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Sean Walter Kelley April 15, 2016

The Effects of Melanotan II on the Social Network Properties in a Group of Male Rhesus Macaques: A Pilot Study

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Abstract

The Effects of Melanotan II on the Social Network Properties in a Group of Male Rhesus Macaques: A Pilot Study

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Oxytocin (OT) is associated with a wide variety of prosocial behaviors including empathy, trust, cooperation, eye gaze and social vigilance. Most current research utilizes either intranasal or aerosolized OT, however it is unknown whether intranasal OT can cross the blood brain barrier. Melanotan II (MTII) is a non-selective melanocortin 4 receptor agonist that can potentially circumvent this problem by increasing endogenous OT in the brain. Our study looked at the effects of MTII (1 mg/kg) on the social network structure of a group of ten male rhesus macaques (*Macaca mulatta*) over two weeks. During the first week, half of the group received MTII and the other half received a placebo; the treatments were reversed the following week. In the third week no treatment was administered in order to establish a baseline social network. We collected a total of 5 hours of MTII/placebo data and 4.5 hours of baseline data. Coders used simultaneous focal sampling to record all instances of proximity and grooming behaviors for each monkey, which were then used to construct social networks. Using social network analysis (SNA), we examined the effects of MTI on four social network metrics: betweenness centrality, closeness centrality, eigenvector centrality and the clustering coefficient. MTII caused a significant increase in the closeness centrality as well as a significant decrease in the frequency of grooming given. Consequently, MTII monkeys were more likely to receive grooming from a greater number of monkeys and less likely to groom than placebo monkeys.

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Introduction

Autism is a disorder associated with deficits in social skills, communication and highly stereotyped behaviors (Baird et al., 2003). According to the Centers for Disease Control and Prevention (CDC) 1 in 68 children in the United States has an autism spectrum disorder (ASD); ASD is five times more prevalent in males than females. Although the diagnosis rate has increased over the last several decades there are currently no pharmacological treatments for the social impairments associated with ASD.

Oxytocin (OT) is a nonapeptide hormone produced in the paraventricular nucleus (PVN) and supraoptic nucleus of the hypothalamus. OT has been shown to be crucial for the formation of pair bonds in monogamous prairie voles due to increased OT receptors in the nucleus accumbens (NAc) and caudate putamen (CP) (Young and Wang, 2004). Administration of an OT antagonist into either the NAc or the CP blocks the formation of partner-preferences in female prairie voles. In humans, OT is primarily administered via an intranasal spray, but in nonhuman primates, it is commonly administered in aerosolized form via a nebulizer (Modi et al., 2013, Guastella et al, 2013). Studies in both human and non-human primates have linked oxytocin to increased prosocial behaviors (Madden et al., 2011; Crockford et al., 2013; Simpson et al., 2014) decreased social vigilance (Ebitz et al., 2013), attenuated attention to negative facial expressions (Parr et al., 2013) along with parochial altruism directed towards one's in-group at the expense of an out-group (De Dreu et al., 2010). OT is also crucial for uterine contraction during birth, as well as the production of milk during lactation (Soloff et al., 1979).

OT may be an effective treatment for people with autism by improving emotional recognition (Guastella et al., 2010; Domes et. al, 2007), decreasing the severity and number of different types of repetitive behaviors (Hollander et al., 2003) and increasing the frequency of

directed gaze towards the eye region (Auyeung et al., 2015). Furthermore, in individuals with high functioning autism, OT increased subjective feelings of trust and increased preference for a cooperative partner in a social decision game (Andari et al., 2010). Along with behavioral effects, OT also produces significant changes in neural activity related to social cognition. OT has been shown to significantly increase activity in the right anterior insula in individuals with ASD (Aoki et al., 2014). The right anterior insula may be involved with attention, salience and the experience of internal bodily states (Craig, 2009; Menon and Uddin 2010). Individuals with ASD have a hypoactivity in the anterior cingulate cortex and right anterior insula compared to neurotypical controls when preforming social cognition tasks (Martino et al., 2009). Along with neuroimaging data, increasing evidence supports the idea that polymorphisms in the OT receptor gene potentially contribute to the development of ASD.

A recent metanalysis found two single nucleotide polymorphisms (rs7632287 and rs2268491) to be significantly associated with social behavior and empathy in humans (LoParo and Waldman, 2015). In women but not men, rs7632287 is associated with pair-bonding, relationship quality, total autism spectrum score, social interactions and communication (Walum et al., 2012). However, although significant, the effect sizes for pair-bonding (d=0.13) and relationship quality (d=0.28) are weak, thus variation in the OT receptor gene does not dramatically effect social behaviors. Cntnap2 knockout (KO) mice, a mouse model of autism spectrum disorder, were treated with a Melancortin 4 Receptor (MC4R) selective agonist, Ro27-3225, and showed improvements in their social behavior (Peñagarikano et al., 2015). Blocking OT receptors using an OT antagonist eliminated the rescuing effect of the Ro27-3225. Consequently, OT is responsible for the improvements in social behavior in cntnap2 KO mice as

result of Ro27-3225 infusion. Consequently, while OT plays a clear role in social cognition it remains unclear whether the social impairments in ASD can be improved by OT treatment.

Along with humans, OT has been shown to play a prominent role in the social behavior of various non-human primates. Within the macaque brain, OT receptors are primarily located in the nucleus basalis of Meynert (NBM), pedunculopontine tegmental nucleus (PPT), the superficial gray layer of the superior colliculus, the trapezoid body, and the ventromedial hypothalamus (Freeman et al., 2014). Previous work in black-pencilled marmosets (*Callithrix pencillata*) showed that intranasal OT increased the frequency of initiation of huddling and close proximity between marmoset partners compared to a control condition (Smith et al., 2010). Marmosets treated with an OT receptor antagonist showed a decreased frequency of initiation for huddling and close proximity. Additionally, marmosets given OT approached their partners more quickly than in the control condition and were less likely to approach an empty cage. An intracerebroventricular (ICV) injection of 1µg OT increased the frequency of autogrooming in both dominant and subordinate male squirrel monkeys (Winslow and Insel, 1991). OT also increased the frequency of aggressive and associative behaviors, however these effects were restricted to the dominant males. These studies support the notion that oxytocin broadly enhances affiliative behaviors in New World primates.

Intranasal aerosolized OT spray, but not intranasal OT spray, has been shown to significantly elevate lumbar CSF levels of OT in adult male rhesus macaques (Modi et al., 2014). However, a recent review of the literature found that studies of intranasal OT in humans produced inconsistent behavioral effects (Walum et al., 2015), possibly because it is unclear how much of the administered intranasal OT crosses the blood-brain barrier and is able to reach brain regions where OT is hypothesized to have its effect (Leng and Ludwig, 2015). In particular,

intranasal OT studies frequently lack substantial statistical power, the probability of detecting an effect when it is present. Walum et al. (2015) showed that intranasal OT studies have a statistical power ranging from 12% in clinical trials to 16% in studies with healthy subjects. As a result, any positive results in these studies would fail to be replicated between 84% and 88% of the time. Additionally, these studies have a low positive predictive value indicating that most positive findings are likely to be false positives. Consequently, methodological improvements are necessary in order to ascertain the causal effects of increased exogenous OT on behavior.

Melanotan II (MTII) may be able to circumvent the problems associated with intranasal OT delivery and directly increase endogenous OT in the brain. MTII is a non-selective agonist for the MC4R associated with food intake, melanogenesis and sexual behavior (Martin and Macintyre, 2004). Recently, MTII has been used to investigate the relationship between the melanocortin system and social bonding in male and female prairie voles. Administration of MTII significantly increased huddling time in the partner preference test in female prairie voles both 6 and 24 hours after cohabitation but had no effect on male partner preference (Barrett et al., 2014). However, Modi et al. (2015) showed an increase in the huddling time in male prairie voles. Male voles also experienced significant decreases in aggressive behaviors compared to saline-injected controls driven by decreases in boxing and wrestling. MTII activates OT neurons in the PVN and also potentiates the release of OT in the nucleus accumbens (NAc), consequently MTII produces its prosocial effects via activation of the OT system (Modi et al., 2015). In another study both MTII and saline significantly activated CRF neurons in neonatal voles although only MTII lead to elevated corticosterone levels (Barrett et al., 2014). Administration of a selective MCR4 agonist into the rat medial amygdala decreased time spend in the opens arms of the elevated plus maze, decreased food intake and increased plasma corticosterone (Liu et al.,

2013). Consequently, the activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis along with anxiogenesis could mitigate any prosocial effects of MTII.

Macaques can potentially be used as a model organism to study the effects of MTII on social behavior and social interactions. Male rhesus macaques live in complex social groups characterized by both affiliative (proximity, grooming) and agonistic (aggressive) behaviors. Macaques have a strict matrilineal dominance hierarchy, inheritable by kin, with females remaining in their natal groups and males dispersing from the group before the onset of puberty. The complex social organization of macaques makes them an excellent model to test the efficacy of MTII at enhancing individual and group sociality.

Social Network Analysis (SNA) is a technique to measure the individual and group-level properties of a social network and is increasingly used to study animal behavior. Studies in animals have used SNA to understand the transmission of disease (Gomez et al., 2013), information transfer (Lusseau, 2007) and likelihood of survival (Stanton and Mann, 2012). Consequently, SNA is a refined and sophisticated method to examine the prosocial effects of MTII as a treatment for ASD. SNA has rarely been used in this context and represents a novel exploratory approach into understanding how MTII might affect behavior in a complex social environment. In order to assess these effects, the behaviors used to construct the social network need to occur frequently to provide enough data for a reliable SNA analysis. Networks can change over time, as a result of activity levels, fights etc., such that behaviors need to be measured reliably during the observation period. This is particularly important for studies such as ours that are designed to examine drug-treatment effects on behavior as the behavior must be sampled within the window of drug efficacy. Previous work has shown that at one hour of

proximity data and six hours of grooming data are necessary to produce a reliable network (Feczko et al., 2015).

There are several ways in which the behavior of a species can be measured, including focal and scan (Altmann 1974). Focal sampling involves noting the behaviors of one individual at a time, so great detail in the onset and duration of behaviors can be acquired. Scan sampling, in contrast, involves a quick estimate of conspicuous behaviors at the group-level. Although this provides information about all individuals at the same time period, detail such as the duration of behaviors and bout frequency can be lost. The majority of primate studies that utilize SNA use serial observations over chronological time of one animal at a time. Simultaneously measuring behaviors, as opposed to serial measurements, will give a clearer picture of how MTII affects individuals and the entire network.

