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Erika Oliver April 15, 2015

Prosocial Effects of MDMA in Nonhuman Primates

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Abstract

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MDMA (3,4-methylenedioxymethamphetamine) is a recreational drug that has profound effects on prosocial behavior. These effects include a sense of increased trust and openness. Due to these unique prosocial effects, MDMA is proposed to have potential therapeutic value for disorders that are characterized by an inability to process relevant social cues, such as PTSD or Autism. Previously, both human and animal studies have shown that MDMA administration results in an increase in prosocial behavior. However, there has not been a study that examines the long-term prosocial effects of MDMA. Thus, the goal of this study was to analyze the long-term effect of MDMA on prosocial behavior in squirrel monkeys. All components of the study were done using male squirrel monkeys (*saimiri sciureus*) as subjects. A single dose of MDMA was given to each monkey twice per week in a lab setting. Behavior on subsequent days following MDMA administration was examined in order to evaluate the long-term off drug effect. Vocalizations and behavior while on the drug in the lab and off the drug in the animal's homeroom were analyzed to understand the acute and long-term increase in prosocial behavior and vocalizations. The experiment was designed in order to examine if multiple exposures to MDMA result in a long-term residual increase in prosocial behavior while the monkeys are off the drug. Behavior in the experimental sessions was compared to the baseline data in both the homeroom and lab setting in order to determine the effect MDMA has on prosocial behavior. MDMA administration resulted in a significant increase in off-drug prosocial behavior, as well as a decrease in locomotion while off-drug. Due to the current findings of this study, further pharmacological experiments may be done in order to develop a drug that lacks the abuse liability, side effects, and neurotoxicity of MDMA.

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Honors Thesis

Hypothesis: This experiment tested the hypothesis that MDMA administration would result in an increase in the amount of prosocial behaviors observed in socially housed squirrel monkeys while on the drug in the lab setting. Additionally, the experimental conditions in the homeroom setting tested the hypothesis that there would be an increase in residual prosocial behavior after multiple exposures to MDMA, therefore indicating that multiple doses of MDMA result in a long-term change of prosocial behavior. Lastly, it was hypothesized that MDMA administration would result in an increase of prosocial vocalizations in the squirrel monkeys.

Purpose and Rationale: This study established a nonhuman primate model for examining the prosocial effects of MDMA. Previous studies have used squirrel monkeys to model neurotoxic and neuropharmacological effects of MDMA, but there has not been a study that modeled the effects on social behavior (Ricaurte et al., 1988; Ricaurte et al., 2009). While previous studies have been done in humans, they are not highly controlled. Often these studies are observational and involve polydrug users. Therefore, there could be confounding variables. In this experimental model, the environment, drug exposure, and time course were all controlled. With the design of this experiment, it was possible to understand how multiple exposures to MDMA result in longitudinal changes in prosocial behavior in monkeys while drug free in their homeroom setting. This portion of the experiment models the long-term effects of MDMA

administration since the animals were observed in their off drug homeroom setting. In addition to understanding the acute and residual increase in prosocial behavior, perhaps this study could be expanded to provide insight as to how long the residual therapeutic effects last and the time of onset. This will improve the current knowledge of the long-term effects of MDMA, which can be translated to a clinical setting. Understanding the acute and long-term prosocial effects will provide a better comprehension of the viability of the therapeutic properties of MDMA. In the future, pharmacological experiments in highly controlled environments can be designed in order to establish a model that illustrates the neurobiological mechanism of MDMA. This will bring the field closer to designing and improving drugs that lack the abuse liability and neurotoxicity of MDMA.

Introduction

Background and History

MDMA was synthesized by Merck in 1912, originally to be used as a clotting agent. Alexander Shulgin resynthesized the compound in the 1960s (Bernschneider-Reif et al., 2006), and after hearing of other people's experience with MDMA, Shulgin tried the compound himself (Benzenhöfer and Passie 2010). He introduced MDMA to his colleague, Leo Zeff, who then used MDMA as an adjunct to psychotherapy for his patients (Benzenhöfer and Passie 2010; Oehen et al., 2012). In the 1970's MDMA gained popularity as a recreational drug in the United States. In 1985 the federal government banned the drug and classified it as a schedule I compound. To be classified as a schedule I compound, there must be a high abuse liability and no redeeming medical benefits. Other schedule I drugs include LSD, marijuana, heroin, and peyote. At the time of its classification, MDMA was believed to have no redeeming medical

value. However, this investigation along with other recent studies, examine the prosocial effects of MDMA that could be of potential therapeutic value (Bedi et al., 2009; Mithoefer et al., 2010; Mithoefer et al., 2012; Morley and McGregor 2000).

MDMA is the primary component of the drug ecstasy. Ecstasy is also known as the "hug drug" or "Molly." In the street form, Ecstasy is typically not pure MDMA; there may be traces of other drugs such as amphetamines or LSD (Green et al., 1995). MDMA is known for causing an increase in social behavior, trust, and openness toward others as well as a euphoric state. It also enhances mood, increases empathy, and makes users feel more extroverted (de la Torre et al., 2004). One study found that users experienced a positive mood state 94% of the time after using MDMA. Users noted that they felt open minded, close to others, happy, accepting, sensual, and euphoric (Solowij, Hall, and Lee 1992). Imaging studies showed that MDMA decreased the amygdala's response to threat, while simultaneously increased the ventral striatum's response to positive social stimuli (Bedi et al., 2009). These data are supported by another study that showed MDMA improved the ability of users to determine positive mental and emotional states in the Reading the Mind in the Eyes Test. This same study showed that MDMA impaired the ability to recognize negative emotional stimuli (Hysek et al., 2012). Taken together, these data illustrate the increased sociability that results from MDMA consumption. In addition to human experiments, MDMA has been shown to increase social interaction, adjacent lying and approach behaviors in rats (Morley et al., 2005; Thompson et al., 2007). This increased social interaction will be a keystone in the development of this study. Due to the fact that it is a weak hallucinogen, MDMA in humans is typically taken orally in tablet form at "rave" parties. This type of environment elaborates on the sensory distortions caused by MDMA and

creates a setting conducive to increased prosocial interaction. Common effects of MDMA include altered perception of colors and sounds and disruption of perception (de la Torre et al., 2004). It is estimated that the average dose in humans is 1-3 mg/kg (Schwartz and Miller 1997; Ricaurte et al., 1988). However, estimates may vary because it is difficult to obtain accurate measures from human drug users and the amount of pure MDMA in an ecstasy tablet is unknown. In humans, it takes approximately thirty to sixty minutes for the effects of MDMA to set in, while the peak effects occur around ninety minutes. These effects may last eight hours or more after consumption (Schwartz and Miller 1997). The half-life of MDMA in humans after a 100 mg dose is 8-9 hours. Similar half-lives are reported for 50, 75, and 125 mg doses (de la Torre et al., 2004).

Neurotoxicity

MDMA has a high abuse liability and there is evidence of neurotoxicity. High doses of MDMA may result in acute adverse effects such as hyperthermia and serotonin syndrome, as seen in rats. (Green et al., 1995). Serotonin syndrome is characterized by increased muscle rigidity, hyperthermia, and hyperreflexia (de la Torre et al., 2004). Other acute side effects include dry mouth, thirst, palpitations, nystagmus, trismus, nausea, muscle aches, tachycardia, and ataxia (de la Torre et al., 2004; Green et al., 1995). Short-term side effects may last up to 24 hours after consumption of MDMA and include fatigue, loss of appetite, insomnia, drowsiness, difficulty concentrating, headaches, and muscle tension (de la Torre et al., 2004). Other side effects may last up to seven days after consumption of MDMA and include anxiety, irritability, insomnia, depressed mood, and muscle tension (de la Torre et al., 2004). Mild toxic symptoms in humans include nausea, vomiting, sweating, tremor, and palpitations. Moderate toxic

symptoms include hyperactivity, aggression, panic attack, psychosis, tachycardia, and increased body temperature. Severe symptoms include delirium, coma, seizures, hyperthermia and renal failure (de la Torre et al., 2004). Long-term effects include changes and damages at serotonergic nerve terminals (Ricaurte et al., 1988). Multiple doses of MDMA per day were found to deplete serotonin in the somatosensory cortex, cerebral cortex, hippocampus, hypothalamus, thalamus, caudate nucleus, and putamen of rhesus monkeys (Ricaurte et al., 1988). However, in this same study, dopamine and norepinephrine concentrations were not affected by MDMA. Additionally, it appears that the effects of MDMA on serotonergic nerve terminals are more severe in nonhuman primates than in rodents (Fantegrossi et al., 2009). Neurodegeneration of the serotonergic system is linked to loss of cognitive function, higher impulsivity, and a greater psychopathology among recreational MDMA users (de la Torre et al., 2004). It is important to note that the majority of neurotoxicity studies administered high doses of MDMA multiple times per day (Ricaurte et al., 1988). In this study, a low dose (0.3 mg/kg) of MDMA was administered twice per week with at least 48 hours between each injection. In addition, it is still not clear whether MDMA or its metabolites are responsible for the neurotoxicity (de la Torre et al., 2004). However, it appears that neurotoxicity and depletion of serotonin and serotonin transporters is a dose dependent relationship (Ricaurte et al., 1988).

