administration , suggesting neurodegeneration of the presynaptic serotonergic terminal. A similar study obtained comparable results and also showed that the same significant MDMA-induced reduction in density of dopamine and noradrenaline uptake sites in the rat brain did not occur (Battaglia et al., 1987), implying a selectively damaging effect of the compound for serotonin terminals. In addition, immunocytochemical analysis of brain slices showed reduced serotonergic axon density in MDMA-administered samples in comparison to control (O'Hearn et al., 1988; Ricuarte et al., 1988b). This reduction showed selectivity for the serotonin axon terminals, specifically, implying that the depletion of neurotransmitter seen post-MDMA use is due to physical damage to the neurons. It is unclear whether the MDMA-induced neurotoxic effects on serotonergic neurons are a direct result of the compound or the free radicals generated from its metabolites (de la Torre et al., 2004; Green et al., 1995). Several studies have supported this latter hypothesis by demonstrating the attenuation of MDMA-induced brain damage by free radical scavenging drugs (Green et al., 2003). Regardless of the direct cause, MDMA-induced neurodegeneration of the serotonergic neural pathways is linked to many detrimental psychological outcomes including impaired cognitive function, increased impulsivity, and psychopathy (de la Torre et al., 2004; Green et al., 2003).

Studies show that the degree of neurotoxicity of MDMA is dose-dependent (Green et al., 2003) and many studies investigating the toxicity of MDMA administered

multiple high doses (Ricuarte et al., 1988a,b; Battaglia et al., 1987; Aguirre et al., 1995). Therefore, it is important to note that the highest dose given in this study was 1.0mg/kg at a maximum frequency of twice per week, with at least 48 hours between each intramuscular injection. Thus the potential for neurotoxicity in the present investigation is lower than in these prior studies.

Materials & Methods

All protocols conform to the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health, and were approved by the Emory University Institutional Animal Care and Use Committee.

Subjects

Subjects used in this study were four adult male squirrel monkeys (*Saimiri boliviensis*) (s-171, s-177, s-181, s-188) of the black cap subspecies. While weights were variable over time, each subject's average weight was between 800-1300 grams. The monkeys have been group housed together in a 1.4x1.8x0.7 meter cage since May 2014 under controlled temperature and lighting (12 hour cycle) conditions. Within their homeroom, the animals were able to hear and make eye contact with twelve other squirrel monkeys in the same room. Each monkey had access to swings, perches, mirrors, and other toys for enrichment. Subjects were fed twice daily (Harlan Teklan monkey chow: Harlan Teklad, Madison, WI, USA; fresh fruits and vegetables) and had

constant access to water. All subjects had two years of abstinence before beginning the current study, but all had prior exposure to stimulant drugs acting on monoaminergic and/or glutamatergic neurotransmitter systems (Fantegrossi et al., 2009; Banks et al., 2009; Kimmel et al., 2009) before that two-year period. Due to the nature of the study, the animals served as their own controls and in behavioral testing were compared to each other rather than to separate groups of monkeys that had never been exposed to psychoactive agents.

Drugs

MDMA was used as the main experimental drug in this study. Methamphetamine was used as a positive control, as it has structural similarities to MDMA but is recognized more for its euphoric and stimulant than prosocial effects. Both MDMA HCl and methamphetamine HCl were acquired from the Research Triangle Institute (RTI), Research Triangle Park, NC and were dissolved in physiological saline. M100907 was synthesized at the Chemical Biology Research Branch of the National Institute on Drug Abuse (NIDA) and National Institute on Alcohol Abuse and Alcoholism at the National Institutes of Health (Bethesda, MD) and was a generous gift from Dr. Kenner C. Rice, Ph.D. M100907 was dissolved in physiological saline and 1.0N hydrochloric acid. WAY163909 was obtained from Pfizer Incorporated (New York, NY, USA). All doses were calculated using salt weights.

Experimental Protocol

Experimental sessions were performed in the subjects' home cage within a laboratory room separate from the rest of the colony. The monkeys' cage was moved to the laboratory and the animals were left alone there for two hours prior to drug administration to allow sufficient time for habituation to the new environment. Animals were given MDMA (0.03-1.0 mg/kg) or methamphetamine (0.01-0.3 mg/kg) through intramuscular injection in a randomized order no more than two times per week with at least two days between each drug administration. Pretreatment with M100907 (0.1-0.3 mg/kg), a selective 5-HT_{2A} receptor antagonist, and WAY163909 (0.03-0.3 mg/kg), a 5-HT_{2C} receptor agonist, was administered one hour and forty-five minutes before MDMA or methamphetamine administration, respectively.

Behavioral Scoring

To observe behaviors, two video cameras were used to record 1-hour periods. The videos were then separated into 20-minute segments. Behavioral scoring through JWatcher software (Blumstein et al., 2000) was performed by a blind participant based on an ethogram created specifically for behaviors of interest for this study (Hopf et al., 1974). Each animal was followed for five minutes during one 20-minute clip, according to a randomized order selected before scoring begins. In this way, each monkey was observed for a total of 15 minutes throughout the hour. Behaviors tracked were affiliation/proximity, locomotion, self-grooming, and residual behavior. Through the ethogram, each of these behaviors corresponded to a specific letter on a keypad. For

example, affiliation/proximity was coded as ‘a’ while residual behavior was coded as ‘s.’ In order to record a behavior, the observer typed the key associated with that behavior. At the end of the scoring session, JWatcher aggregated the amount of time each monkey engaged in each behavior. Affiliation/proximity had been identified as a prosocial behavior. Unlike other species of non-human primate, squirrel monkeys do not social groom (Baldwin & Baldwin, 1981), and thus we did not look for this behavior when analyzing affiliation. We defined affiliation/proximity, also recognized as huddling, as the monkeys sitting next to each other, usually in parallel orientation and often with tails wrapped over their shoulders (Hopf et al., 1974). Locomotion included whole body motions such as walking, running, jumping, and swinging (Hopf et al., 1974). Monkeys self-groomed by scratching or lightly biting themselves for the purpose of cleaning. Residual behavior occurred when the animal was not engaged in a particular action.

Vocalization Scoring

Auditory files were recorded for the entire one-hour session and were converted to spectrogram files using MATLAB software (Mathworks). A blind participant categorized vocalizations based on the shape of the spectrogram. Vocalizations of note in this study were chucks, purrs, pulsed calls, growls, and peeps. Chucks, purrs, and pulsed calls were categorized as affiliative. Growls are heard in association with displays of threat or aggression (Winter et al., 1966; Jürgens, 1979), whereas peeps are heard in a wider variety of situations including separation from the group after a change in the environment (Winter et al., 1966; Jürgens, 1979).

Statistical Analysis

Behavioral data were analyzed using SPSS Statistical Software. A one-way repeated measures analysis of variance (ANOVA) was used to analyze behaviors following separate administration of only MDMA and methamphetamine. This test aimed to determine if there was a statistically significant difference in the mean percent of time spent engaged in each behavior between each dose (0, 0.03, 0.1, 0.3, 1.0 mg/kg for MDMA and 0, 0.01, 0.03, 0.1, 0.3 mg/kg for methamphetamine). A post-hoc pairwise comparison using a Bonferroni adjustment for multiple comparisons was used to analyze which doses, if any, resulted in the largest change in behavior.

A two-way repeated measures ANOVA was used to analyze the differences in behaviors following administration of MDMA with a pretreatment of either M100907 or WAY163909. This test aimed to determine if there was a statistically significant difference in the mean percent of time spent engaged in each behavior among increasing doses of pretreatment (0, 0.1, 0.3 mg/kg for M100907 and 0, 0.03, 0.3 mg/kg for WAY163909). A post-hoc pairwise comparison using a Bonferroni adjustment for multiple comparisons was used to analyze which doses, if any, resulted in the largest change in behavior.

To analyze vocalizations R (R Core Team, 2012) and *lme4* (Bates, Maechler, & Bolker, 2012) were used to generate a regression model of the relationship between number of affiliative calls and drug dosage. Vocalizations on methamphetamine were analyzed using a linear regression with ln-transformed vocalizations. Vocalizations on MDMA and M100907 with MDMA were analyzed using a negative binomial regression

model. Dosage for MDMA, methamphetamine, and MDMA and M100907 as well as their interaction term were entered as fixed effects into the model. Dose and dose^2 were used as independent predictors in all models due to the possibility that the outcome changes either linearly or quadratically with dose. P-values were obtained by using residual maximum likelihood (REML) tests with Satterthwaite approximations of degrees of freedom.

Results

Behavioral Results

MDMA induced an increase in social behavior, as indicated by duration of time spent huddling, in the immediate in-laboratory setting ($p<0.05$; Table 1). These data support the original hypothesis that MDMA administration would increase the amount of affiliative behavior. A post-hoc pairwise comparison using a Bonferroni adjustment for multiple comparisons did not yield any significant differences between group means and thus we cannot firmly conclude a dose-response relationship between MDMA and affiliative behavior. However, in the post-hoc analysis, the difference between saline and the two highest doses of MDMA trended more closely to significance than any other post-hoc comparisons performed. In addition, when looking at the data visually, the amount of time spent huddling increases consistently with each rising dose of MDMA (Figures 2 and 3). There was also a significant decrease in locomotion ($p<0.05$), and residual behavior ($p<0.05$) (Table 1). A post-hoc pairwise comparison using a Bonferroni adjustment for multiple comparisons did not yield any significant differences

between group means, yet visual representation of the data shows that the frequency of both behaviors decreased with increasing doses of MDMA. These findings fit with the hypothesis, because as the percentage of time spent huddling increases, the percentage of time engaged in other behaviors must decrease. There was not a statistically significant difference in amount of time spent self-grooming ($p>0.05$) across doses of MDMA (Table 1).