In order to investigate the effects of MTII on proximity and grooming, we chose four individual network metrics: betweenness centrality, closeness centrality, eigenvector centrality and the clustering coefficient (Table 1). We chose these metrics in order to quantify social facilitation, likelihood of interaction, influence within the network and integration within the network respectively. As a way to understand what these metrics mean, imagine going to a party with a friend. You do not know anyone at this party but your friend knows everyone there. Your friend easily strikes up conversations, however, since you do not know anyone it would be odd to walk up to a stranger and start a conversation. Instead of attempting to start conversations with random people, your friend introduces you to his friends. In this scenario, your friend acts as a social facilitator connecting you to this new group of people. In SNA terms your friend has a high betweenness centrality (Figure 1). Furthermore, since your friend can readily interact with everyone at the party he has a high closeness centrality (Figure 2). As a stranger, you are not as

close to the people at the party resulting in a lower closeness centrality. In the process of meeting new people through your friend you establish direct new connections with these former strangers. Your friend's introductions enable you to increase your own closeness centrality. Additionally, the people you meet are likely to be the most gregarious members of your friend's group. Meeting the most popular friends increases your eigenvector centrality (Figure 3) because these friends also have lots of friends. Finally, becoming friends with your friend's friends increases your clustering coefficient (Figure 4) because you are integrating yourself into their social network.

Understanding the relationship between MTII and changes in network properties, will provide more information on whether MTII enhances the sociability of individuals within a group setting. Consequently, the prosocial effects of MTII could potentially provide a treatment for individuals with social impairments by increasing their interactions and connectedness with other members of their social network. Graph theory metrics provide a rich and powerful method of analyzing social behavior at the individual and group levels in response to a potentially prosocial drug treatment.

Aims and Hypotheses

Aim: To determine the effect of MTII on individual roles in the network (proximity and grooming)

Hypothesis 1: Betweenness centrality

We expect MTII to increase the average betweenness centrality of the group compared to baseline. A higher betweennness centrality means that monkeys facilitate social relationships between monkeys in the group that do not frequently interact.

Hypothesis 2: Closeness centrality

We expect MTII to increase the average closeness centrality of the group compared to baseline. The increase in social facilitation should increase the number of connections between previously disparate parts of the network causing an increase in the closeness centrality. Consequently, individual monkeys become more likely to interact with more monkeys in the group.

Hypothesis 3: Eigenvector centrality

We expect MTI to increase the average eigenvector centrality of the group compared to baseline. The increase in closeness centrality will cause the eigenvector centrality of the group to increase as individuals become more social and connect with other social group members.

Hypothesis 4: Clustering coefficient

We expect MTII to increase the average clustering coefficient of the group compared to baseline. The increase in the clustering coefficient reflects a greater integration of the social network, more monkeys interacting that did not previously interact.

Hypothesis 5: Total duration of proximity and grooming (received and given)

We expect no difference between the total duration of proximity and grooming (received and given) between MTII and baseline. The absence of a difference in total duration between MTII and baseline indicates that MTII did not decrease the activity levels of the monkeys.

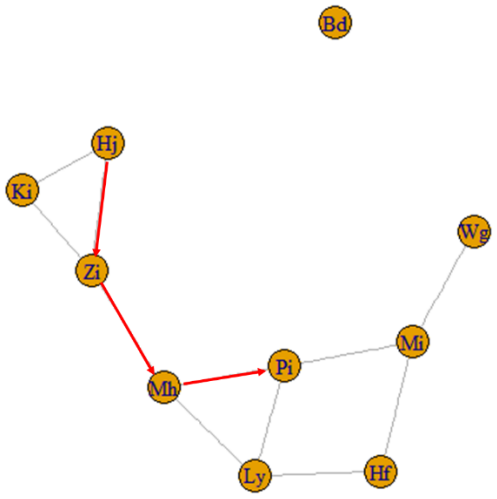

Figure 1: Betweenness centrality of Mohair (Mh) in an MTII proximity duration social network of 25% edge density. Each monkey is represented by a node with connections indicated by lines. Happy Jack (Hj) wants to interact with Pi, however, Happy Jack cannot directly contact Pi. The shortest path from Happy Jack to Pi goes through Mohair and the shortest paths from Kingsley (Ki) and Zaire (Zi) to Pi also pass through Mohair. As a result, Mohair has a high betweenness centrality because all of the shortest paths from Happy Jack, Kingsley and Zaire to other monkeys in the network pass through Mohair.

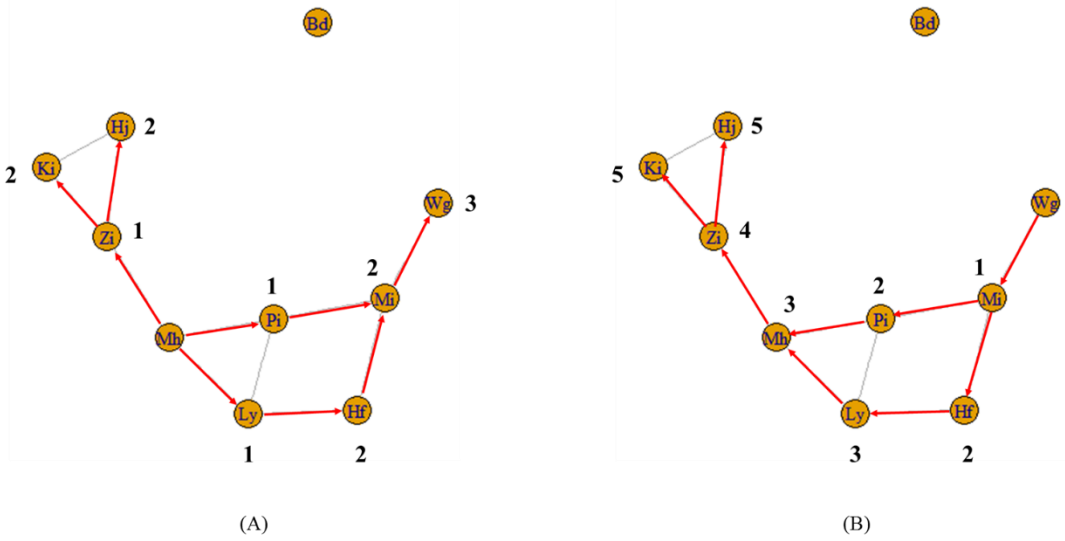

Figure 2: Closeness centrality for Mohair (A) and Willie G. (B) in an MTII proximity duration social network of 25% edge density. Numbers indicate the shortest path length from either Mohair or Willie G to every other monkey in the group. Bernard does not have a path length because he is not connected to any other monkey in the group. Willie G is in a more distal location in the network compared to Mohair. Consequently, Willie G has a smaller closeness centrality than Mohair.

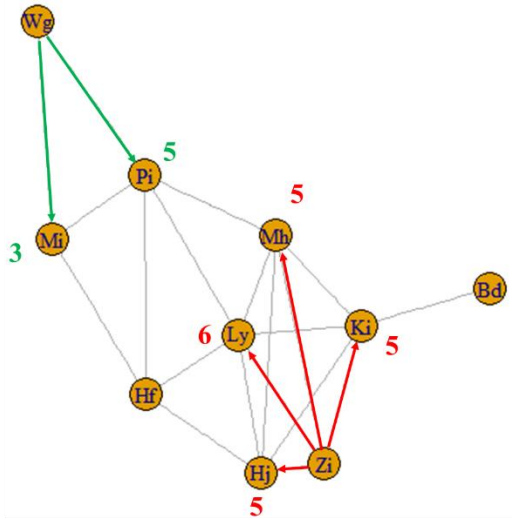

Figure 3: Eigenvector centrality for an MTII proximity duration social network of 45% edge density for Willie G. and Zaire. Red indicates the degree centrality of monkeys connected to Zaire, while green indicates the degree centrality of monkeys connected to Willie G. Since, Zaire is connected to monkeys with higher degree centralities than Willie G., he has a higher eigenvector centrality.

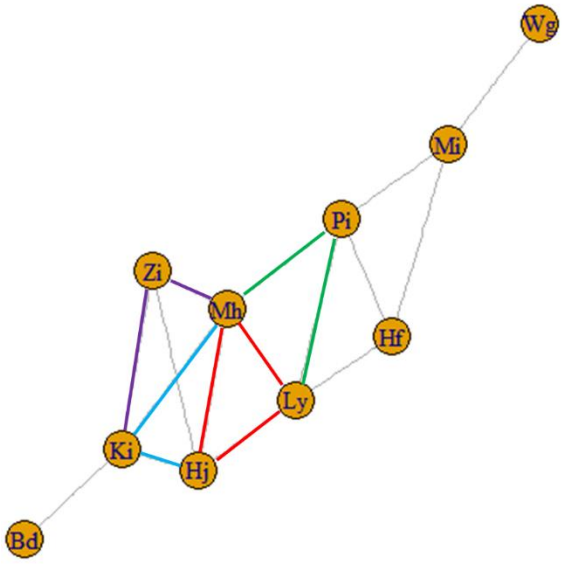

Figure 4: Clustering coefficient of Mohair in an MTII proximity duration social network. Green, red, blue and purple represent four different triples that are centered on Mohair. Since Mohair is the center of the most triples in the network, he has the highest clustering coefficient of the group.

SNA metric	Description
Betweenness Centrality	Number of shortest paths that connect any two nodes that involves passing through a particular node
Closeness Centrality	Average shortest path length between one node and all other nodes in the network
Eigenvector Centrality	Generalization of degree centrality, a node's centrality is proportional to the sum its neighbors centralities
Clustering Coefficient	Number of connected triples within the network divided by the total number of possible triples

Table 1: Quantitative descriptions of betweenness centrality, closeness centrality, eigenvector centrality, and the clustering coefficient.

Methods

Experimental Design

This study used 10 peer housed male rhesus macaques (average age 3.5 years) at the Yerkes Field Station in Lawrenceville, GA (Figure 5). All 10 monkeys were mother-reared in

large social groups for the first three years of life before being peer housed together in a single group. Monkeys were randomly divided into two groups of 5 monkeys each (group A and B). Group A first received a dose of MTII (1 mg/kg) dissolved in 2ml of saline, while group B first received the placebo, which was the same volume injection but with sterile saline. The following week, the treatments were reversed. We gave each drug condition separated by one week apart, so the experiment lasted at total of 3 weeks with two weeks of drug conditions and one week of baseline (Figure 6). The baseline condition was recorded after the second week of the MTII condition. Post injection, subjects were filmed for 3 hours using 4 cameras mounted inside the upper corners of the home cage giving us 4 viewing angles so that subjects were visible at all times. All observations were later coded in Noldus Observer XT10.1. Video recordings occurred between 11am and 2pm.

