Effects of Pharmacological Manipulations on Natural Social Interaction in Rhesus Macaques: A Pilot Investigation

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Abstract

The neuropeptide oxytocin has been shown to have prosocial effects in both monkeys and humans, leading to its proposed use as a treatment for social disorders in humans such as Autism Spectrum Disorder (ASD). The opioid system interacts with the oxytocinergic system in an antagonistic manner, so that inactivation of the opioid system causes an increase in oxytocin levels. In this experiment, we administered either oxytocin alone, the opioid antagonist naloxone alone, the combination of the two, or a saline control to two non-human primate subjects (one female, one male) to examine the effects on natural social behaviors of rhesus macaques. We recorded the behavior of the subject monkey in the cage for 90 minutes and used a computer scoring software to mark specific behavior types. We found that oxytocin increased huddling and prosocial behavior in the male subject and increased the mean duration of grooming given in the female subject. Naloxone was found to increase prosocial behavior in the male subject and decrease antisocial behavior in the female subject. The combination of oxytocin and naloxone slightly increased prosocial behavior in the female subject but showed no clear effect in the male subject. Further studies with a larger sample size are needed to provide helpful insight into how to more effectively translate lab-based results from controlled studies into natural behavioral settings.

Introduction

Social interactions and exchanges are central to our daily lives. They occur in just about every social environment, which encompasses locations such as the workplace, school, and home. Humans are naturally social beings and the countless interactions that occur with others are essential to one's survival and well-being. For some individuals, however, social deficits impede their ability to communicate and interact with others effectively. Social deficits underlie a wide variety of psychological and neurodevelopmental disorders. One such example is Autism Spectrum Disorder, or ASD. This disorder causes affected individuals to exhibit difficulties in social communication and to engage in repetitive, restricted behaviors. Individuals with ASD pay less attention to social cues compared to individuals without this deficit. For example, researchers have found that there is diminished gaze fixation, or less time looking at the eyes of another, among those affected with ASD (Dalton et al., 2005). The eyes are the most socially informative part of the face, and less time spent looking at the eyes indicates decreased social competency. Additionally, individuals with ASD experience a heightened emotional response, with correlates in activity of the amygdala, associated with gaze fixation (Dalton et al., 2005). This reinforces the notion that recognizing social cues is an impaired and challenging behavior for these individuals. Researchers have been interested in various pharmacological interventions that can potentially alleviate the effects of ASD and provide therapeutic benefits to affected individuals. In recent years, one substance that has become a prominent subject of investigation for these researchers is oxytocin.

Oxytocin is a neuropeptide that has been shown to promote social behavior both in humans and in monkeys (Campbell, 2010; Chang, Barter, Ebitz, Watson, & Platt, 2012). Oxytocin is produced in the hypothalamus and released by the pituitary gland (Colaianni et al.,

2011). It is involved in a wide variety of functions ranging from sexual reproduction to social bonding (Gimpl & Fahrenholz, 2001). Both human and non-human primate experiments have been conducted examining the effects of increasing the amount of oxytocin present in the brain. A common route of administration for these experiments is intranasal inhalation of the neuropeptide. Inhaled oxytocin can bypass the blood brain barrier and be transported to the brain (Chang & Platt, 2014; Chang, Barter, Ebitz, Watson, & Platt, 2012). This was initially shown by taking cerebro-spinal fluid draws in monkeys and measuring the oxytocin levels after intranasal exposure. This shows that inhalation of oxytocin is a dependable method to increase oxytocin levels in the brain and can therefore be used to study the effects of this neuropeptide.

Human studies have indicated that intranasal oxytocin (spray form) enhances a multitude of social behaviors. One of its effects include increasing trusting behavior towards others (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005; Theodoridou, Rowe, Penton-Voak, & Rogers, 2009). In the Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr (2005) study, participants played a trust game involving the transfer of money. Oxytocin increased trust in treated participants in this social game. Theodoridou et al. (2009) showed that oxytocin increased ratings of trustworthiness and attractiveness of male and female targets in raters of both sexes, suggesting that higher levels of oxytocin may enhance affiliative behavior towards unfamiliar people. Oxytocin has also been shown to have the effect of maintaining trust levels in others even after treated individuals learn that their trust had been breached several times (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008). Controls receiving the placebo in this study decreased their trust after learning it had been violated. Trust, which is a key component of positive social interactions, is greatly enhanced by increased levels of oxytocin in the brain.

In addition, oxytocin promotes cooperation within group settings (Dreu et al., 2010). Cooperation was quantified in the Dreu et al. (2010) study by a participant's behavior in prisoner's dilemma-type games, in which treatment of oxytocin increased helpful action. Additionally, studies have found that this drug increases the number of fixations and total gaze time toward the eye region, as compared to untreated controls (Guastella, Mitchell, & Dadds, 2008). This may be one mechanism by which oxytocin enhances emotion recognition and social approach in humans.