Involvement of Serotonin

MDMA releases dopamine and norepinephrine, but primarily results in the release of serotonin (5-hydroxytryptamine, 5-HT) (Koch and Galloway 1997; Verrico et al., 2007). The increased prosocial behavior may be a result of increased synaptic serotonin concentration. MDMA directly binds to the serotonin transporter and is taken back up into the synaptic

terminal, resulting in an increase of synaptic serotonin (Rudnick and Wall 1991). Once MDMA is taken up into the presynaptic neuron, it disrupts serotonin vesicle stability resulting in an increase of synaptic serotonin levels. Previous studies have indicated the role that 5-HT plays in the positive effects of MDMA. For example, when users were given MDMA they noted extroversion, heightened mood, increased sensory perception, and increased self-confidence. However, when citalopram, a 5-HT uptake inhibitor was given, these effects were significantly diminished (Liechti et al., 2000). This indicates that 5-HT release may be responsible for the positive effects of MDMA. Additionally, another study found that a 5-HT_{1A} receptor antagonist prevented the increased social behavior caused by MDMA when co-administered (but not pretreated). In this same study, $5\text{-HT}_{2B/2C}$ receptor antagonists also prevented MDMA prosocial effects in rats when co-administered. (Morley et al., 2005). Pretreatment with a 5-HT_{1A} receptor antagonist reversed the effects of serotonin syndrome in rats. This indicates that the 5-HT_{1A} receptor plays a role in MDMA induced serotonin syndrome. This study also reported that MDMA increased generalized anxiety-like behaviors in an emergence test. However, 5-HT_{1A}, 2A, and 2B/C receptor antagonists did not reduce the anxiety in the emergence test. This suggests that another receptor such as 5-HT_3 may be involved in the anxiety-induced behavioral effects of MDMA. Additionally, in another study, subjects were given MDMA, and many of the positive effects of MDMA such as elation and positive mood, were attenuated by administration of fluoxetine, a serotonin reuptake inhibitor (Tancer and Johanson 2007). Taken together, this provides support for the crucial role of 5-HT and 5-HT receptors in MDMA induced prosocial behavior. However, the fact that not all of the behaviors were attenuated suggests that there are other neurotransmitter systems involved.

The role of Oxytocin

In addition to serotonin, MDMA is a potent releaser of the neuropeptide/neurohormone oxytocin. Oxytocin is known to play a role in maternal bond formation and pair bonding. A previous study has shown that MDMA induced the release of oxytocin from the supraoptic and paraventricular nuclei of the hypothalamus, and that MDMA elevated plasma oxytocin levels (Thompson et al., 2007). However, this study also illustrated that MDMA may act on 5-HT_{1A} receptors to mediate oxytocin release. Current studies are examining the role of oxytocin in the prosocial effects of MDMA (Ramos et al., 2015). However, as discussed above, there is substantial evidence that supports the involvement of 5-HT in inducing the positive effects of MDMA.

Structure and Mechanism of Action

The unique effects of MDMA stem from the fact that it has hallucinogenic and psychostimulant properties. By definition, a hallucinogen is a drug that causes hallucinations, changes in thought or mood, or perceptual distortions of reality. Psychostimulants are drugs that increase the mood-state as well as psychomotor activity. MDMA does not cause true hallucinations but it alters sensory perceptions in a way that psychostimulants do not. The stimulant properties of MDMA are most likely due to the molecular structure, which is very similar to amphetamine (Figure 1). MDMA is a ring substituted amphetamine molecule, therefore the effects would be predictably similar to amphetamine. There is no other drug that changes prosocial behavior in the same manner as MDMA. As a result, it has been classified into a separate category apart from hallucinogens and psychostimulants. MDMA falls into a class called entactogens (Vollenweider 2001; Nichols and Oberlender 1990). Entactogens

usually induce very modest psychedelic effects but not true hallucinations. More specifically, MDMA is an empathogen-entactogen because of the particular way it affects social behavior.

Once MDMA is present in the brain, it causes an increase in the extracellular concentration of monoamines by inducing their release (de la Torre et al., 2004). MDMA interacts with membrane transporters to reverse the direction of transport. It also destabilizes vesicles that store monoamines, resulting in an increase in extracellular concentration. MDMA has a high affinity for the serotonin transporter (Rudnick and Wall 1992). MDMA is taken up by the 5-HT transporter and reverses the transport of 5-HT. This results in a release of intracellular serotonin, thus causing an increase in the amount of serotonin in the synaptic cleft (Figure 2). MDMA is particularly potent at serotonin receptors and has been found to be a direct agonist at 5-HT_{1A} receptors (Thompson et al., 2007 and Morley et al., 2005). The pathways in the brain that MDMA targets include those related to mood, appetite, and perception (de la Torre et al., 2004). Therefore an increase in serotonin activity in these pathways would explain why there is an increase in sensory perception and an elevated mood state.

Human Studies

MDMA was used in the 1970s and 1980s in psychotherapy due to its ability to enhance the effectiveness of therapy (Oehen et al., 2012). A pilot study examined the safety and efficacy of MDMA-assisted psychotherapy in treatment-resistant Post Traumatic Stress Disorder (PTSD) patients. MDMA induces a positive cognitive-emotional state and in psychotherapy, exposure to traumatic memories must occur in an “optimal arousal zone” or “window of tolerance.” This study proposed that MDMA-assisted psychotherapy may be beneficial because of the ability of MDMA to increase prosocial effects and thereby make the “optimal arousal zone” a larger

window (Mithoefer et al., 2010, Oehen et al., 2012). Mithoefer et al. (2010) proposed that since MDMA causes a decreased fear response, it would be useful in treating PTSD, which is characterized by uncontrolled fear responses. Additionally, psychotherapy for PTSD includes revisiting the trauma; however many patients are unable to tolerate this exposure and the feelings that are elicited. Therefore, Mithoefer et al. (2010) proposed that MDMA may reduce fear and increase trust between the therapist and patient, thereby increasing the effectiveness of therapy. In the original study by Mithoefer, the group of patients that received MDMA in the Stage 1 double blind portion or the experiment, experienced a significant change in CAPS (Clinican Administered PTSD Scale) scores compared to inactive placebo. The study by Oehen et al. (2012) sought to confirm the findings of the study by Mithoefer et al. (2010). The Oehen study suggests that MDMA-assisted psychotherapy is safe for clinical use, while the Mithoefer study found significant efficacy of MDMA-assisted psychotherapy. Additionally, Mithoefer et al. (2012) completed a long-term follow up study and found that 16 of 19 patients had maintained the improvement in their CAPS scores from the initial study. As mentioned in the discussion, there are negative side effects of MDMA. However, our study seeks to understand the prosocial benefits of MDMA with the long-term goal of developing a novel therapeutic that mimics these prosocial effects.

Methods

Subjects

All subjects used in this study had extensive exposure to stimulant drugs that either acted on monoaminergic and/or glutamatergic systems (Kimmel et al., 2007; Banks et al., 2009; Bauzo et al., 2009; Fantegrossi et al., 2009; Kimmel et al., 2009). However, the monkeys were compared to one another, not to animals that were never exposed to drugs. Additionally this can translate to humans since past drug exposure in humans is hard to control for and most people who consume MDMA are polydrug users. The last exposure to drugs in all animals was in 2012. The animals had not been tested or administered any drug for two years. 4 monkeys weighing between 940-1070 grams were used in this study. Published data has shown that four subjects are enough to obtain statistically reliable results. Monkeys were fed twice daily (LabDiet 5045 High Protein Monkey Chow, PMI Nutrition International, Brentwood, MO; fresh fruit/vegetables; cereal) and had constant access to water. The subjects also had access to perches, toys, and swings for enrichment purposes. Prior to this study, monkeys were individually housed. In order to acclimate them to group housing, a pair of monkeys was introduced. Once they were determined to be stable for two weeks, meaning there was no aggressive behavior, a third monkey was introduced. Once the group of three was determined to be stable, a fourth monkey was introduced to the group. All four monkeys were from the common squirrel monkey, green cap subspecies. In the homeroom, the monkeys were able to make visual contact with other squirrel monkeys in the same room. All aspects of this study were in compliance with the National Institute of Health's Guide for Care and Use of Laboratory

Animals and the American Accreditation of Laboratory Animal Care. The procedure was also approved by the Institutional Animal Care and Use Committee of Emory University.

Behavioral Scoring and Vocalizations

To score social behavior, two cameras were used to video one-hour segments of behavior. One camera videoed the top half of the cage while the other videoed the bottom half of the cage. Using JWatcher software, a blind observer scored the randomized videos based on an established ethogram (Hopf et al., 1974). A randomized order was created to score individual monkeys. Each individual monkey was watched according to this order in five-minute segments. Therefore, each animal was observed for a total of 15 minutes per hour. Each behavior was coded with a certain letter, for example "affiliation/proximity" was coded as 'a.' Whenever the behavior occurred, the blind observer simply typed the key that corresponded to the behavior. The JWatcher program totaled the recorded amount of time each monkey spent doing each behavior. This setup provided insight into how long each behavior lasted during the one-hour segment of video. Behaviors that were scored included affiliation/proximity, self-huddling, play behavior, aggression, locomotion, self-grooming, fly-catching, genital display, and residual behavior. Affiliation/proximity and play behavior are indicators of prosocial behaviors. Affiliation/proximity is defined as when the monkeys sit very close and parallel to one another, often with their tails thrown over their shoulders (Hoft et al., 1974). Fly catching is an indicator of hallucinations. During this behavior the animals appear to be grabbing flies with their hands; however, no flies are present. Self-grooming is when a monkey picks or scratches at his fur or a part of his body. Self-huddling may be an indication of anxiety. During self-huddling, the animal sits by itself with its tail thrown over its shoulder. Residual behavior is

defined as when the monkey is not doing any particular behavior, but is typically sitting and looking around. Play behavior consists of play fighting or wrestling play. Locomotor play includes running, jumping, and swinging (Biben 1998). Typically play behavior is only seen in juvenile monkeys; therefore, observance of play behavior in adult monkeys may indicate increased sociality. Swinging is defined as "hanging by feet or by clasping the end of the tail that is looped around a perch" (Hopf et al., 1974). Wrestling can be characterized by grasping and embracing another monkey, pushing, and mock-biting. Play behavior also includes chasing, which is seen when a monkey quickly follows another monkey around the cage. This portion of the experiment tests the hypothesis that after administration of MDMA, the time spent doing a prosocial behavior such as play or affiliation/proximity will increase over time.

