

VARIATION IN RHESUS MACAQUE (*Macaca mulatta*) VOCALIZATIONS: SOCIAL AND  
BIOLOGICAL INFLUENCES

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## **Abstract**

Rhesus macaques (*Macaca mulatta*) are the most widely studied nonhuman primate. While some work has been done on both vocal communication and the role of the neuropeptides, oxytocin, and vasopressin in the behavior of these highly social primates, key questions remain unanswered. In this study, seven rhesus macaques (four adult females and three adult males) were given a dose of either saline (control), oxytocin, or vasopressin. After being given this treatment, they were placed in close proximity to a conspecific who had not received any such treatment and the two monkeys were allowed to interact for five minutes. A variety of data, including the number of vocalizations that occurred in each session was recorded. Analysis showed that there were significant relationships between the number of vocalizations the female macaques produced and both the sex of the other individual in the room, and whether the female macaque had received saline, oxytocin, or vasopressin prior to the trial. These results have implications on the study of rhesus macaque communication and the study of the effects of oxytocin and vasopressin on promoting social behavior

## Introduction

Other than our own genus, *Homo*, the genus *Macaca* has the most widespread geographic distribution of all the primates (Thierry, 2007). This expansion occurred approximately seven million years ago, and led to a radiation that spanned across North Africa and across Asia into northern Japan. Presently, the genus consists of 22 extant species of macaques (Thierry, 2007). Broadly, macaques are characterized by species living in permanently multi-male, multi-female groups, with male post-natal dispersion.

Of particular note are the social relationships among female macaques. Across *Macaca*, because of the stability of female group membership, several generations of females tend to coexist in one group, allowing females to form kin-based matrilineal dominance hierarchies (Thierry, 2007). The stringency with which the dominance status of each individual female characterizes every social relationship and interaction has been shown to vary across the genus following phylogenetic trends (Thierry et al., 2009).

The rhesus macaque (*Macaca mulatta*) is not only the most widely studied macaque, but perhaps the most widely studied nonhuman primate (Maestripieri, 2007). Rhesus macaques' popularity among researchers can be attributed to their high level of social complexity, their adaptability to a wide range of habitats, and their broad geographic range and availability. This species typically lives in groups of 20 to 40 individuals, although observations of groups of 100 to 200 individuals are not unheard of as well (Thierry, 2007). Rhesus macaques' natural range includes parts of Central, South, and Southeast Asia. Within these regions rhesus macaques occupy a diversity of habitats from cities, to forests, to grasslands. Their ecological adaptability is highlighted by their diversity of diet across these varying habitats; rhesus macaques have been

observed to be primarily frugivorous in some environments and climates, while primarily folivorous in others (Thierry, 2007). The adaptability and plasticity of rhesus macaques across a wide range of ecological situations is truly impressive.

This feat is at least partially due to the rhesus macaque's unique social structure. This structure is framed by the broad trends that characterize the rest of the genus: multi-male, multi-female groups, with male post-natal dispersion, and female matrilineal dominance hierarchies. While there is great variability and plasticity when considering each individual's social outcomes, a species-wide behavioral archetype certainly emerges. An aggressive temperament, asymmetrical dominance relationships, mothers strongly influencing their infants' behaviors, a strong preference for maternal kin, strict rules for rank inheritance among matriline, and age-inverse dominance ranks characterize this archetype (Thierry, 2007).

Rhesus macaques have been observed in both wild and in experimental settings to exhibit a wide range of complex social behaviors. They have also shown capabilities thought, at one time, to be unique to humans (Frith & Frith, 2007). For example, rhesus macaques seem to be able to understand a fear response in another monkey, and use that information to protect themselves (Cook & Mineka, 1989). Similarly, rhesus macaques have been shown to understand the perceptions of human experimenters. In an experiment conducted by Flombaum & Santos (2005), rhesus macaques were more likely to steal grapes from experimenters who could not see the grapes than experimenters who could, suggesting that the monkeys had an understanding of what the experimenters could see, and, furthermore, how the experimenter would react to their grapes being taken.

Rhesus macaque's impressive suite of socially complex behaviors does not stop at the understanding of others' behaviors and perceptions. These monkeys have also been shown to

react to situations flexibly. For example, Washburn et al. (2006) found that rhesus macaques used an uncertain response in an experimental task when they lacked adequate information to perform with more confidence. Furthermore, studying rhesus macaques has shown that these monkeys are capable of imitation from a young age. One study found that rhesus macaque infants were able to imitate facial gestures, and that certain monkeys were more skilled at this imitation than others (Ferrari et al., 2009). There is also evidence that rhesus macaques engage in prosocial behaviors. Chang et al. (2011) concluded that a group of rhesus macaques were engaging in vicarious reinforcement, and suggested that this behavior indicates a promotion of learning and group coordination.

Rhesus macaques garner this level of attention from researchers, in part, with the hope that understanding their complex social capabilities can be informative about the history and foundation of human's social capabilities. This goal is particularly important in light of the social brain hypothesis, which suggests that the demands of increasingly complex social lives was the primary driving force behind the evolution of human's exceptional cognitive complexity (Dunbar, 2003). In this context, understanding the roots and extent of social complexity in primates can help answer greater questions about the origin and nature of what some consider to be the distinguishing characteristics of human behavior.

One way that researchers have tried to operationalize their study of social complexity and the social brain hypothesis is through the study of communicative complexity. The link between the social brain hypothesis and the dynamics of social communication systems was formalized by the social complexity hypothesis. According to Freeberg et al. (2012:1785), "The 'social complexity hypothesis' for communication posits that groups with complex social systems require more complex communicative systems to regulate interactions and relations among

group members.” In other words, as the demands of social complexity increase, communication systems are also more likely to become more varied, nuanced, and complex in order to facilitate the maintenance of this increasingly intricate web of social connections. Thus as the pressure of increased social demands drives cognitive evolution, one manifestation of this process should be seen in the increasing complexity of communication modalities. From this perspective, studying the communication systems of our socially complex primate relatives, like rhesus macaques, can provide crucial information about more central questions in human evolution. Accordingly, researchers have attempted to develop an understanding of rhesus macaque vocal communication in terms of its biological and cognitive foundations, its variability across age classes and sexes, and its utility in different social and ecological contexts.

Rhesus macaques have a varied and complex vocal repertoire, producing approximately 18 acoustically distinct vocalizations (Hauser & Marler, 1992). These vocalizations are generally used flexibly, though some are used in specific contexts, for what seem to be specific purposes. Some calls, for instance, are used in food acquisitions, while others are used in mother-infant interactions, or for social cohesion. For example, Gouzoules et al. (1984) examined a subset of the rhesus macaque vocal repertoire: those that are used in agonistic or aggressive encounters. Vocalizations in this context are thought to be important for ally recruitment, specifically among immature monkeys. They found, in a population of free-ranging rhesus macaques, that five acoustically distinct scream vocalizations are used regularly in these interactions. After conducting a playback experiment, in which the screams of immature monkeys were played to their mothers, Gouzoules et al. (1984) concluded that these screams each carry specific messages potentially regarding the opponent or extent of physical aggression.

There is also evidence that rhesus macaques can match the vocalizations of their peers to the faces of their peers. In a study of six captive rhesus macaques, researchers found that the monkeys were able to correctly assign the vocalizations of both humans and other macaques with whom they were familiar to pictures of the vocalizer (Sliwa et al., 2011). In this context, rhesus macaque vocalizations could play a more nuanced role in inter-individual social relationships. This evidence demonstrates that rhesus macaque vocal communication plays a significant, complex role in their multifaceted social lives.

Beyond their diversity of use, rhesus macaque vocalizations are also known to be sexually dimorphic. Across various contexts, females are observed as vocalizing significantly more than their male counterparts. For example, in the Gouzoules et al. (1984) study discussed previously, they were only able to record a few screams from adult males because they are used only rarely. In fact, they observed that male rhesus macaques are much more likely to be silent during bouts of aggression. Others have also noticed this disparity, with Bernstein & Ehardt (1985:128) noting that, “adult males thus may be seen as seldom vocalizing, avoiding animals engaged in agonistic vocalization, and likely to ‘ignore’ approaching others.” Furthermore, they found that males are far less frequently observed as responding to any kind of provocation with vocal scream sequences (Bernstein & Ehardt, 1985). Tomasycki et al. (2001) made similar observations, noting that this sex discrepancy in vocalization rate also extends beyond agonistic encounters, with females generally using a wider variety of calls at more frequent intervals than their male counterparts.

Variation in vocalization frequency in rhesus macaques also exists across age class, with immature monkeys vocalizing more often than their adult counterparts. Gouzoules et al. (1984) observed juveniles utilizing scream vocalizations far more frequently than adults. Similarly,

Bernstein & Ehardt (1985) observed adolescent and juvenile monkeys vocalizing more frequently than adults. Interestingly, even with juveniles of both sexes vocalizing at higher rates than mature monkeys, the sexual dimorphism in call rates appears to manifest early in life. Tomaszycski et al. (2001:274) found that separation-rejection vocalizations, as well as the maternal response to these vocalizations, become sexually dimorphic within the first eight months of a rhesus macaque's life, which "supports the notion that rhesus macaque vocalizations sexually differentiate very early in life during a period of intense mother-infant interaction." They found this early pattern continues to develop as the monkeys age, with females continuing to use vocalizations socially through adulthood, while males become increasingly strong silent types.

A number of researchers have hypothesized about the root cause of these variations across age and sex classes in vocalization frequency. Some think that these differences are tied to differences in social life between females and males, with females staying in their native groups and forming stable, linear, matrilineal hierarchies, while males leave their natal groups, and have less structure to their social interactions. Tomazycki et al. (2001: 267) suggest that, "differences in male and female life histories may reflect the fact that, in rhesus macaques, as well as other cercopithecines, social structure is based upon stable, linearly ranked matrilines with strong bonds among female kin, coupled with male-biased post pubertal dispersal." Bernstein & Ehardt (1985) also acknowledge the role that these differing social histories may play in the sexual dimorphism of vocalization rates. However, they also states that these disparities could not be explained solely by differences in size and relatedness among males and females, and instead they suggest that there must be a, "greater ontogenetic process in operation" (Bernstein & Ehardt, 1985:129). Certainly, further studies on the extent and nature of variation in



vocalization frequency between the sexes and across age classes will need to be conducted in order to fully elucidate their potential evolutionary significance and social importance.

These trends that characterize the rhesus macaque vocal repertoire on the whole also manifest themselves in their most frequently used vocalization, the “coo” (Hauser & Marler, 1992). Sometimes referred to as clear calls as well, the “coo” is utilized in a variety of contexts. Hauser (1992) suggests that the main purpose of the “coo” is as a social contact call to maintain cohesiveness between group members that may be dispersed over a wider area. On the other hand, Erwin & Mitchell (1973:463) suggest that, “while clear calls occur in many contexts other than mother-infant separation, few situations seem to be as closely correlated with these sounds as those involving separation.” In their view, then, the “coo” is most closely associated with mother-infant interactions. Still, the “coo” has been associated with even more contexts including food. In a study on free-ranged rhesus macaques on the island of Cayo Santiago, Puerto Rico, Hauser & Marler (1992) found that the “coo” is used as a food call, but only in the presence of less desirable chow (like the provisioned chow, in this case). While the main purpose of the “coo” may not be entirely apparent, it is clear that the “coo” is an integral part of the rhesus macaque vocal repertoire, and used in a variety of social contexts.

