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Weak effects of common genetic variation in oxytocin and vasopressin receptor genes on rhesus macaque social behavior

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Short title: Weak effects of OT/AVP genetic variants on social behavior

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Abstract

The neuropeptides oxytocin (OT) and arginine vasopressin (AVP) influence pair bonding, attachment, and sociality, as well as anxiety and stress responses in humans and other mammals. The effects of these peptides are mediated by genetic variability in their associated receptors, *OXTR* and the *AVPR* gene family.. However, the role of these genes in regulating social behaviors in non-human primates is not well understood. To address this question, we examined whether genetic variation in the OT receptor gene *OXTR* and the AVP receptor genes *AVPR1A* and *AVPR1B* influence naturally-occurring social behavior in free-ranging rhesus macaques -- gregarious primates that share many features of their biology and social behavior with humans. We assessed rates of social behavior across 3,250 hours of observational behavioral data from 201 free-ranging rhesus macaques on Cayo Santiago island in Puerto Rico, and used genetic sequence data to identify 25 *OXTR*, *AVPR1A*, and *AVPR1B* single-nucleotide variants (SNVs) in the population. We used an animal model to estimate the effects of 12 SNVs (n=3 *OXTR*; n=5 *AVPR1A*; n=4 *AVPR1B*) on rates of grooming, approaches, passive contact, contact aggression, and non-contact aggression, given and received. Though we found evidence for modest heritability of these behaviors, estimates of effect sizes of the selected SNVs were close to zero, indicating that common *OXTR* and *AVPR* variation contributed little to social behavior in these animals. Our results are consistent with recent findings in human genetics that the effects of individual common genetic variants on complex phenotypes are generally small.

Keywords: oxytocin, vasopressin, behavioral genetics, social behavior, rhesus macaques

Introduction

The neuropeptides oxytocin (OT) and arginine vasopressin (AVP) regulate social behaviors across a variety of mammalian species. In various non-human primate (NHP) species, introducing exogenous OT into the central nervous system promotes affiliative social relationships and pair bonding behaviors (Smith, Agmo, Birnie, &

French, 2010; Snowdon et al., 2010), increases social interaction (Parr, Modi, Siebert, & Young, 2013), and inhibits social aversion (Parr et al., 2013). Vasopressin has been less studied in NHPs, but may play a role in promoting paternal care in tamarins (Kozorovitskiy, Hughes, Lee, & Gould, 2006). In humans, OT has been implicated in a wide variety of social behaviors, ranging from trust and altruism in economic games (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005) to eye contact and social attention (Auyeung et al., 2015), as well as a role in reducing anxiety (Bartz, Zaki, Bolger, & Ochsner, 2011). However, a common limitation of both human and NHP research into the role of OT and AVP is the reliance on laboratory tasks in relatively small samples, with correspondingly less variable and dynamic social stimuli than in natural environments. Humans and many NHP species live in large social groups where maintaining relationships and navigating hierarchies are crucial to biological success (L. J. N. Brent, Ruiz-Lambides, & Platt, 2017; Fedigan, 1983; Silk, Alberts, & Altmann, 2003; Steptoe, Shankar, Demakakos, & Wardle, 2013). Yet to what extent laboratory findings regarding OT and AVP generalize to richer, more realistic social environments remains an open question. In this paper, we attempt to address this question by comparing variability in social behavior in free-ranging rhesus macaques (*Macaca mulatta*) in a naturalistic setting, to variability in the genes that encode OT and AVP receptors, OXTR and AVPR.

The *OXTR* and the *AVPR* family of genes encode the OT and AVP receptors respectively. While *OXTR* is the only oxytocin receptor gene, three AVP genes encode three different vasopressin receptors: *AVPR1A*, *AVPR1B*, and *AVPR2*. *AVPR1A* and *1B*, but not *2*, are expressed in the brain (Freeman, Inoue, Smith, Goodman, & Young, 2014). Since OT and AVP share similar amino-acid sequences, they can bind to each other's receptors, albeit with different affinities (Freeman et al., 2014; Young & Flanagan-Cato, 2012). This structural commonality may contribute to similarities in the range and type of processes that the two neuropeptides mediate.