In the first week, three hours of video footage was recorded to establish a baseline social network several days prior to the experimental manipulations. A total of 19 videos, each 30 minutes in length, were coded for three different experimental conditions: baseline, placebo, and MTII. In 10 of the 19 videos, half the group received MTII and the other half received a placebo treatment, while the other 9 videos were of the baseline social network. Proximity and grooming behaviors were used to construct the macaque social networks. Proximity is a non-directed, duration of A→ B is the same as the duration of B→A, behavior defined as the duration that two monkeys spend within arm's length of one another for a minimum of three seconds. Grooming is a directed, duration of A→B is not the same as the duration of B→A, behavior defined as one monkey moving its hands through the fur of another monkey while actively paying attention to the monkey being groomed. A bout of grooming needed to last at least five seconds in order to be counted. This study had 5 and 4.5 hours of video in the drug/placebo and baseline conditions

respectively. Consequently, we felt confident there were sufficient data to create reliable social networks for both proximity and grooming. Coders used simultaneous focal sampling to record all the behaviors of each of the ten monkeys throughout the video. Coders were blind to the treatment condition throughout the coding process.

Data Analysis

Each set of coded data was extracted from Observer. Spatial proximity and grooming behaviors were then converted into discrete 10 x 10 adjacency matrices using in-house scripts via MATLAB 2012a. The rows and columns reflect the identities of the monkeys with each cell representing either the duration (proximity or grooming) in seconds or frequency (grooming) (Figure 7). In the grooming matrices, the rows indicate the monkey giving the grooming while the columns are the monkeys receiving the grooming. The strongest connections represent the core structure of the network while weaker connections could be the results of insignificant random interactions, so the protocol is to threshold the networks to only include the strongest connections, before any centrality measures are calculated (Borgatti et al., 2013). Proximity matrices were thresholded at 25%, 35% and 45% edge density while grooming matrices were already sparse (Figure 7B) and thus did not need to be thresholded. In order to threshold the matrices all proximity durations, excluding zeroes along the matrix diagonal, were listed from the longest to the shortest duration (Figure 8). Once the durations were in descending order, only the top 25%, 35% or 45% of durations were kept while the rest of the proximity bouts were set to zero. The resultant matrices were then converted into graphs (Figure 9 & 10) from which all the weighted SNA metrics were calculated. The threshold values were chosen to capture three different levels of community structure. Network statistics were all calculated in R (version 3.2.3) using in house scripts along with the igraph package.

Using a linear mixed effects model, we first preformed linear model fitting analyses to determine the best model for our data. Akaike Information Criterion (AIC) values were used to compare the fits for two linear models, one dependent only on drug and the other dependent on both drug and placebo controlling while controlling for individual random intercepts. Across all models, the linear model with only drug outperformed the linear model with both drug and placebo, indicating there was no significant difference between baseline and placebo groups. Consequently, the model only including drug (comparing drug to the combination of baseline and placebo) was chosen for generating the coefficients for significance testing. The beta coefficient, here presenting the mean value difference between the drug and combined baseline and placebo groups, for the effect of drug was calculated while controlling for random individual effects. Permutation tests, resampling without replacement 10,000 times, were then conducted to determine the statistical significance of the effect of drug on network metrics by disassociating monkeys from their respective condition (drug or no drug). A symmetric distribution of drug beta coefficients centered at zero was then found for each network metric. The absolute values of the beta coefficients were used to make a distribution of all positive beta coefficients. P-values were calculated by finding the number of values in the distribution greater than the observed point estimate of the drug beta coefficient. That value was then divided by the number of resamples (10,000) to give a p-value. This value is a two sided p-value since absolute values of beta coefficients were used to generate the resampled distribution. Along with the permutation tests, we used bootstrapping with replacement to calculate 95% confidence intervals for the effect size. Additionally, we corrected for multiple comparisons using the Bonferroni method. As a result, the two-sided alpha level was set at 0.002 (0.05 divided by 28 comparisons).

Furthermore, we calculated the mean and standard deviation of each SNA metric in the drug and Baseline+Placebo conditions. Using these values, we calculated the Cohen's d effect size by taking the mean difference between the drug and Baseline+Placebo groups along divided by the pooled standard deviation. An effect size of 0.2 is considered small, 0.5 is medium and 0.8 is large; the effect size measures the number of standard deviations between the two means (Rosnow and Rosenthal, 1996). For example, 0.6 is a medium effect size and indicates that the means of the metric distributions differ by 0.6 standard deviations. Consequently, as the means of the metric distributions become further apart the effect size increases and there is less overlap between the distributions. Only individual metrics were included in the analysis to maximize the statistical power of the study. Group based metrics would only have a maximum sample size of 10 while by using individual metrics the sample size increases to 190 (N multiplied by the number of "trials"). Consequently, the statistical power for individual metrics is much greater than that of group based metrics.

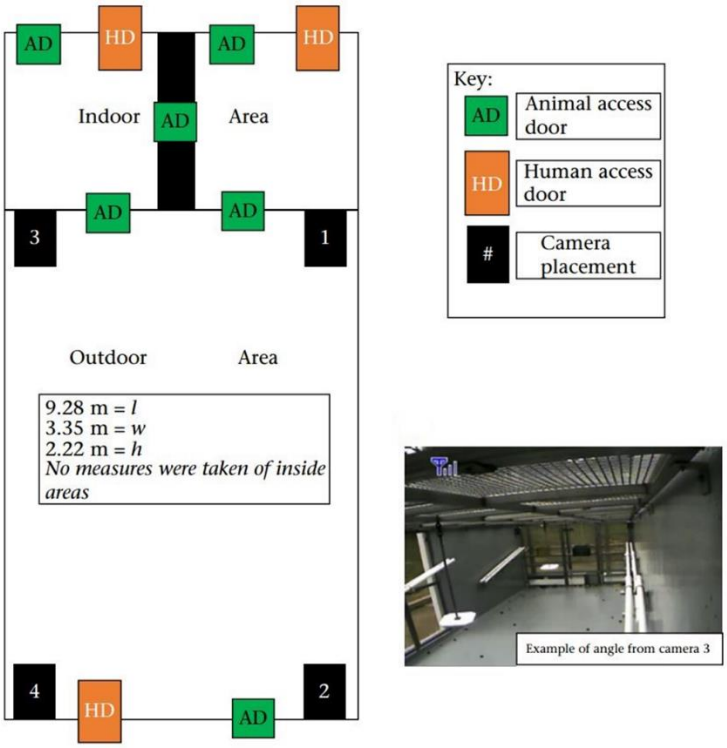

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Figure 5: Schematic of the outdoor enclosure at the Yerkes Field Station where all video recordings took place. Four cameras where placed in the upper corners of the enclosure to continually monitor the location of each monkey. (Reproduced from Feczko et al., 2015)

	Group	M	Tu	W	Th	F	Sat	Sun
WK 1	A	MTII	Washout	Washout	Baseline	Baseline	Baseline	-
	B	Placebo	Washout	Washout	Baseline	Baseline	Baseline	-
WK 2	A	Placebo	Washout	Washout	Baseline	Baseline	Baseline	-
	B	MTII	Washout	Washout	Baseline	Baseline	Baseline	-

Figure 6: Group A received MTII in week 1 while group B received the placebo treatment. In week 2, group A received the placebo treatment and group B received MTII. The baseline proximity and grooming social networks were recorded over a period of three days, two days after the administration of MTII.

Subjects	Bd13	Hf14	Hj14	Ki14	Ly13	Mh14	Mi14	Pi14	Wg14	Zi14
Bd13	0	8.23	13.3	0	5.1	14.24	35.74	36.34	20.5	5.57
Hf14	8.23	0	8.7	6.03	298.63	273.44	73	87.44	18.07	0
Hj14	13.3	8.7	0	389.56	76.66	77.08	14.04	201.77	0	226.57
Ki14	0	6.03	389.56	0	15.13	249.37	11.9	291.5	0	981.08
Ly13	5.1	298.63	76.66	15.13	0	1013.57	4.17	70.3	0	0
Mh14	14.24	273.44	77.08	249.37	1013.57	0	12.2	17.57	0	180.02
Mi14	35.74	73	14.04	11.9	4.17	12.2	0	481.56	71.53	0
Pi14	36.34	87.44	201.77	291.5	70.3	17.57	481.56	0	58.1	32.37
Wg14	20.5	18.07	0	0	0	0	71.53	58.1	0	0
Zi14	5.57	0	226.57	981.08	0	180.02	0	32.37	0	0

(A)

Subjects	Bd13	Hf14	Hj14	Ki14	Ly13	Mh14	Mi14	Pi14	Wg14	Zi14
Bd13	0	0	0	0	0	0	0	0	0	0
Hf14	0	0	0	0	0	0	0	0	0	0
Hj14	0	0	0	63.03	0	0	0	0	0	7.6
Ki14	0	0	0	0	0	0	0	0	0	82.67
Ly13	0	0	0	0	0	0	0	0	0	0
Mh14	0	0	0	0	68.84	0	0	0	0	0
Mi14	0	0	0	0	0	0	0	0	0	0
Pi14	0	0	0	0	0	0	215.27	0	0	0
Wg14	0	0	0	0	0	0	0	0	0	0
Zi14	0	0	32.13	13.63	0	0	0	0	0	0

(B)

Figure 7: Example of a proximity duration adjacency matrix (A) and grooming duration adjacency matrix (B), each cell represents the total amount of time, in seconds, two monkeys were in contact with each other throughout the 30 minute session. The diagonal of the matrix is always zero because a monkey cannot be in contact with itself. The proximity, but not the grooming, matrix is symmetric about the diagonal. The high number of zeroes in the grooming duration adjacency matrix indicates that grooming is a relatively infrequent behavior, especially when compared with proximity.

subjects	variable	value
Bd13..1.	Bd13..1.	0.00
Hf14..2.	Bd13..1.	8.23
Hj14..3.	Bd13..1.	13.30
Ki14..4.	Bd13..1.	0.00
Ly13..5.	Bd13..1.	5.10
Mh14..6.	Bd13..1.	14.24
Mi14..7.	Bd13..1.	35.74
Pi14..8.	Bd13..1.	36.34
Wg14..9.	Bd13..1.	20.50
Zi14..10.	Bd13..1.	5.57
Bd13..1.	Hf14..2.	8.23
Hf14..2.	Hf14..2.	0.00
Hj14..3.	Hf14..2.	8.70
Ki14..4.	Hf14..2.	6.03
Ly13..5.	Hf14..2.	298.63
Mh14..6.	Hf14..2.	273.44
Mi14..7.	Hf14..2.	73.00
Pi14..8.	Hf14..2.	87.44
Wg14..9.	Hf14..2.	18.07
Zi14..10.	Hf14..2.	0.00

Figure 8: Example of the conversion of an adjacency matrix into a vector as the first step of the thresholding procedure. In order to threshold the adjacency matrices at 25%, 35% and 45%, the matrix is first converted into a vector. The rows of the adjacency matrix (figure 5A) were converted into columns, then the value column was ordered from the largest to the smallest value, excluding the zeroes of the diagonal. From this ordered column, the top 25%, 35% or 45% of values were chosen and the rest of the values were set to zero. The vector was then reconverted into an adjacency matrix and the centrality measures were calculated using this new matrix.