Following methamphetamine administration, there was a significant increase in affiliation ($p<0.05$) (Table 1), which does not support the hypothesis that increases in prosocial behavior would be unique to MDMA and not be induced by methamphetamine. A post-hoc pairwise comparison using a Bonferroni adjustment for multiple comparisons did not yield any significant differences between group means. In addition, the amount of time spent huddling is variable with increasing doses of methamphetamine and does not show a consistent increase with rising doses of the drug (Figures 6 and 7). No significant differences were observed in the amount of time spent engaged in locomotion ($p>0.05$), residual behavior ($p>0.05$), and self-grooming ($p>0.05$) (Table 1).

Co-administration of M100 one hour prior to MDMA blocked the increase in prosocial behavior seen with administration of just MDMA ($p<0.05$) (Table 2). A post-hoc test using a Bonferroni adjustment for multiple comparisons yielded a statistically significant difference in the mean amount of time spent huddling while on the highest dose of MDMA (0.3mg/kg) between doses of M100907 ($p<0.05$), suggesting a dose-response decrease in prosocial behavior with increasing doses of M100907. These data support the initial hypothesis that the 5-HT_{2A} receptor subunit would be necessary for

the increase in prosocial behavior associated with MDMA. Following co-administration with M100907 across all doses of MDMA, there was not a statistically significant change in locomotion ($p>0.05$) or self-grooming ($p>0.05$) across all doses of MDMA (Table 2). There was a statistically significant change in the amount of time spent engaged in residual behavior ($p<0.05$) (Table 2). A post-hoc test did not yield significant results between group means. This finding is not surprising, given that as higher doses of M100907 reduced the amount of time spent huddling, a greater proportion of time must be spent engaged in another behavior.

Co-administration of WAY163909 forty-five minutes prior to MDMA presented inconclusive results. There was not a statistically significant difference in the amount of time spent huddling following administration of MDMA alone and administration of WAY163909 together with MDMA ($p>0.05$) (Table 2). These results do not support our hypothesis that the 5-HT_{2C} receptor would have contrasting effects compared to the 5-HT_{2A} receptor, and thus activation via WAY163909 would inhibit the prosocial behavior induced by MDMA. There were no statistical differences in the amount of time spent engaged in any other behaviors ($p>0.05$ for all three) following pretreatment with WAY163909 (Table 2).

Vocalization Results

Analysis of vocalizations categorized chucks, twitters, and purrs as affiliative calls. There was a statistically significant dose-dependent increase in affiliative calls following MDMA administration ($p<0.05$) (Table 3). The relationship between dose and number of

affiliative calls changed linearly, so dose was used as the independent predictor. These findings are in line with our behavioral data showing a significant increase in prosocial huddling with these same doses of MDMA.

Methamphetamine also induced a significant increase in the amount of affiliative calls ($p<0.05$) (Table 3), which does not support our initial hypothesis that the prosocial effects would be unique to MDMA. The relationship between dose and number of affiliative calls changed quadratically, so dose^2 was used as the independent predictor. This relationship indicates that, similar to the behavioral data, the amount of affiliative vocalizations does not show a consistent increase with rising doses of methamphetamine, but rather formed a convex curve with a maximum for dose effects (Figure 8). Additionally, the degree of increase was not as extreme as that observed following MDMA administration (Figure 8).

Co-administration of M100907 with MDMA blocked the increase in prosocial vocalizations seen with just MDMA. This result was indicated by a statistically significant decrease in the number of affiliative calls following pretreatment with M100907 in comparison to administration of MDMA alone ($p<0.05$) (Table 3). The relationship between dose and number of affiliative calls changed linearly, so dose was used as the independent predictor. These data are in line with the corresponding behavioral dataset and support the initial hypothesis that the 5-HT_{2A} receptor would be necessary for the prosocial effects associated with MDMA, specifically indicating that this receptor subunit is essential for the increase in affiliative vocalizations.

Due to technical problems and time constraints in our lab, vocalization data collected on subject animals following co-administration of WAY163909 with MDMA have not yet been analyzed.

Discussion

We used a nonhuman primate model to investigate the prosocial effects of MDMA on behavior and vocalizations, as measured by amount of time spent huddling and number of affiliative calls made, respectively. In line with our hypothesis, MDMA induced an increase in social behavior in our squirrel monkey sample. Specifically, MDMA increased both the amount of time subjects spent huddling and the number of affiliative calls they made in the immediate in-laboratory setting. These findings supplement many prior studies demonstrating that, following administration of MDMA, humans experience enhanced feelings of sociability and closeness to others (Bedi et al., 2009, 2010, 2014; Kirkpatrick et al., 2014a,b; Hysek et al., 2012, 2014a; Wardle and de Wit, 2014), while experimental rodents also exhibit increased social interaction (Morley and McGregor, 2000; Morley et al., 2005; Thompson et al., 2007; Ando et al., 2006). While not directly comparable, the observed increase in chucks, purrs, and pulsed calls made by our monkeys are in line with human studies that found an increase in use of social language while on MDMA (Bedi et al., 2014; Wardle and de Wit, 2014; Baggot et al., 2015). While research has investigated the pharmacological and neurotoxic effects of MDMA in nonhuman primates (Ricuarte et al., 1988a,b; Mechan et al., 2006; Mueller

et al., 2009), this is the first study to show an MDMA-induced increase in sociability in a nonhuman primate model.

It is interesting to note that methamphetamine also had an effect on the social behavior of our nonhuman primate model. However, the response was not as clear as that induced by MDMA. The maximum average amount of time spent huddling during the hour immediately after any dose of methamphetamine was approximately three times less than the average amount of time spent huddling while on MDMA. In line with this outcome, the maximum number of affiliative calls made while on methamphetamine was substantially lower than that induced by MDMA. Further, the increase in both prosocial huddling and affiliative vocalizations did not show a clear relationship with increasing doses of methamphetamine, whereas observations of both showed a consistent increase with rising doses of MDMA. The fact that the time spent engaged in all other behaviors was not statistically different on methamphetamine further illustrates that the degree of increase in prosocial behavior was not as great as it was with MDMA. If the time spent huddling had increased to the same degree, there would be a statistically significant decrease in the amount of time engaged in other behaviors, as was seen in the analysis of behaviors following MDMA administration. These findings are not unexpected, as methamphetamine has been found to induce a low degree of sociability in humans (Bedi et al., 2010; Halkitis et al., 2005; Kirkpatrick et al., 2012). Together, these trends suggest that while methamphetamine may have social properties, the affiliative behavioral effects of MDMA are more robust and well-defined than those of methamphetamine.

Additionally, pretreatment with M100907, a 5-HT_{2A} receptor antagonist, blocked the prosocial effects of MDMA. At the highest dose of MDMA (0.3mg/kg), in particular, there was a statistically significant decrease in the amount of time spent huddling as M100907 dosage was increased from 0.1mg/kg to 0.3mg/kg. The observed effects on affiliative vocalizations are in line with these behavioral findings. Increasing doses of M100907 attenuated the number of prosocial calls made by subjects in comparison with the number induced by MDMA alone. Activity of the 5-HT_{2A} receptor within the amygdala may help explain these effects. Research suggests that MDMA enhances reactivity to positive socioemotional signals while reducing emotional responding to negative social stimuli and threats. One study indicated that MDMA attenuated amygdala activation in response to angry faces and enhanced ventral striatum activation in response to happy faces in comparison to placebo (Bedi et al., 2009). In subsequent experiments, MDMA reduced accurate recognition and decoding of negatively valenced emotions such as fear, anger, and sadness (Hysek et al., 2012, 2014a,b; Kirkpatrick et al., 2014b), and even enhanced the recognition of and reactivity to positive social and emotional stimuli (Hysek et al., 2014a; Schmid et al., 2014). Together, these studies suggest that MDMA increases the reward value of, as well as the response to, positive social signals while decreasing responses to negative and potentially threatening social signals, collectively decreasing social fear and leading to increased sociability. The 5-HT_{2A} receptor is found throughout regions of the amygdala on both excitatory and inhibitory neurons (Bombardi and Giovanni, 2013). While the exact role of 5-HT_{2A} receptors on excitatory neurons in the amygdala is not well known,

(Bombardi and Giovanni, 2013), studies have demonstrated that the 5-HT_{2A} receptor activates GABAergic neurons and thus increases inhibitory post-synaptic currents (iPSCs) (Jiang et al., 2009) in this region. These neurons provide inhibitory innervation of adjacent glutamatergic neurons known to be involved in emotional memories (Bombardi and Giovanni, 2013). For this reason, it may be suggested that 5-HT_{2A} receptor inhibitory neurons regulate the generation of emotional, possibly fear-inducing, memories. One study showed that, in humans, binding of medial prefrontal cortex (mPFC) 5-HT_{2A} receptors is inversely correlated with amygdala reactivity to angry and fearful faces (Fisher et al., 2009). In mouse models, it has been demonstrated that the 5-HT_{2A} receptor mediates anxiolytic effects (Dhonnchadha et al., 2003). These studies suggest that the 5-HT_{2A} receptor may be part of a mechanism mediating the reduced social anxiety associated with increased sociability and closeness to others following administration of MDMA. At the same time, 5-HT_{2A} receptor stimulation within the mesolimbic system enhances dopamine release (Howell and Cunningham, 2014), and this has been linked to reinforcing properties of MDMA (Orejarena et al., 2011). This research suggests a mechanism whereby the 5-HT_{2A} receptor mediates enhanced reward associated with the positive effects of MDMA.