To test the hypothesis that MDMA administration increases the amount of prosocial vocalizations, vocalizations were also recorded and scored. Squirrel monkeys produce a distinct set of calls that may be classified as prosocial, neutral, or aggressive/aversive. Analysis of vocalizations can provide insight into prosocial changes caused by MDMA administration. Additionally, there has not yet been a study that examined the effect of MDMA on prosocial communication in nonhuman primates. Monkeys were recorded for one-hour while in the lab setting during administration of MDMA. The Audacity program was used to segment the files into shorter .wav files. These files were imported into MATLAB, where spectrograms of the calls were generated. When scoring vocalizations, the frequency of how often the calls occurred rather than how long the calls lasted was of importance for this study. Calls include chucks, peeps, caws, growls, purrs, and twitters. Prosocial calls include pulsed calls such as twitters and chatter (Jürgens, 1979). Twitters appear to be the only calls associated with a pleasurable

emotional state, while chatter is typically heard in relation to feeding. In addition, chucks and purrs are also considered prosocial. Purrs may be emitted during a calm state of contentment. Neutral calls include peeps and isolation peeps, while aggressive calls are growls and caws. Growls may be heard when dominance gestures are exchanged while caws may be heard during food stealing, unwanted bodily touching or before and after fights.

Duration and Experimental Design

In order to evaluate the long-term therapeutic potential of MDMA, the monkeys were monitored in two situations: the lab and their homeroom. The homeroom is where the monkeys are housed. There are other squirrel monkeys housed in cages in the same room. In the lab, baseline behavior videos obtained during the control period were randomized with experimental videos obtained during MDMA administration in order to examine changes in behavior. The homeroom environment served as a model to evaluate the long-term effects of MDMA. As in the lab, baseline behavior in the homeroom was randomized with experimental behavior. The monkeys were given MDMA in the lab on Mondays and Thursdays, then videoed in the homeroom on Wednesdays and Fridays (Figure 3). This portion of the experiment tested the hypothesis that there would be an increase in residual prosocial behavior seen in the absence of drug while the monkeys were in their homeroom, and that multiple exposures would be required to observe significant changes in homeroom behavior.

Control Period

Due to the nature of the experimental design, subjects served as their own controls. To begin, the four monkeys were acclimated to the lab for four weeks (Sept. 24-Oct. 22). The cage from their homeroom was transported to the lab twice per week and remained there for three

hours between the hours of 9 am and 1 pm. During this time, they were videoed for the last hour of their time in the lab. The monkeys were videoed for an hour in the homeroom on subsequent days between 11 am and 12 pm. These videos provided baseline data for both environments. The baseline data in the homeroom was compared to the experimental data from the homeroom, while the baseline data in the lab was compared to the experimental data from the lab. However there was never a cross comparison between the lab and the homeroom as it would be an experimental confound. After the monkeys were fully acclimated, there was a saline control period from Oct. 23-Nov. 12. During this period, the same protocol outlined above was followed. However, during the last hour in the lab, saline injections were given in order to obtain behavior while allowing the monkeys to acclimate to the procedure. 0.35 mg 0.9% saline was administered to each monkey during every session in the lab.

Experimental Period

From Nov. 13 to Dec. 12, MDMA was administered no more frequently than twice per week with at least two days separating each day that MDMA was administered. During this period the animals were videoed in the lab (on-drug) and the homeroom (off-drug), but again, these situations were never directly compared. The monkeys were brought into the lab and left to acclimate to the environment for two hours. After two hours, 0.3 mg/kg of MDMA was administered via intramuscular injections alternating between the right and left upper thighs of the squirrel monkeys in order to minimize discomfort. After administration, the animals were videoed for one hour. The only dose of MDMA given was 0.3 mg/kg. This dose was selected because it is a relatively low dose and elicits the most amount of prosocial behavior as previously determined by studies in our lab (Elizabeth Pitts, unpublished data). The monkeys

were videoed in the homeroom on days following MDMA administration. The schedule was as follows: Monday-administration of MDMA, video in lab setting while monkeys are on-drug; Tuesday-no drug administration, Wednesday-no drug administration, video in homeroom; Thursday-administration of MDMA, video in lab setting; Friday-no drug administration, video in homeroom (Figure 3). In order to control for bias, both the baseline and experimental videos were blinded and randomized within the homeroom pool and within the lab pool. A blinded observer scored the videos using JWatcher 1.0 software. There was a video key created to differentiate between baseline and experimental data. Once all videos had been scored, they were unblinded. This experiment was focused on the long-term effects of MDMA on prosocial behavior, therefore the increase in prosocial behavior while the monkeys were off the drug in their homeroom may represent a translational match to what would be observed in a clinical setting.

Follow Up Studies

Interestingly, after analyzing the raw data gathered from the initial experiment it appeared that in the homeroom, the animals experienced an immediate off drug effect after a single exposure to MDMA (data not shown). The original hypothesis was that multiple exposures to MDMA would results in a *gradual* increase in off drug prosocial behavior. However, according to the current results, it appeared there was an immediate increase in prosocial behavior maintained across multiple administrations. The current experiment did not reveal whether multiple administrations were needed to maintain the prosocial behavior or if a single dose was sufficient. In order to test this, a single dose of 0.3 mg/kg of MDMA was administered to all four animals simultaneously. The animals were videoed in their homeroom

every other day to observe changes in prosocial behavior for two weeks. After this period, the videos were blinded and randomized in order to determine the effect of single dose administration. This study began on Feb. 9 and continued through Feb. 20. It was hypothesized that there would be a gradual decline in the off drug prosocial behavior, and that multiple administrations were needed in order to maintain this off drug effect. There appeared to be no trend of sensitization in the current data. However, perhaps a future experiment may be designed to analyze a sensitization effect.

Statistical Analysis

Group data in both the homeroom and the lab were analyzed in GraphPad Prism software. A one tailed paired t-test (Table 1) was used to analyze affiliation both in the homeroom and the lab, while a two tailed paired t-test was used to analyze the rest of the behaviors. It was originally hypothesized that there would be an *increase* in affiliation/proximity, which is why the t-test was one tailed. A one-way ANOVA was used to analyze the single dose data. The analysis is a one-way ANOVA because it is only comparing by day since there is only one drug condition, and no saline control.

To analyze vocalizations, R (R Core Team, 2012) and *lme4* (Bates, Maechler, & Bolker, 2012) were used to perform a linear mixed effects analysis of the relationship between affiliative calls and drug dosage. Time and dosage, 0 for saline and 0.3 for MDMA, as well as their interaction term were entered as fixed effects into the model. As a random effect, there was an intercept for testing day. P-values were obtained by using ImerTes REML t-tests with Satterthwaite approximations of degrees of freedom.

Results

Behavioral Results

In the homeroom while off-drug, there was a significant increase in affiliation/proximity ($p<0.05$), and a decrease in locomotion ($p<0.05$) (Figure 4). These data support the original hypothesis that MDMA administration would increase the amount of off-drug prosocial behavior. While there was not a significant difference in play behavior in the homeroom, a p-value of 0.09 suggests the behavior was trending toward significance (Table 1). In the lab, no significant differences were observed in any of the behaviors. There was a trending increase in affiliation/proximity ($p=0.07$), and a trending decrease in residual behavior ($p=0.08$). However, there was no significant change in any of the other behaviors (Figure 5), which does not support the original hypothesis. However, a p-value of 0.07 for affiliation indicates there was an increasing trend of affiliation/proximity. Additionally, a p-value of 0.07 was obtained for residual behavior, which indicates a trend toward decreased residual behavior when the animals received MDMA. In this group of monkeys, there is a social hierarchy. The most subordinate animal appeared to be a non-responder based on evaluations of individual subject data (Figure 6 and 7). This is important to note since his behavior could explain why behavior in the lab was not statistically significant, but rather was trending.

In the single dose experiment, changes in affiliation were not significant ($p=0.114$), locomotion was not significant ($p=0.06$), nor was self-huddling ($p=0.187$). However, there was a significant change in the amount of time spent self grooming ($p<0.05$, 0.042). This provides evidence that multiple administrations of MDMA may be needed in order to obtain a significant amount of off-drug prosocial behavior. When comparing data from this portion of the

experiment, it appears as though there was an increase followed by a decrease in prosocial behavior. However, this was observed when evaluating group data. It is possible that individual monkeys reacted differently, as seen by the non-responding animal.

Vocalization Results

There were no effects of time ($t=-1.375$, $p=0.18$), dose ($t=0.125$, $p=0.9$), or their interaction ($t=0.44$, $p=0.662$) on prosocial affiliative calls (Figure 8, 9, and 10). The analysis grouped purrs, twitters, and chucks into prosocial affiliative calls. There was not a significant increase in the amount of prosocial calls when saline and MDMA conditions were compared. Figure 8 represents the average total amount of affiliative calls over the course of an hour in both saline and MDMA conditions. Figure 9 represents the average frequency of affiliative calls over the course of an hour in both MDMA and saline conditions. Figure 10 illustrates the difference in the total amount of each type of call between saline and MDMA conditions. As represented, there was not a significant effect of time, dosage, or an interaction between the two variables.