One can appreciate from the above findings the potential oxytocin has as a possible treatment of disorders characterized by social processing deficits such as ASD. In the previously discussed literature, the research subjects were normally-functioning individuals who did not have a social disorder. What was found that compared to controls not exposed to oxytocin, oxytocin-treated participants exhibited increased social behavior. In addition to testing the effects of oxytocin on normally-functioning individuals, studies have been conducted testing this drug's effects on those affected by social deficits such as ASD. In one such study, participants played a simulated ball game on a computer in which they interacted with fictitious partners. After oxytocin was inhaled, stronger interactions with the most socially cooperative partner were exhibited and higher feelings of trust and preference were reported (Andari et al., 2010). In the same study, a free-viewing task involving looking at images on a screen and following the eye's focus with an eye-tracker found that those treated with oxytocin spent more time gazing at the eyes, a socially informative region of the face. This replication of the previous finding of increasing eye-gaze time in normally-functioning individuals implies that the effects found in such studies may also carry over to those affected by ASD and other social deficits. Another study found that intranasal oxytocin improves emotion recognition for youth with ASD

(Guastella et al., 2010). In this study, males with Autism or Asperger's Disorder between 12 and 19 years old who received oxytocin nasal spray improved emotion recognition as measured by the Reading the Mind in the Eyes Task. This evidence supports the notion that the prosocial effects of oxytocin seen in normally-functioning individuals is also seen in those with ASD.

Aside from human studies, oxytocin research using monkey subjects have also shed light on the socially-enhancing abilities of this neuropeptide. As is the case in humans, oxytocin increases attention to the eye region in rhesus macaques (Dal Monte, Noble, Costa, & Averbeck, 2014). In a free-viewing task with images of monkeys exhibiting three different facial expressions, eye-tracking revealed increased gaze fixations to the eye region relative to the mouth region. This once again shows a more socially informative approach to observing another animal after treatment with oxytocin. Furthermore, oxytocin treatment increases affiliative behavior and interactions with caregivers among monkeys (Chang, Barter, Ebitz, Watson, & Platt, 2012; Simpson et al., 2014). What is important to note about these studies are that the effects of oxytocin were measured in highly controlled lab settings, leaving uncertain how the peptide is influencing naturalistic behaviors.

Monkeys make for an ideal model organism to study the effects of oxytocin and its potential as a social disorder treatment in humans. Specifically, monkeys share more than 90% of their genes with humans, making them very closely related to us. Research using lab animals, including monkeys, is behind countless medical treatments in existence. The knowledge we can gain from primate research in this field is essential for the advancement of treatment for humans with ASD and other social disorders. For many of these experiments, chronic studies have the ability to provide valuable information regarding the long-term effects of oxytocin treatment. In human studies, these types of longitudinal drug studies are not typically possible. Therefore, by

using the model organism of monkeys, we can gain insight into long-term effects of this type of treatment due to the similarity of neural mechanisms between these animals and humans.

The story of oxytocin may not be as one-sided as it seems from the evidence provided above, however. Although this neuropeptide has been shown as a socially enhancing substance, studies have also found that oxytocin may also trigger non-social or antagonistic behaviors. For example, one study found that oxytocin increased envy and gloating during an experiment that involved unequal monetary gains and losses (Shamay-Tsoory et al, 2009). This shows that the oxytocinergic system may be involved in modulating selfish emotions and actions such as envy and gloating and is not just associated with prosocial behaviors. Oxytocin may also play a role in intergroup conflict and violence. De Dreu et al. found that oxytocin administration increases in-group favoritism as well as out-group derogation (Dreu, Greer, Kleef, Shalvi, & Handgraaf, 2011). The effect of escalation of out-group derogation was not as great as that of in-group favoritism, but the effect was still significant. These findings point to a more general conclusion that can be made about oxytocin. The social effects of this drug in humans depend largely on the situation and the individual (Bartz, Zaki, Bolger, & Ochsner, 2011). The behavioral and emotional effects that an individual experiences as a result of oxytocin are not universal and are heavily influenced by what that person is currently engaged in and the unique character of that person. One study demonstrated how individual qualities may affect the results of oxytocin administration by examining memories of maternal care and closeness. Individual differences in anxiety levels affected the role of oxytocin. These experimenters found that less anxiously attached individuals remembered their mother as more caring and close after oxytocin administration while more anxiously attached individuals remembered their mother as less caring and close after oxytocin administration (Bartz et al., 2010). In addition to individual qualities

affecting oxytocin's influence, the effects of oxytocin between genders have also been shown to vary. In one study investigating prairie voles, researchers found that after a brief exposure to a male, females treated with oxytocin became more aggressive and less social than control females (Bales & Carter, 2003). This alteration in behavior was not observed in oxytocin-treated male prairie voles.

These findings point to the variety of effects oxytocin can have and how situation and personal characteristics may alter these effects. The somewhat contradictory findings about oxytocin's role in social behavior reveal its complexity in effect. Still it is apparent that this neuropeptide plays a key role in social behavior, which is why it is heavily studied in a social context. Its precise and specific role is still being uncovered and is part of what was examined in the present study. It is important to acknowledge, however, that the oxytocin system is not the only factor in regulating social behavior.