Pretreatment with WAY163909, a 5-HT_{2C} receptor agonist, did not have a significant influence on the affiliative effects of MDMA, which does not support our initial hypothesis. Due to this result, we did not run trials using a combination of both M100907 and WAY163909 as a pretreatment to MDMA. Initial belief that WAY163909 would attenuate the social effects of MDMA was influenced by the 5-HT_{2C} receptor's opposing

properties to the 5-HT_{2A} receptor within the mesolimbic system. 5-HT_{2C} receptors are expressed mainly on GABAergic inhibitory neurons throughout the mesolimbic system, and thus stimulation of these receptors inhibits dopamine release (Howell and Cunningham, 2014). If the prosocial effects of MDMA are mediated largely by a dopaminergic system that enhances the reward value of positive social stimuli, it may be hypothesized that inhibition of this dopamine release via a 5-HT_{2C} receptor agonist would reduce the reward associated with social signals and thus reduce sociability. Our results do not show any such reduction in the social behavior induced by MDMA following pretreatment with WAY163909, a 5-HT_{2C} receptor agonist. This finding suggests that the sociability induced by MDMA may not, in fact, be mediated by a dopaminergic reward system. This idea is supported by data collected after administration of methamphetamine, a potent releaser of dopamine. If dopamine mediates the increase in social behavior, one would expect to observe a dramatic increase in huddling in our squirrel monkey model post methamphetamine administration. While methamphetamine did induce a statistically significant increase in the amount of time spent huddling, the effect was clearly not as robust as that induced by MDMA (Figures 2 and 6). Together, these findings, in combination with the decrease in time spent huddling after pretreatment with M100907, a 5-HT_{2A} receptor antagonist, suggest that MDMA's specific social effects may be more strongly mediated by a system involving activation of 5-HT_{2A} receptors in the amygdala to reduce social fear. However, this does not negate findings that the reinforcing properties of MDMA may be driven by a dopaminergic system (Orejarena et al., 2011).

Our nonhuman primate model provided knowledge of the neurobiological mechanisms underpinning the prosocial effects of MDMA. This model holds potential for future studies in this area and in psychotherapy. MDMA was first used as an adjunct to psychotherapy because of its positive social and emotional effects in the late 1900s (Greet and Tolbert, 1986; Greer and Tolbert, 1998), and its effectiveness in treatment of social disorders continues to be studied (Mithoefer et al., 2010; Oehen et al., 2013; Danforth et al., 2016). Knowledge about the role of the 5-HT_{2A} and 5-HT_{2C} receptors can provide focus for studies aiming to develop novel therapeutics that both isolate increased in social behavior and have reduced negative side effects.

Future Directions

A future study should continue to explore the role of a system that involves anxiety reduction mediating the prosocial effects of MDMA. Evidence supports a role of the medial prefrontal cortex (mPFC) in fear regulation via interaction with the amygdala (Arruda-Carvalho and Clem, 2015; Cho et al., 2013). Fisher et al. (2009) demonstrated that binding of medial prefrontal cortex (mPFC) 5-HT_{2A} receptors is inversely correlated with amygdala reactivity to angry and fearful faces in humans. In line with these data, a positron emission tomography (PET) study found that MDMA increased activity in the mPFC and decreased activity in the amygdala (Gamma et al., 2000). Thus, perhaps MDMA's action in the reduction of social fear occurs throughout this circuit, specifically stimulating 5-HT_{2A} receptors expressed on excitatory neurons within the mPFC and 5-HT_{2A} receptors on inhibitory neurons in the amygdala. A future experiment should aim to

trace the binding of these receptors while also observing social behavior in order to detect a possible direct relationship between the two.

Preliminary research has implicated both the 5-HT_{1A} receptor and the neuropeptide oxytocin in the effects of MDMA, and thus a future study might further this investigation into the role of the 5-HT_{1A} receptor and oxytocin in the MDMA-induced prosocial effects in this group of black top squirrel monkeys. Research in humans has investigated the correlation between MDMA-induced subjective prosocial feelings and an increase in plasma oxytocin levels (Dumont et al., 2009; Hysek et al., 2012, 2014a; Kirkpatrick et al., 2014a). However, the results from these studies are not congruent and thus the findings are inconclusive. Thompson et al. (2007) found a positive correlation between increased prosocial behaviors in male Wistar rats and plasma oxytocin levels following administration of MDMA. This study subsequently noted that pretreatment with either an oxytocin receptor antagonist or a 5-HT_{1A} receptor antagonist attenuated the increased in prosocial behaviors induced by the drug. These results suggest that the increase in affiliative behaviors seen after use or administration of MDMA may be related to increased oxytocin levels via interactions with the 5-HT_{1A} receptors. It would therefore be interesting to attempt to duplicate the results of Thompson et al.'s (2007) study using a 5-HT_{1A} receptor antagonist, such as WAY100635, in our group of black top squirrel monkeys. In addition, a study might investigate behaviors following pretreatment with a 5-HT_{1A} receptor agonist, such as 8-OH-DPAT, to determine if sociability changes in the same way as it does following administration of MDMA. These

experiments, further investigating the role of the 5-HT_{1A} receptor, would provide deeper understanding into the mechanisms by which MDMA induces prosocial behaviors.

Another important avenue for further investigation might test for the effects of physical and/or social context when receiving the drug. Initial studies suggests that the prosocial effects of MDMA may be enhanced by social settings or stimuli, so altering the social arrangement of the monkeys prior to drug administration could provide insight into MDMA's effects. Recreational users of MDMA often report an increase in sociability after taking the drug in a social setting (Siegel, 1986; Weir, 2000; Sumnall et al., 2006). In a controlled human study, Kirkpatrick and de Wit (2015) found that MDMA increased feelings of confidence only in subjects who were accompanied by at least one other participant also under the influence of MDMA during experimental testing. It would be interesting to keep the monkeys separate from each other during the two hours prior to administration of MDMA and compare the rates of prosocial behavior and affiliative calls after this condition with those from the present study in which the animals were always housed together. Similarly, a future experiment might test the behavioral effects of MDMA when given to only one or two of the monkeys rather than all four. It is interesting to note that in early trials with MDMA alone and methamphetamine, one subject did not huddle nearly as much as the other three (Figures 3 and 7). It appeared that over time, with repeated administration of MDMA, this monkey became integrated into the group and began to huddle more. This suggests a role of ongoing MDMA exposure in sociability and alteration of social groups. These studies could provide a better

understanding of the conditions in which MDMA given for clinical human use might be most effective.

Limitations

Due to time constraints, we were only able to collect a limited amount of data using the WAY163909 pretreatment. Specifically, we observed the combination between two doses of WAY163909 (0.03 and 0.3 mg/kg) in conjunction with only one dose of MDMA (0.3 mg/kg), rather than the two doses of MDMA (0.03 and 0.3 mg/kg) used in combination with M100907. More trials with the addition of a 0.03mg/kg dose of MDMA might have resulted in more complete results. Control data, using saline as the main treatment to show that WAY163909 did not have significant effects on its own, was collected for only one day at each dose of WAY163909 (0.03 and 0.3 mg/kg). A greater amount of data with WAY163909 pretreatment and saline treatment would provide a more valid control comparison. Time constraints in combination with technical difficulties also prevented us from analyzing the vocalization data collected from the WAY163909 trials. As a result, the findings from this section of the study are not complete. A future study should expand the timeline of this experiment to ensure enough days to collect a more substantial amount of data and fully analyze all results.

Two monkeys used in this study (s-171 and s-177) had prior exposure to MDMA (Fantegrossi et al., 2009). Additionally, all four animals had prior exposure to stimulant drugs acting on monoaminergic and/or glutamatergic neurotransmitter systems (Fantegrossi et al., 2009; Banks et al., 2009; Kimmel et al., 2009). While the last drug

exposures for all animals was at least two years before the start of this experiment, it is possible that they affected the subjects' present behavior. Although the subjects in this experiment served as their own controls in order to mitigate any bias that may have arisen as a result of their previous exposures, another investigation might use a drug-naïve subject sample. Adding to the findings of the present study with such new data could help lead to more conclusive results.

MDMA's acute effect on body temperature may be interpreted as a reason for the increase in prosocial huddling that we observed following administration of MDMA. Most studies report an MDMA-induced increase in body temperature, however research has also reported hypothermic reactions in experimental animals, which may be dependent on ambient temperature and the age of subjects (Docherty and Green, 2010; Malberg and Seiden, 1998). The increase in prosocial huddling that we found following administration of MDMA may be interpreted as a way to conserve body heat. Preliminary research has found a similar reduction in core body temperature following methamphetamine administration in some cases (Myles et al., 2008), yet the increase in huddling following administration of this compound was not as pronounced as that induced by MDMA. In addition, there was a statistically significant increase in MDMA-induced affiliative vocalizations that was tightly coupled with the increase in prosocial behavior. This pattern of vocalizations conceivably would not have been observed if the motivation for huddling was non-social. Together, these findings suggest that the increased time spent huddling observed in the present study was, in fact, due to enhanced sociability rather than an attempt to make up for a decrease in core body

temperature. Measurement of the body temperature of subjects following each drug treatment would mitigate doubt regarding this issue.