Elizabeth Pitts, a graduate student in this lab, has completed similar behavioral and vocalization analysis with a completely different set of monkeys. Her animals are of the *Saimiri Boliviensis* (black cap) subspecies, while the animals used in this study are *Saimiri Sciureus* (green cap). The black cap and green cap monkeys are two different subspecies of squirrel monkeys. In comparing the data obtained in this experiment to Elizabeth Pitts data, a very obvious difference was observed (Figure 11A and 11B). This difference is likely explained by variability between species. For example, in evaluating prosocial behavior in the lab after giving the same dose of MDMA, the black cap animals exhibited a much more robust increase in

prosocial behavior. However, the green cap monkeys used in this study did not exhibit significant changes in prosocial behavior in the lab setting. This interspecies variation is significant in the comparison of results obtained from this experiment and her experiment. She also found a significant increase in prosocial vocalizations in the black cap group. The green cap animals used in this study did not exhibit the same robust increase that the black cap animals did. In comparing baseline behavior between the two subspecies, the green cap animals used in this study seem to have a much higher baseline of social interaction. Therefore, while there was an increase in prosocial behavior, it was not as robust of a change as seen in the black cap animals (Figure 11A and 11B).

Discussion

In this experiment, significant results were obtained showing an increase in prosocial behavior and a decrease in locomotion while off the drug in the homeroom. There was also a trend toward significance for increased play behavior while off the drug. This finding is important for understanding the long-term positive effects of MDMA. Since this experiment demonstrated an off drug prosocial effect, multiple exposures to MDMA may result in long term changes to prosocial behavior that are beneficial to patients experiencing social anxiety. This is a very important novel finding. To date, there have been no other studies that illustrate the off-drug effect our experiment has shown. Additionally, the original experimental data showed that the monkeys exhibited an off-drug prosocial effect after only one dose of MDMA (data not shown). There was no clear trend of sensitization across days. Therefore, the data was averaged across all days to represent changes in behavior between saline and MDMA

conditions. This is an important point because it indicates a single exposure to MDMA may result in an off-drug prosocial effect. Clinically, this means that one dose of a therapeutic equivalent may cause an immediate prosocial response. In analyzing data from the lab, no significant changes in prosocial behavior were seen. However, the trend of increased affiliation approached significance. Perhaps with a larger sample size or longer observation periods, significance can be obtained. Importantly, there was one animal that was a non-responder. Therefore, his behavior influenced the significance of the entire experiment. While on MDMA in the lab setting, the other three animals huddled together and expressed an obvious increase in prosocial behavior. In contrast, the non-responder was seen self-huddling and making many vocalizations, many of which were isolation peeps. This animal did not participate in prosocial behavior with the group while in the lab. If he attempted to huddle with the rest of the group, his behavior was countered by aggression from the other three animals. The other three animals have obvious changes in prosocial behavior. While in the homeroom, interestingly, the non-responding monkey seemed to follow the trend of increased prosocial behavior, whereas in the lab he did not. Perhaps this animal is more susceptible to the stress of being transferred from the homeroom to the lab and therefore MDMA does not have as great of an effect on his behavior. Additionally, since this study investigates the prosocial effects of MDMA, the behavior of the non-responding animal is interesting. His acute prosocial behavior is not increased, yet his residual off drug prosocial behavior is increased. Perhaps there must be some degree of sociality in order to respond to MDMA and experience the acute prosocial effects or perhaps if an animal is in a different yet familiar environment, the stress of the procedure hinders the acute prosocial effects in this animal. It is imperative to note that according to dominance

rankings, the non-responder monkey is one of the most subordinate animals. Due to his subordinate ranking, it is not all that surprising that he has the least amount of prosocial interaction. However, the hypothesis that MDMA would increase prosocial interaction included all animals. Perhaps a future study could look at single administration to this animal and examine changes in prosocial behavior with the rest of the group.

The results from this study did not indicate a significant change in prosocial vocalizations after administration of MDMA. Vocalizations and behavior were obtained in the lab setting and thus, the lack of significance in vocalizations parallels a lack of significance in behavioral changes. There was an increase in the average amount of twitters and purrs, both prosocial calls, while the animals were on MDMA, but this was not a significant change (Figure 10). Perhaps a higher dose of MDMA is needed in order to elicit a significant number of prosocial vocalizations. The lack of significance in prosocial vocalizations parallels the lack of significance in prosocial behaviors. The non-responding animal appeared to make numerous isolation peeps. The average number of peeps was much greater than the average number of other calls. Therefore, the high number of peeps could have had a minimizing effect on the number of other calls. If the number of peeps were similar to the number of other calls, perhaps a significant effect may have been obtained.

Additionally, the two most social animals in the group are from a wild colony, while the two least social animals were raised in captivity. This difference in animal history could be a significant variable that affected the results of our study. Interestingly, the two animals that are the most locomotive are from the captivity colony. As mentioned in the Methods, these animals have all had previous exposure to psychostimulant compounds and two of the animals

have had exposure to MDMA. However, they have not received MDMA since 2009. Animal 178 received the S(+) enantiomer of MDMA in 2009. The doses were 0.3 mg/kg, 1.0, and 1.7 and the exposure lasted two weeks. Animal 194 received 18 exposures to both the S(+) and R(-) enantiomers over a period of 3 months. It has been five years since previous experiments with MDMA. Additionally, three of the animals have been on a drug vacation since 2012. This was the last time they had exposure to a psychostimulant, which was in all three cases, cocaine. The fourth animal has been drug free since 2011, when he received cocaine. While most humans that use ecstasy are polydrug users, the target population that would need therapy to increase prosocial behavior may not have this extensive drug history. Therefore, the behavior in humans that haven't had exposure to drugs could be significantly different than humans that have used multiple drugs. It's possible that humans who have no previous exposure to MDMA or psychostimulant compounds could experience more robust effects after using MDMA in psychotherapy.

Future Studies

Since one animal was a non-responder, it would be interesting to administer MDMA to only this animal. Perhaps then, an increase in prosocial interaction with the rest of the group may be seen. It is possible that by increasing the prosocial behavior of this animal, an increase in prosocial interaction from the rest of the group, or more aggressive behavior from the other three animals may be observed. If the non-responder is made more prosocial and attempts to interact more with the dominant monkeys, they may become more aggressive in preventing this interaction since he is one of the most subordinate animals. However, this experimental set up may be more indicative of results in a clinical setting. The results from this experiment

indicated an increase in off-drug prosocial behavior. However, it is unknown how long this off-drug effect lasted once MDMA administration was complete. An additional experiment that is designed to understand how long it takes for prosocial behaviors to attenuate would add to the understanding of long-term effects of MDMA.

Additionally, in scoring vocalizations there were many long peeps. From anecdotal evidence, it appears that the more subordinate non-responder is making the majority of these peeps. The specific type of call he is making is known as an isolation peep. Since this animal was not able to huddle or interact with the rest of the group, it makes sense that he was making isolation peeps. In the future it would be interesting to examine individual vocalizations. With our current design it is indistinguishable which animal was making each specific call, however a future study that looks at the vocalizations by animal could be beneficial. Perhaps along with isolation peeps, this non-responding animal also exhibited an increase in prosocial vocalizations. It is important to note the disconnect between vocalizations and behavior. In the wild, male animals typically do not exhibit much prosocial interaction; therefore a modest increase in prosocial behavior does not necessarily mean that MDMA only had a modest effect. For this reason, vocalizations were also examined in this study.

Additionally, the 0.3 mg/kg dose was based off a pilot study completed using the black cap subspecies. Since the green cap subspecies have a different behavioral repertoire and vocalizations, a pilot study should be done with only the green cap species in order to determine the most effective dose of MDMA. There are three more green cap animals in the lab that are currently being introduced to social housing. These animals will be used to examine the same variables in this study: changes in prosocial behavior and prosocial vocalization after

MDMA administration. Since the green cap animals used in this experiment differed markedly from the black cap animals, repeating this experiment with another set of green cap animals will be beneficial to understanding the interspecies variation.

After an experiment that examines individual changes in behavior, administration of a 5-HT_{1A} agonist (8-OH-DPAT) could be beneficial to this study. Previous studies have illustrated a role for the 5- HT_{1A} receptor (Thompson et al., 2007); therefore, the purpose of this study would be to determine if prosocial behavior is increased in the same manner as MDMA. There have been inconsistent findings as to whether the prosocial behavior caused by MDMA is due to changes in 5-HT or oxytocin levels. It would also be interesting to analyze compare the prosocial effects caused by a 5- HT_{1A} agonist and oxytocin. These experiments could provide a greater understanding of the neurobiological mechanism by which MDMA causes increases in prosocial behavior.

Limitations:

The control period for habituation was approximately one month. However, due to time constraints within the semester the saline control period could only last approximately two weeks in order to collect one month of MDMA data. Ideally, each period of the experiment would last the same length of time in order to obtain the same amount of data points for each period. Additionally, some videos in the homeroom had to be taken out of the data set. Upon review, animal care workers entered the room, and this significantly changed the behavior of the animals. This variance caused spikes in behavior during the control period, and was taken out of the data set. A paired t-test was used for the statistical analysis of the data obtained from this experiment. However, a repeated-measures ANOVA would have been a useful

analysis, as it would have accounted for the variability by day, which a paired t-test did not account for. Due to technical problems with the video cameras and the timeline of the project, the number of days that the monkeys received saline was not the same as the number of days the monkeys received MDMA. To do a proper repeated-measures ANOVA, these two conditions must have equal data points. While a paired t-test is a completely valid analysis, a repeated-measures ANOVA would have provided more information about the variability by day across all monkeys used in the study.