Researchers also have reason to believe that the opioid system plays a role in social behavior. The opioid system is most known for its role in pain relief and addiction, but it has also been connected to social behavior and cognition. It is thought that individuals with ASD exhibit excess levels of circulating opioids (Panksepp, 1979). These high opioid levels may contribute to the social deficiencies associated with ASD and may explain why these individuals lack interest in social communication and interaction. As a result, scientists have introduced opioid antagonists into the mix of possible treatments for social deficits such as ASD. One such opioid antagonist is naloxone. Naloxone is a competitive antagonist that binds most selectively to mu-opioid receptors with a high affinity. This effectively blocks the receptor, preventing the body from responding to opioids and endorphins at these receptors. As such, one of its common uses is to treat or prevent opioid abuse in humans and for reversing opioid overdose. Naloxone is potent

and long-lasting with minimal side effects, making it an appropriate opioid antagonist for this research. In humans, naloxone has been shown to reduce symptoms of ASD such as stereotypies as well as promoting social behaviors by increasing eye contact, social play, communication, and attention (Leboyer et al., 1992). Another study found that naloxone treatment in healthy adults increased attention to emotional expressions (Wardle, Bershad, & de Wit, 2016). These results reveal the potential role of naloxone in increasing social behavior.

Researchers have also examined the effects of naloxone administration in monkeys. One study found that naloxone increases grooming and grooming solicitations in these animals (Fabre-Nys, Meller, & Keverne, 1982). Additionally, another experiment revealed that naloxone increased social contact of monkeys with their caregivers (Martel, Nevison, Simpson, & Keverne, 1995). These findings suggest that naloxone may cause subjects to seek further affiliative comfort and as a result increase their social behavior.

The positive social effects of naloxone make it a suitable candidate in the treatment of social disorders. As oxytocin has also been proven to at times enhance social behaviors, one could imagine that administration of these two drugs may have even more powerful results than either on its own. Besides both having positive effects on social behavior, the oxytocin system and opioid system have been shown to interact with each other. The opioid system has been shown to have a regulatory relationship with the oxytocin system. High opioid activity inhibits oxytocin release from the pituitary gland (Bicknell & Leng, 1982). On the other hand, introducing an opioid antagonist into the system strongly increases oxytocin levels. Therefore, one could predict that introducing oxytocin as well as naloxone would more effectively enhance the levels of oxytocin than by administering oxytocin by itself.

The current study explores the idea of using a combination treatment of oxytocin and naloxone together in rhesus macaques. The reasoning is that by using the opioid antagonist naloxone, we can reduce opioid processing and can endogenously increase oxytocin release coupled with the exogenously increased level of oxytocin during natural social interaction between rhesus macaques. The central question we were trying to answer was if intranasal drug administration of naloxone and oxytocin together significantly improves prosocial behavior in rhesus macaques as compared to administering naloxone or oxytocin alone. There are several possible outcomes when combining drugs. The combined drug may have a greater effect than either individual effect. On the other hand, the drugs can have an eliminative effect in which one cancels out or eliminates the effect of the other so there is no net biological effect. We predicted that a combined naloxone and oxytocin treatment would show stronger changes in social behaviors in that the social interaction would be enhanced in this treatment more so than the effects that either drug on its own would show. This would improve the effect of oxytocin in that its levels would be further increased. Although both naloxone and oxytocin have been studied in rhesus macaques in previous studies, these have all been in very controlled laboratory settings. This pilot experiment with one male and one female monkeys, on the other hand, examined non-human primate behavior in-cage and not in a controlled situation. This novel test aimed to determine the effect of oxytocin, naloxone, and their combination on naturalistic and ecological behavior.

Materials and Methods

Subjects

The two monkeys tested in this experiment were a 5-year-old male (Jodorowsky) and a 10-year-old female (Campion) rhesus macaque (*Macaca mulatta*) (N=2). The monkeys reside in

a colony room in cage pairings with varying numbers of other monkeys of the same sex. The cage unit is raised off the ground and includes removable floors and dividers. Prior to video recording, the subject monkey and one other cage partner were enclosed in two top units of the cage so all their behaviors would be captured by the video camera. The cage partner was kept consistent throughout the entire experiment. Once recording was complete, the monkeys were given access to all units and both floors contained within their cage unit. Monkeys exhibit social hierarchies in their living groups and the subject monkey was the subordinate monkey in the dominance hierarchy.

Conditions

The various drugs were administered to the monkeys intranasally through a nebulizer. The monkeys had received prior training using a nebulizer and were familiar with this drug administration method. The four drug conditions tested were saline only, naloxone only, oxytocin only, and combined naloxone and oxytocin. Each monkey participated in 16 testing days, with each condition being tested 4 times in a randomized order, making a total of 32 testing days between the two monkeys tested.

Drug Preparation and Administration

Before beginning experimental trials, the drugs were prepared and placed into vials. The drug dosages were 24 IU of oxytocin, 1 mg of naloxone, and a combination of 1 mg of naloxone and 24 IU of oxytocin. The prepared vials were labeled and stored in a freezer and were only removed just before being used in testing. A dose of 2.5 ml of a saline stock solution, measured with a syringe, was used as the control condition. A randomized order of drug administration was created before testing to ensure that a drug condition was never repeated the following day.

The drug conditions were the independent variables and the observations of the monkeys' behavior from the videos served as the dependent variable.

On each testing day just before drug administration, the drug vial was removed from the freezer. If that day was a control day, the syringe was prepared with the saline solution. Vials from the freezer were given time to defrost completely. Next, the nebulizer was assembled. This nebulizer was equipped with a mask that covered the monkey's mouth and nose when held up to the monkey's face. This mask was held in place during drug administration to ensure that the monkey received proper drug inhalation. The drug was poured into the capsule of the nebulizer and the nebulizer was switched on. The start time of drug administration was recorded in the monkey data chart. The mask was held up to the monkey's face and the drug was administered for 10 minutes. After this time, the mask was removed and the end time was recorded in the data chart.