Conclusion

The present study showed an increase in prosocial behavior and vocalizations in black cap squirrel monkeys following administration of MDMA. The extent of this effect can be interpreted as unique to MDMA, as methamphetamine induced a much smaller increase in affiliative behavior and vocalizations with no clear trend over increasing doses. This MDMA-induced effect was attenuated by a 5-HT_{2A} receptor antagonist, implicating a mechanism via this receptor subtype in the drug's effects. The findings of this study provide a greater understanding of the mechanisms by which MDMA increases sociability and will hopefully help contribute to the development of a novel therapeutic that provides the same prosocial and anxiety-reducing benefits to aid in psychotherapy without the risks associated with MDMA.

Figures and Graphs

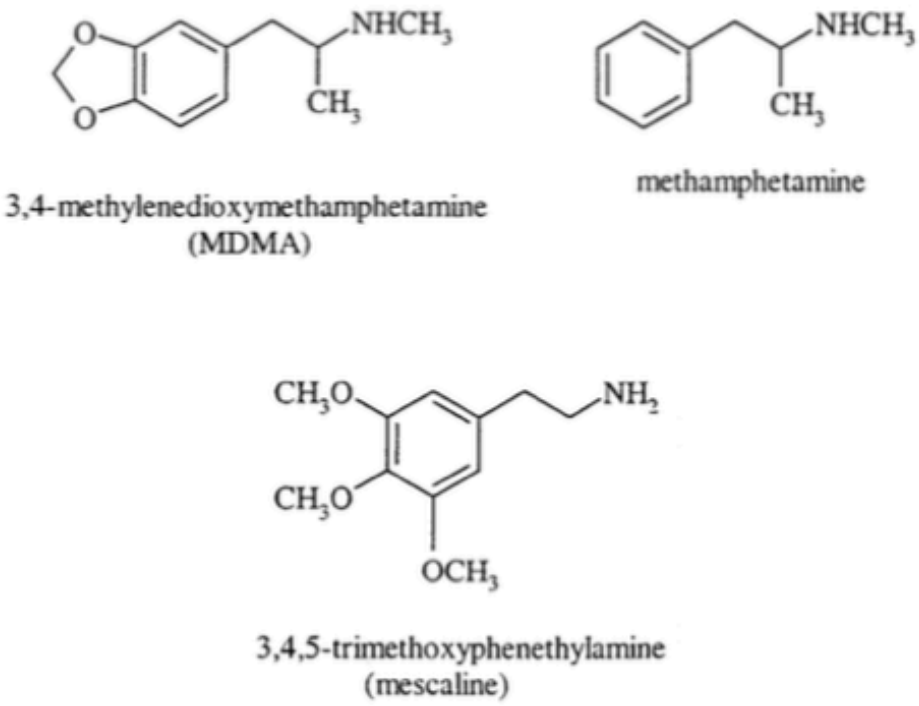

Figure 1. The above image compares the structure of MDMA, methamphetamine and mescaline. Methamphetamine is a traditional stimulant drug and was used as the positive control in this study. Mescaline is a hallucinogenic compound. The similarity between these two compounds and MDMA most likely accounts for the comparable increase in psychomotor behavior and mild hallucinations, respectively, seen after administration of MDMA.

Behavior	MDMA	Methamphetamine (not shown)
Affiliation/Proximity	F(4,12) = 15.112, p = 0.000*	F(4,12) = 5.630, p = 0.009*
Locomotion	F(4,12) = 6.487, p = 0.005*	F(4,12) = 0.969, p = 0.459
Residual	F(4,12) = 4.911, p = 0.015*	F(4,12) = 1.423, p = 0.285
Self-Grooming	F(4,12) = 2.062, p = 0.149	F(4,12) = 0.661, p = 0.631

* - indicates statistical significance

Table 1. The table above is a collection of descriptive statistics from the behavioral analysis following MDMA and methamphetamine administration. Behaviors were analyzed using a one-way repeated measures ANOVA at a 0.05 level of significance.

Behavior	M100907 + MDMA	WAY163909 + MDMA
Affiliation	F(4,12) = 8.666, p = 0.002*	F(2,6) = 4.862, p = 0.056
Locomotion	F(4,12) = 0.843, p = 0.524	F(2,6) = 4.010, p = 0.078
Residual	F(4,12) = 4.920, p = 0.014*	F(2,6) = 2.123, p = 0.201
Self-Grooming	F(4,12) = 0.550, p = 0.702	F(2,6) = 1.566, p = 0.284

* - indicates statistical significance

Table 2. The table above is a collection of descriptive statistics from the behavioral analysis following MDMA administration with pretreatment of either M100907 or WAY163. Behaviors were analyzed using a two-way repeated measures ANOVA at a 0.05 level of significance.

	Beta	SE	Test Statistic	p-value	R^2
Methamphetamine Dose^2	-61.8	26.5	t = -2.32	0.033*	24.3%
MDMA Dose	5.75	2.20	z = 2.61	0.009*	
M100 Dose	-3.58	1.60	z = -2.23	0.026*	

* - indicates statistical significance

Table 3. The table above is a collection of descriptive statistics from the analysis of affiliative vocalizations following administration methamphetamine, MDMA, and M100907 with MDMA. Vocalizations on methamphetamine were analyzed using a linear regression with ln-transformed vocalizations. Vocalizations on MDMA and M100907 with MDMA were analyzed using a negative binomial regression model.

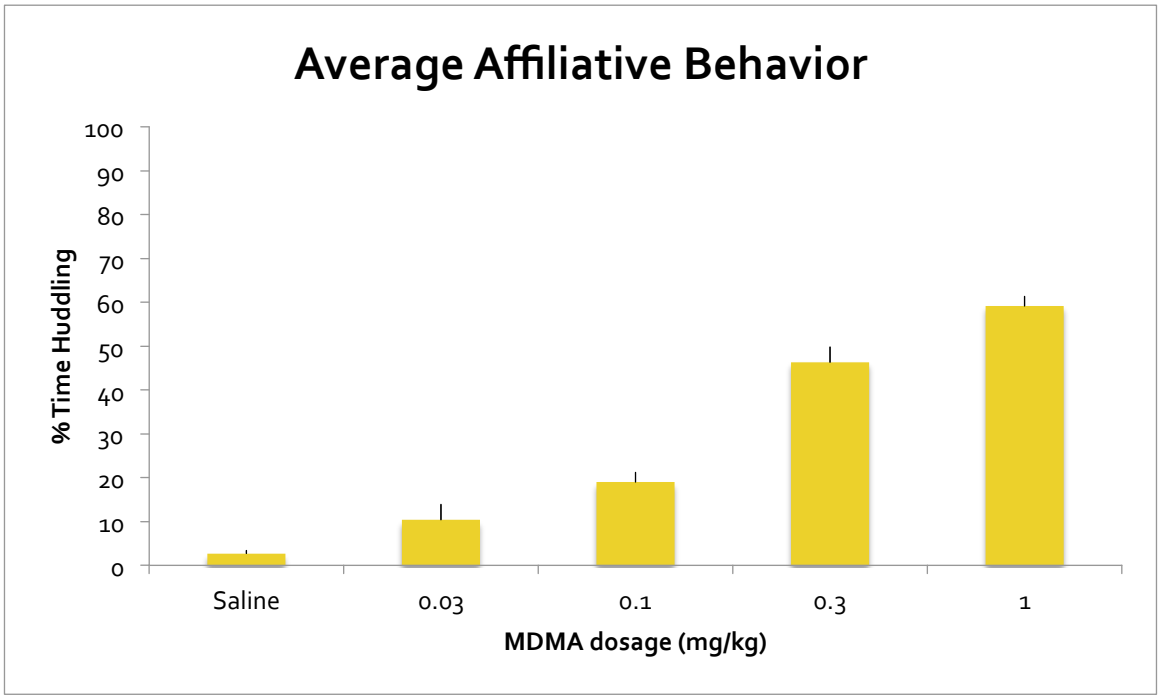

Figure 2. The figure above represents the average amount of time spent huddling among the four subjects across all doses of MDMA used. There was a significant difference in the mean amount of time spent huddling across all doses of MDMA ($p=0.000$). However, a post-hoc pairwise comparison using a Bonferroni adjustment for multiple comparisons did not yield significant differences between group means. Error bars represent the standard error of the mean.