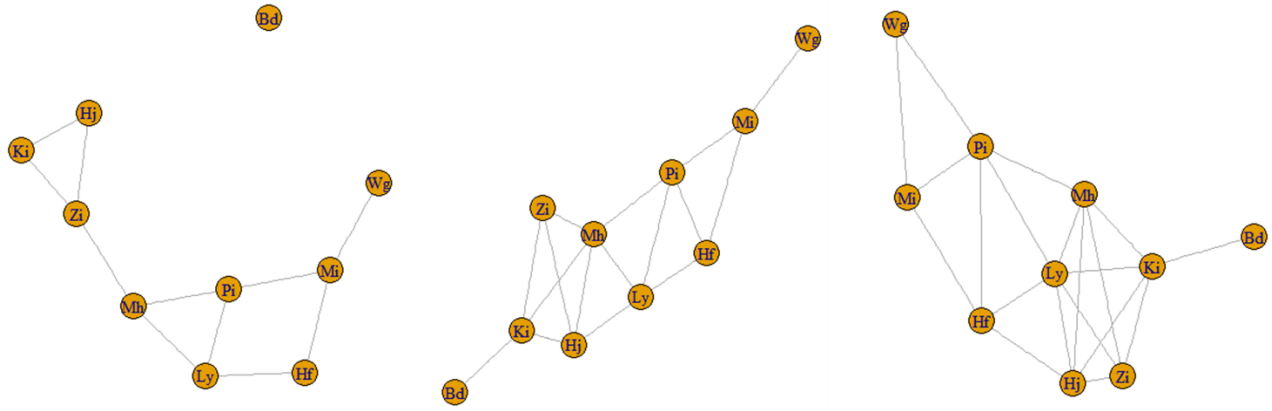

Figure 9: Average weighted proximity social network for the drug/placebo conditions for week 1 at 25%, 35% and 45% edge densities. The number of edges increases between 25% and 45% as more connections are systematically added to the network.

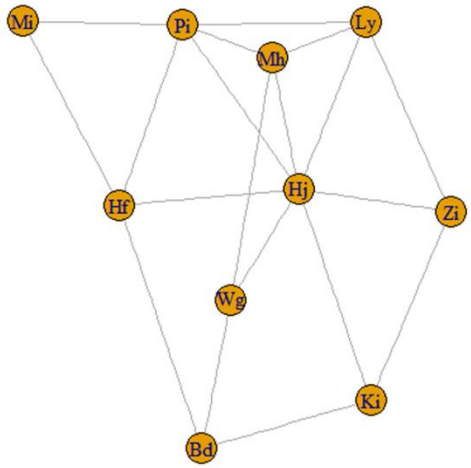

Figure 10: Average weighted grooming social network for the drug/placebo conditions for week 1 with no threshold.

Results

Proximity Duration

Total Proximity Behavior

The total proximity duration did not differ between treatment groups (Table 2; Figure 11).

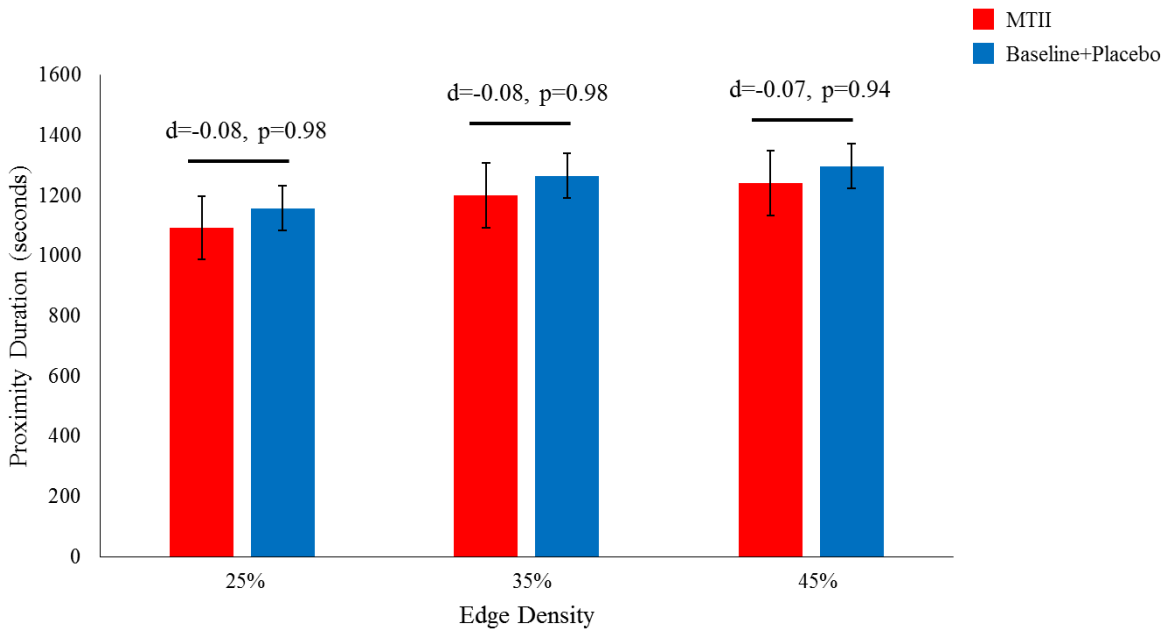

Figure 11: Average proximity duration +/- SEM for 25%, 35% and 45% edge densities. There is no significant difference in the average proximity duration between MTII and Baseline+Placebo at 25%, 35% and 45%.

SNA Measures

The betweenness centrality, closeness centrality, eigenvector centrality and the clustering coefficient did not differ significantly between treatment groups at 25%, 35% and 45% edge density (Table 2; Figure 12, 13, 14, and 15).

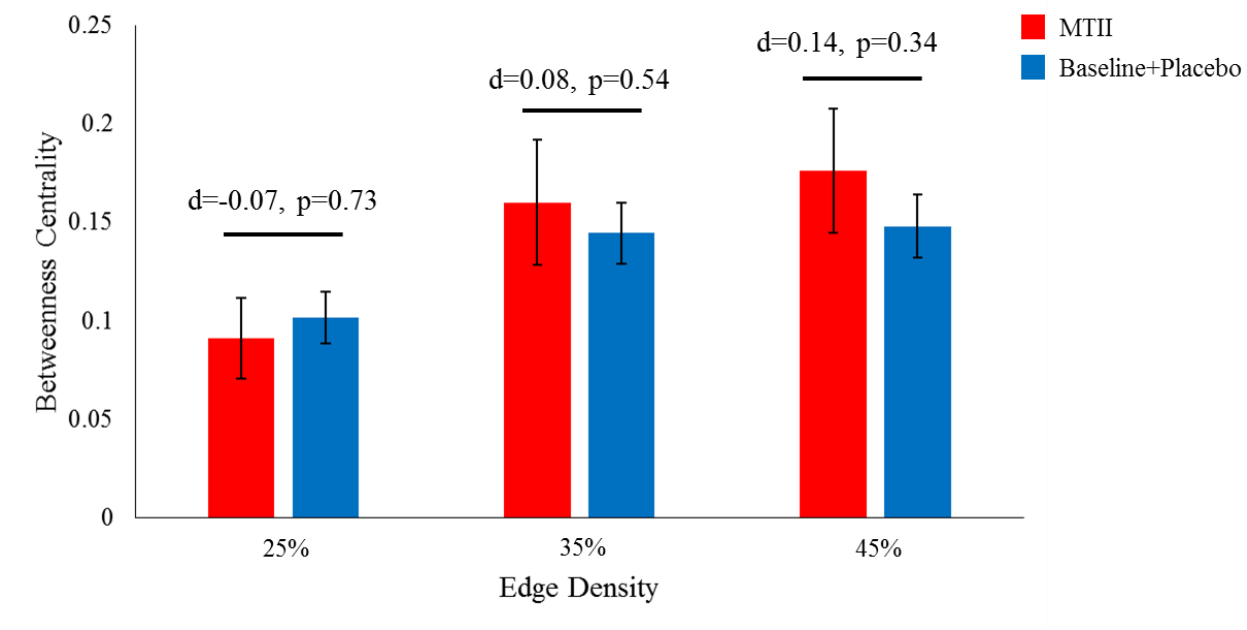

Figure 12: Betweenness centrality +/- SEM for the average proximity duration social network at 25%, 35% and 45% edge densities. There is no significant difference in the betweenness centrality between MTII and Baseline+Placebo at 25%, 35% and 45%.

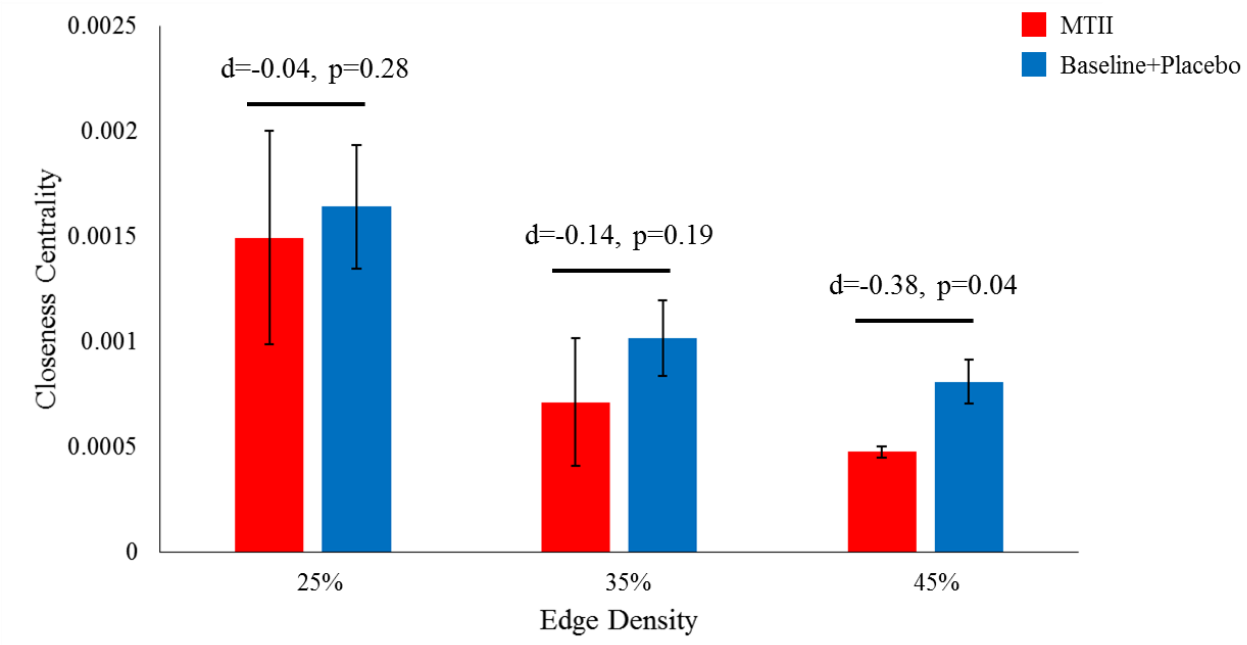

Figure 13: Closeness centrality +/- SEM for the proximity social network at 25%, 35% and 45% edge densities. There is no significant difference in the closeness centrality between MTII and Baseline+Placebo at 25%, 35% and 45%.