Researchers have consistently cited *AVPR1A* as the *AVPR* gene most relevant to social functions (Freeman & Young, 2016). In humans, genetic variants of *AVPR1A* are associated with behaviors ranging from altruism in an economic game (Israel et al., 2008) to self-reported partner bonding (Walum et al., 2008). *AVPR1B* variation, while not implicated as directly in social behavior, is associated in humans with stress responses (Keck et al.,

2008) and mood disorders (Dempster et al., 2007). *OXTR* polymorphisms in humans have been linked to variation in a wide range of social behaviors and phenotypes, ranging from attachment style (Costa et al., 2009) and prosociality in economic games (Israel et al., 2009) to emotion perception and stress reactivity (Rodrigues, Saslow, Garcia, John, & Keltner, 2009) (see (Ebstein, Knafo, Mankuta, Chew, & Lai, 2012) for a more thorough review).

The role of variation in these genes is less studied in NHPs than in humans, though several studies have investigated an indel polymorphisms in the 5' flanking region of *AVPR1A* in the social behavior of captive great apes (Hopkins, Donaldson, & Young, 2012; Latzman, Hopkins, Keebaugh, & Young, 2014; Staes et al., 2015; V. A. D. Wilson et al., 2017). (Hopkins et al., 2012), (Latzman et al., 2014), and (V. A. D. Wilson et al., 2017) each examined the relationship between the long versus short allele and personality, as measured by observer questionnaires, in captive chimpanzees (*Pan troglodytes*). Hopkins et al. (2012) found a sex-by-genotype interaction whereby "dominance" and "conscientiousness" personality traits differed between males and females, but only among animals carrying a copy of the long allele, while Wilson et al. (2017) found using the same personality dimensions a Hopkins et al. (2012), that the long allele predicted decreased extroversion but with no interaction with sex. Latzman et al. (2014), using a similar questionnaire-based data set, but a different decomposition into personality dimensions, also reported a sex-by-genotype interaction wherein males carrying the long allele were higher for "dominance" and "stability" personality dimensions. (Staes et al., 2015)) also looked at captive chimpanzee personality, but used observed rates of specific behaviors to assess personality rather than questionnaire ratings, and reported that the long allele was associated with total time spent giving and receiving grooming. (Staes et al., 2016) reported a study of the same polymorphism in captive bonobos rather than chimpanzees, using both observer questionnaires and behavioral rate observations, and found the long allele associated with higher "attentiveness" and lower "openness". To our knowledge only Staes et al. (2015) has examined *OXTR* in NHPs social behavior, which reported no effect of an intronic SNV.

In the present study, we drew on a large set of behavioral and genetic sequence data from the free-ranging rhesus macaque colony on Cayo Santiago Island off the coast of Puerto Rico. Rhesus macaques are an excellent

model organism for studying biological and environmental influences on social behavior due to their extensive use in laboratory and field research, and complex social behaviors that are critical to their survival and reproductive output (Lauren J. N. Brent et al., 2013; L. J. N. Brent et al., 2017; Massen et al., 2012). The large macaque population and minimal human intervention on Cayo Santiago provide a unique opportunity to study the genetic influence of *OXTR* and *AVPR* variation in a naturalistic setting where social environment more closely resembles conditions in the wild and social behavior can directly impact biological success. Demographic, life history, and pedigree data are also available for the Cayo population, which allows us to disentangle the effects of specific genetic regions from the influence of environment and overall genetic similarity. Though OT and AVP have been implicated in a variety of behaviors that include both social and non-social, here our interest lay specifically with social interactions between monkeys that indicate the quality and nature of their social relationships. Accordingly, we focused on rate of giving and receiving grooming, approaches towards another macaque (approach), being in non-grooming physical contact (passive contact), of aggression that resulted in physical contact between macaques (contact aggression), aggressive actions and threats that did not result in physical contact.

Methods

Study site

The studied population is a colony of rhesus macaques living on the island of Cayo Santiago, a 15-hectare island located 1km off the southeastern coast of Puerto Rico. This is a free-ranging, freely-breeding population with known pedigrees, rich data on life histories and fitness, and extensive genetic and observational data on behavior. The colony was founded in 1938 with a population of 409 Indian-origin rhesus macaques and is currently maintained by the Caribbean Primate Research Center (CPRC; University of Puerto Rico Medical Sciences Campus). The population as of July 2017 numbered 1571 animals self-organized into six different social groups. 537 of the animals are adults of age six or above, and 758 are juveniles between the ages of one

and five. Researcher and caretaker intervention in the population is minimal. Animals in the colony are provided commercially available monkey chow daily and unlimited access to water. Animals are handled only during designated annual trapping periods, during which infants are tagged for identification. Despite the lack of immigration since its founding there is little evidence for high rates of inbreeding on Cayo Santiago (Blomquist, 2009; Widdig et al., 2016). All procedures described below were approved by the University of Puerto Rico's Institutional Animal Care and Use Committee (IACUC #A6850108) and adhered to the legal requirements of the United States of America and the American Society of Primatologists' Principles for the Ethical Treatment of Primates.