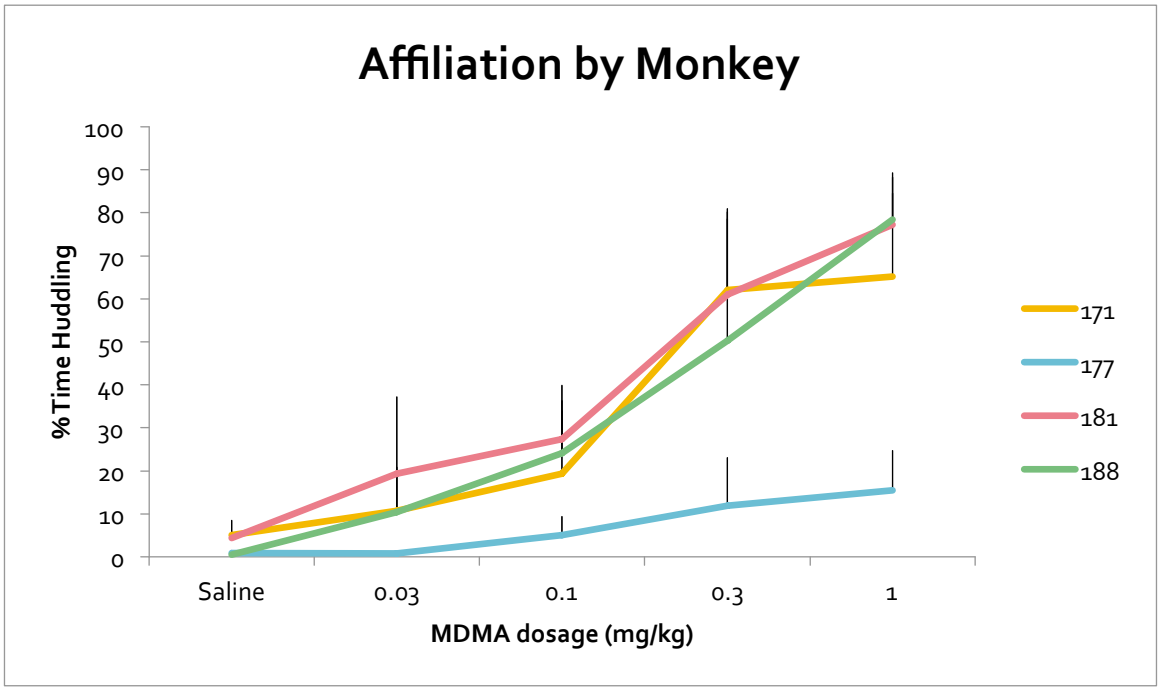

Figure 3. The figure above shows the percentage of time spent huddling across all doses of MDMA, separated by monkey. Error bars represent the standard error of the mean. As represented, s-177 spent visibly less time huddling than the other three subjects across all doses of MDMA.

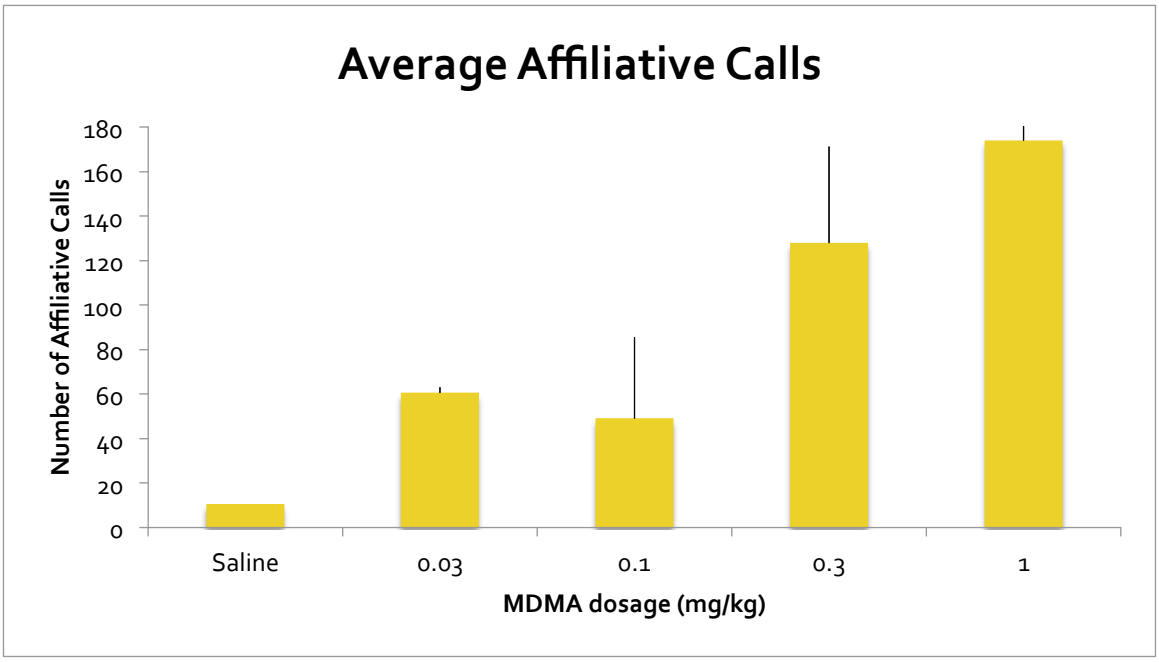

Figure 4. The figure above is a representation of the average number of affiliative vocalizations made by all monkeys across all doses of MDMA used. Chucks, purrs, and pulsed calls were categorized as affiliative. Error bars represent the standard error of the mean. Due to the way in which vocalizations are recorded, calls produced by each individual monkey could not be collected. As seen in the figure, there was a statistically significant increase in the number of affiliative vocalizations ($p=0.009$) following MDMA administration.

Figure 5A.

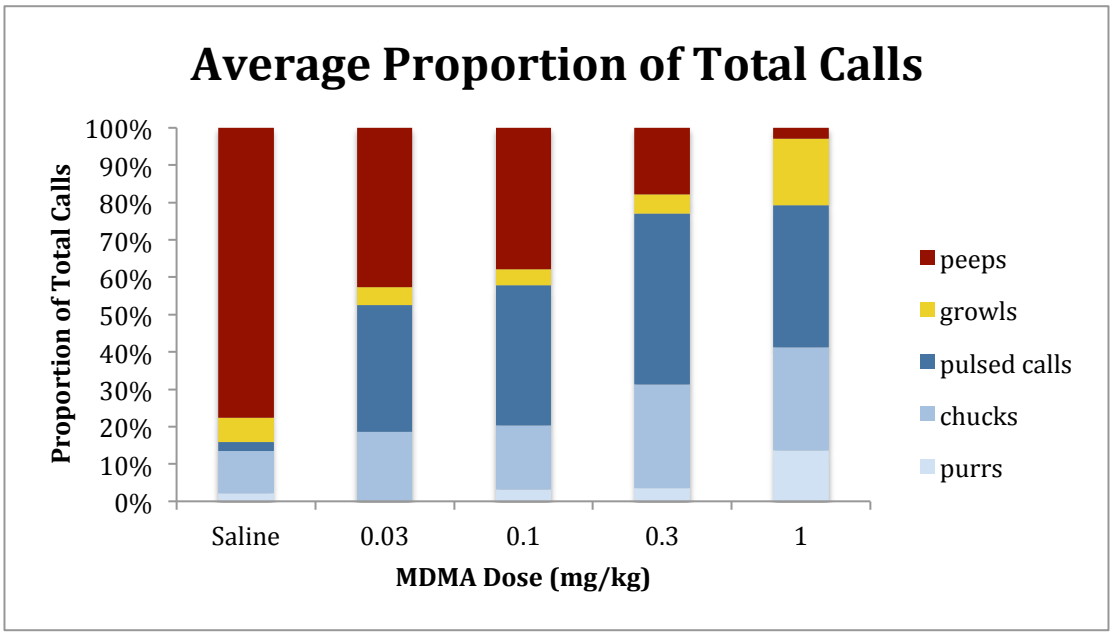

Figure 5B.

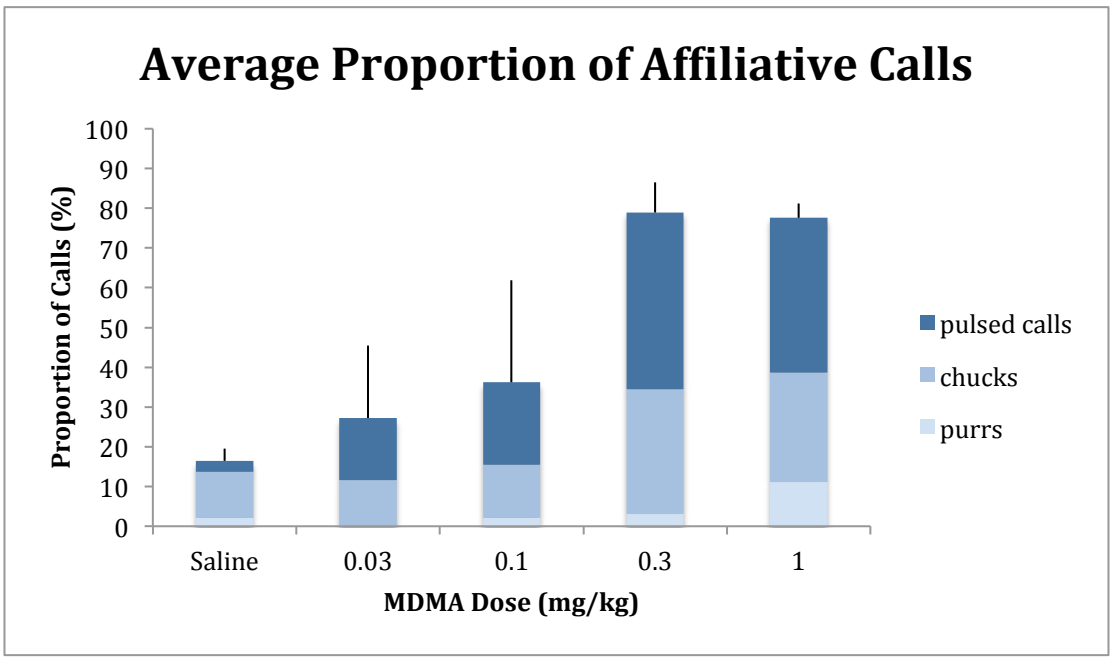

Figure 5. The figure above is a representation of the average frequency of each call type across all doses of MDMA used. All vocalizations studied are represented in figure 5A. Only the three calls categorized as affiliative (chucks, purrs, and pulsed calls) are represented in figure 5B. Error bars represent the standard error of the mean. As seen in the figure, MDMA induced an increase in the proportion of affiliative calls ($p=0.009$).