Several researchers have attempted to understand the extent to which rhesus macaques use the “coo” flexibly. Studies by Newman & Symmes (1974), and Owren, et al. (1993) each took different approaches to this issue. Newman & Symmes (1974) analyzed potential structural differences in the vocalizations of two male and two female rhesus macaques that were raised in partial social isolation. They found that the “coos” of these rhesus macaques had acoustic abnormalities, including, “abrupt pitch changes, harmonic emphasis shifts, temporal discontinuity, and lack of the characteristic inflection” found normally in “coos” (Newman &

Symmes, 1974:351). Owren, et al. (1993), on the other hand, looked for potential differences in the frequency of vocalizations in rhesus macaques that were cross-fostered and raised by Japanese macaques (*Macaca fuscata*). They found that, while the rhesus macaques often exhibited species typical rates of “coo” vocalization, there were some cases where their vocalization rates were more similar to that of the Japanese macaques surrounding them.

This evidence suggests that the development and use of the “coo” is likely somewhat influenced by rhesus macaques’ early social environment. A study by Sutton et al. (1973) aimed to see if human experimenters could successfully intentionally manipulate the “coo”. They were able to train three rhesus macaques to emit longer than average calls. Furthermore, they were able to train the monkeys to produce the call in response to a provided stimulus. These results suggest that rhesus macaques’ “coos” are not purely emotive, and are under at least some degree of intentional control.

Another potential sign of the flexibility of the “coo” is the evidence suggesting that it may be distinct based on the matriline to which an individual belongs. Hauser (1992) found that the matriline he was studying produced an acoustically distinct “coo”, which has an enhanced nasal tone compared to the “coos” produced by other monkeys. He suggested that this modification is likely learned, and could serve as a means of personal identification based on vocalization, given the likelihood that the rhesus macaques also perceived these calls as distinctive.

While it is unclear whether or not the “coo” has the potential to universally contain information about the familial origins of the caller, there is evidence that it may provide information about the body size of the caller. Researchers once held that the fundamental frequency ( $F_0$ ) of rhesus macaque “coos” reliably correlated to the body size of the caller.

However, Fitch (1997) suggests that the  $F_0$  is not a reliable indicator of the body size of the caller, but, instead, claims that formant dispersion has the potential to reliably carry this information. Formants are vocal tract resonance frequencies, and formant dispersion is, “the averaged difference between successive formant frequencies” (Fitch, 1997:1213). This measurement correlates with vocal tract length, and has been shown to correlate with the body size of the rhesus macaque producing the “coo” (Fitch, 1997). Simply put, this indicates that the “coo” reliably carries information about the body size of the sender. However, it is unclear that other rhesus macaques can perceive or use this information.

Variations in the frequency of the “coo” have also been observed between the sexes and across age classes. Adult females frequently utilize the call, although, it is rarely heard coming from adult male macaques (Rowell & Hinde, 1962; Erwin & Mitchell, 1973). The sexual dimorphism of the “coo” becomes more pronounced with age, although evidence suggests that it is present, if to a lesser degree, from early age. Tomaszycski et al. (2001) point out that less is known about earlier differences in “coo” frequency between the sexes, but females use more “coos” than do males in response to maternal separation starting at two months of age.

Erwin & Mitchell (1973) also described how “coo” production rate changed over the course of rhesus macaque lifetime. For both sexes, they described a major increase in “coo” frequency during the first year of life, followed by a decline in the second year, with males’ production continuing to decline into the third year. Generally, juvenile macaques “coo” the most, both in the field and the lab. Another stark decline in “coo” production is observed around the time of puberty, but this decline is more closely associated with puberty in males than in females.

Despite production rates following broadly similar trends, females consistently “coo” more than males through development and into adulthood (Erwin & Mitchell, 1973). The “coo” clearly plays an integral role to rhesus macaque sociality, and more work is needed to understand what underlies its variation across contexts, between sexes, and through development and adulthood.

In trying to develop a greater understanding of the biological basis of these vocalization trends, researchers may start looking at how hormones and neuropeptides influence rhesus macaque vocalizations. Oxytocin and vasopressin are neuropeptides, each containing nine amino acids. They are closely related, with only two amino acids differing between the two (Chang & Platt, 2014). These neuropeptides developed early on in vertebrate history, with their homologs dating back 700 million years, and are highly evolutionarily conserved. In mammals, oxytocin and vasopressin are produced primarily in the hypothalamus, and then travel to the pituitary for release into the rest of the body or brought to other regions of the brain itself (Donaldson & Young, 2008).

Broadly, neuropeptides are thought to be integral to the regulation of sociality, both cognitively and behaviorally (Donaldson & Young, 2008). These roles are due in part to the fact that neuropeptides, like oxytocin and vasopressin, can act as both neurotransmitters (agents that travel through synapses to receptors), and neurohormones (agents that travel through the bloodstream to potentially more distant receptors). For this reason, they are seen as evolutionarily flexible. Oxytocin and vasopressin specifically are thought to regulate social and reproductive behaviors in a wide variety of animals, though Chang & Platt (2014:57) point out, “in highly social animals, these ancestral functions have been co-opted to serve social functions,

such as promoting maternal behavior, fostering pair-bonding and affiliative behaviors, encouraging in-group bias, reducing social vigilance, and amplifying other-regarding behaviors.”

The observed roles of oxytocin and vasopressin are made even more interesting, for they seem to serve in similar contexts of macaque vocalizations and specifically the “coo”. For example, Feldman et al. (2010) found that oxytocin played an important role in the mother-infant bonding of humans, while Bosch et al. (2005) found that intercerebral patterns of oxytocin release play a role in maternal defense of offspring. These are similar to the role the “coo” plays in mother-infant separation as described by Erwin & Mitchell (1973). Oxytocin is also associated with the cooperative activity of food sharing. Wittig et al. (2014) measured levels of oxytocin in chimpanzees after a bout of food sharing and found a significant increase in levels of oxytocin regardless of the relatedness of those consuming the food. The “coo” also plays a role in food sharing in rhesus macaques, as described by Hauser & Marler (1993). Because both the “coo” and the neuropeptides oxytocin and vasopressin all play significant roles in the social lives of rhesus macaques, it is no surprise that they share these functions. However, the relationship between social and emotional vocalizations has not yet been well elucidated.

There’s a more limited body of literature looking specifically at the relationship between these neuropeptides and vocalizations. While some work has been done with rodents (e.g. Marlin et al., 2015), human studies are of more relevance because macaques share more of our complex social relationships and organization. De Dreu et al. (2016) examined how oxytocin influences the ability to decode the emotional contexts of vocalizations of both those with similar cultural background as well as those with differing cultural background. They found that oxytocin enhanced the ability to decode emotional vocalizations, but had a more significant impact on the decoding the vocalization of those from the cultural out-group (De Dreu et al., 2016). In another

human study looking at ties between vocalizations and neuropeptides, Riem et al. (2011:291) found that, “oxytocin promotes responsiveness to infant crying by reducing activation in the neural circuitry for anxiety and aversion in regions involved in empathy.”

Seltzer et al. (2010) also examined how vocalizations influenced oxytocin in the context of mother-child relationships. Their research found that children comforted by just their mothers voice exhibited only slightly lower levels of oxytocin than those who received physical contact care in addition to vocal comfort. This result suggests that vocalizations are likely crucial to neuroendocrine regulations of socialization. However, this work focused on the impact of oxytocin on interpreting vocalizations, and did not evaluate the influence of oxytocin or vasopressin on those producing the vocalizations.

Developing a greater understanding of the link between vocalizations and neuropeptides like oxytocin and vasopressin could elucidate important information about the mechanisms underlying socialization and communication in humans and other social animals. As De Dreu et al. (2016:837) point out, “Although the accurate assessment of, and responding to, emotional vocalizations is well documented and understood, its neuroendocrine underpinnings remain elusive.” Understanding the relationship between levels of these neuropeptides and vocalizations could therefore contribute greatly to this discourse. Rhesus macaques, with their well-studied social complexity and dynamic use of vocal communication provide a prime opportunity to attempt to develop this understanding, especially given the similar social contexts in which the “coo” and oxytocin and vasopressin seem to play integral roles. This paper begins to determine how these neuropeptides influence the dynamics of rhesus macaques’ use of the “coo”.

## **Methods**

The vocalization data were collected as part of a larger study concerned with the impact of oxytocin and vasopressin on a variety of social behaviors in rhesus macaques. The experimental design of that study is described below:

### *Experimental Design*

Rhesus macaques were administered doses of saline (control), vasopressin, and oxytocin in order to understand the impact of these neuropeptides on their behavior. Seven adult rhesus macaques housed in the Smilow Center for Translational Research at the University of Pennsylvania participated in the study. The group consisted of four females—Bales (15 years old, 8 lbs.), Curry (15 years old, 7.4 lbs.), Feinstein (20 years old, 10.2 lbs.), and Schroeder (20 years old, 8 lbs.)—and three males—Dart (20 years old, 10.4 lbs.), Oskar (18 years old, 17 lbs.), and Solly (20 years old, 13 lbs.). These monkeys were housed in the same room, with cages along the wall facing the center of the room. A rough illustration of the exact arrangement of cages is provided in Figure 1. Bales and Curry shared an enclosure.

Each trial consisted of two monkeys facing each other head-on in an empty room for a period of five minutes. One monkey, M1, had been treated with either saline, oxytocin, or vasopressin, while the other monkey, M2, had not. Monkeys sat in Crist Instruments primate chairs that were positioned close together, but not touching. Each session was recorded with a Logitech (60 fps) video camera, which was placed to the right side of M1 (the left side of M2) so it could capture both monkeys' behavior.

Doses of the treatment solutions were delivered via a pediatric nebulizer mask, which each of the monkeys were conditioned to accept prior to experimental trials. The nebulizers covered the monkeys' nose and mouth, as pictured below along with an image of the experimental set up in Figure 2.

A more detailed account of intranasal delivery procedure in rhesus macaques can be found in Chang et al. (2012), and Ebitz et al. (2013). Doses of 1 mL of oxytocin (25 IU/mL in saline; Sigma Aldrich), 1 mL of vasopressin (25 IU/mL in saline; Sigma Aldrich), or 1 mL of saline were delivered via the nebulizer at a constant rate of 0.2 mL/minute over the course of five minutes. Behavioral trials began half an hour after the treatment dose was given to M1, and continued for one to two hours.

Each monkey received each treatment or saline, and sat across every possible M2, and each of these potential combinations were repeated five times. Additionally, each M1 also faced an empty chair for five minutes after receiving each treatment or saline to provide another control. These control trials were also repeated five times. Monkeys had an equal likelihood of being M1 or M2, and the saline and neuropeptide treatments were alternated daily, with no monkey receiving more than five treatments per week. Furthermore, counterbalancing the treatments both across and within monkeys mitigated potential order effects.

### *Data Analysis*

One to three independent viewers who were not aware of the treatment conditions coded videos from each trial. Those coding were able to play and pause the video, as well as adjust the speed of the videos. The data from the video reviews were put into MATLAB, and subsequently broken down into ethograms, including one for the vocalizations. Example ethograms can be seen in Figure 3.

Those coding classified the vocalizations as “coos” (therefore, for the purposes of this paper, “coo” and vocalization will be used interchangeably going forward). No males were observed vocalizing, so there were no male data to code and subsequently analyze. Additionally, only three of the four females (Bales, Curry, and Feinstein) were observed vocalizing.



Once the vocalization data were coded, MATLAB was used to perform further analysis of them. A number of analyses and statistical tests were performed in an attempt to gain a better understanding of factors that might influence vocalization rates. Factors that were investigated included (a) the potential influence of the oxytocin and vasopressin treatments, (b) the potential influence of facing different monkeys during the trial (specifically the potential difference between facing a male versus facing a female, and facing a monkey that the individual lived in closer proximity to versus a monkey that lived in a more distant enclosure), (c) the potential influence of receiving any treatment as M1 or no treatment as M2, and (d) the potential influence of the other monkey's vocalization rate (i.e. a call and response effect). The results of these analyses are presented in the subsequent sections.