Genetic data

Animals were captured by CPRC staff and technicians during annual trapping procedures. Following capture, subjects were caged and anesthetized for blood draws using an intramuscular injection of ketamine HCl, 10mg/kg body weight. Blood was drawn by animal health technicians, and animals were released after full recovery from anesthesia. DNA was immediately isolated from blood in the field using commercially available QIAGEN extraction kits (QIAamp DNA Blood Mini Kit). Extracted DNA was stored frozen until shipment to the Genomics and Microbiology Research Lab at the North Carolina Museum of Natural Sciences, where libraries were prepared for next-generation sequencing, and a catalog of variants were genotyped. We used standard tools to identify single nucleotide variants (SNVs) by aligning sequence reads to the three genes of interest (*OXTR*, *AVPR1A* and *AVPR1B*) from the two most recent published rhesus macaque reference assemblies (Rhesus Macaque Genome Sequencing and Analysis Consortium et al., 2007; Zimin et al., 2014), as well as the current reference assembly (rheMac8 or Mmul_8.0.1).

Our variant calling pipeline integrated read alignment using bwa-mem (H. Li & Durbin, 2009), PCR duplicate removal using picard, as implemented within SAMtools (H. Li et al., 2009) and simultaneous SNV discovery using GATK (McKenna et al., 2010). We excluded SNVs with minor allele frequency <0.05, where the minor (vs major) allele refers to the allele that is less (vs more) frequent in the sampled population. If genotype coverage depth fell below a minimum of two reads, then genotypes were imputed using standard default

parameters in the software Beagle version 4.1 (Browning & Browning, 2016). All variants were annotated using the software SNPeff (Cingolani et al., 2012), and SNVs of interest were those predicted as having high, moderate, or low impact. Genotypes and their predicted impact results were then compared across all three reference assemblies. All reported genotypes were denoted using rheMac8 genomic coordinates. Next, designated missense variants were assessed for predicted functional impact (e.g., protein structure, protein stability, binding affinity, etc.) using the SNAP2 browser (Hecht, Bromberg, & Rost, 2015). Finally, human orthologues of the macaque SNVs were identified using KAVIAR and the UCSC Genome Browser (Glusman, Caballero, Mauldin, Hood, & Roach, 2011; Kent et al., 2002). We used dbSNP (build 150) to determine whether any human orthologues had known clinical significance (Sherry et al., 2001).

Nearby SNVs are often highly correlated to the level of redundancy, which can cause issues in interpreting and estimating phenotypic effects. Accordingly, we iteratively identified pairs of the SNVs with a correlation >0.9 and randomly removed one of the SNVs, repeating the process until no such pairs existed. Only the SNVs which survived this process were included in the behavioral analyses.

Behavioral data

The data is comprised of ten-minute focal samples(Altmann, 1974). The order in which animals were observed was semi-randomized to equalize the times of day and year of each animal's observation periods. Observers recorded the times at which the monkey engaged in any behaviors specified by a rhesus macaque-specific ethogram consisting of both social and non-social behaviors (Lauren J. N. Brent, 2010). A total of 201 macaques (123 females, 78 males) were both represented in the behavioral data set and had genotype data available. The behavioral data used in this study were 19,501 ten-minute focal observations collected from adult (age $\geq$ 6 years) male and female macaques from five social groups, F, R, V, HH, and KK. Observational data was collected from group KK in 2014, from group F during 2011 through 2016 , from group V during 2015 and 2016, and from group HH and R during years 2014 and 2015, respectively. If an animal had an unusually small number of focal observations taken for their group in a given year, their focal observations from that year were removed from the data set. The threshold for removal was two standard deviations below the mean number of

focal observations across animals for their social group in that year. The total number of focal observations per animal across all years ranged from 174 (approximately 29 hours) to 11 (approximately 1.83 hours), with an average of 79.99 focal observations per animal (approximately 13.16 hours).