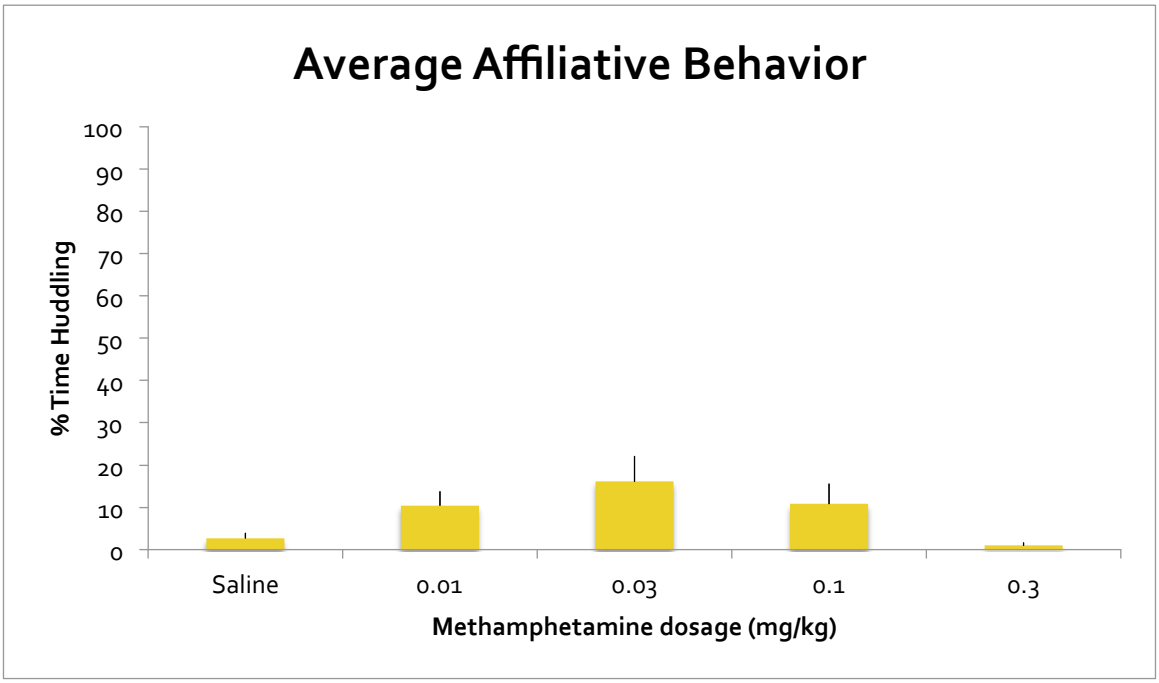

Figure 6. The figure above represents the average amount of time spent huddling among the four subjects across all doses of methamphetamine used. Error bars represent the standard error of the mean. There was a significant difference in the mean amount of time spent huddling across all doses of methamphetamine ($p=0.009$). However, the peak proportion of time spent huddling at any dose was approximately three times less than that induced by MDMA. A post-hoc pairwise comparison using a Bonferroni adjustment for multiple comparisons did not yield significant differences between group means.

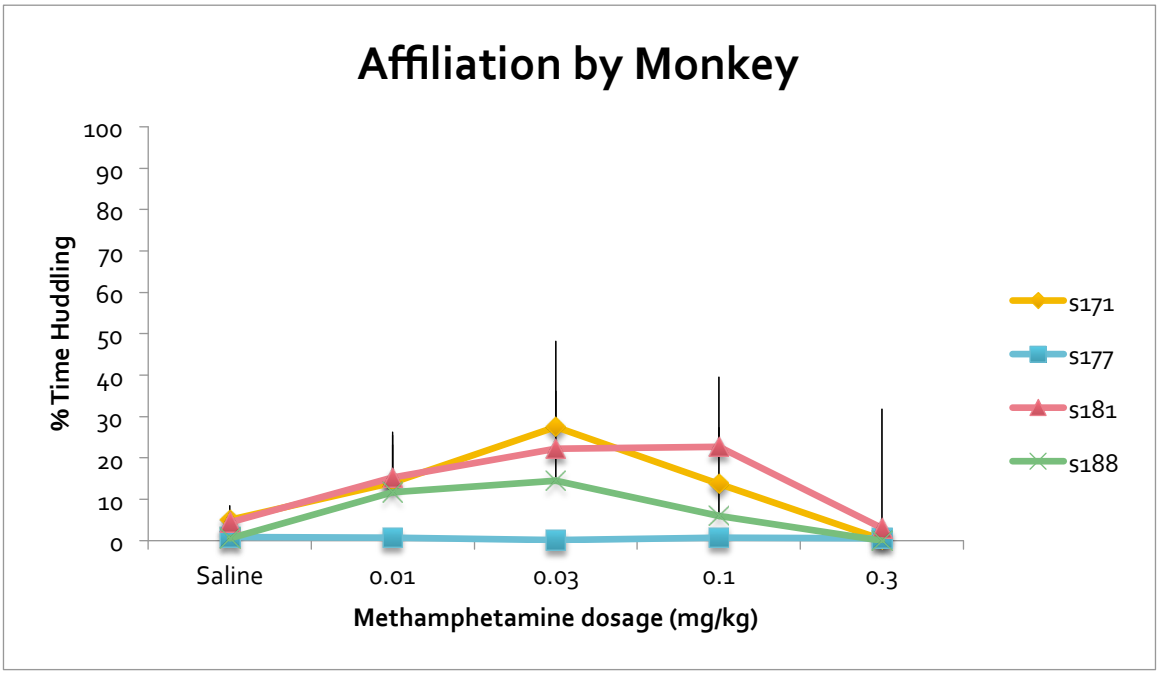

Figure 7. The figure above shows the percentage of time spent huddling across all doses of methamphetamine, separated by monkey. Error bars represent the standard error of the mean. As represented, s-177 spent visibly less time huddling than the other three subjects across all doses of methamphetamine.

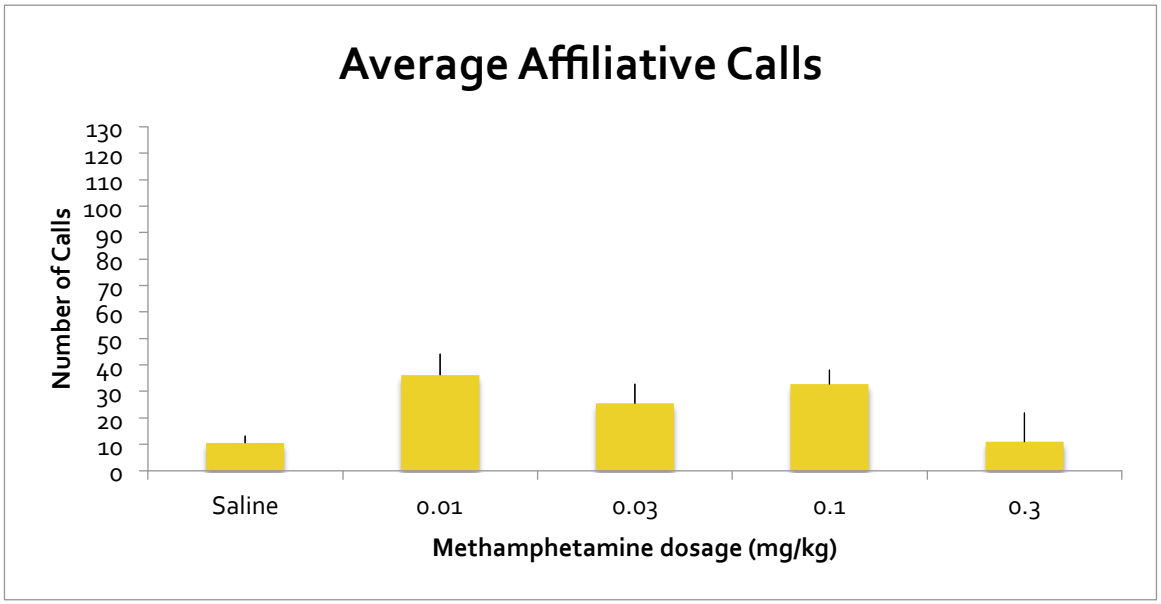

Figure 8. The figure above is a representation of the average number of affiliative vocalizations made by all monkeys across all doses of methamphetamine used. Chucks, purrs, and pulsed calls were categorized as affiliative. Error bars represent the standard error of the mean. Due to the way in which vocalizations are recorded, calls produced by each individual monkey could not be collected. There was a statistically significant increase in the number of affiliative vocalizations ($p=0.033$) following methamphetamine administration. However, the peak number of affiliative calls made at any dose of was approximately five times less than that induced by MDMA.

Figure 9A.