## **Results**

A larger study was conducted in order to understand the impact of oxytocin and vasopressin on rhesus macaque behavior. Five-minute videos were recorded of two monkeys in close proximity and facing each other, but without the ability to make physical contact. Before these sessions, one rhesus macaque (M1) was administered a dose of saline, oxytocin or vasopressin through a pediatric nebulizer, while the other rhesus macaque (M2) was not administered any treatment. After these experimental trials were conducted, one to three independent viewers, who were blind to M1's treatment type, coded the videos. These codes were converted into ethograms, of which vocalizations (all of which were determined to be "coos") were one of the recorded behaviors. The three male rhesus macaques, and one female rhesus macaque (Schroeder) were never observed vocalizing. Because only three individuals

(Bales, Curry, and Feinstein) vocalized it was feasible to segregate data by individual. Results were displayed by M2 in order to determine the potential impact of the M2 each individual was facing.

Bales' results for saline control treatments, as M1, can be seen in Figure 4. Furthermore, we evaluated to see, grossly, how the drugs impacted Bales' vocalizations with respect to the M2 she was facing with her results for oxytocin and vasopressin appearing in Figure 5. Bales consistently vocalized more in front of male M2s, both in the saline control trials, and the oxytocin and vasopressin treatment trials. Table 1 displays the raw data depicted in Figures 4 and 5. It noteworthy that the raw vocalization rates between the "Saline" and "Treatment (OT and VP)" cannot be compared directly because the "Treatment (OT and VP)" totals resulted from double the number of trials as the "Saline" totals (i.e., "Treatment (OT and VP)"  $n$  is double "Saline"  $n$ ). While oxytocin and vasopressin may change the frequency at which Bales vocalized, it does not change which M2s Bales vocalized more frequently when in front of, as seen in Table 1. Bales consistently vocalized in front of Curry the least of the females, and Oskar the least of the males. This was interesting because Bales shares an enclosure with Curry, and Bales' enclosure faces Oskar's enclosure.

Curry's results for saline control treatments, as M1, can be seen in Figure 6. Furthermore, to determine how the drugs impacted Curry's vocalizations with respect to the M2 she was facing, Curry's results for oxytocin and vasopressin can be seen in Figure 7. Curry consistently vocalized more in front of male M2s, both in the saline control trials, and the oxytocin and vasopressin treatment trials. Table 2 displays the raw data depicted in Figures 6 and 7. It is of note that the raw vocalization rates between the "Saline" and "Treatment (OT and VP)" cannot be compared directly because the "Treatment (OT and VP)" totals resulted from double the

number of trials as the “Saline” totals (i.e., “Treatment (OT and VP)”  $n$  is double “Saline”  $n$ ). While oxytocin and vasopressin may change the frequency at which Curry vocalized, it does not change which M2s Curry vocalized more frequently in front of, with the exception of Dart and Oskar. In both the saline and oxytocin and vasopressin treatment trials, Curry vocalized least in front of Bales, with whom she shares an enclosure. This is similar to the pattern seen with Bales’, who also vocalized least in front of Curry.

Feinstein’s results for saline control treatments, as M1, can be seen in Figure 8. Furthermore, to determine how the drugs impacted Feinstein’s vocalizations with respect to the M2 she was facing, we present Feinstein’s results for oxytocin and vasopressin (Figure 9). Feinstein does not seem to follow the same pattern that Bales and Curry do with respect to vocalizing more in front of male M2s than in front of female M2s. In Feinstein’s saline trials, the opposite pattern emerges, with Feinstein consistently vocalizing more in front of females than males. Conversely, in the oxytocin and vasopressin trials, Feinstein’s pattern more closely resembles that of Bales’ and Curry. Table 3 displays the raw data depicted in Figures 8 and 9. It is of note that the raw vocalization rates between the “Saline” and “Treatment (OT and VP)” cannot be compared directly because the “Treatment (OT and VP)” totals resulted from double the number of trials as the “Saline” totals (i.e., “Treatment (OT and VP)”  $n$  is double “Saline”  $n$ ). Again, Feinstein did not seem to follow the same patterns as Bales and Curry. Generally, Feinstein generally seems to have vocalized less than Bales and Curry. Additionally, the M2s Feinstein to which vocalized is not consistent with the saline and oxytocin and vasopressin treatment trials, unlike the patterns observed in Bales and Curry.

Analyses were run in order to understand if, and if so, how, the sex of the M2, and the treatment given to M1s impacted the number of vocalizations in the experimental trials. The

average number of vocalizations by female M1s (Bales, Curry, Feinstein, and Schroeder) divided by treatment and sex are displayed in Figure 10. A two-way analysis of variance was conducted on the influence of M2 sex and M1 treatment type (saline, oxytocin, or vasopressin) on the number of vocalizations produced by the M1. All effects were statistically significant at the 0.05 significance level. The M2 sex main effect produced an F ratio of  $F(1, 354)=14.63, P=0.000$ , indicating a significant difference between the average number of vocalizations produced in front of a male M2, and the average number of vocalizations produced in front of a female M2. The treatment type main effect produced an F ratio of  $F(2, 354)=17.9, P=0.000$ , indicating a significant difference between the average number of vocalizations produced by M1s who had been administered saline, the M1s who had been administered oxytocin, and the M1s who had been administered vasopressin. Furthermore, there was a significant interaction effect ( $F(2, 354)=4.88, P=0.008$ ), suggesting that oxytocin and vasopressin may have exacerbated already existing differences between the rates of vocalization in front of male M2s and female M2s. Generally, females vocalized significantly more in front of male M2s than in front of female M2, and oxytocin and particularly vasopressin increased the frequency of vocalization when compared to the saline treatment.

There was also interest in seeing if the rates of vocalization were affected by whether the individual was acting as M1 or M2. Figure 11 illustrates the mean vocalization rate of the females Bales, Curry, and Feinstein as M1, and the mean vocalization rate of the females Bales, Curry, and Feinstein as M2. The M1 data do not include the vocalizations from the empty chair control trials, for there is no equivalent data for M2 data. The mean M2 vocalization frequency was significantly higher than the M1 vocalization frequency ( $P=0.000$ ; unpaired T-test).

Analyses were run to explore if and how the sex of the M1, and the treatment given to M1s, impacted the number of vocalizations the M2s produced in the experimental trials. The average number of vocalizations by female M2s (Bales, Curry, Feinstein, and Schroeder) broken down by treatment and sex are displayed in Figure 12. A two-way analysis of variance was conducted on the influence of M1 sex and M1 treatment type (saline, oxytocin, or vasopressin) on the number of vocalizations produced by the M2. All effects were not statistically significant at the 0.05 significance level. The M1 sex main effect produced an F ratio of  $F(1,354)=.02$ ,  $P=0.890$ , indicating an insignificant difference between the average number of vocalizations produced in front of a male M1, and the average number of vocalizations produced in front of a female M1. The treatment type main effect produced an F ratio of  $F(2,354)=.58$ ,  $P=.561$ , indicating an insignificant difference between the average number of vocalizations produced by M1s who had been administered saline, the M1s who had been administered oxytocin, and the M1s who had been administered vasopressin. Furthermore, there was no significant interaction effect ( $F(2,354)=.11$ ,  $P=.893$ ).

Another representation of the impact of the treatments on the frequency of vocalization can be seen in Figure 13, which illustrates a histogram of the number of trials in which a certain vocalization frequency for female M1s, broken down by the treatment received. Though trials with zero vocalizations were excluded from representation in this figure, it shows a clear trend of higher vocalization rates among those M1s who received vasopressin treatments.

To determine if there was any positive feedback relationship between the vocalizations of M1 and M2, a correlation between M1 and M2 vocalization frequencies was performed (Figure 14). Each data point represents an experimental trial session. Correlation analysis revealed no significant correlation between the number of times the M1 vocalized compared to the number of

times M2 vocalized ( $R=-0.101$ ,  $P=0.176$ ). This lack of relationship suggests that the M1s were not simply increasing their vocalization frequency in response to the vocalization activity of the M2 they were facing.

Fixation refers to the behavior when the individual was facing and focusing on the individual seated across from him/ her in the experimental trial. Several analyses were performed to see if there was any relationship between fixation and vocalization behaviors. Figure 15 shows the average number of vocalizations M1 females (Bales, Curry, Feinstein, and Schroeder) performed in fixation, and the average number of vocalizations M1 females performed while not in fixation. There was a significant difference between these averages, with female M1s vocalizing more while not in fixation than while in fixation ( $P= 0.006$ ; unpaired T-test). This indicates that the M1s were more likely to vocalize while facing away from the M2 during the experimental trial, rather than vocalizing directly at the individual.

Figure 16 illustrates the relative proportion of female M1 vocalizations that occurred in fixation versus the proportion of female M1 vocalizations that occurred while not in fixation, broken down by sex of the M2. Similarly, Figure 17 illustrates the relative proportion of female M1 vocalizations that occurred in fixation versus the proportion of female M1 vocalizations that occurred while not in fixation, broken down by the treatment of the M1. Both across sex of M2, and across treatment types, it appears that the relative proportion of vocalizations in fixation versus not in fixation is fairly consistent. The result indicates that while there may be a significant difference between the number of vocalizations produced while in fixation and the number of vocalizations produced while not in fixation, there is likely no interaction effect between each of these variables.

## Discussion

This analysis revealed several interesting patterns that are consistent with existing work on the “coo”, and potentially reveal patterns that previous research has yet to fully explore. There were significant differences in the vocalization rate of female M1s based on the sex of the M2 they were facing, as well as the treatment (saline, oxytocin, or vasopressin) that they had received prior to the experimental trial. Additionally, there were interesting results with regard to the lack of male vocalizations, the relationship between fixation and vocalization behaviors, and how the living proximity of the females affected the number of vocalizations they produced in the experimental sessions. These observations, as well as some potential explanations for their occurrence, are discussed further below.

One finding that was consistent with existing the literature was the lack of observed vocalizations produced by the male rhesus macaques. As described before, rhesus macaque vocalizations are widely recognized as sexually dimorphic, with males vocalizing far less than females of the same age class (Gouzoules et al., 1984; Bernstein & Ehardt, 1985; Tomasycki et al., 2001). Researchers have also recognized that the “coo” specifically is sexually dimorphic (Rowell & Hinde, 1962; Erwin & Mitchell, 1973), and this dimorphism becomes more pronounced with age (Tomasycki et al., 2001). The observations made in this study reinforce the findings of previous work, with the adult females vocalizing far more frequently than their adult male counterparts.

Another finding consistent with existing literature is the fact that Curry and Bales vocalized less frequently than Schroeder and Feinstein. Curry and Bales are both 15 years old, while Feinstein and Schroeder are 20 years old. Several studies have noted how macaque vocalizations

decline with age (Gouzoules et al., 1984; Bernstein & Ehardt, 1985), and specifically how the “coo” declines with age (Erwin & Mitchell, 1973). The fact that Curry and Bales are five years younger than Feinstein and Schroeder could help explain why they vocalized at higher rates than the others.

A significant amount of vocalization occurred while the female M1s were not in fixation; in fact, the majority of the vocalizations occurred while the monkeys were facing away from the M2 seated across from them. One explanation for this result is that the M1s were attempting to contact those outside the experimental room enclosure. This would be consistent with Hauser’s (1992) description of the “coo” as a social contact call. He describes that, “the main function of this call [the “coo”] is to maintain cohesiveness between members of the group,” (Hauser, 1992:2175). Furthermore, Hauser & Marler (1992) specifically suggest that the “coo” serves to increase group size. In this regard, the proportion of vocalizations in fixation did not seem to change based on the sex of the M2, or whether the M1 was administered saline, oxytocin, or vasopressin. In other words, the rhesus macaques were vocalizing more while not in fixation regardless of the sex of the M2 or the treatment they had received. The majority of the vocalizations occurring while the rhesus macaques were looking away from the other monkey in the room is compatible with the framework that they were attempting to contact monkeys outside the experimental trial room with a call that is thought to be used to maintain social contact.