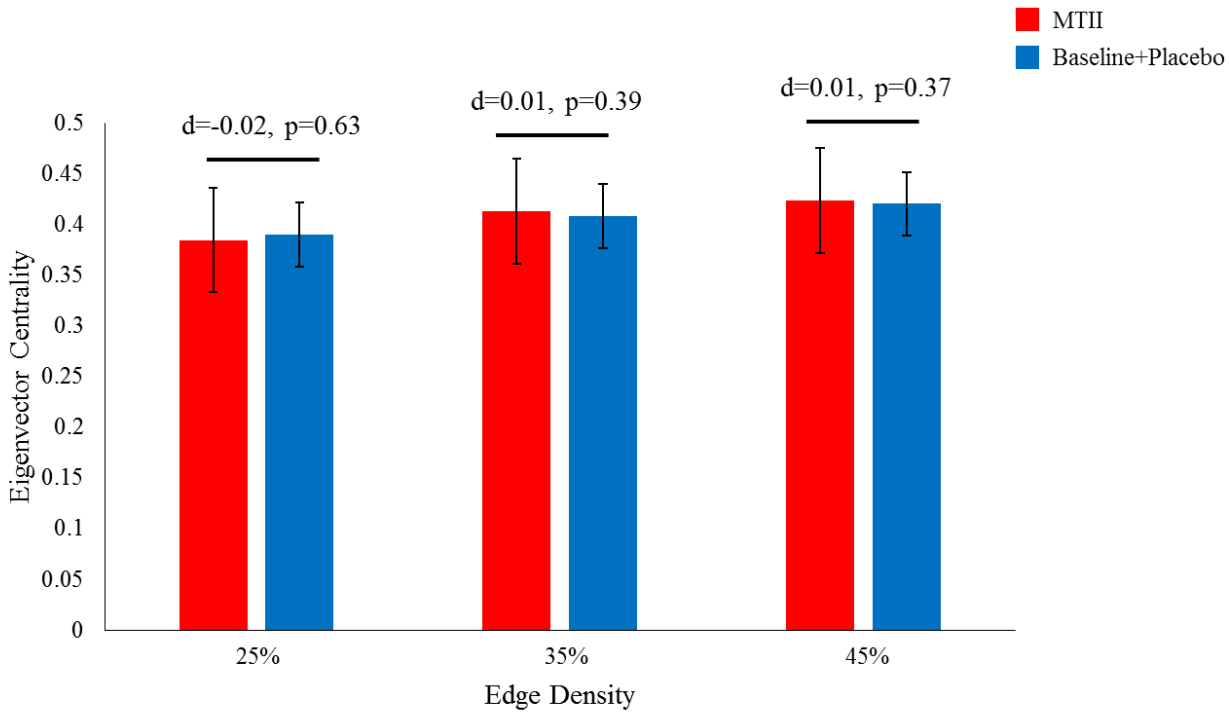

Figure 14: Eigenvector centrality +/- SEM for the proximity duration social network at 25%, 35% and 45% edge densities. There is no significant difference in the eigenvector centrality between MTII and Baseline+Placebo at 25%, 35% and 45%.

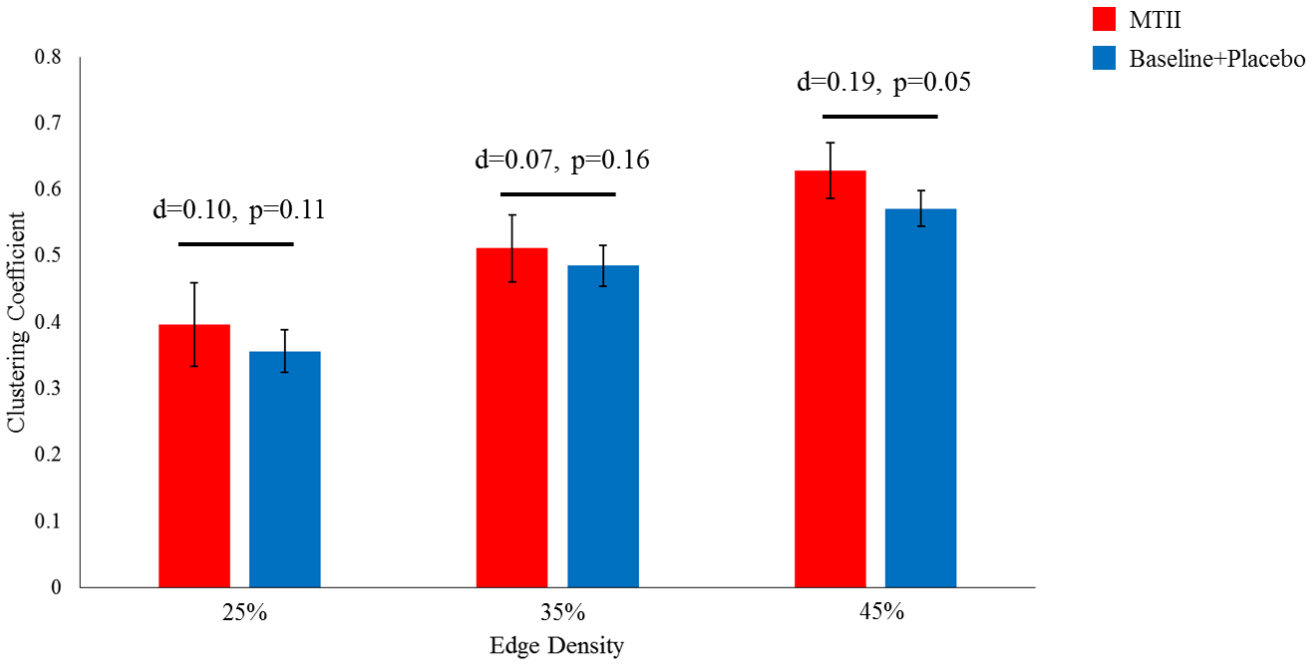

Figure 15: Clustering coefficient +/- SEM for the proximity duration social network at 25%, 35% and 45% edge densities. There is no significant difference in the clustering coefficient between MTII and Baseline+Placebo at 25%, 35% and 45%.

Betweenness							AIC	
Threshold Percentage	Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
25	0.0911	0.1452	0.1018	0.1539	-0.0714	0.7333	-166.1170	-165.8719
35	0.1600	0.2239	0.1444	0.1808	0.0764	0.5381	-75.3397	-68.6345
45	0.1761	0.2223	0.1480	0.1899	0.1359	0.3401	-63.3701	-56.4562
Closeness							AIC	
Threshold Percentage	Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
25	0.0015	0.0036	0.0016	0.0035	-0.0416	0.2775	-1648.8540	-1633.4860
35	0.0007	0.0021	0.0010	0.0021	-0.1429	0.1876	-1782.4620	-1768.4010
45	0.0005	0.0002	0.0008	0.0012	-0.3779	0.0411	-2023.4640	-2015.5300
Eigenvector							AIC	
Threshold Percentage	Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
25	0.3846	0.3659	0.3902	0.3736	-0.0152	0.6295	75.9806	80.9457
35	0.4127	0.3665	0.4083	0.3730	0.0119	0.3915	47.8653	53.3440
45	0.4237	0.3666	0.4204	0.3685	0.0090	0.3742	39.3476	45.1254
Clustering							AIC	
Threshold Percentage	Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
25	0.3960	0.4454	0.3560	0.3813	0.0966	0.1110	137.3197	141.0836
35	0.5113	0.3590	0.4850	0.3609	0.0732	0.1632	101.3703	100.3419
45	0.6290	0.2968	0.5713	0.3149	0.1885	0.0452	61.0360	64.7131
Total duration (sec)							AIC	
Threshold Percentage	Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
25	1092.3640	748.0840	1158.2430	888.1497	-0.0802	0.9794	3007.5120	2998.0840
35	1199.7960	758.3166	1265.8310	889.8810	-0.0799	0.9828	3010.7810	3001.2070
45	1241.1930	758.6578	1297.5820	887.4901	-0.0683	0.9415	3010.9070	3001.3060

Table 2: Network metrics and total proximity duration for the proximity duration social network for MTII and Baseline+Placebo conditions.

Grooming Received

Duration

The total grooming received duration did not significantly differ between treatment groups (Table 3; Figure 16).

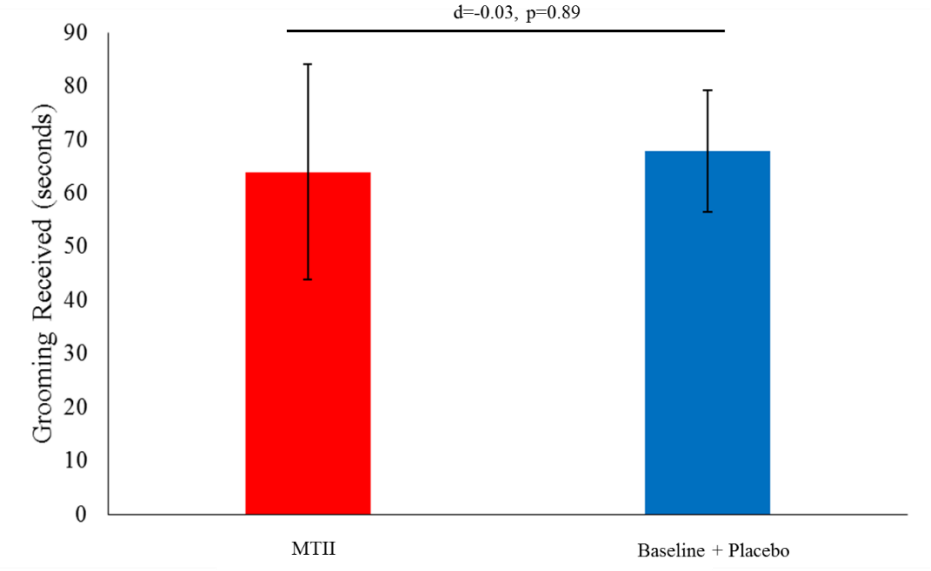

Figure 16: Grooming received +/- SEM in the grooming duration social network. There is no significant difference in grooming received between MTII and Baseline+Placebo.