Video Recording

Following drug administration, the monkey was transported back to the colony room. The monkey was placed back into its cage and the video recording was started. The monkey was re-paired with its cage mate. The video camera was positioned on a tripod and placed to the side of the cage unit in a location that would not be disturbed by the monkeys. The location of the camera remained constant throughout the testing of each respective monkey. Each recording session lasted for 1 hour and 30 minutes. We chose to record for this length of time because most behavioral studies of oxytocin test 30-60 minutes or more after administration (Churchland & Winkielman, 2012). Video recording start and stop times were recorded in the monkey data chart. After recording, the camera was removed and the video was uploaded to the lab Mac desktop computer, where they could be watched and scored.

Ethogram and Tinbergen Software

Before starting experimental trials, an ethogram was designed that identified and assigned code to different behavior categories. The three categories of behaviors analyzed were prosocial (affiliative), antisocial (aggressive), and nonsocial (neutral). Prosocial behaviors included lip smack given and received, prosocial vocalization given and received, social play, huddling, and grooming given and received. Antisocial behaviors included bared teeth given and received, hitting given and received, biting given and received, ear flattening, aggressive vocalization given and received, attack and chase, cage shake, and threat face given and received. Nonsocial behaviors included exploration, eating, foraging, and stereotypies. The behaviors of grooming given, grooming received, grooming self, and huddling were coded both for frequency and duration. The remaining behaviors were coded only for frequency.

To score the videos, we used the behavior scoring software Tinbergen, which uses the coding language Python. While watching the video, the video scorer tracked only the behaviors of the monkey who was administered the drug. The video scorer watched the subject monkey and marked whenever an ethogram-specified behavior occurred with that behavior's three-character code. The software time-logged the entry and entered the behavior into the observation list. The video scorer recorded behaviors for the entirety of the video. During any point of the video scoring, the video scorer could observe the order and time that different behaviors occurred. The date and the drug of the video were not revealed before watching the video so as to prevent any bias on the part of the viewer.

Once behavior observations were complete for a given video, Tinbergen produced a comprehensive behavior observation output code for the corresponding video. This output code

served as the basis of the data analysis. MATLAB software was used to analyze prosocial, antisocial, and non-social behaviors across each condition.

Results

When analyzing the data, we separated the results by monkey as we found varying trends between the two subjects. In this way, we could examine if certain tendencies were general or specific to an individual monkey or gender.

Jodorowsky

Below are some of the results gathered for the male monkey tested, Jodorowsky. In examining these effects, we were most interested in either prosocial or antisocial behaviors, as opposed to nonsocial behaviors. Specifically, for the duration-measured behaviors, the most informative conducts were grooming given and huddling, as these are prosocial behaviors on the part of the subject monkey. We only investigated the behavior of our tested monkey in this pilot study.

The preliminary data for mean duration spent in these behaviors across conditions reveals that huddling mean duration was greater for oxytocin than for the other conditions (Fig. 1). The mean duration of grooming given did not vary greatly between conditions, being only slightly smaller in the oxytocin condition.