This reasoning would also be consistent with the trends observed in terms of the vocalization rates in front of female M2s versus the male M2s. Female M1s were vocalizing significantly more in front of male M2s rather than female M2s. One possible explanation for this trend can be found in the rhesus macaque social organization. As described previously, rhesus macaques are known to have strong matrilineal hierarchical organizations that dominate virtually every aspect



of their social lives (Thierry, 2007). The development of these structured hierarchies in this population of rhesus macaques is limited by the fact that these rhesus macaques are not in a free or semi-free environment. However, broad similarities to these relationships likely exist. Given the likelihood that the females were calling to individuals not in the experimental trial room, it would also seem likely that they would call less frequently when there are other females in the room with whom they have stronger social relationships. By contrast, when they are facing male M2s, they may be more likely to seek contact with other females they are more comfortable socially, and therefore “coo” more frequently.

These trends could also provide an explanation for some of the vocalization frequency patterns in relation to the rhesus macaques’ living proximity that were observed. Specifically, Bales and Curry, who are normally housed in the same enclosure, both vocalized least in front of each other when compared to trials facing any other M2. As a result of their shared enclosure, Bales and Curry are more generally comfortable with each other than the others. Under these circumstances, it would follow that Bales and Curry would “coo” less frequently when in experimental trials together, for the individual they are most comfortable socially with is already in the room. Bales and Curry reducing their number of vocalizations when in experimental trials with each other is consistent with the strength of female social relationships among rhesus macaques, and the idea that the vocalizations are intended to address individuals not present in the room.

Further substantiating these ideas is the fact that, among the males, Bales vocalized least frequently when in experimental trials with Oskar. This is interesting because Bales’ enclosure faces Oskar’s, which may have made her more comfortable with Oskar socially. However, the same pattern is not observed in the Curry’s vocalization counts, despite the fact that Curry shares

the enclosure with Bales that faces Oskar. While it seems likely that the social relationships between females had the effect of reducing the number of vocalizations produced, it is unclear whether familiarity between females and males actually affected female the vocalization rate.

Another interesting observation is the fact that the females vocalized more frequently when they were acting as M2s than when they were acting as M1s. One explanation for this trend may lie in the experimental procedures. Because M1s sat through several experimental trials in a row, they were seated in the experimental trial room for long periods of time. On the other hand, M2s only sat through the one experimental trial at a time. As a result, M2s had less time to become accustomed to the room, and may have been more anxious as a result. This difference could explain why M2s were vocalizing more frequently. Researchers have noted that vocalizations can be a sign of anxiety in nonhuman primates. Coleman & Pierre (2014:335) note, “vocalizations may also indicate anxiety: young macaques often “coo” when separated from their mother.” The significant increase in average number of vocalizations in M2s when compared to M1s could be the result of M2s being more anxious due to less continuous time in the experimental trial room.

The M2 data are also of interest because significant patterns that were observed in the M1 data are not significant within this set. For example, there is no significant difference between the vocalization averages when the M2 is facing a male M1 versus facing a female M1. Again, this outcome may be related to the increased anxiety the M2s may feel, as they have less time to acclimate to the new environment when they enter the room for the experimental trials. This arrangement could lead to a higher overall vocalization rate, which does not discriminate based on the sex of the M1 being faced.

This lack of correlation could also be attributed to the M2s not being administered any kind of treatment through the nebulizer. Analysis revealed a significant increase in the number of vocalizations produced when comparing the M1s who were administered saline to those administered oxytocin or vasopressin, as well as a difference in the vocalization averages of female M1s based on the sex of the M2 they were facing. Evaluation of the data also revealed a significant interaction effect, suggesting that there is some connection between these observations. Perhaps then, the M2 data shows a lack of significant difference because they were not administered any nebulizer treatment, which had a significant impact on the results of the female M1 data.

It is worth discussing the impact of the neuropeptide treatments on the vocalization rates further. Vasopressin and oxytocin treatments produced significantly higher vocalization rates than the saline treatments in female M1s. This finding makes sense in light of the way that both oxytocin and vasopressin have been implicated in the forming and maintaining of social relationships across a variety of species. As Lim & Young (2006:507) describe, “Both oxytocin and vasopressin are heavily involved at each of the conceptual levels of social bonding: The initial approach and affiliation, the recognition of social cues required for individual recognition, and finally the formation of the bond itself.” This is true not only in primates like humans and rhesus macaques, but also other mammals like mice and rats, as well as some birds and fish (Engelmann et al., 2000; Storm & Tecott, 2005; Goodson & Bass, 1999; DeVries & Panzica, 2006).

Despite the evidence suggesting that oxytocin and vasopressin are involved in these social behaviors, the categorization of these neuropeptides as strictly promoting prosocial behaviors remains controversial. Chang et al. (2012) found that oxytocin, when administered through a

pediatric nebulizer, only increased rhesus macaques' tendency to reward conspecifics only when the alternative was to reward no one. In fact, in trials where there was an option to reward themselves, inhalation of oxytocin increased rhesus macaques' tendency towards selfish choices. As a result, "OT does not appear to have a universal prosocial influence on behavior, but rather amplifies ongoing social information processing, perhaps by influencing already existing preferences" (Chang et al., 2012:962).

Other work done with human subjects also seems to indicate a more complex relationship between these neuropeptides and prosocial behaviors. A study done by DeVries et al. (2011) found that administering oxytocin to humans increased in-group favoritism, as well as out-group derogation. This work then also suggested that oxytocin seems to merely reinforce existing predispositions towards sociality rather than indiscriminately promoting these prosocial behaviors. Furthermore, Bartz et al. (2011) advocate for an understanding of oxytocin's impact on social behavior that more strongly considers contextual factors, like preexisting social relationships, and environments. They suggest, "that the view of exogenous oxytocin as broadly and invariantly improving social cognition or prosocial behavior is incorrect, and could impede progress in understanding the function and potential utility of it in treatment" (Bartz et al., 2011:307).

There is a large body of research about how oxytocin and vasopressin influence various social behaviors, yet the literature elucidating how these neuropeptides impact vocal communication remains limited. Considering the social importance of vocal communication, and specifically the "coo", in rhesus macaques, as well as the significant correlation found between the administration of these neuropeptides and vocalization rates in this study, perhaps more work should be done to further elucidate the nature of this relationship. Vocalizations are more easily

quantifiable than other measures of sociality, and could provide insight into whether oxytocin and vasopressin strictly promote prosocial behavior. While the exact role of oxytocin and vasopressin in social behavior has not yet been illuminated, this work seems to indicate that the neuropeptides increased the rate of vocalization among female rhesus macaques. Taking this work further to gain a better understanding of how oxytocin and vasopressin influence nonhuman primate vocalization could add crucial perspective to this debate.

Regardless of this discussion, vasopressin seemed to have an even greater effect on the number of vocalizations than did oxytocin. This could be due to the number and distribution of oxytocin versus vasopressin receptors in the rhesus macaque brain. While oxytocin and vasopressin are highly conserved evolutionarily, the distribution of their receptors across the animal kingdom has been shown to vary quite a bit (Freeman & Young, 2016). In an analysis done on 25 primate and rodent species, Babb et al. (2015) found that there were significant differences both in the genes coding for the oxytocin receptor OXTR as well as its resulting structure of the receptor itself. The neurogeography of OXTR, as well as the vasopressin receptor AVPR1a, have been mapped in a variety of animals. For example, in rodents OXTRs are distributed along cortical structures that play a role in olfactory behavior like anogenital sniffing and sent marking (Freeman & Young, 2016). Contrastingly, in primates, OXTRs are more densely present in subcortical regions involved in visual processing (Freeman et al., 2014). Cortical regions of the brain are largely implicated in social behaviors, like vocalization. In fact, Eliades & Wang (2008) found that cortical neurons are involved in complex feedback mechanisms when marmoset monkeys (*Callithrix jacchus*) both hear and produce vocalizations.

Therefore, this lack of OXTR density in the cortex could play a role in understanding why oxytocin did not have as much of an effect as vasopressin (Freeman et al., 2014). This idea is

further substantiated by the fact that, “the rhesus macaque brain in general exhibits extremely sparse OXTR expression relative to AVPR1a, with most dense OXTR binding seen in the nucleus basalis and superficial gray layer of the superior colliculus” (Freeman & Young, 2016:8). In other words, among rhesus macaques, the distribution of AVPR1a is far less restricted than the distribution of OXTR. Based on this research, the relative number of oxytocin and vasopressin receptors could be key in explaining the differential effects of these neuropeptides on vocalization frequency.

## **Conclusion**

Rhesus macaques are the most widely studied nonhuman primate, yet major questions about aspects of their behavior remain unanswered. For example, major work has yet to be done aiming to understand the nature of rhesus macaque communication across modalities. While the rhesus macaque vocal repertoire has been catalogued, research has yet to elucidate key questions about how they use these vocalizations, and what biological and social factors may influence their use.

This study revealed significant differences in the vocalization rate of female rhesus macaques based on both the sex of other conspecifics in their immediate environment, as well as the administration of oxytocin, and vasopressin through a pediatric nebulizer. Despite the fact that these results present interesting preliminary findings, the ability to make greater generalizations based on these findings is limited by the sample size. With only four female rhesus macaques in the sample, and only three of them vocalizing at all, it cannot be said that this sample population is well representative of rhesus macaques as a whole. Furthermore, the sample is limited by the artificiality of the experimental setting, as well as the fact that these monkeys have been raised in

a laboratory setting. These factors likely limit expression of certain social behaviors that would be more readily expressed in free or semi-free environments.

Regardless of these limitations, the results of this work are valuable, as they indicate potentially promising results for further research conducted on these subjects. Particularly, understanding likely connections between rhesus macaque vocalizations and oxytocin and vasopressin could have the dual value of revealing more information about the nature of vocal communication itself, as well as the role of these neuropeptides in promoting social behavior more broadly. Understanding the neurological underpinnings of vocal communication in rhesus macaques has the potential to aid in the uncovering of biological adaptations that possibly predisposed primates to developing more complex forms of communication, like human language use. Further research on the relationship between oxytocin, vasopressin and rhesus macaque vocalizations could also help in apprehending the exact role of these neuropeptides in the promotion of prosocial behaviors, which remains controversial in the current literature.

Adult female rhesus macaque vocalizations seem to be influenced by a variety of social, environmental, and biological factors. Elucidating the nature of these correlations in future research could significantly contribute to developing a greater understanding of primate communication and the broader role of neuropeptides in sociality.