Frequency

The total grooming received frequency did not significantly differ between treatment groups (Table 4; Figure 17).

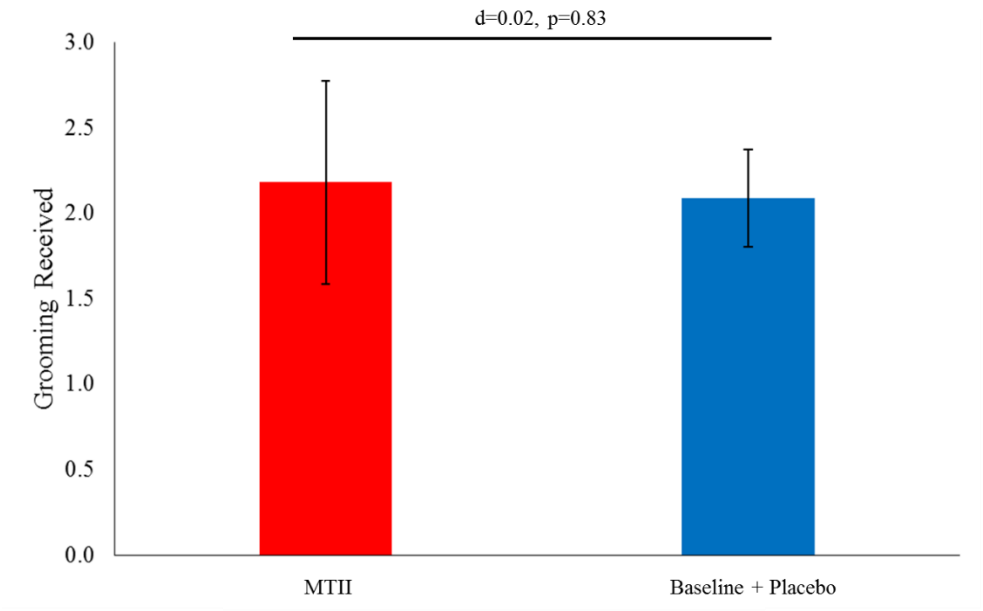

Figure 17: Grooming received +/- SEM in the grooming frequency social network. There is no significant difference in the grooming received between MTII and Baseline+Placebo.

Grooming Given

Duration

The total grooming given duration did not differ significantly between treatment groups (Table 3, 95% CIs; -0.86,-0.49; Figure 18). However, there is a large difference in the grooming given between treatment groups. Monkeys on MTII groomed for an average of 15.42 (SD 37.43) seconds per session while monkeys in the Baseline+Placebo condition groomed for an average of 85.17 (SD 139.45) seconds per session.

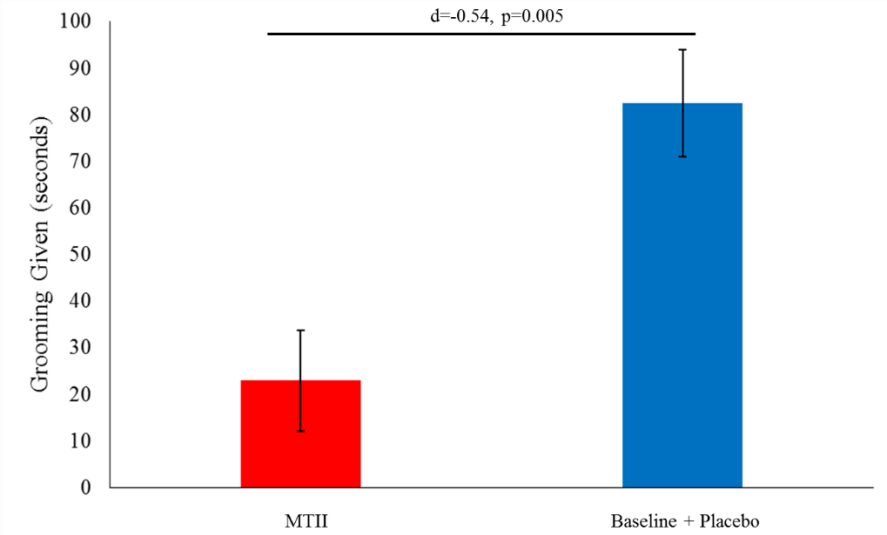

Figure 18: Grooming given +/- SEM in the grooming duration social network. There is no significant increase in grooming given between MTII and Baseline+Placebo with a moderate positive effect size.

Frequency

The total grooming given frequency (Table 4; Figure 19) did differ significantly between treatment groups. Monkeys on MTII initiated grooming an average of 0.84 (SD 1.75) times per session while monkeys in the Baseline+Placebo condition initiated grooming an average of 2.56 (SD 3.44) times.

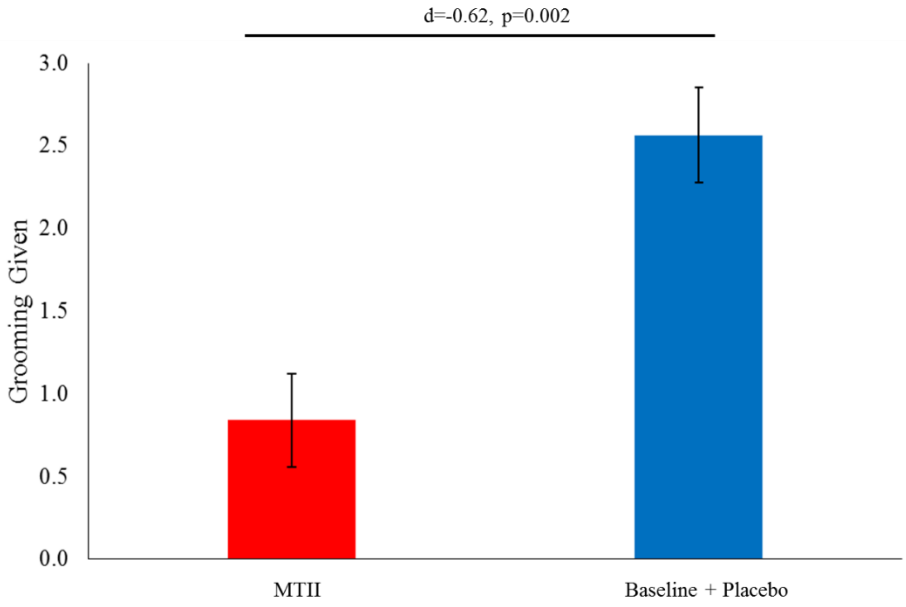

Figure 19: Grooming given +/- SEM in the grooming frequency social network. There is a significant difference in the grooming given between MTII and Baseline+Placebo.

Grooming Duration: SNA Measures

We found no significant difference between treatment groups on betweenness centrality, eigenvector centrality and the clustering coefficient for the grooming duration social network (Table 3; Figure 20, 22, 23). However, closeness centrality significantly differed between MTII and Baseline+Placebo (Table 2, 95% CIs; 0.34, 1.02; Figure 21). Monkeys given MTII had a higher closeness centrality, 0.0099 (SD 0.0026), compared to the Baseline+Placebo group, 0.0076 (SD 0.0039) suggesting MTII increased the likelihood that placebo monkeys would groom MTII monkeys.

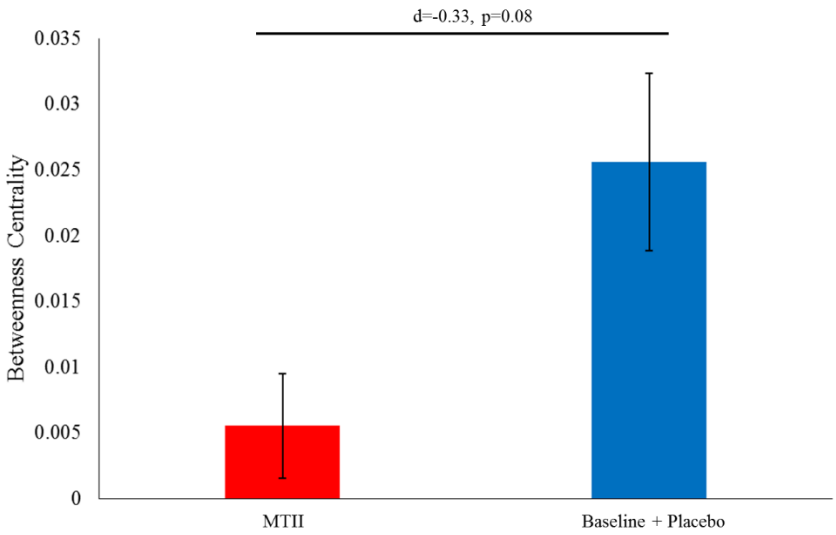

Figure 20: Betweenness centrality +/- SEM for the grooming duration social network. There is no significant difference in the betweenness centrality between MTII and Baseline+Placebo.

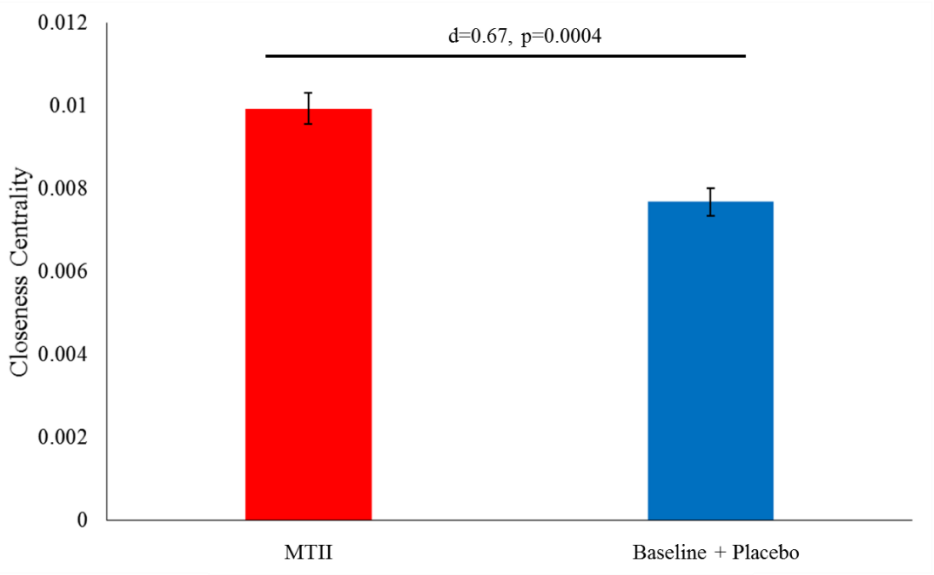

Figure 21: Closeness centrality +/- SEM for the grooming duration social network. There is a significant increase in the closeness centrality between MTII and Baseline+Placebo with a moderate positive effect size.