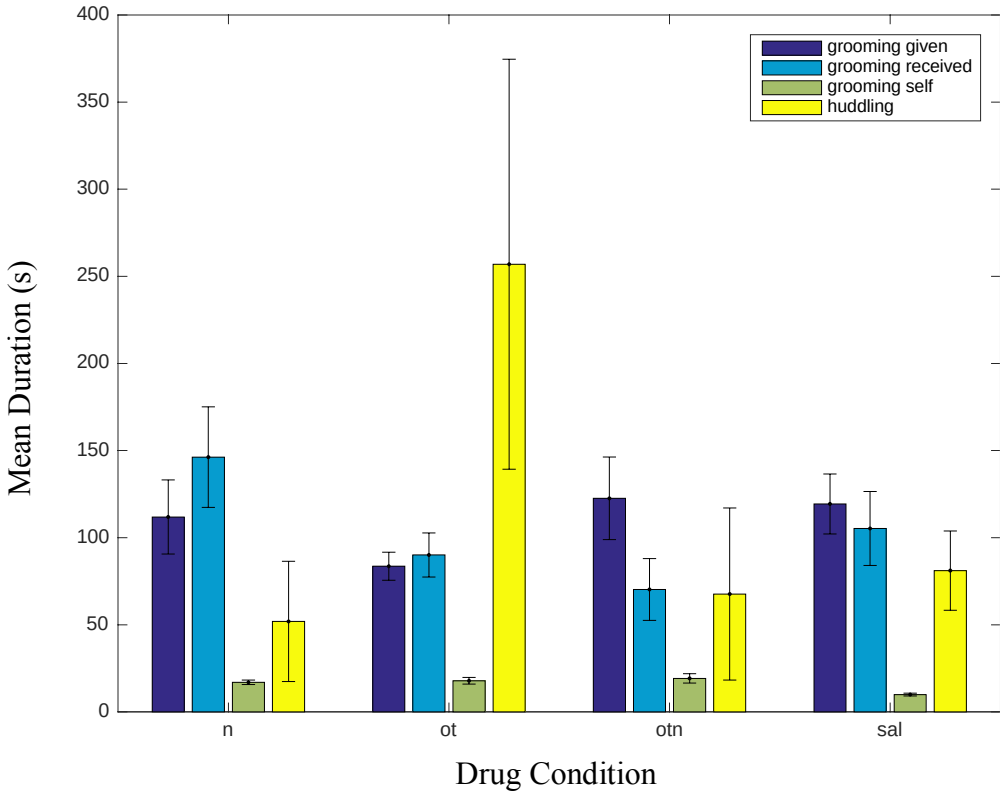

Figure 1. Mean Duration Spent in Grooming and Huddling Behaviors for Jodorowski This graph analyzes the mean amount of time spent engaged in a specific duration-recorded behavior for Jodorowski, the male monkey tested. This represents the average amount of time a behavior lasted each time it occurred across the different drug conditions. The drug conditions are naloxone (n), oxytocin (ot), combined naloxone and oxytocin (otn), and saline (sal). Each color is associated with a different grooming or huddling behavior. Grooming given refers to the subject monkey grooming his cage mate, and grooming received refers to the cage mate grooming the subject monkey. Grooming self refers to when the subject monkey grooms himself. Huddling refers to the subject monkey being intentionally close to his cage mate during which the subject monkey sits near its cage mate and does not engage in any other behavior. Saline served as the control condition and is the baseline of comparison. This graph includes data from the 16 sessions conducted on one monkey, Jodorowski.

When analyzing the frequencies of behavioral categories for Jodorowski, we normalized to our control of saline administration. In this way, we could more easily visualize the effects that these neuropeptides had on social behavior in comparison to our baseline condition. The line at 0 represents frequencies of the behavioral categories in the saline condition. There was an increase in prosocial behavior in the oxytocin condition. There were no other significant variances for either antisocial or prosocial behavior for the other conditions.

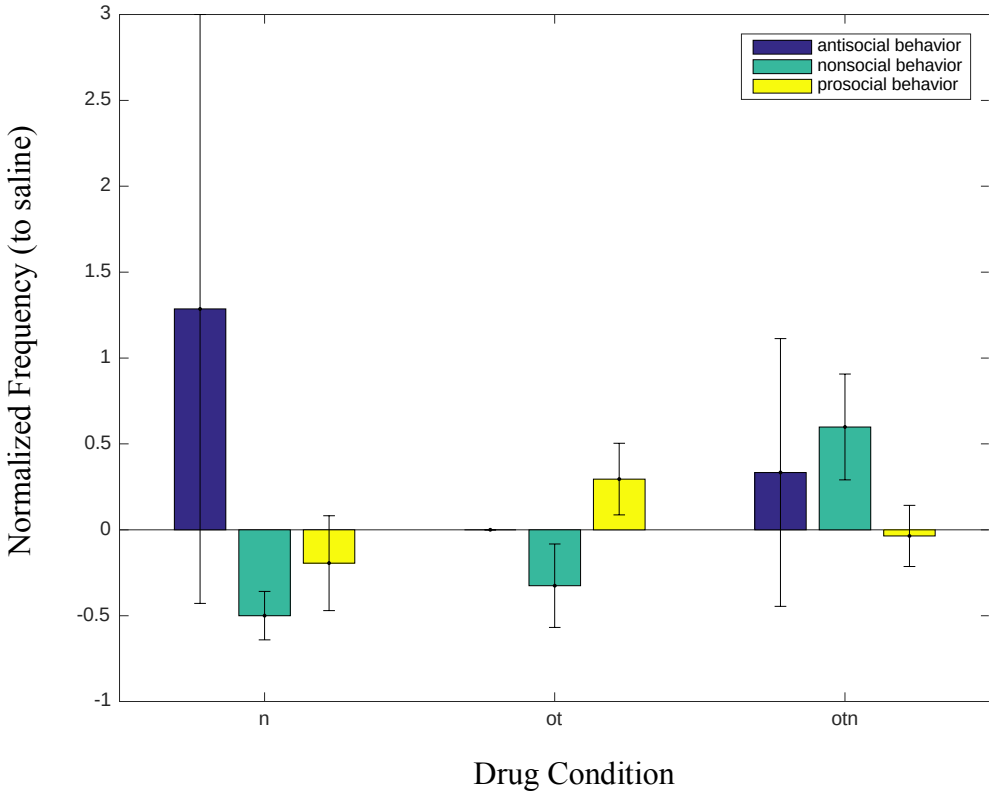

Figure 2. Normalized Frequency to Saline of Behavioral Categories for Jodorowski This graph depicts the frequency of the three categories of behavior by condition normalized to saline. The baseline at 0 denotes the frequencies of these behavioral categories in the saline condition. Bars above this line represent frequencies that surpassed this baseline while bars below this line represent frequencies below this baseline frequency level. Different colored bars signify different categories of behavior. Antisocial behaviors are aggressive, nonsocial behaviors are neutral, and prosocial behaviors are affiliative. The drug conditions are naloxone (n), oxytocin (ot), combined naloxone and oxytocin (otn), and saline (sal). Saline was used as the control condition.