## References Cited

- Babb, P. L., Fernandez-Duque, E. & Schurr, T. G., 2015, 'Oxytocin receptor gene sequences in owl monkeys and other primates show remarkable interspecific regulatory and protein coding variation', *Molecular Phylogenetics and Evolution* 91, 160-177.
- Bartz, J. A., Zaki, J., Bolger, N. & Ochsner, K. N., 2011, 'Social effects of oxytocin in humans: context and person matter', *Trends in Cognitive Sciences* 15(7), 301-309.
- Bernstein, I. S. & Ehardt, C. L., 1985, 'Age-Sex Differences in the Expression of Agonistic Behavior in Rhesus Monkey (*Macaca mulatta*) Groups', *Journal of Comparative Psychology* 99(2), 115-132.
- Bosch, O. J., Meddle, S. L., Beiderbeck, D. I. Douglas, A. J. & Neumann, I. D., 2005, 'Brain oxytocin correlates with maternal aggression: Link to anxiety', *Journal of Neurosciences* 25, 6807-6815.
- Chang, S. W. C., Winecoff, A. A. & Platt, M. L., 2011, 'Vicarious reinforcement in rhesus macaques', *Frontiers in Neuroscience* 5, Article 27.
- Chang, S. W., Barter, J. W., Ebitz, R. B., Watson, K.K. & Platt M. L., 2012, 'Inhaled oxytocin amplifies both vicarious reinforcement and self reinforcement in rhesus macaques (*Macaca mulatta*)', *Proceedings of the National Academy of Sciences* 109(3), 959-964.
- Chang, S. W. C. & Platt, M. L., 2014, 'Oxytocin and social cognition in rhesus macaques: Implications for understanding and treating human psychopathology', *Brain Research* 1580, 57-68.
- Coleman, K. & Pierre, P. J., 2014, 'Assessing Anxiety in Nonhuman Primates' *ILAR Journal* 55(2), 333-346.
- Cook, M. & Mineka, S., 1989, 'Observational conditioning of fear to fear-relevant versus fear-irrelevant stimuli in rhesus monkeys', *Journal of Abnormal Psychology* 98(4), 448-459.
- De Dreu, C. K. W., Greer, L. L., Van Kleef, G. A., Shalvi, S. & Handgraaf, M. J. J., 2011, 'Oxytocin promotes human ethnocentrism', *PNAS* 108(4), 1262-1266.
- De Dreu, C. K. W., Kret, M. E. & Sauter, D. A., 2016, 'Assessing Emotional Vocalizations From Cultural In-Group and Out-Group Depends on Oxytocin', *Social Psychological and Personality Science* 7(8), 837-846.
- DeVries, G.J. & Panzica, G. C., 2006, 'Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: different mechanisms, similar endpoints', *Neuroscience* 138, 236-254.

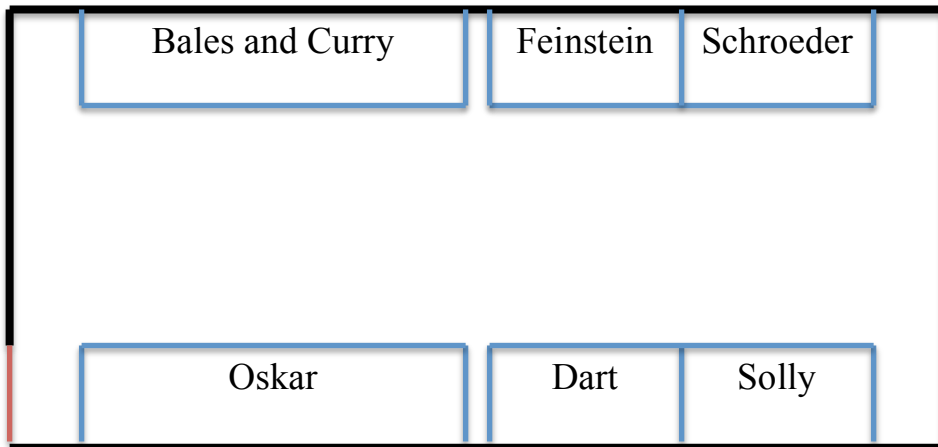


- Donaldson, Z. R. & Young, L. J., 2008, 'Oxytocin, Vasopressin, and the Neurogenetics of Sociality', *Science* 322(5903), 900-904.
- Dunbar, R. I. M., 2003, 'The Origin and Subsequent Evolution of Language', in M. H. Christiansen & S. Kirby (eds.), *Language Evolution*, pp. 219-234. Oxford University Press, New York.
- Ebitz, R. B., Watson, K. K. & Platt M. L., 2013, 'Oxytocin blunts social vigilance in the rhesus macaque', *Proceedings of the National Academy of Sciences* 110(28):11630-11635.
- Eliades, S. J. & Wang, X., 2008, 'Neural substrates of vocalizations feedback monitoring in primate auditory cortex', *Nature* 453, 1102-1106.
- Engelmann, M., Wotjak, C. T., Ebner, K. & Landgraf, R., 2000, 'Behavioural impact of intraseptally released vasopressin and oxytocin in rats', *Experimental Physiology* 85, 125S-130S.
- Erwin, J. & Mitchell, G., 1973, 'Analysis of Rhesus Monkey Vocalizations : Maturation-related Sex Differences in Clear-call Frequency', *American Journal of Physical Anthropology* 38(2), 463-467.
- Feldman, R., Gordon, I., Schneiderman, I., Weisman, O. & Zagoory-Sharon, O., 2010, 'Natural variations in maternal and paternal care are associated with systematic changes in oxytocin following parent–infant contact', *Psychoneuroendocrinology* 35(8), 1133-1141.
- Ferrari, P. F., Paukner, A., Ruggiero, A., Darcey, L., Unbehagen, S. & Suomi, S. J., 2009, 'Interindividual Differences in Neonatal Imitation and the Development of Action Chains in Rhesus Macaques', *Child Development* 80(4), 1057-1068.
- Freeberg, T. M., Dunbar, R. I. M. & Ord, T. J., 2012, 'Introduction: Social complexity as a proximate and ultimate factor in communicative complexity', *Philosophical Transactions of the Royal Society B* 367(1597), 1785-1801.
- Freeman, S. M., Inoue, K., Smith, A. L., Goodman, M. M., Young, L. J., 2014, 'The neuroanatomical distribution in the male rhesus macaque (*Macaca mulatta*)', *Psychoneuroendocrinology* 45, 128-141.
- Freeman, S. M. & Young, L. J., 2016, 'Comparative Perspectives on Oxytocin and Vasopressin Receptor Research in Rodents and Primates: Translational Implications', *Journal of Neuroendocrinology* 28(4).
- Frith, C. D. & Frith, U., 2007, 'Social Cognition in humans', *Current Biology* 17(16), 724-732.
- Fitch, W. C., 1997, 'Vocal tract length and formant dispersion correlate with body size in rhesus macaques', *Journal of the Acoustical Society of America* 102(2), 1213-1222.

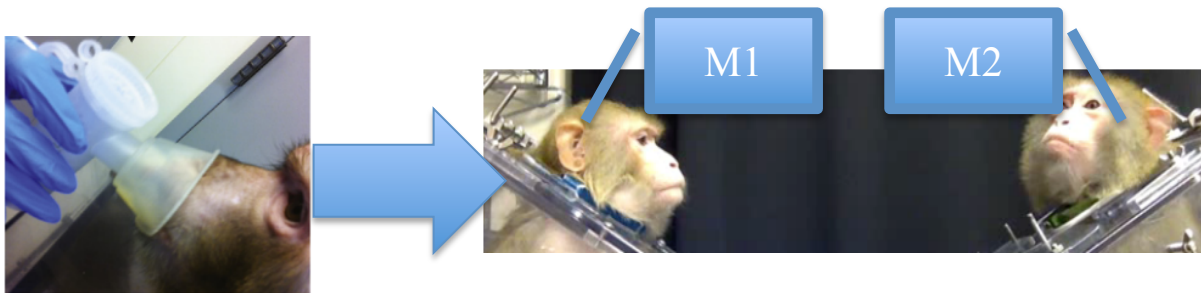
- Flombaum, J. I. & Santos, L. R., 2005, 'Rhesus Monkeys Attribute Perceptions to Others', *Current Biology* 15(5), 447-452.
- Goodson, J. L. & Bass A. H., 2001, 'Social behavior functions and related anatomical characteristics of vasotocin/ vasopressin systems in vertebrates', *Brain Research Review* 35, 246-265.
- Gouzoules, S., Gouzoules, H. & Marler, P., 1984, 'Rhesus monkey (*Macaca mulatta*) screams: representational signaling in the recruitment of agonistic aid', *Animal Behavior* 32(1), 182-193.
- Hauser, M. D., 1992, 'Articulatory and social factors influence the acoustic structure of rhesus monkey vocalizations: A learned mode of production?' *Journal of the Acoustical Society of America* 91(4), 2175-2179.
- Hauser, M. D. & Marler, P., 1993, 'Food-associated calls in rhesus macaques (*Macaca mulatta*): I. Socioecological factors', *Behavioral Ecology* 4(3), 194-205.
- Lim, M. M. & Young, L. J., 2006, 'Neuropeptidergic regulation of affiliative behavior and social bonding in animals' *Hormones and Behavior* 51(2), 506-517.
- Maestriperi, D., 2007, *Macchiavellian Intelligence: How Rhesus Macaques and Humans Have Conquered the World*, University of Chicago Press, Chicago.
- Marlin, B. J., Miltre, M., D'amour, J. A., Chao, M. V. & Froemke, R. C., 2015, 'Oxytocin enables maternal behavior by balancing cortical inhibition', *Nature*, 520, 499-503.
- Newman, J. D. & Symmes, D., 1974, 'Vocal Pathology in Socially Deprived Monkeys', *Developmental Psychobiology* 7(4), 351-358.
- Owren, M. J., Dieter, J. A., Seyfarth, R. M. & Cheney, D. L., 1993, 'Vocalizations of Rhesus (*Macaca mulatta*) and Japanese (*M. fuscata*) Macaques Cross-Fostered between Species Show Evidence of Only Limited Modification', *Developmental Psychology* 26(7), 389-406.
- Riem, M. M. E., Bakermans-Kranenburg, M. J., Pieper, S., Tops, M., Boksem, M. A. S., Vermeiren, R. R. J. M., van IJzendoorn, M. H. & Rombouts, S. A. R. B., 2011, 'Oxytocin Modulates Amygdala, Insula, and Inferior Frontal Gyrus Responses to Infant Crying: A Randomized Controlled Trial', *Biological Psychiatry* 70, 291-297.
- Rowell, T. E. & Hinde, R. A., 1962, 'Vocal communication by the rhesus monkey (*Macaca mulatta*)' *Proceedings of the Zoological Society of London* 138(2), 279-294.
- Seltzer, L. J., Zeigler, T. E. & Pollak, S. D., 2010, 'Social vocalizations can release oxytocin in humans', *Proceedings of the Royal Society B—Biological Sciences*, 277(1694), 2661-2666.

- Sliwa, J., Duhamel, J., Pascalis, O. & Wirth, S., 2011, 'Spontaneous voice-face identity matching by rhesus monkeys for familiar conspecifics and humans', *PNAS* 108(4), 1735-1740.
- Storm, E. E. & Tecott, L. H., 2005, 'Social circuits: peptidergic regulation of mammalian social behavior', *Neuron* 47, 483-486.
- Sutton, D., Larson, C., Taylor, E. M. & Lindeman, R. C., 1973, 'Vocalization in Rhesus Monkeys: Conditionability', *Brain Research* 52, 225-231.
- Thierry, B., Iwaniuk, A. N., Pellis, S. M., 2000, 'The Influence of Phylogeny on the Social Behaviour of Macaques (Primates: Cercopithecidae, genus *Macaca*)', *International Journal of Behavioural Biology: Ethology* 106(8), 713-728.
- Thierry, B., 2007, 'Unity in Diversity: Lessons From Macaque Societies', *Evolutionary Anthropology*, 16, 224-238.
- Tomaszycki, M. L., Davis, J. E., Gouzoules, H. & Wallen, K., 2001, 'Sex Differences in Infant Rhesus Macaque Separation-Rejection Vocalizations and Effects of Prenatal Androgens', *Hormones and Behavior* 39(4), 267-276.
- Wallen, K., 1996, 'Nature Needs Nurture: The Interaction of Hormonal and Social Influences on the Development of Behavioral Sex Differences in Rhesus Monkeys', *Hormones and Behavior* 30(4), 364-378.
- Washburn, D. A., Smith, J. D. & Shields, W. E., 2006, 'Rhesus Monkeys (*Macaca mulatta*) Immediately Generalize the *Uncertain* Response' *Journal of Experimental Psychology: Animal Behavior Processes* 32(2), 185-189.
- Wittig, R. M., Crockford, C., Descher, T., Langergraber, K. E., Zeigler, T. E. & Zuberbuhler, K., 2014, 'Food sharing is linked to urinary oxytocin levels and bonding in related and unrelated wild chimpanzees', *Proceedings of the Royal Society B—Biological Sciences*, 281(1778), 1-10.