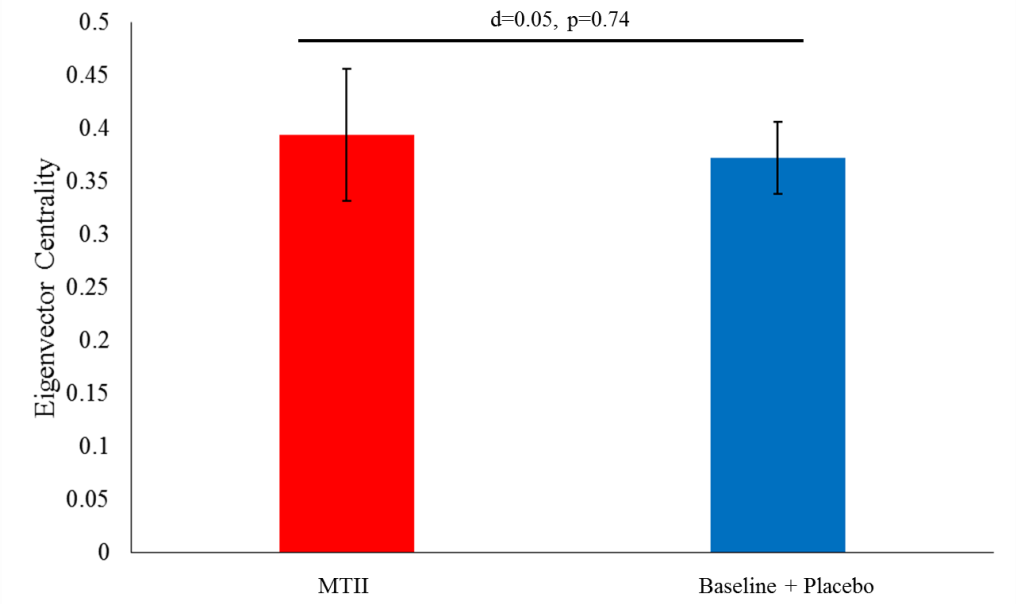

Figure 22: Eigenvector centrality +/- SEM for the grooming duration social network. There is no significant difference in the eigenvetor centrality between MTII and Baseline+Placebo.

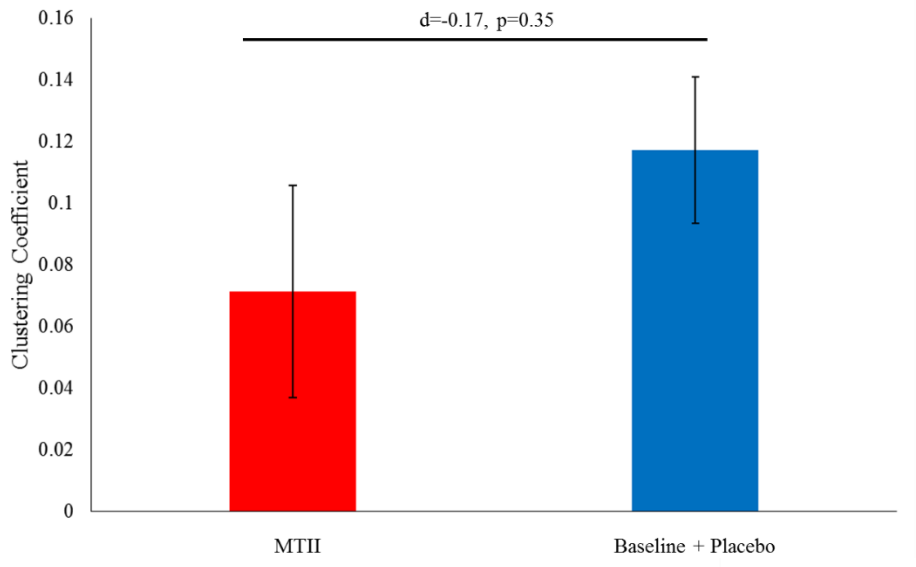

Figure 23: Clustering coefficient +/- SEM for the grooming duration social network. There is no significant difference in the clustering coefficient between MTII and Baseline+Placebo.

Betweenness						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
0.0056	0.0281	0.0256	0.0797	-0.3354	0.0796	-449.5555	-443.2780
Closeness						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
0.0099	0.0027	0.0077	0.0040	0.6654	0.0004	-1562.2000	-1547.7120
Eigenvector						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
0.3941	0.4414	0.3724	0.4003	0.0516	0.7414	216.3461	221.4151
Clustering						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
0.0713	0.2426	0.1171	0.2805	-0.1747	0.3518	55.5775	56.5831
Grooming - Given (sec)						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
15.4150	37.4347	85.1738	139.4531	-0.5427196	0.0049	2355.789	2349.274
Grooming - Received (sec)						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
51.9596	123.2013	72.1221	140.6117	-0.0279631	0.8905	2397.38	2385.594

Table 3: Network metrics and total grooming given and received (seconds) for the grooming duration social network for the MTII and Baseline+Placebo conditions.

Grooming Frequency: SNA Measures

For the grooming frequency social network the betweenness centrality, closeness centrality, eigenvector centrality and the clustering coefficient did not differ significantly between treatment groups (Table 4; Figure 24, 25, 26, 27).

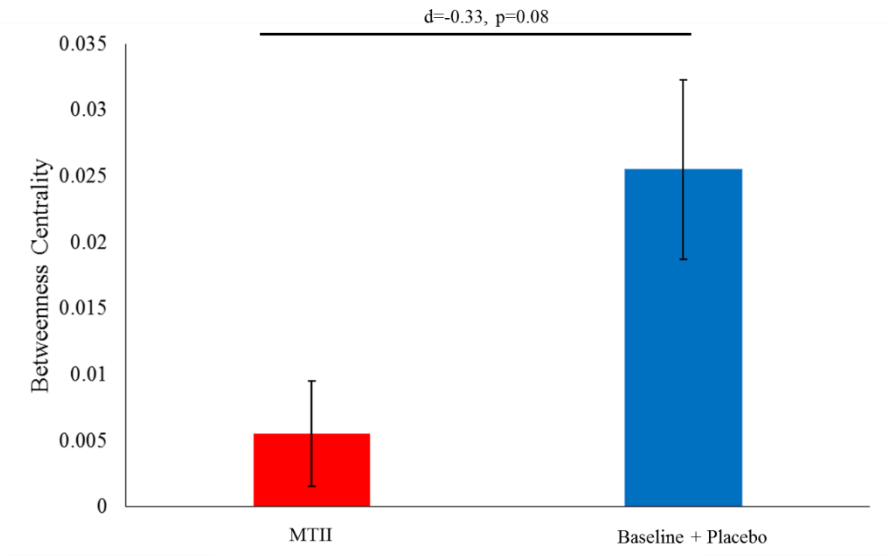

Figure 24: Betweenness centrality +/- SEM in the grooming frequency social network. There is no significant difference in the betweenness centrality between MTII and Baseline+Placebo.

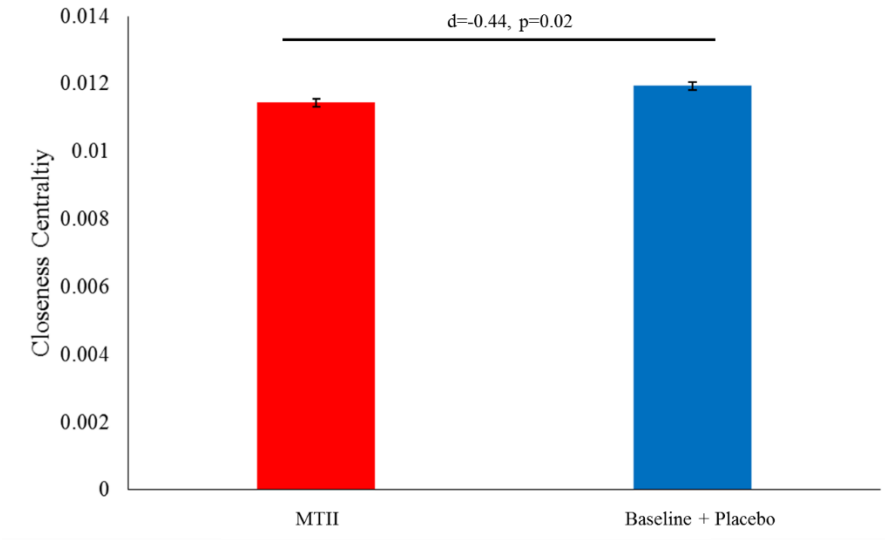

Figure 25: Closeness centrality +/- SEM in the grooming frequency social network. There is no significant difference in the closeness centrality between MTII and Baseline+Placebo.

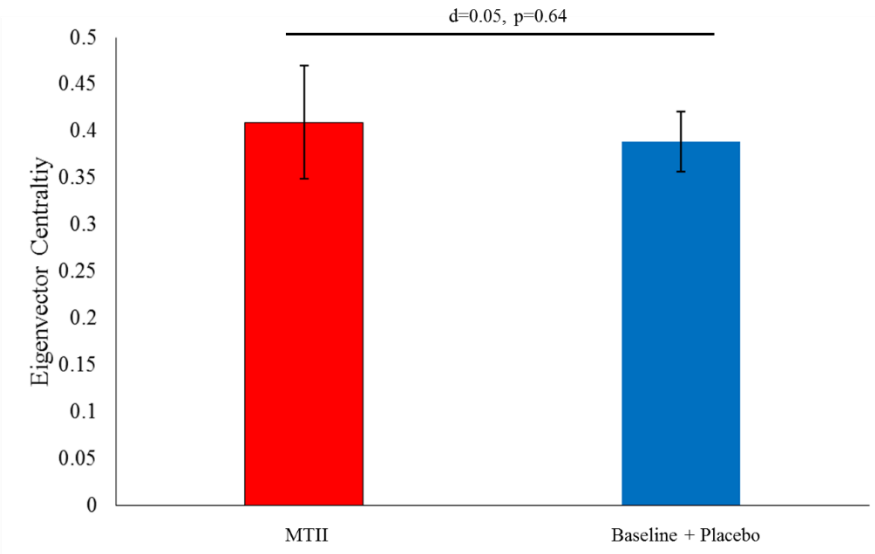

Figure 26: Eigenvector centrality +/- SEM in the grooming frequency social network. There is no significant difference in the eigenvector centrality between MTII and Baseline+Placebo.