The following behaviors were analyzed in this study:

1. Grooming: Running the hands or mouth through the hair of another monkey for at least 5 s.
2. Passive contact: Sitting or lying in physical contact with another animal without grooming.
3. Approach: One individual approaches another to within arms' reach (2 m) without physical contact, and remains within that distance for at least 5 s.
4. Contact aggression: Direct physical contact such as a bite, hit, push, or grab.
5. Noncontact aggression: A lunge, charge, or chase that does not result in direct physical contact, or a threatening gesture that entails some combination of staring, barks, head bobs, and opening one's mouth with covered teeth.

Each behavior except for passive contact was further divided into whether the focal animal performed the action or received the action from another animal, for a total of nine interaction types. Only social interactions involving another adult macaque were used in this study; interactions with infant or juvenile macaques were not used.

Pedigree data

We obtained animal pedigrees from a long-term database maintained by the CPRC. From the founding of the population up through 1992, maternal identity was ascertained by behavioral observations, such as nurturing behaviors and lactation. For most macaques born after 1992, both maternal and paternal identity were ascertained genetically through the analysis of 29 microsatellite markers (Lauren J. N. Brent et al., 2013). In this study, maternity was known from genetics for 195 macaques (97%), while paternity was known from genetics for 197 macaques (98%). When maternity was not known from genetics, maternity assignments from behavior were used. The population pedigree was used to generate a kinship matrix across animals via R package kinship2. We multiplied each element of the kinship matrix by two to create the genetic covariance matrix in

order to measure the heritability of social behaviors. Maternal identity was known on average for 6 (1.4 sd) previous generations, and paternal identity was known on average for 2 (0.8 sd) previous generations.

Data processing and model specification

We used individual 10-minute focal observations as the basic unit of analysis rather than aggregating those focal observations into rates of behaviors for each animal across longer periods of time. The motivation for this approach is that when animals have different numbers of focal observations being aggregated into a single rate, the rates of animals with fewer focal observations will be intrinsically more noisy and less precise than those of animals with more focal observations, and thus should be weighed less. By using individual focal observations as data points, the number of focal observations itself for a given animal in a given year provides the appropriate weighting.

We represented each focal observation in terms of the total amount of each behavior that occurred during that focal observation. For behaviors with durations, such as grooming, that amount corresponded to the total time spent engaged in that behavior, while for events such as approaches, it was the number of times that behavior occurred during an observation. The distributions of behavior amounts across focal observations were highly right-skewed and zero-inflated for each of the behaviors examined in this study. Furthermore, some behavior amounts were continuous (e.g., amount of time grooming), while others were discrete (number of times aggressive acts occurred). These issues rendered ordinary linear regression inappropriate. Therefore, for each focal observation, each behavior was discretized into one of three ordered categories, or levels. The levels corresponded to a behavior not occurring at all (none), occurring at a low rate (low), and occurring at a high rate (high) during a given focal observation. Focal observations in which the behavior did occur were assigned to the "none" category, and we divided the remaining observations into the "low" and "high" categories by performing a median split on the behavior amounts. Note that the median used for the median split was calculated using only the focal observations not in the "none" category. The end result is that each focal observation was represented as a vector of category labels (none, low, or high), one for each behavior. This approach preserved

information about relative amounts of behaviors while avoiding problems arising from mismatches between an assumed distribution of observations (e.g. normal or poisson) and the true distribution.

We assessed the contribution of the genetic variants to social behaviors using multivariate ordered logistic regression. We included age (linear and quadratic), sex, dominance rank (linear and quadratic), age-by-sex (linear and quadratic) and rank-by-sex (linear and quadratic) interactions as fixed effects covariates in the model. Nonlinear terms were included for age because the effect of aging one year likely changes across the lifespan, and for dominance rank because the difference between low and middle-ranked macaques may not be the same as the difference between middle and high ranked macaques. Dominance rank was represented on an ordered categorical scale: low-ranking animals outranked less than 50% of their social group, medium-ranking animals outranked between 50% and 80%; and high-ranking animals outranked greater than 80%. All covariates were mean-centered, and the linear and quadratic age terms were orthogonalized against each other and z-scored.