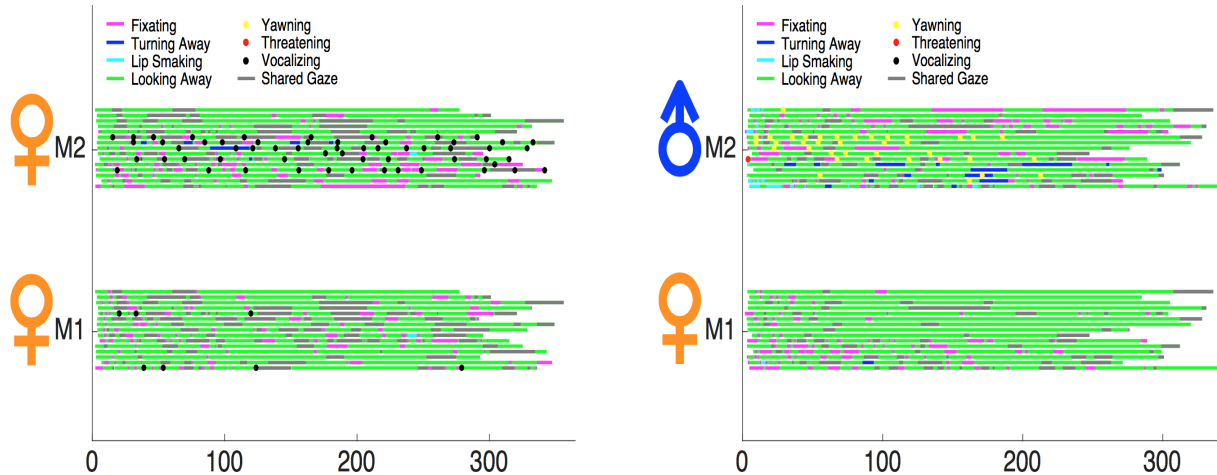
## Tables and Figures



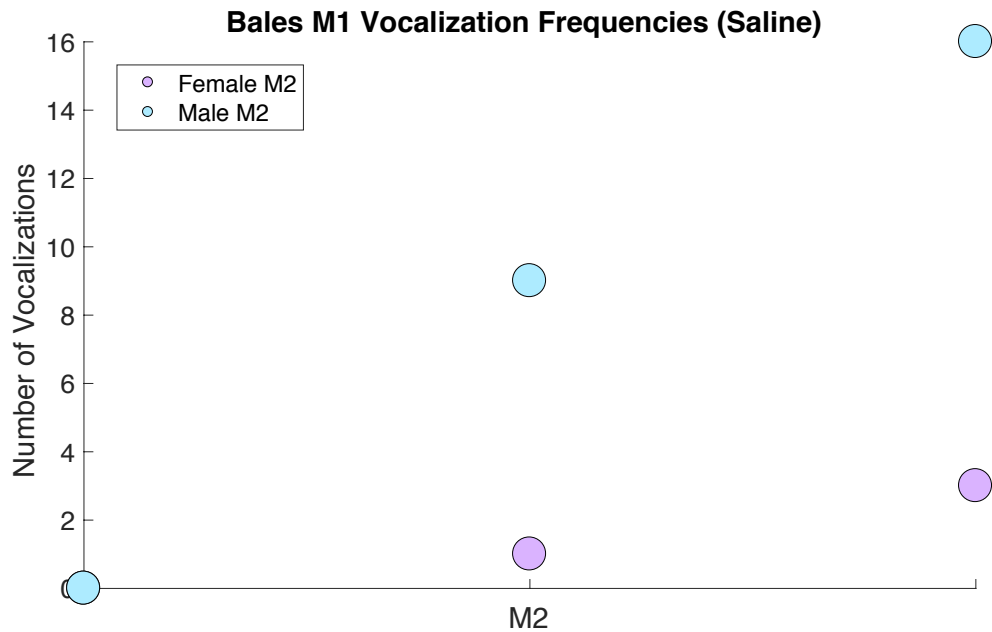
**Figure 1:** Diagram of the enclosure where the monkeys participating in the experiment are regularly held. Each box, outlined in blue, represents a separate, individual cage. Bales and Curry are housed together. The red line on the left represents the door, where researchers and caretakers frequently enter and exit. Not drawn to scale.



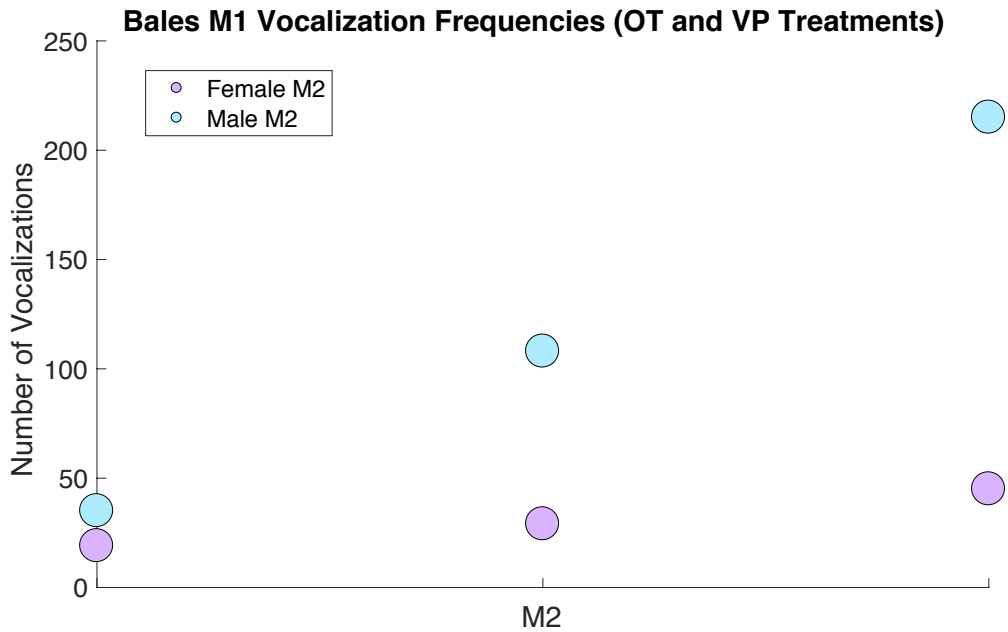
**Figure 2:** Picture demonstrating a rhesus macaque being administered its treatment via the pediatric nebulizer (left). Picture of the experimental set up with M1 and M2 monkeys labelled (right). Photo credit: Chang & Platt, 2014, and Yaoguang Jiang.



**Figure 3:** Sample ethogram displaying how behavioral data was coded and transformed during the data analysis process. Vocalizations are indicated by black dots.



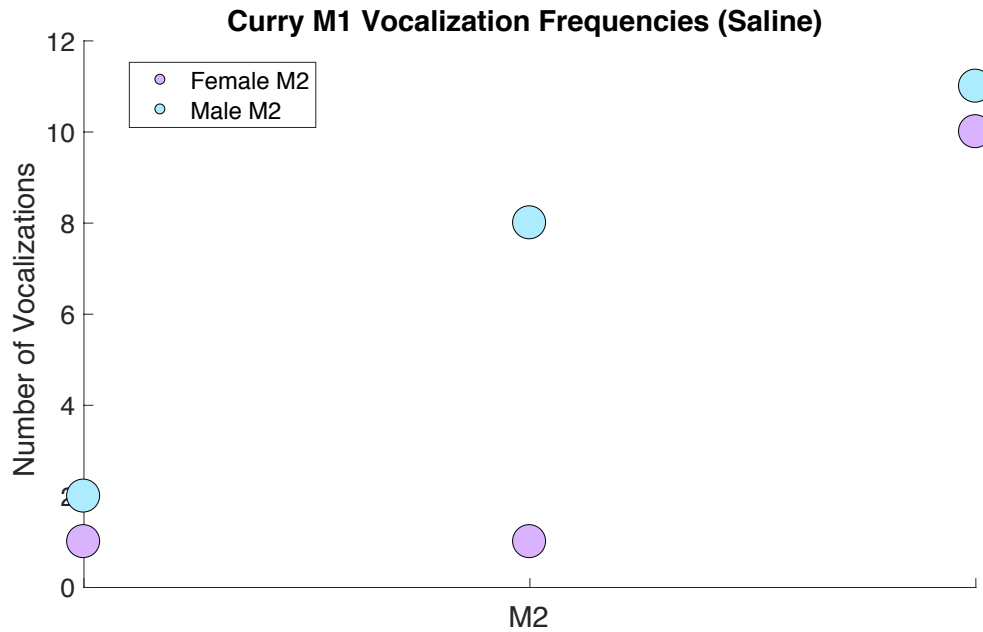
**Figure 4:** Graph showing Bales' vocalization frequencies as M1 after being administered the saline control treatment. Data points are sums broken down by the M2 Bales was facing.



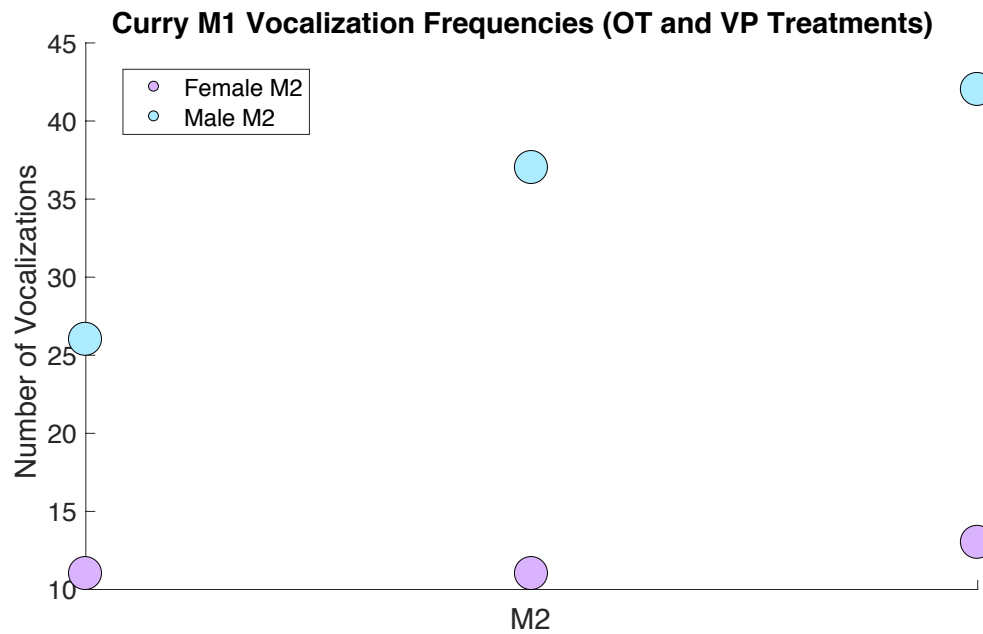
**Figure 5:** Graph showing Bales’ vocalization frequencies as M1 after being administered either the oxytocin (OT) or vasopressin (VP) treatment. Data points are sums broken down by the M2 Bales was facing.

Bales				
	<b>Saline</b>			
	Female M2	Curry, 0	Feinstein, 1	Schroeder, 3
	Male M2	Oskar, 0	Solly, 9	Dart, 16
	<b>Treatment (OT and VP)</b>			
	Female M2	Curry, 19	Feinstein, 29	Schroeder, 45
	Male M2	Oskar, 35	Solly, 108	Dart, 215

**Table 1:** Table showing raw data sums of Bales’ vocalization frequencies after being administered the saline control treatment, or either the oxytocin (OT) or vasopressin (VP) treatment. Data is broken down by the M2 Bales was facing. *Note:* Saline and Treatment numbers should not be compared directly, as the Treatment totals represent the sums of double the number of trials than the Saline sums (i.e. Treatment  $n$  is double Saline  $n$ ).