Lastly, two animals in this study have had previous exposure to MDMA. While the last exposure to MDMA was over five years ago, it is possible that this previous exposure affected their current behavior. In the future, this experiment should be repeated with another set of green cap animals as well as another set of black cap animals. Perhaps by obtaining more data from both groups of animals, more conclusive data can be obtained.

Conclusion:

Ultimately this study exhibited a significant increase in the amount of off-drug prosocial behavior in squirrel monkeys. In the lab, there was not an observable significant change in prosocial behavior after administration of MDMA, nor was there a significant increase in the amount of prosocial affiliative vocalizations. The novel discovery of off-drug prosocial behavior contributes to the understanding of the behavioral effect of MDMA and supports the original hypothesis that there would be an increase in off-drug prosocial behavior following MDMA administration.

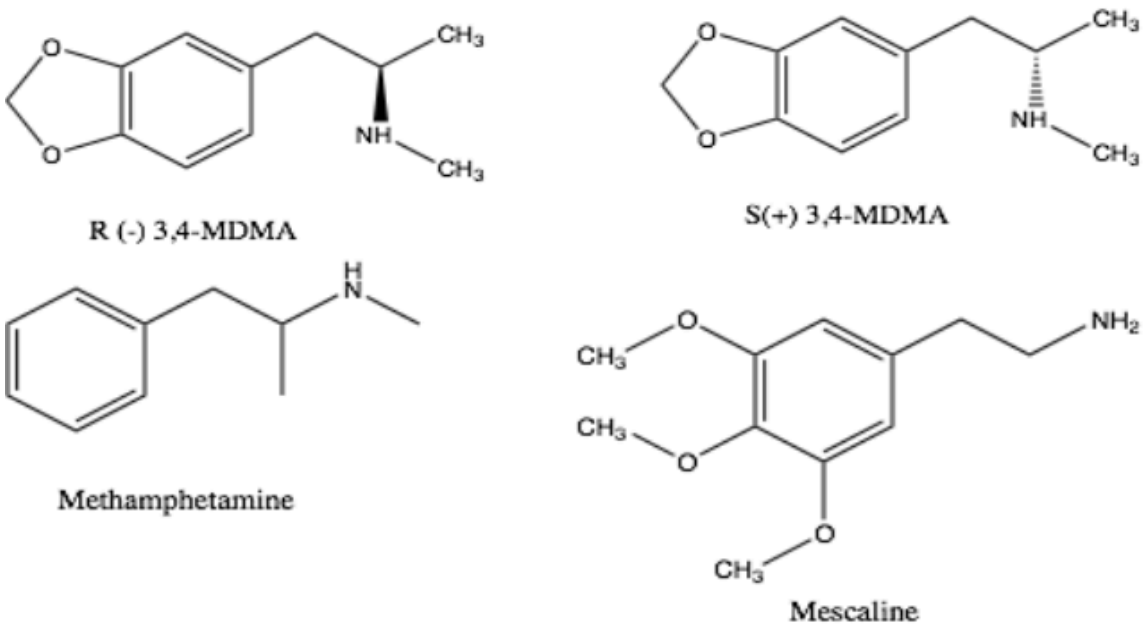

Figure 1- A comparison of MDMA, methamphetamine, and mescaline molecules. The top left molecule exhibits the structure of the R (-) enantiomer while the top right illustrates the S (+) enantiomer. The bottom structure of methamphetamine demonstrates the similarity between MDMA and amphetamine molecules, which account for the psychomotor stimulant behaviors induced by MDMA.

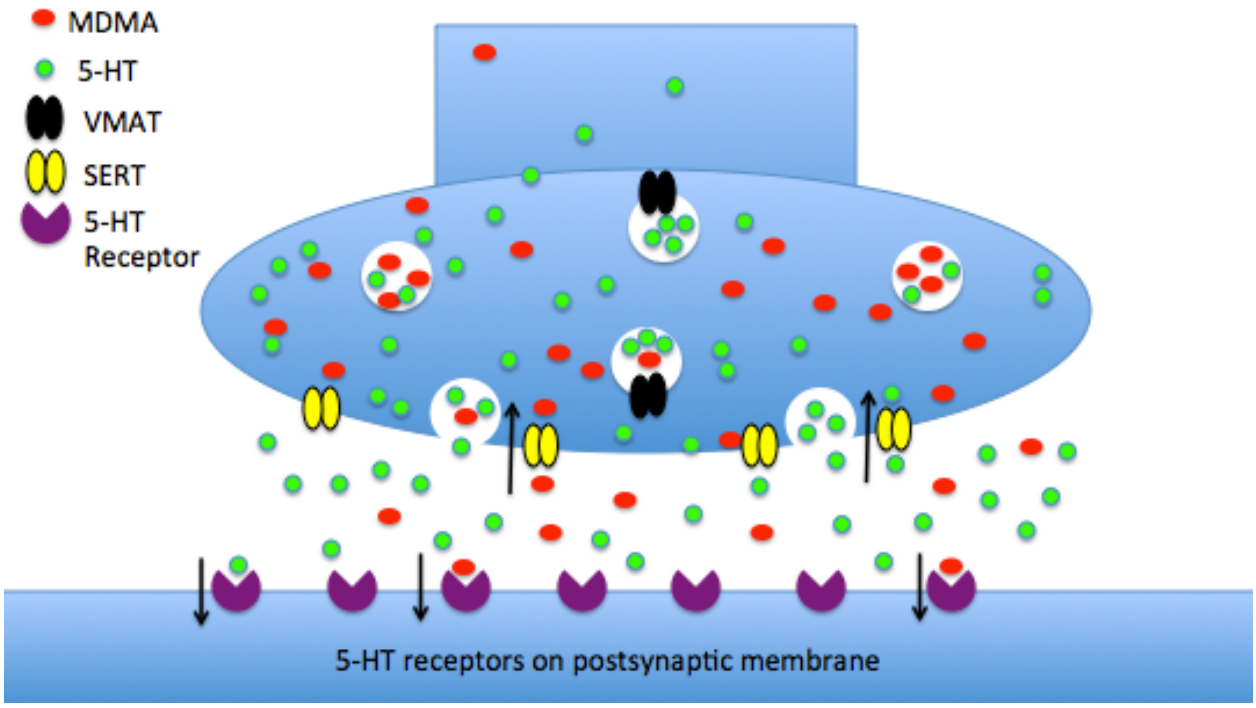

Figure 2-The figure above illustrates the mechanism of action by which MDMA causes an increase in the amount of synaptic serotonin. Once MDMA enters the synaptic cleft, it is taken back up by the serotonin transporter (SERT), and reverses the transport of serotonin. MDMA also occupies the vesicular monoamine transporter (VMAT) and results in an increase of the release of intracellular serotonin.

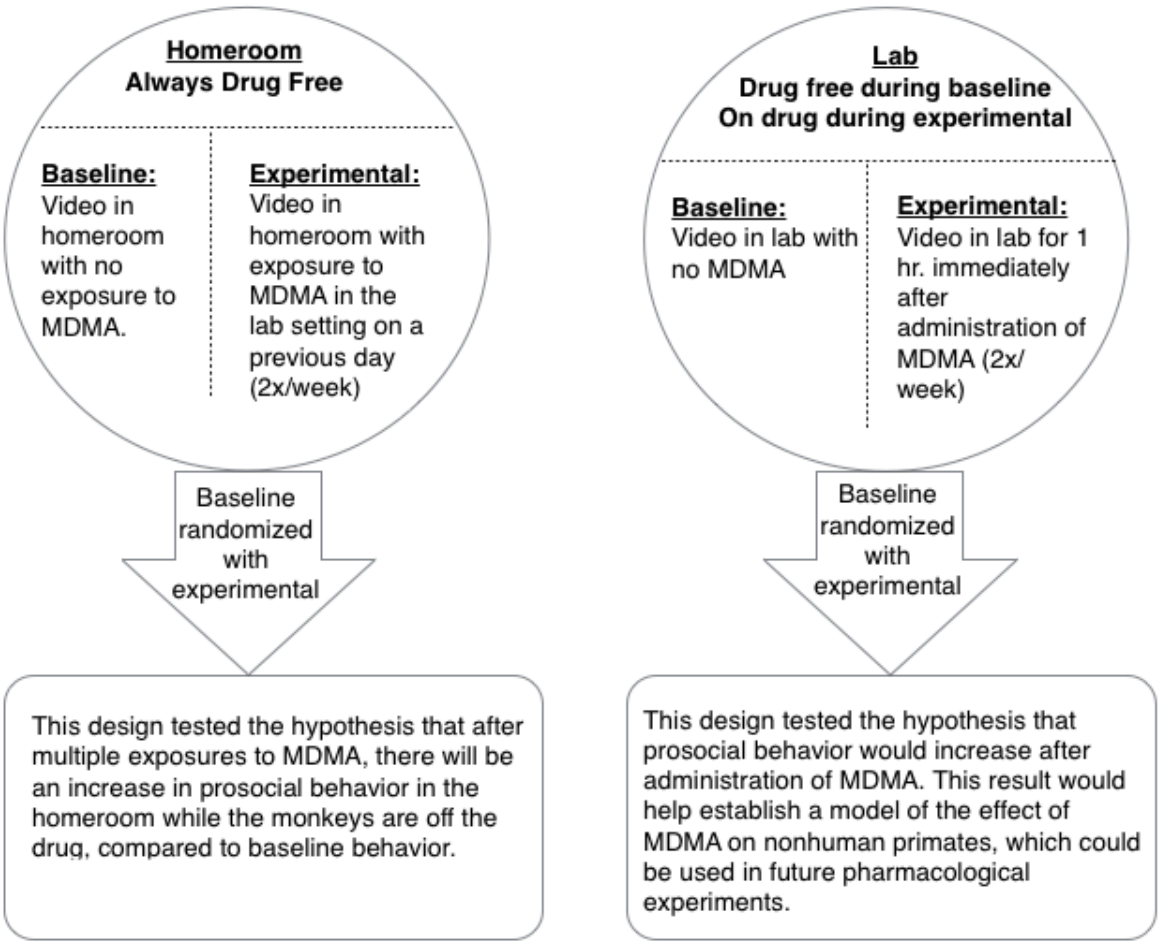

Figure 3-The figure above explains the experimental design in both the homeroom and the lab setting.