Campion

Below are the findings from the female monkey tested, Campion. Figure 3 shows the mean duration of the duration-measured behaviors across conditions, as was shown in Figure 1

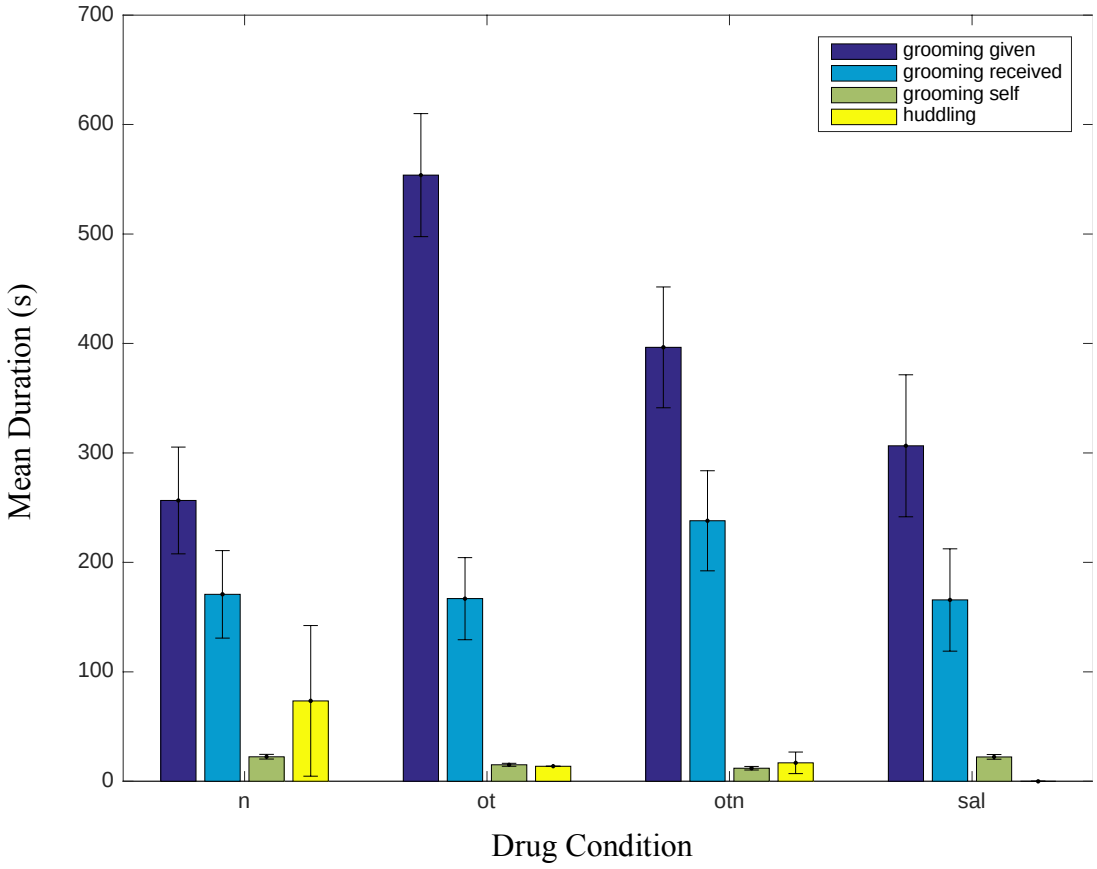

Figure 3. Mean Duration Spent in Grooming and Huddling Behaviors for Campion This graph analyzes the mean amount of time spent engaged in a specific duration-recorded behavior for Campion, the female monkey tested. This represents the average amount of time a behavior lasted each time it occurred across the different drug conditions. The drug conditions are naloxone (n), oxytocin (ot), combined naloxone and oxytocin (otn), and saline (sal). Each color is associated with a different grooming or huddling behavior. Grooming given refers to the subject monkey grooming his cage mate, and grooming received refers to the cage mate grooming the subject monkey. Grooming self refers to when the subject monkey grooms himself. Huddling refers to the subject monkey being intentionally close to his cage mate during which the subject monkey sits near its cage mate and does not engage in any other behavior. Saline served as the control condition and is the baseline of comparison. This graph includes data from the 16 sessions conducted on one monkey, Campion.

for Jodorowski. This graph reveals that the mean duration for grooming given was highest in the oxytocin condition and next highest in the combination condition. Mean duration for huddling was highest in the naloxone condition compared to the other conditions. Once again, we were mainly interested in grooming given and huddling for these graphs because these are the most social behaviors.