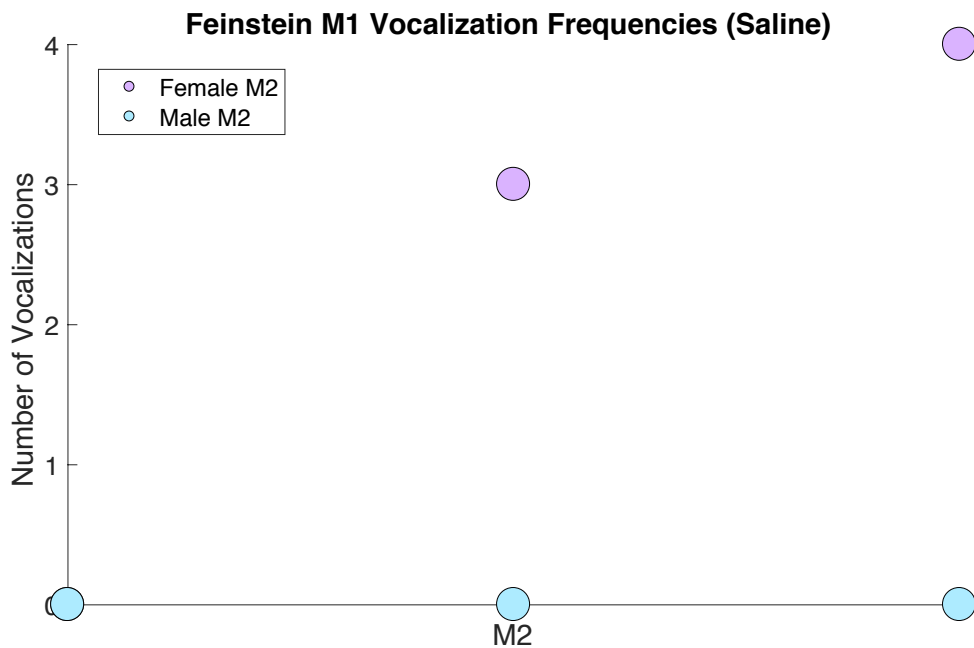
**Figure 6:** Graph showing Curry’s vocalization frequencies as M1 after being administered the saline control treatment. Data points are sums broken down by the M2 Curry was facing.



**Figure 7:** Graph showing Curry’s vocalization frequencies as M1 after being administered either the oxytocin (OT) or vasopressin (VP) treatment points are sums broken down by the M2 Curry was facing.

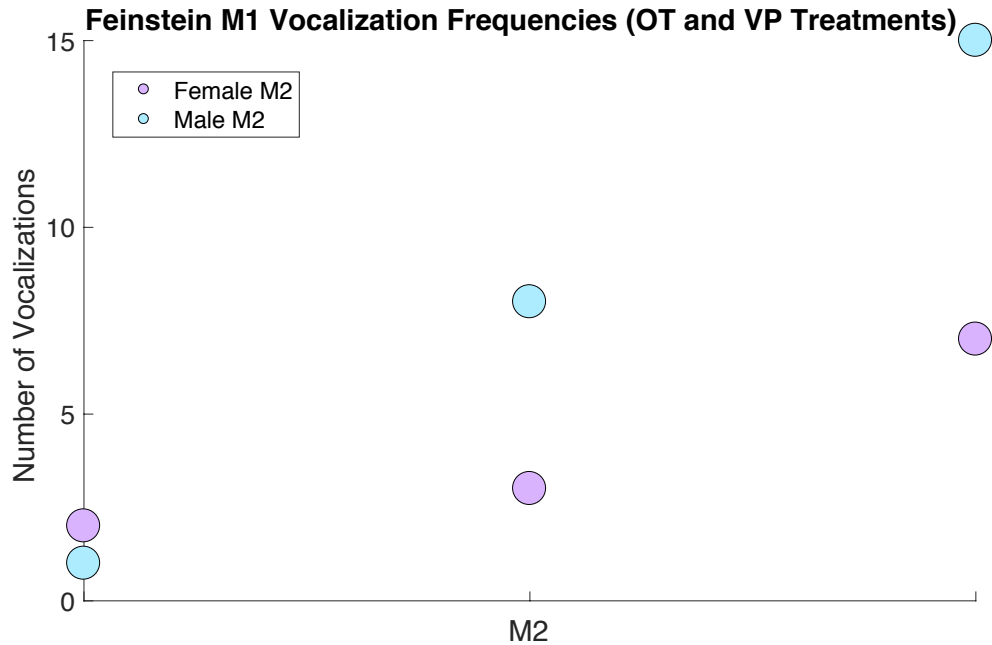
Curry				
<b>Saline</b>				
Female M2	Bales, 1	Schroeder, 1	Feinstein, 10	
Male M2	Solly, 2	Dart, 8	Oskar, 11	
<b>Treatment (OT and VP)</b>				
Female M2	Bales, 11	Schroeder, 11	Feinstein, 13	
Male M2	Solly, 26	Oskar, 37	Dart, 42	

**Table 2:** Table showing raw data sums of Curry’s vocalization frequencies after being administered the saline control treatment, or either the oxytocin (OT) or vasopressin (VP) treatment. Data is broken down by the M2 Curry was facing. *Note:* Saline and Treatment numbers should not be compared directly, as the Treatment totals represent the sums of double the number of trials than the Saline sums (i.e. Treatment  $n$  is double Saline  $n$ ).



**Figure 8:** Graph showing Feinstein’s vocalization frequencies as M1 after being administered the saline control treatment. Data points are sums broken down by the M2 Feinstein was facing.

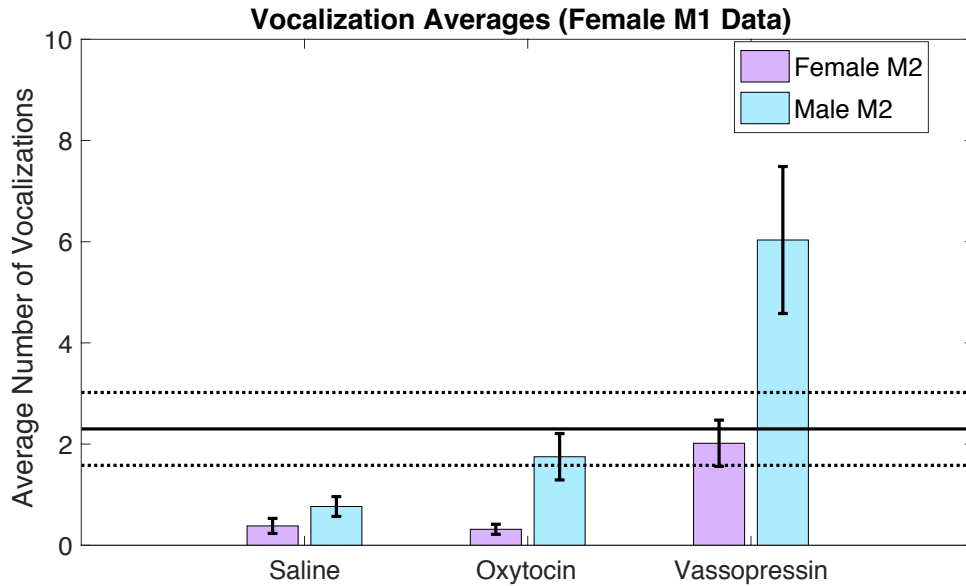




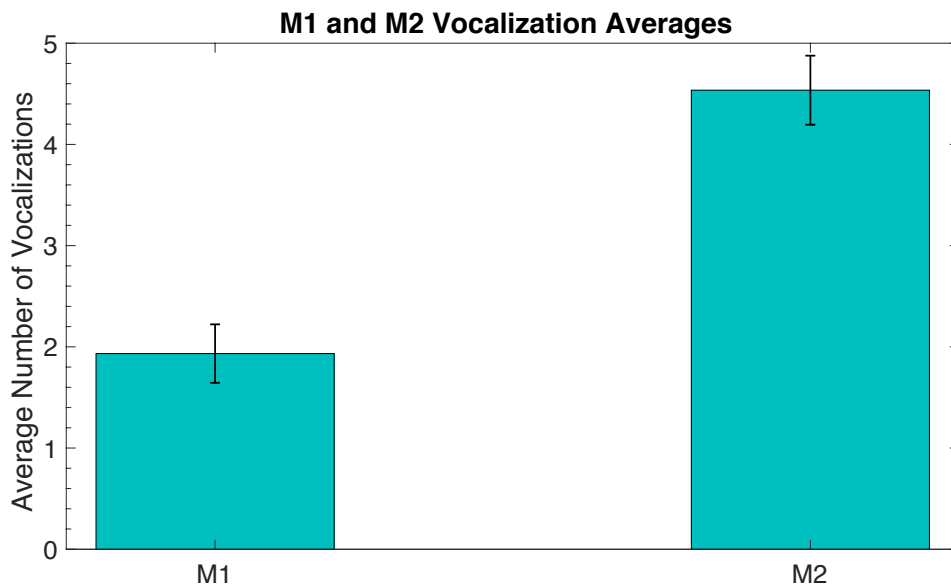
**Figure 9:** Graph showing Feinstein’s vocalization frequencies as M1 after being administered either the oxytocin (OT) or vasopressin (VP) treatment. Data points are sums broken down by the M2 Feinstein was facing.

Feinstein				
	<b>Saline</b>			
	Female M2	Curry, 0	Schroeder, 3	Bales, 4
	Male M2	Dart, 0	Oskar, 0	Solly, 0
	<b>Treatment</b>			
	Female M2	Bales, 2	Curry, 3	Schroeder, 7
	Male M2	Solly, 1	Oskar, 8	Dart, 15

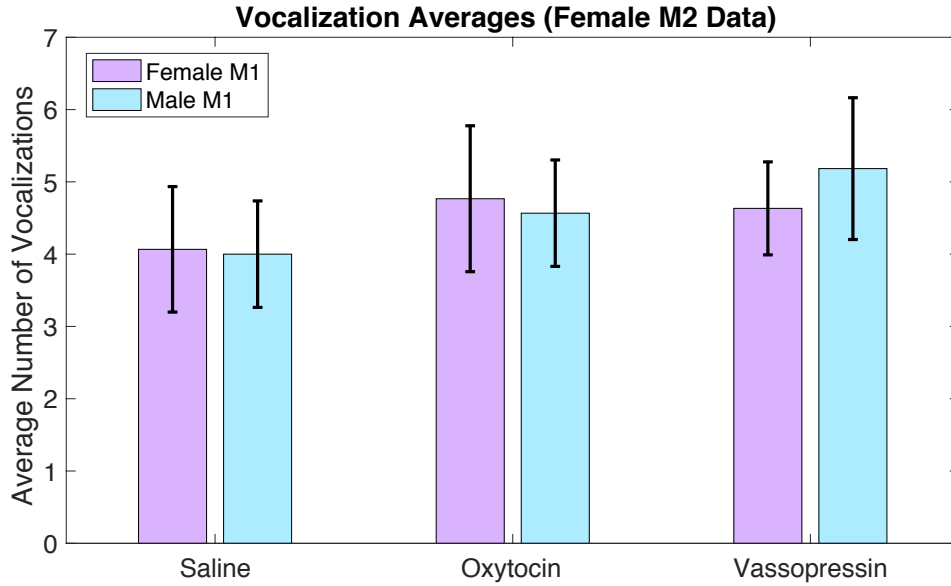
**Table 3:** Table showing raw data sums of Feinstein’s vocalization frequencies after being administered the saline control treatment, or either the oxytocin (OT) or vasopressin (VP) treatment. Data is broken down by the M2 Feinstein was facing. *Note:* Saline and Treatment numbers should not be compared directly, as the Treatment totals represent the sums of double the number of trials than the Saline sums (i.e. Treatment  $n$  is double Saline  $n$ ).