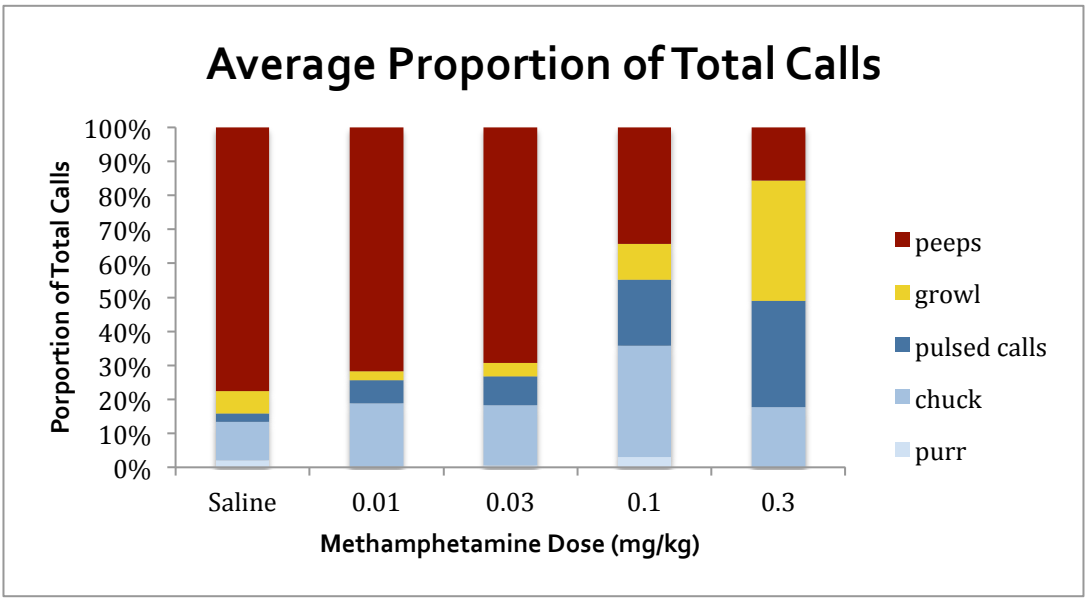

Figure 9B.

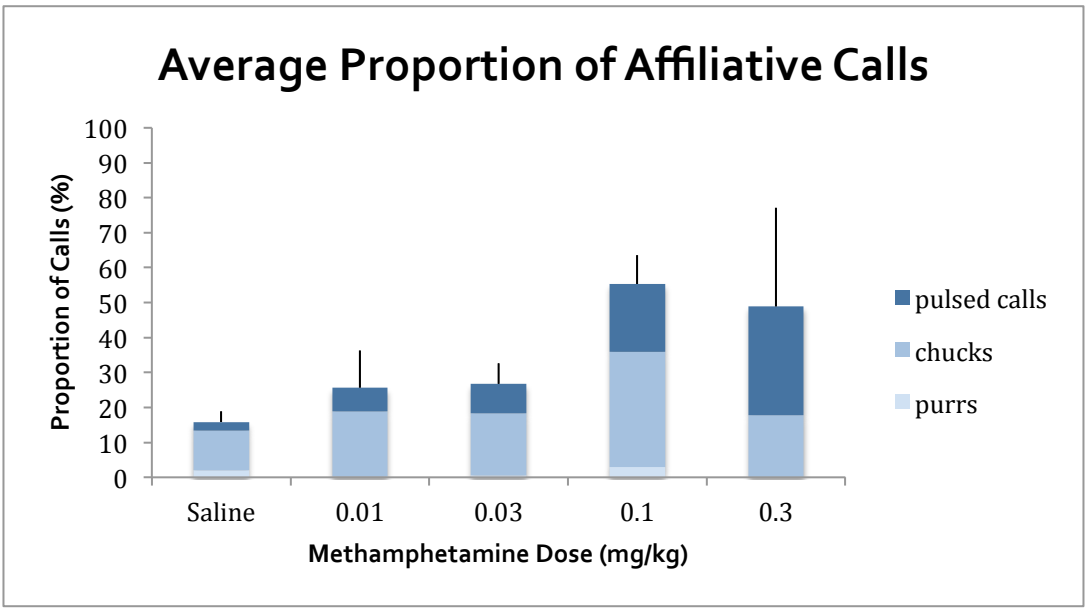

Figure 9. The figures above show the proportion of each type of vocalization across increasing doses of methamphetamine. All vocalizations studied are represented in figure 9A. Only the three calls categorized as affiliative (chucks, purrs, and pulsed calls) are represented in figure 9B. Error bars represent the standard error of the mean. As seen in the figure, there was an increase in the proportion of affiliative calls ($p=0.033$). However, the peak average proportion of affiliative calls across any dose is substantially less than that induced by MDMA.

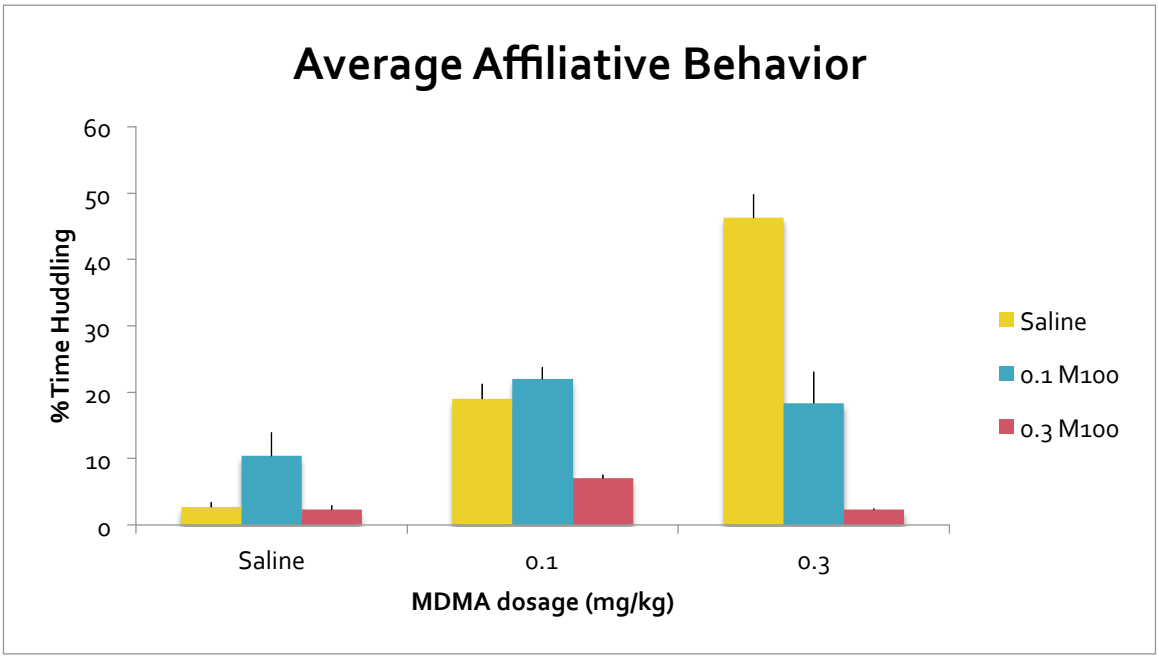

Figure 10. The figure above represents the average percentage of time spent huddling among the four subjects following pretreatment of increasing doses of M100907 with increasing doses of MDMA. Error bars represent the standard error of the means. There was a statistically significant overall interaction between M100907 and MDMA ($p=0.002$). Specifically, at the highest dose of MDMA (0.3mg/kg), M100907 blocked the increase in affiliative behavior (post-hoc test using a Bonferroni adjustment for multiple comparisons, $p=0.048$ between 0.1mg/kg M100907 and 0.3mg/kg M100907).

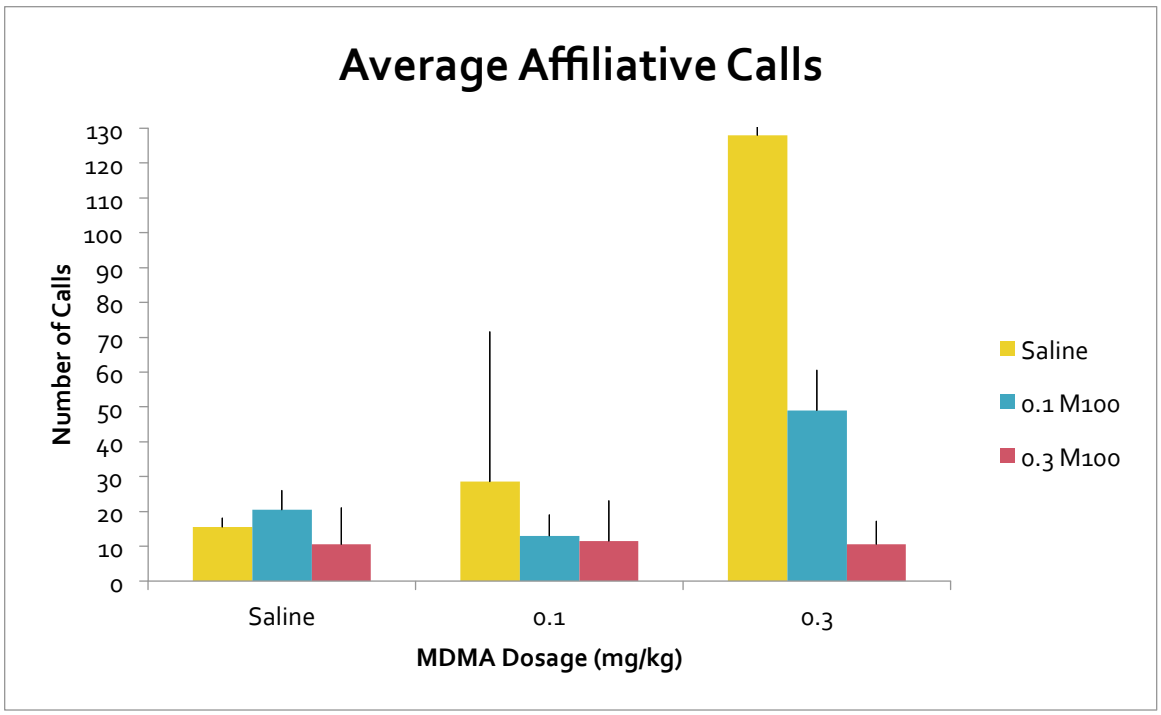

Figure 11. The figure above is a representation of the average amount of affiliative vocalizations made by all monkeys across all doses of M100907 and MDMA used. Chucks, purrs, and pulsed calls were categorized as affiliative. Error bars represent the standard error of the mean. Due to the way in which vocalizations are recorded, calls produced by each individual monkey could not be collected. There was a statistically significant interaction between M100907 and MDMA ($p=0.026$), such that the amount of affiliative calls made after administration of M100907 was less than that induced by MDMA alone.