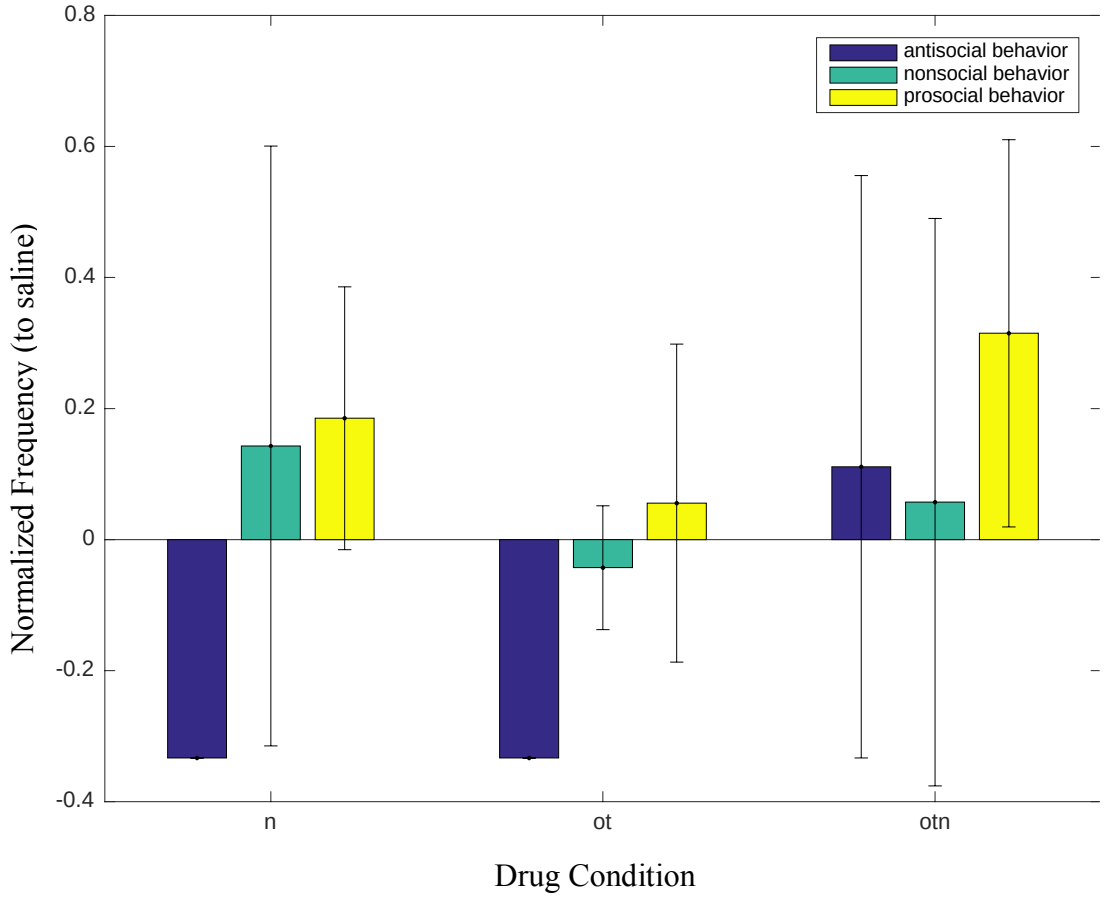

Figure 4. Normalized Frequency to Saline of Behavioral Categories for Campion This graph shows the frequencies of the different behavioral categories normalized to saline. The baseline at 0 represents the frequencies of these behavioral categories in the saline condition. Bars above this line represent frequencies that surpassed this baseline while bars below this line represent frequencies below this baseline frequency level. Different colored bars signify different categories of behavior. Antisocial behaviors are aggressive, nonsocial behaviors are neutral, and prosocial behaviors are affiliative. The drug conditions are naloxone (n), oxytocin (ot), combined naloxone and oxytocin (otn), and saline (sal). Saline was used as the control condition.

When comparing the frequencies of the behavioral categories for Campion, we again normalized to saline to be able to better observe the differences from baseline (Fig. 4). The line at 0 represents the frequencies for the different behavioral categories in the saline condition, which was the control. There was a slightly significant increase in prosocial behavior, represented by the yellow bar, in the combination condition. In addition, there were decreases in antisocial behavior in both the naloxone and oxytocin conditions.

Discussion

The main goal of this pilot investigation was to determine the impact of oxytocin, naloxone, and a combination of the two on in-cage, naturally occurring social interactions. We tested this by administering different combinations of drugs to rhesus macaques as described above and observing their behavioral conduct with their cage mate. We found that oxytocin alone increased some levels of huddling and prosocial behavior in the male subject and that the combination of oxytocin and naloxone somewhat increased prosocial behavior in the female subject. The two monkeys tested showed different trends from one another, leading us to examine their results separately. This could be due to sex differences or due to individual variations. When interpreting our preliminary results, we focused on being descriptive rather than conducting statistical analyses with the notion that with our small sample size, we were vastly underpowered to interpret any of our data with statistics. The current study serves as a pilot test and is not conclusive due to the small sample size.

Duration Behaviors

Out of all the behaviors observed and listed on the ethogram, four were marked for duration. In other words, their start and end times were recorded in the video scoring software. These behaviors were grooming given, grooming received, grooming self, and huddling. Out of these behaviors, we were most interested in grooming given and huddling since these represent social acts on the part of the subject monkey who was administered the drug. In examining Jodorowski's results for these behaviors, huddling was significantly higher in the oxytocin condition than in any of the other conditions. Previous studies have shown that oxytocin increases huddling frequency in primates (Smith, Ågmo, Birnie, & French, 2010). Here, we suggest that this neuropeptide also increases mean duration of huddling, as well as frequency. It is interesting that this same effect was not found in the combination naloxone and oxytocin

condition as the same dose of oxytocin was also present in this condition. However, our limited dataset cannot draw any conclusions in a statistically meaningful manner. Across the drug conditions, there is no clear variation in mean duration of grooming given. According to our prediction, the combination condition should be the highest bar while saline should be the lowest. This is not what we found in the results, as the data reveal the mean duration of saline to even be higher than that of the oxytocin condition. It is unclear why we would obtain these results suggesting that drug condition had no effect on this part of social behavior. This may be due to noise in the data sampling or scoring, coupled with the low number of test days obtained.