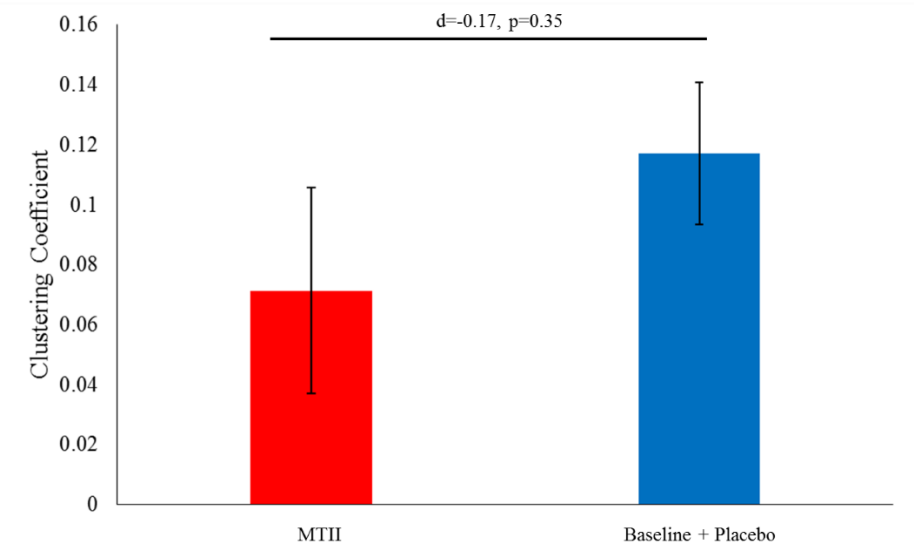

Figure 27: Clustering coefficient +/- SEM in the grooming frequency social network. There is no significant difference in the clustering coefficient between MTII and Baseline+Placebo.

Betweenness						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
0.0056	0.0281	0.0255	0.0800	-0.3326	0.0836	-448.2150	-442.1213
Closeness						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
0.0114	0.0008	0.0119	0.0014	-0.4428	0.0181	-1958.7050	-1941.7870
Clustering						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
0.0713	0.2426	0.1171	0.2805	-0.1747	0.3508	55.5775	56.5831
Eigenvector						AIC	
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
0.4093	0.4279	0.3887	0.3836	0.0506	0.6377	188.1654	192.4171
Grooming - Given							
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
0.8400	1.7538	2.5643	3.4479	-0.6304	0.0023	971.5186	972.8016
Grooming - Received							
Drug	SD	Baseline + Placebo	SD	Effect size	P-value	Drug	Drug+Placebo
1.8600	3.7743	2.2000	3.5527	-0.0928	0.7107	1026.9520	1025.2090

Table 4: Network metrics and total grooming given and received frequency for the grooming frequency social network for the drug and Baseline+Placebo conditions.

Since we observed mean value differences regarding the amount of duration grooming given, we further investigated this effect by breaking the data apart and looking at the amount of grooming across treatment groups not controlling for baseline. Data are shown in Table 5. The average grooming bout duration given by monkeys in the placebo condition to the monkeys in the MTII condition, Placebo-MTII, was 9.95 (SD 49.79) seconds. Furthermore, the average grooming bout duration given by monkeys in the placebo condition from other placebo monkeys, Placebo-Placebo, was 4.75 (SD 25.63) seconds. The effect size of the mean difference between the grooming given between the Placebo-MTII and Placebo-Placebo conditions was 0.13 but was not statistically significant (95% CIs -0.04, 0.26). The total sum of grooming given was 2488.98 seconds in the Placebo-MTII condition and 1180.3 seconds in the MTII-MTII condition. Consequently, although not statistically significant (d=0.13, p=0.57) the placebo group groomed the MTII group 2.1 times as long as the MTII group groomed itself.

	MTII	Placebo
MTII	2.83 (31.70) Total: 709.18	1.75 (15.14) Total: 438.49
Placebo	9.95 (49.79) Total: 2488.98	4.75 (25.63) Total: 1180.3

Table 5: Grooming given and received in the MTII grooming duration social network for monkeys on MTII and placebo. Within each cell, the top row indicates the average grooming duration with the standard deviation in parentheses. The rows indicate the monkey giving the grooming while the columns are the monkeys receiving the grooming.

Discussion

Our study found a significant increase in the closeness centrality in the MTII group compared to the Baseline+Placebo group in the grooming duration social network. We also found a significant decrease in the grooming given in the MTII group compared to the Baseline+Placebo group in the grooming frequency social network However, we found no significant differences between treatment groups in the proximity duration, grooming duration and grooming frequency social networks across any of the network metrics. The increase in closeness centrality within the grooming duration social network indicates that MTII monkeys are more likely to receive grooming from a greater number of monkeys than the placebo monkeys. Consequently at least in our study, grooming seems to be more useful for measuring prosocial behavior since proximity could simply reflect passive tolerance rather active engagement.

These results raise the question of why MTII monkeys have fewer grooming interactions than placebo monkeys. Since there are no proximity differences, the MTII monkeys are not simply avoiding others so that they do not have an opportunity to groom. They may solicit more grooming, e.g., by approaching and presenting to others, and therefore receive more grooming. This interpretation is plausible because our findings showed that MTII does not groom MTII very much, and placebo does not groom placebo very much. The main direction of the effect is that placebo grooms MTII more. However, we cannot determine whether this effect is driven by placebo monkeys being attracted to MTII monkeys or MTII monkeys soliciting more grooming. MTII macaques could also could have been less aggressive towards other macaques, which led to increases in grooming. Another alternative is that MTII macaques were more indifferent to other macaques, leading to greater social tolerance (but not social preference), which resulted in more grooming from a greater number of partners.

Anxiety

MTII is associated with yawning, stretching, penile erections and excessive grooming in rats in a dose dependent relationship (Argiolas et al., 2000). Administration of MTII directly into the PVN of the hypothalamus affects the expression of yawning, stretching and grooming; an MC4R antagonist significantly reduces the occurrence of these behaviors. In wild type (WT) mice, MTII decreases social interaction and MCR4 null mice spend more time in the open arms of an elevated plus maze compared to WT controls (Chuang et al., 2010). Acute restraint stress activates MC4R expressing neurons in the medial amygdala and restraint stress caused a significant decrease in the open arm entries and open arm time in the elevated plus maze (Liu et al., 2013). Administration of 1nmol of an MC4R agonist (Cyclo (β-Ala-His-D-Phe-Arg-Trp-Glu)-NH2 into the medial amygdala significantly decreased the number of open arm entries and

open arm time. The MC4R agonist also caused a significant increase in plasma corticosterone 30 minutes after infusion into the medial amygdala. Anxiogenic effects of acute restraint stress were reversed with 1nmol of an MC4R antagonist (SHU 9119). MTII has been shown to cause high increases in MC4R mRNA expression in the medial amygdala (Modi et al., 2015) as well as significant increases in the corticosterone response of neonate voles (Barrett et al., 2014). A potential increases in anxiety behavior could explain the substantial decrease in grooming given among MTII monkeys, while not affecting the amount of grooming received. Although it is possible the monkeys were more anxious, as some of the literature reports, this is counter to the supposed effects of MTII. We also have no indication that they were anxious and did not measure anxiety behavior.

Locomotion

Another possible explanation is that MTII decreased the locomotor activity of the monkeys. Alpha melanocyte hormone (α-MSH) has been shown to significantly increase the immobility time in the forced swim test compared to a vehicle control but did not affect mean locomotor activity (Goyal et al., 2006). High doses of OT reduce locomotor activity while low doses reduced the time spent in the periphery of an open-field (anxiolytic-like effect) in male rats (Uvnäs-Moberg et al., 1994). A subcutaneous injection of 1 mg/kg of OT in the male rats produced the strongest reduction in locomotor activity while a dose of 0.004 mg/kg of OT caused the strongest anxiolytic effect. A decrease in locomotor activity due to sedative effects could have prevented the MTII monkeys from actively engaging in grooming while still receiving the same amount of grooming as the placebo monkeys. If the monkeys laid down more they could have received grooming from a greater number of monkeys. The small decrease in the betweenness centrality in the grooming duration social network could possibly be evidence of

reduced locomotor activity since less grooming would "flow" through the network if MTII monkeys are not giving as much grooming. However, we measured neither locomotor activity nor laying down and do not know whether MTII affected either one.

Limitations/Future Studies

The baseline videos of the group were recorded one week after the administration of MTII for both weeks. Therefore, the baseline social network may not accurately reflect the social interactions of the group prior to MTII. As drug response study, we have a limited window of drug efficacy in which to collect data. Examining changes in the SNA metrics over time may help to determine when the drug produces its largest effects on group dynamics. We also averaged the drug groups over two weeks, first half MTII and placebo and then second half placebo and MTII. This could potentially lead to a loss of information about the social network structure and may be just as bad as current animal behavior studies averaging behavior for each individual across a long time period. Additionally, we could look at drug effects between subjects instead of within subjects. Measuring the locomotor activity of the macaques would help to determine whether the SNA metric changes are driven by changes in locomotion. In future studies, we could possibly give everyone MTII or get a larger sample size by increasing the number of weeks over which the drug is administered. Future studies could also examine the dose effects of MTII on SNA measures and the prosocial behavior of the monkeys.

MTII Effects on Prosocial Behavior

Our study showed that MTII decreased rather than increased the prosocial behavior of male macaques. More research is needed to determine whether MTII could potentially enhance prosocial behavior in male macaques.

References

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Appendix 1: SNA measures

Individual nodes

1. Betweenness centrality: number of shortest paths that connect any two nodes that involves passing through a particular node (Newman, 2005)

$$b_i = \frac{\sum_{s<t} g_i^{(st)}/n_{st}}{\left(\frac{1}{2}\right) n(n-1)}$$

2. Closeness centrality: average shortest path length between one node and all other nodes in the network (Sabidussi, 1966)

$$C_{C(v)} = \frac{1}{\sum_{I \epsilon V \setminus v} d_{G(v,t)}}$$

3. Eigenvector Centrality (Newman, 2008)

$$x_v = \frac{1}{\lambda} \sum_{t \epsilon M(v)} a_{v,t} x_t$$

$$x_t = \frac{1}{\lambda} \sum_{t \epsilon G} a_{v,t} x_t$$

$$A\mathrm{X} = \lambda \mathrm{X}$$

4. Clustering coefficient: degree of cliquishness within a network (Newman, 2003)

$$C_i = \frac{Number\ of\ triangles\ connected\ to\ node\ i}{Number\ of\ triples\ centered\ on\ node\ i}$$

$$C = \frac{1}{n}\sum_{i} C_i$$