Figure 12A.

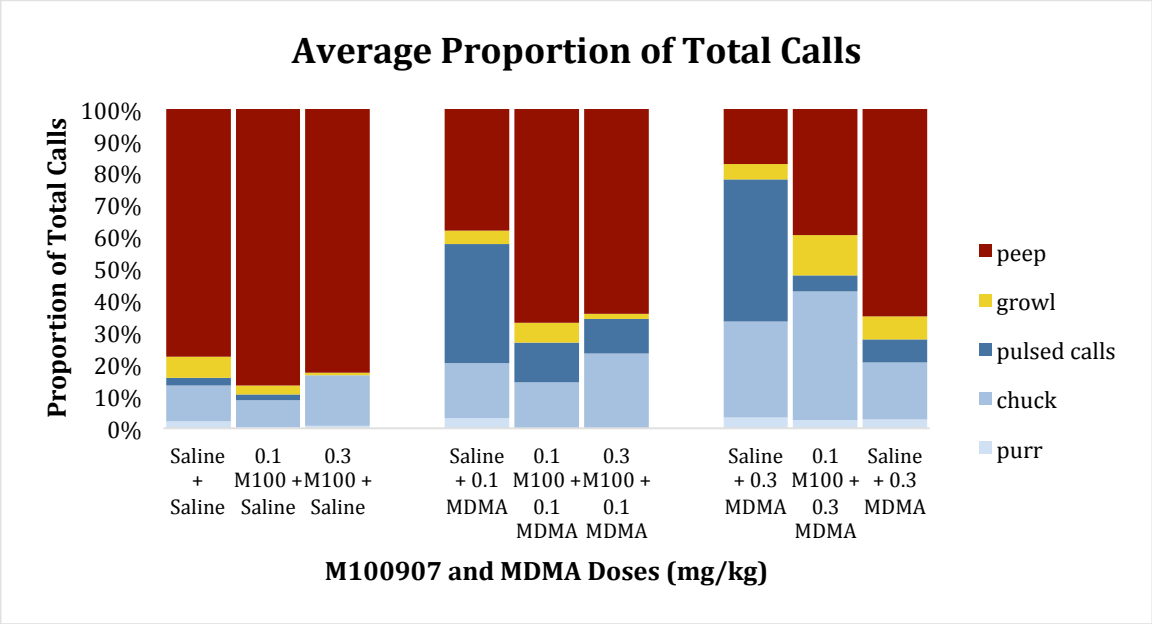

Figure 12B.

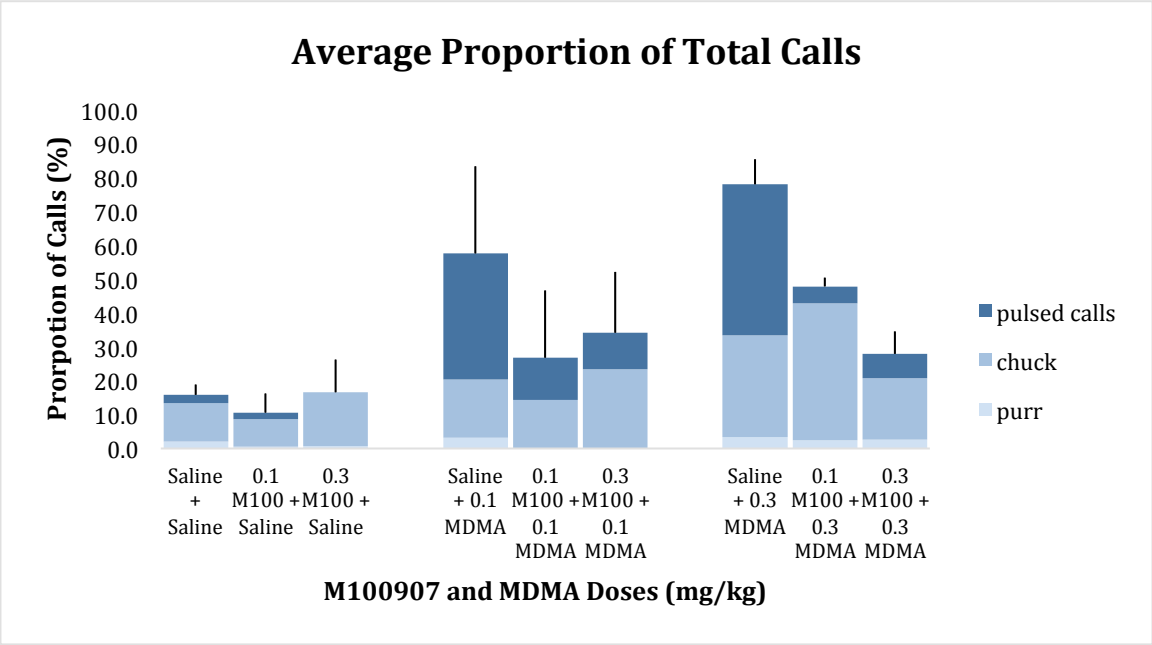

Figure 12. The figures above show the proportion of each type of vocalization across increasing doses of M100907 in combination with MDMA. All vocalizations studied are represented in figure 12A. Only the three calls categorized as affiliative (chucks, purrs, and pulsed calls) are represented in figure 12B. Error bars represent the standard error of the mean. There was a statistically significant interaction between M100907 and MDMA ($p=0.026$), such that M100907 blocked the increase in the proportion of affiliative calls induced by MDMA alone.

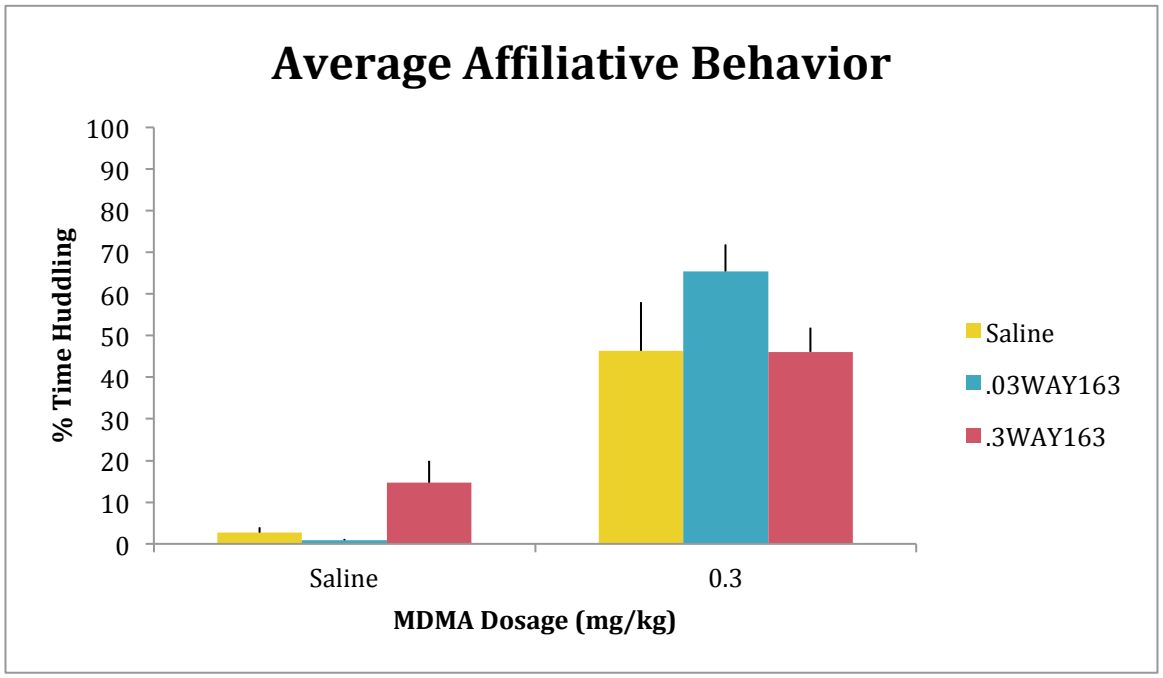

Figure 13. The figure above represents the average percentage of time spent huddling among the four subjects following pretreatment of increasing doses of WAY193909 with MDMA. Error bars represent the standard error of the means. There was not a statistically significant overall interaction between WAY163909 and MDMA (p=0.056).

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