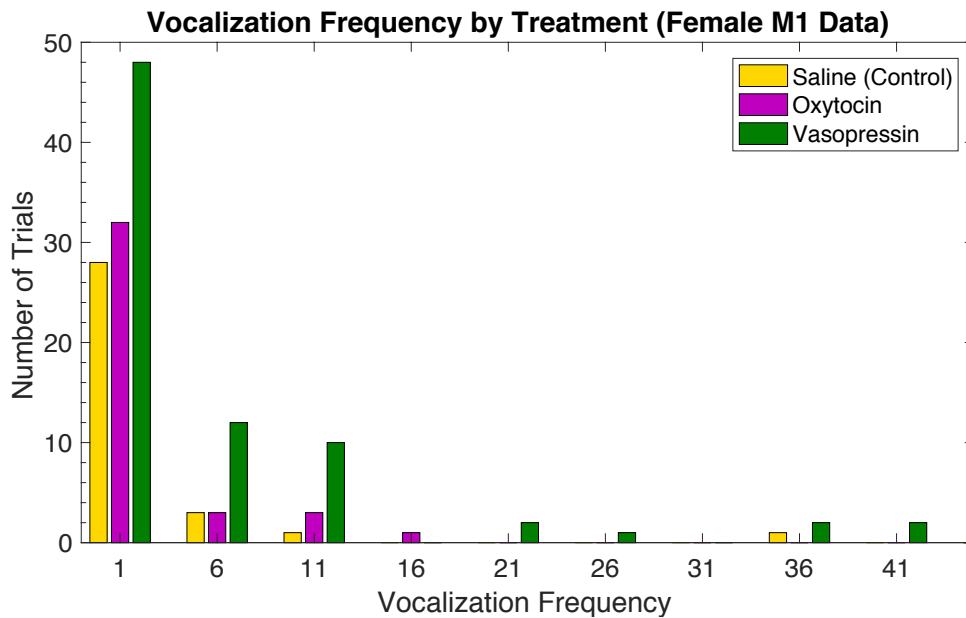
**Figure 10:** Graph showing the average number of vocalizations of all female M1s (Bales, Curry, Feinstein, and Schroeder) broken down by the treatment and the sex of M2. Error bars represent the standard error of the mean. The solid black line ( $y=2.3$ ) represents the average number of vocalizations during the empty chair control trials (all treatments included). The dotted lines represent the empty chair average plus or minus one standard error of the mean ( $y=1.58$ ,  $y=3.02$ ).



**Figure 11:** Graph showing the average of all vocalizations of Bales, Curry, Feinstein, and Schroeder as M1, and the average of all vocalizations of Bales, Curry, Feinstein, and Schroeder as M2. Error bars represent the standard error of the mean. *Note:* Vocalizations facing the empty chair as a control are excluded from the M1 sum, as there is not

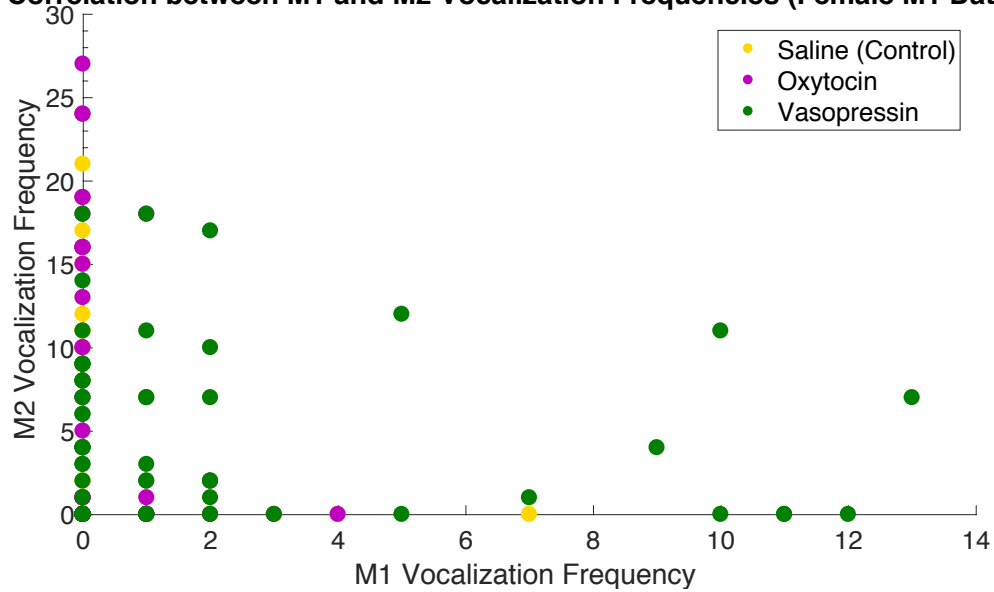


**Figure 12:** Graph showing the average number of vocalizations of all female M2s (Bales, Curry, Feinstein, and Schroeder) broken down by the treatment and the sex of M1. Error bars represent the standard error of the mean.

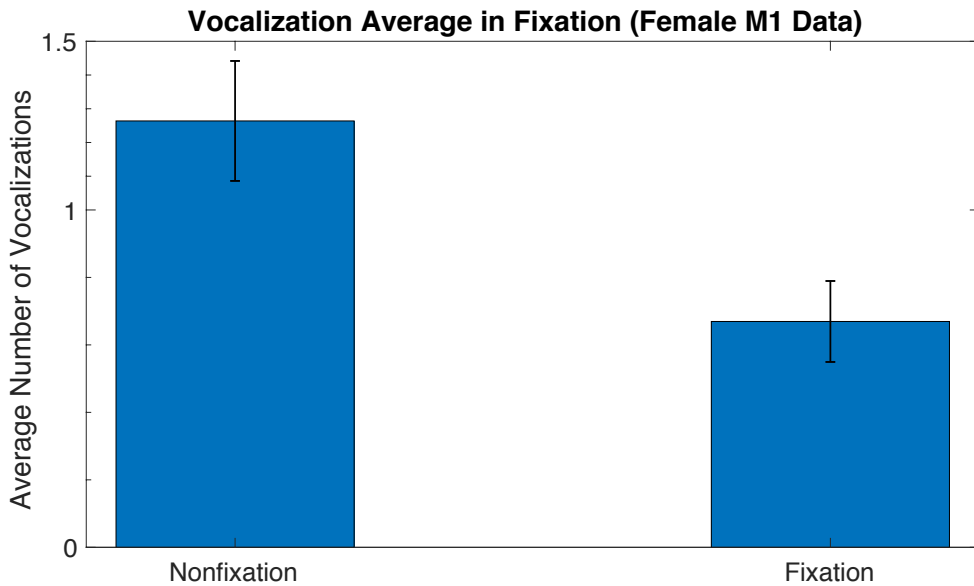


**Figure 13:** Histogram showing the number of trials relative to certain vocalization frequencies broken down by treatment for female M1s. *Note:* trials with zero vocalizations were not included in this representation.

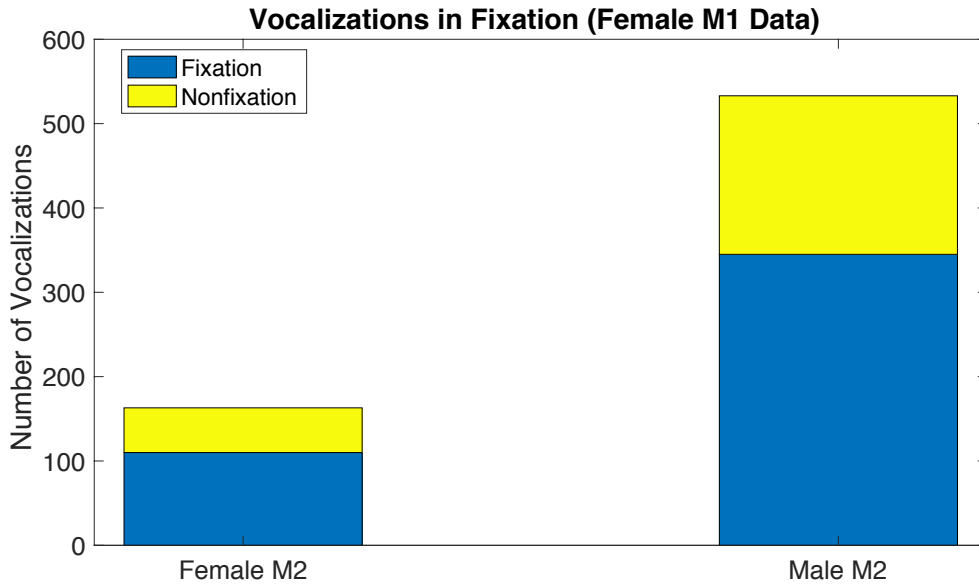
**Correlation between M1 and M2 Vocalization Frequencies (Female M1 Data)**



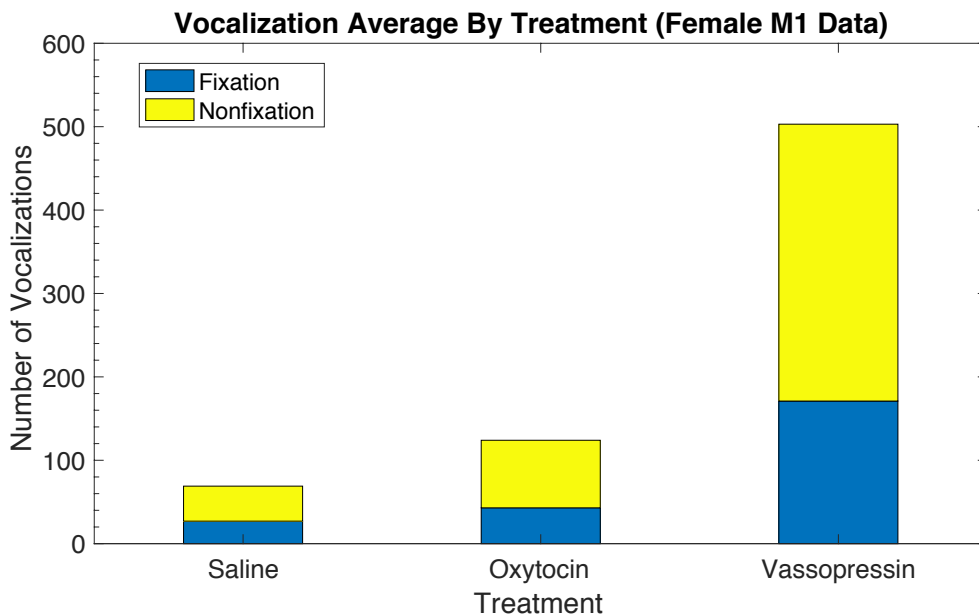
**Figure 14:** Plot showing the correlation between the M1 and M2 vocalization frequency broken down by treatment. Each dot represents one experimental trial session.



**Figure 15:** Graph showing the average of all vocalizations of Bales, Curry, Feinstein and Schroeder while not in fixation, and the average of all vocalizations of Bales, Curry, Feinstein, and Schroeder while in fixation. Error bars represent standard error of the mean.



**Figure 16:** Graph showing the proportion of female M1 vocalizations produced while in fixation against the proportion that were produced while not in fixation broken down by the sex of the M2. The data represented are sums.



**Figure 17:** Graph showing the proportion of female M1 vocalizations produced while in fixation against the proportion that were produced while not in fixation broken down by the treatment administered to the M1. The data represented are sums.