The data from these duration-measured behaviors reveal a different story in the results from Campion. Mean duration for huddling was slightly higher in the naloxone condition, differing from Jodorowski's results. Additionally, grooming given mean duration was highest in the oxytocin condition and second highest in the combination condition. This may support the notion of some sort of cancellation effect on the effects of oxytocin as a result of naloxone, as was hinted at in the huddling mean duration data from Jodorowski. More variation in this measure was found in Campion's data than in Jodorowski's data. It is also curious that the baseline condition of saline showed a higher mean duration for grooming given than naloxone. This may suggest some nonsocial effects of naloxone. However, with our limited dataset, it is difficult to draw any firm conclusions without further studies with a larger sample size with more testing days.

Behavioral Categories

All the behaviors recorded were in one of three behavioral categories – prosocial, nonsocial, and antisocial behaviors. We visualized the data by these groupings in order to

examine the effects of these drugs on entire behavior collections. We were most interested in prosocial and antisocial behaviors.

In analyzing the data collected from Jodorowsky, we decided to normalize the frequency of these various behavioral categories to saline in order to more easily compare the effects of the various conditions against the control. Prosocial behavior was most clear in the oxytocin condition. For Campion's results in these behavioral categories, we analyzed the data by normalizing frequency to a saline baseline. This was so that the frequencies could be clearly compared to the control baseline condition. We expected that the prosocial behavior frequencies would be higher than baseline in all conditions and highest in the combination condition. Additionally, we expected that antisocial behavior frequencies would be lower than the saline baseline for all conditions and lowest in the combination condition. For prosocial behaviors, only the combination condition was greater than the baseline condition, while the naloxone and oxytocin conditions were only slightly greater than the baseline level. These trends agree with our prediction; however, the differences were small. For antisocial behaviors, the naloxone and oxytocin frequencies were below baseline while the combination condition frequency was slightly greater than baseline. It is interesting that the combination condition is greater than baseline for this measure, although our limited dataset prevents us from interpreting the data in a meaningful manner.

Conclusions

The different trends we saw in social behavior across drugs between monkeys may be a result of gender differences or a result of individual differences between the monkeys. More studies examining this relationship need to be conducted to affirm these findings. In the current experiment, we tested whether these effects of a combination of naloxone and oxytocin could

translate to more gross level changes in overall naturalistic behaviors, such as grooming. Based on this preliminary dataset with our limited sample size, the answer to this question remains unclear. Although previous studies have shown that the combination of naloxone and oxytocin has a strong effect on social gaze, this effect may be restricted to specific social contexts or interactive settings (Bartz, Zaki, Bolger, & Ochsner, 2011). For example, one study found that these effects may be limited to mutually engaging events such as mutual eye contact or reward (Dal Monte et al., 2017), which could not be detected from scoring video recordings of in-cage behaviors. It is difficult to depict these modulations in a free environment, such as the monkey colony room that was used in the current study. Additionally, the study by Dal Monte et al. revealed that these effects might be restricted to specific parts of the face depending on the context (Dal Monte et al., 2017). Measuring behaviors on a larger scale in a less detailed manner may have made it difficult to detect significance in behavior change.

It is possible that we found no systematic patterns of results not because the combination condition did not work in this in-cage experiment, but rather because we could not detect the effect based on our sample size. Perhaps with more subjects, the effects would be more easily identified and substantial. Additionally, it is possible that our method of video scoring was not sensitive enough to detect any effects that require mutual interactions at high temporal resolution. In order for the drug to show clear effect, the interactive social setting may need to be more controlled. If this is the case, this has implications for the use of this combination of drugs in individuals with ASD. It might be most beneficial to use the drug while encouraging interactive effect in some controlled manner.

Limitations and Future Directions

Although we believe we conducted this experiment thoroughly and precisely, there are a few limitations that should not be ignored. The first is the sample size. Testing only two monkeys, one of each gender, is not sufficient to obtain robust and meaningful evidence of significance in behavioral differences. Trends were detected, but more monkeys need to be tested to allow statistical tests and to obtain assurance in what the true effects of these drugs are. Additionally, the size or hierarchy status of the tested monkeys could be manipulated in further studies to examine the effects of these variables. The second limitation is the process of video scoring used in this study. Only one person viewed and scored all the videos on the computer software. The viewer may have been biased to be more or less lenient on certain behaviors. In order to account for any such possible bias, it would be beneficial to have multiple scorers whose results could be compared and averaged to eliminate any variances caused by viewer bias.

Future experiments examining these effects could alleviate these limitations. For example, more monkeys could be tested and there could be more video scorers in future similar studies. Additionally, since we observed noteworthy effects of naloxone on social behavior, other opioid antagonists could be tested in combination with oxytocin to examine if they act in the same way as naloxone. Testing the effects of pharmacological manipulations in naturalistic settings in addition to more controlled experimental settings would be helpful in advancing potential treatments for social disorders in humans.

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