Behavior	Homeroom (p-value)	Lab (p-value)
Affiliation/Proximity	p=0.0272, t=3.074, df=3 one-tailed	p>0.05, trending, p=0.0718 one-tailed
Locomotion	p=0.0036, t=8.372, df=3	p>0.05
Self-Grooming	p>0.05	p>0.05
Self-Huddling	p>0.05	p>0.05
Play Behavior	p>0.05, trending, p=0.0942	--
Locomotion	p>0.05	p>0.05, trending, p=0.0785

Table 1-The table above is a collection of the descriptive statistics from the behavioral analysis. Affiliation/proximity was analyzed using a one-tailed paired t-test, while the remaining behaviors were analyzed using a two-tailed paired t-test. There was no play behavior in the lab during saline and MDMA administration, therefore no p-value was reported.

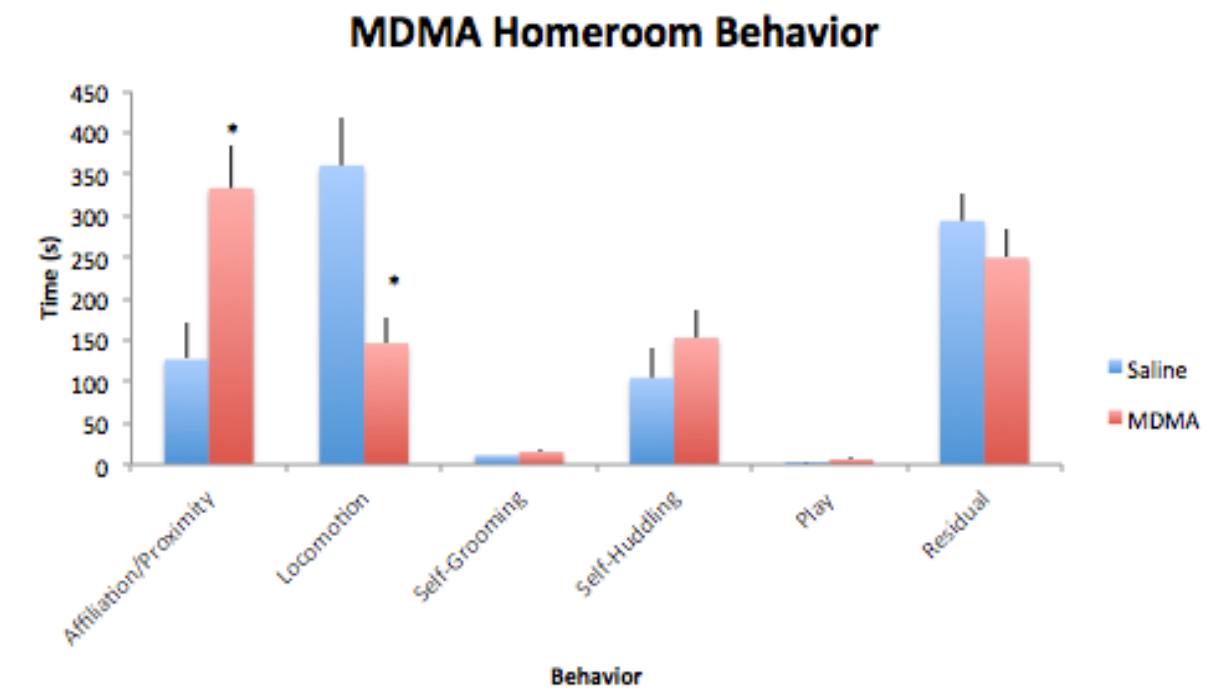

Figure 4-The figure above illustrates average across all monkeys and all days under each condition in the homeroom. The average amount of time per behavior was averaged across all days under both saline and MDMA conditions. Then these averages were averaged across monkeys to create the mean of means. The mean of means for saline are represented in blue, while the mean of means for MDMA are represented in red. The error bars represent the standard error of the mean. The amount of time spent in affiliation was significantly greater when the monkeys received MDMA (one-tailed paired t-test, $p<0.05$), and the amount of time spent in locomotion was significantly less after MDMA administration (two-tailed paired t-test $p<0.05$). There was also a trend toward increased play behavior ($p>0.05$, $p=0.0942$). The other behaviors were not significantly different between the two conditions.

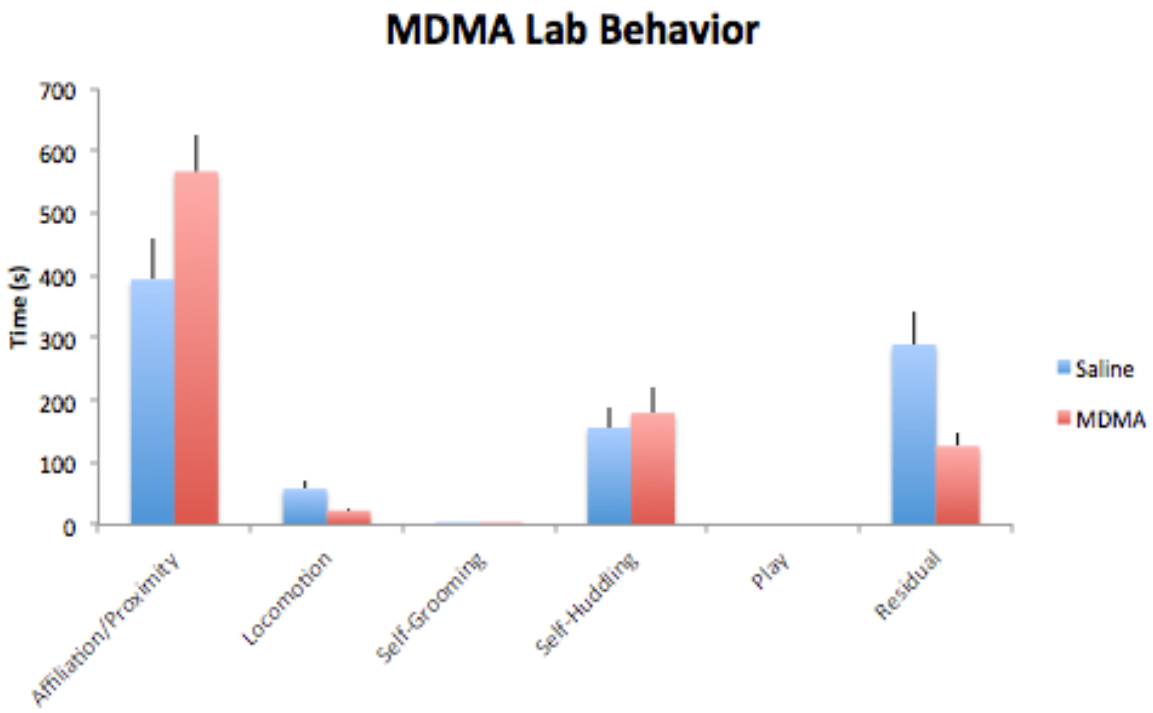

Figure 5-The figure above represents the average of all behaviors across all days and all monkeys in the lab condition. There was not a significant difference in the amount of affiliation when saline and MDMA conditions were compared (one-tailed paired t-test, $p>0.05$, $p=0.0718$). However, there was a trend toward increased affiliation. The amount of time spent in locomotion, self-grooming, and self-huddling was not significantly different between the two conditions ($p>0.05$). There was a trend toward decreased residual behavior, but significance was not obtained ($p>0.05$, $p=0.0785$). The error bars represent the standard error of the mean.

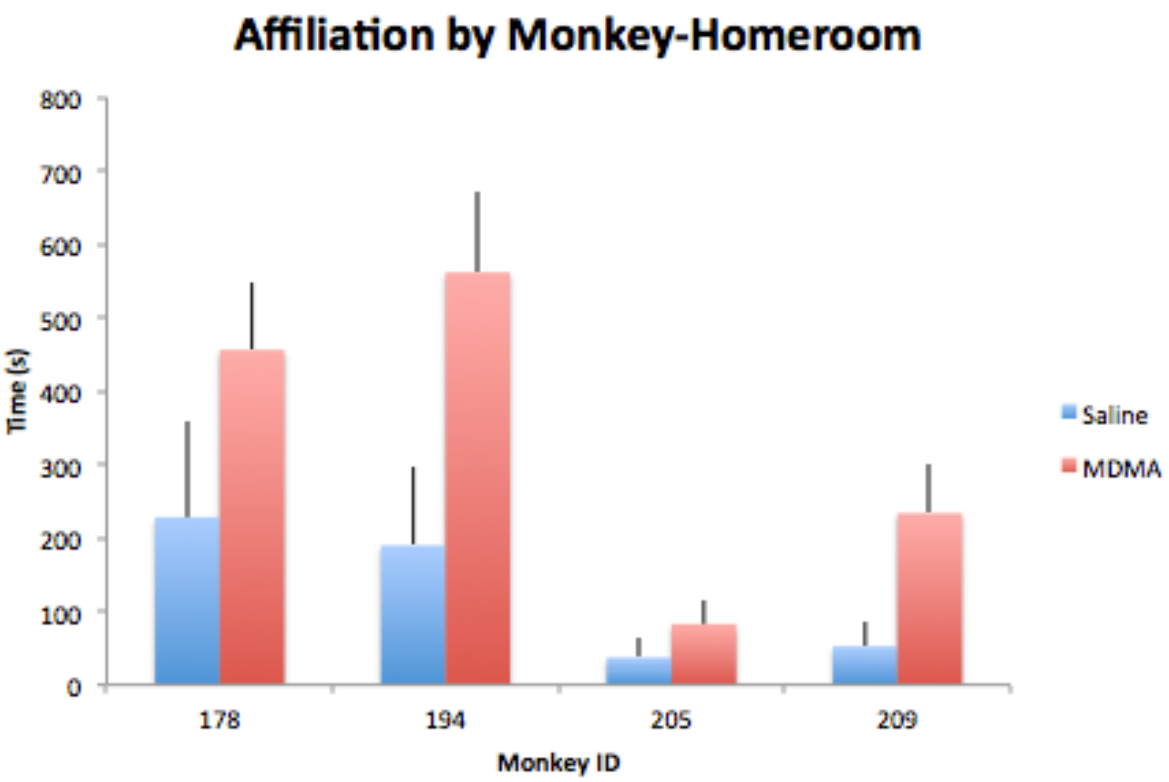

Figure 6-The figure above represents the amount of time spent in affiliation averaged across all days in each condition by monkey. The error bars represent the standard error of the mean. This graph represents the variability between the four monkeys used in the study. As represented by the graph, 205 spent much less time in affiliation as compared to the other three animals.

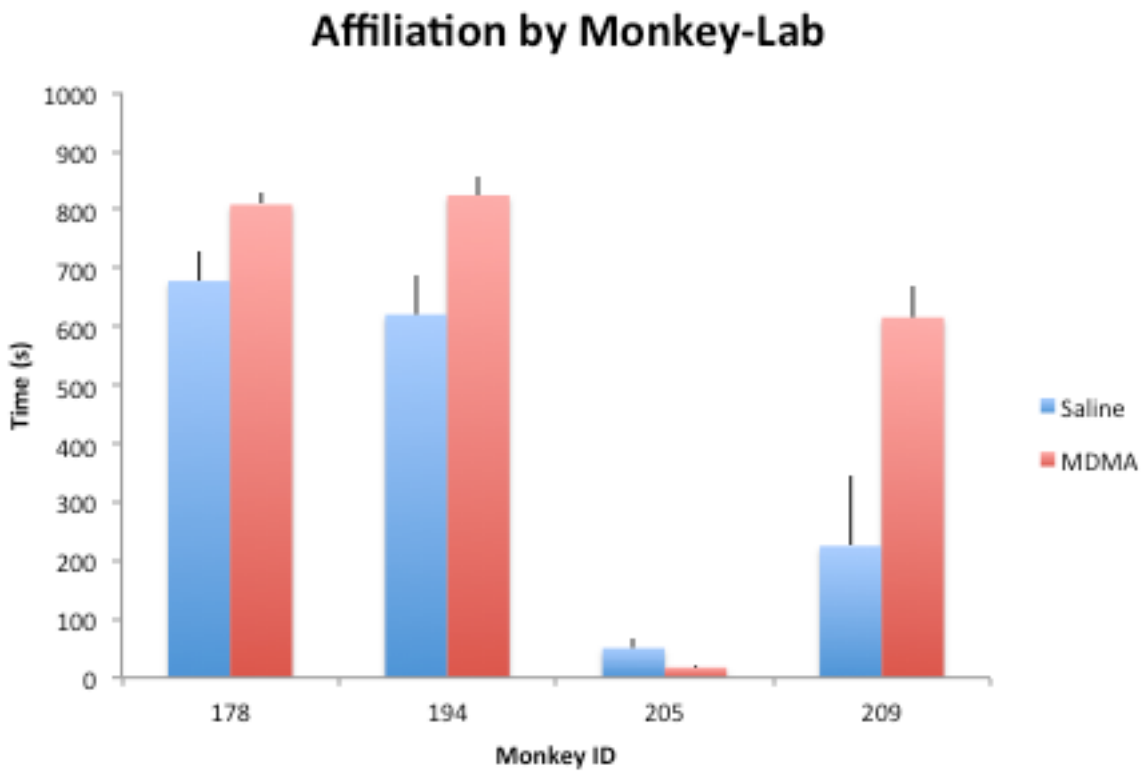

Figure 7-The figure above illustrates the amount of average affiliation across the two conditions by monkey. This graph represents the variability between the four monkeys used in the study. As seen above, monkey 205 exhibited much less affiliation compared to the other three animals. This behavior likely brings down the significance of the rest of the group. Additionally, this graph illustrates the high amount of baseline prosocial behavior observed in these animals. There was not a significant difference in the amount of time spent in affiliation after comparing saline and MDMA conditions (one-tailed paired t-test $p>0.05$, $p=0.0718$).

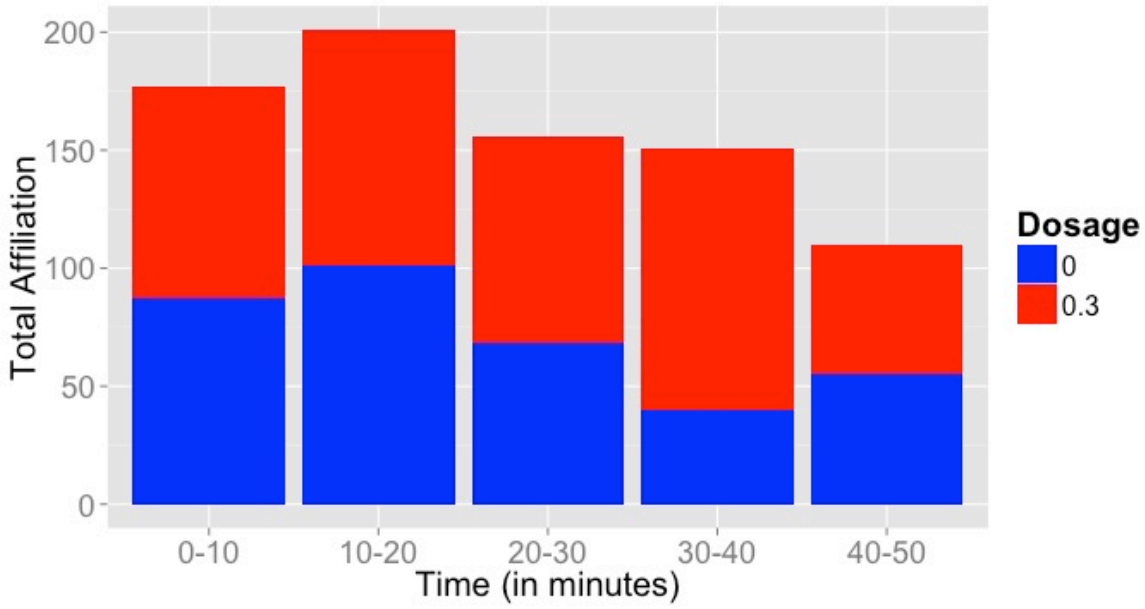

Figure 8-The figure above represents the average of the amount of total affiliative prosocial calls over the course of an hour in both saline and MDMA conditions. The average of all affiliative calls in all saline days was compared to the amount of all affiliative calls in MDMA days. There was not a significant effect of time or dosage on the amount of affiliative prosocial calls.

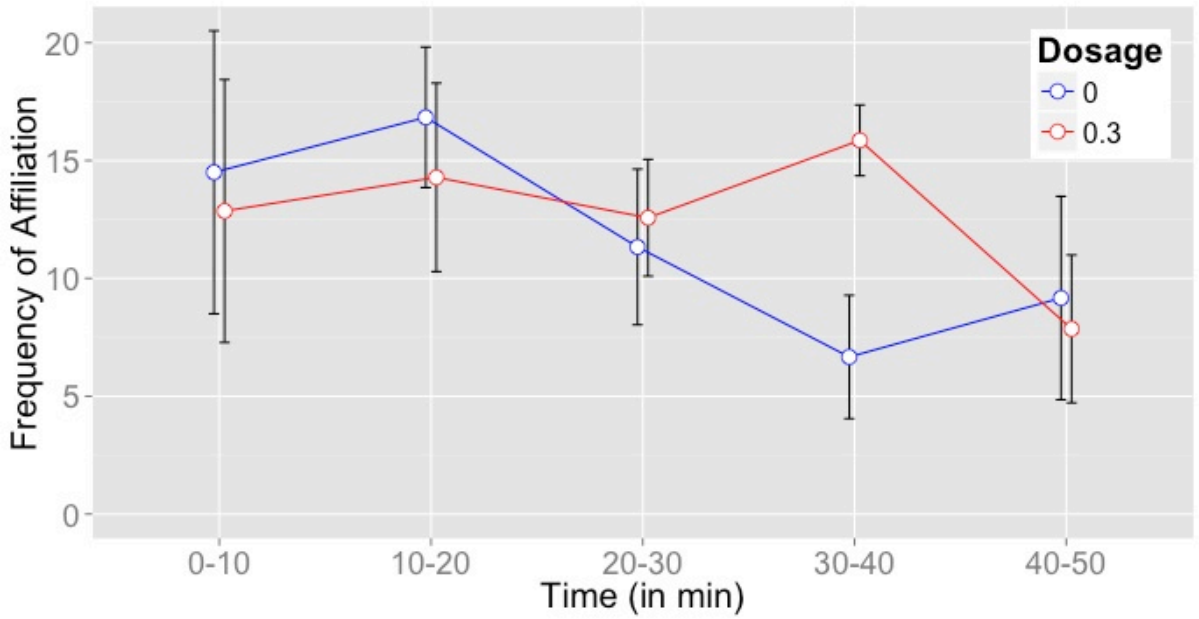

Figure 9-The figure above represents the average frequency of affiliative calls across the hour in both saline and MDMA conditions. There was not a significant effect of time, dosage, or their interaction on the amount of increased prosocial affiliative calls.

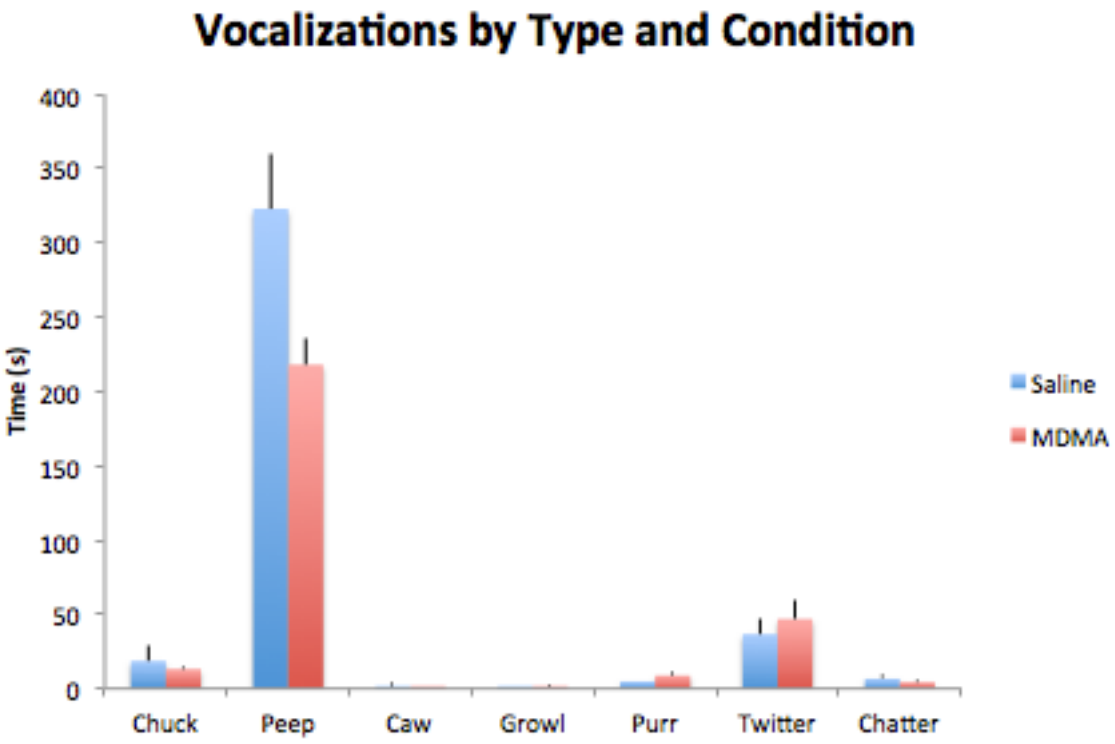

Figure 10-The figure above represents all types of vocalizations observed during the experiment. As seen in the figure, there was a decrease in chucks, peeps, and chatters, as well as a slight increase in purrs and twitters. However, there was not a significant effect of dosage ($p=0.90$, $t=0.125$) on prosocial calls.

Figure 11A

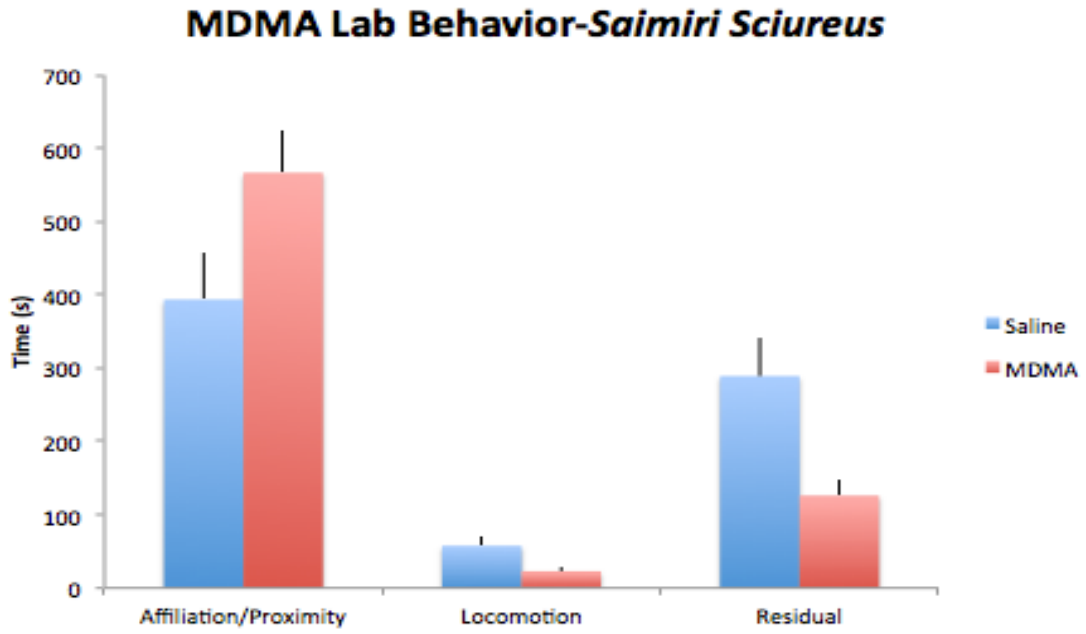

Figure 11B

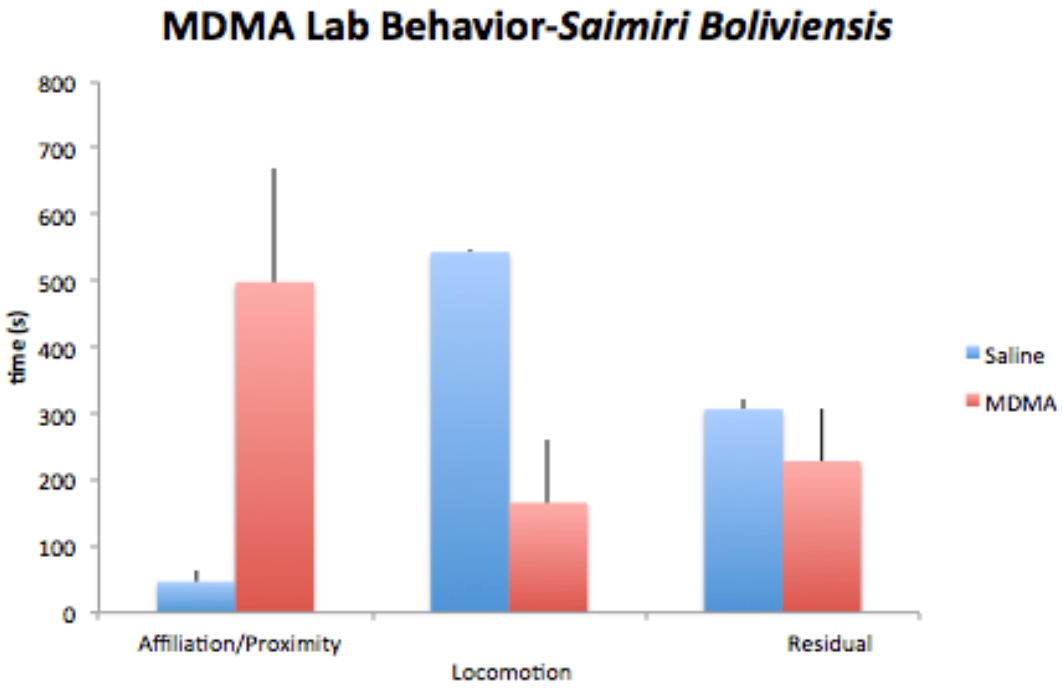

Figure 11-The figures above are a comparison of data obtained by the graduate student Elizabeth Pitts and data obtained in this experiment. The green cap species, *Saimiri Sciureus* is represented in figure 11A. The black cap species, *Saimiri Boliviensis* is represented in figure 11B. As illustrated in these graphs, the animals used in this study and Elizabeth Pitts' study exhibited

very different changes in prosocial behavior. The green cap species exhibited an increase in affiliation/proximity and a decrease in locomotion, but the black cap species exhibited a much more robust increase in prosocial behavior and a decrease in locomotion.

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