Additive genetic effects, permanent environment effects, maternal effects, and the year and group during which the observation took place (that is, observations from each year-group pair being grouped together) were included as random effects. We defined genetic and permanent environment effects as those associated with particular animals that were consistent across focal observations of the same animal, but they differed in whether the effects were correlated across animals. Additive genetic effects refer to animal-specific effects that were assumed to be correlated across animals according to their kinship, here calculated using the Cayo pedigree, while permanent environment effects were assumed to be independent across animals (Fisher, 1918; Kruuk, 2004). We also note that permanent environment effects are “permanent” in the sense that they are consistent within an animal across the full timespan that the animal was studied. Finally, maternal effects were effects consistent across all focal observations of animals with the same dam (A. J. Wilson et al., 2010). The magnitude of the additive genetic variance component relative to the other sources of variation determined the narrow-sense heritability of a phenotype.

The effects of genotypes on behaviors was modeled as random effects with a heavy-tailed distribution, details of which are described in the section below. The motivation for treating genotypic effects as random rather than fixed is to provide regularization and prevent false positives (Gelman, Hill, & Yajima, 2012). This approach is also consistent with the approach used by genomic prediction tools, which generally assume that for complex phenotypes, many genetic variants have some small effect that comes from a common distribution that is estimated directly (Yang et al., 2011; Zhou et al., 2013). Animal genotypes were coded as the number of minor alleles at each locus (Balding, 2006; Yang, Lee, Goddard, & Visscher, 2011).

Regression model and fitting procedure

We used a multivariate ordinal logistic version of the animal model:

$$\begin{aligned} \eta_{i,j} &= x_i'\beta_j + z_i'u_j + s_{a(i)}'\lambda_j + g_{a(i),j} \\ \mathrm{p}(y_{i,j} > 0|\eta_{i,j}) &= \mathrm{logit}^{-1}(\eta_{i,j} - \alpha_j^{(0)}) \\ \mathrm{p}(y_{i,j} > 1|\eta_{i,j}) &= \mathrm{logit}^{-1}(\eta_{i,j} - \alpha_j^{(1)}) \\ \mathrm{logit}^{-1}(x) &= \frac{1}{1+\exp(-x)} \end{aligned}$$

where $y_{i,j}$ is the level (0, 1, or 2) of behavior j that occurred during focal observation i, x_i is a vector of fixed effects covariates, and z_i is a vector of random effects covariates. The vector s_a is the vector of SNVs belonging to the animal a, while $a(i)$ is the focal animal followed during observation i.

The parameters β_j, u_j and λ_j are the regression weights for behavior j associated with the fixed effects, random effects, and SNV effects respectively. The parameters g_a represent the overall additive genetic component to the phenotype of animal a, as in the traditional animal model (Henderson, Kempthorne, Searle, & von Krosigk, 1959; Kruuk, 2004). The parameters α_j, where $\alpha_j^{(0)} < \alpha_j^{(1)}$, are offsets that determine the baseline probabilities of each level of behavior j.

To avoid false positives when estimating the genetic effects, we regularized the effect estimates using a flexible sparsity-inducing prior:

$$\lambda_{j,l} \sim \text{Student's t}(4, 0, \sigma_\lambda^2)$$
$$\sigma_\lambda \sim \text{N}^+(0, 2)$$

where $\lambda_{j,l}$ is the effect of a minor allele at locus l on behavior j. The genetic effects are given a Student's t distribution centered at zero with four degrees of freedom, with a width σ_λ that is estimated from the data and is given a normal prior truncated at zero. This and similar priors have been used frequently in the estimation of genetic effects and predicting genetic values for animal breeding (T. Meuwissen & Goddard, 2010; T. H. Meuwissen, Hayes, & Goddard, 2001; Resende et al., 2012; Zhou, Carbonetto, & Stephens, 2013). This prior has the property that effects for which the evidence is weak will be penalized and pooled towards zero, preventing overfitting, but because of the *t*-distribution's heavy tails, large effects for which there is strong evidence will be preserved.

Finally, the fixed effects, random effects terms are given weakly-informative priors (Gelman, 2006):

$$\beta_j \sim \text{N}(0, 2)$$
$$u_j \sim \text{N}(0, \sigma_j^2)$$
$$g_j \sim \text{N}(0, \gamma_j^2 A)$$
$$\sigma_j \sim \text{N}^+(0, 2)$$
$$\gamma_j \sim \text{N}^+(0, 2)$$

Where A is the relatedness matrix among animals, and σ_j^2 and γ_j^2 are the random effect variance components and the additive genetic variance components respectively. Note that although for brevity only one random effect covariance component is listed in the equations, separate variances were fit for the animal identity and observer identity random effects terms.

Following (S. W. Davies, Scarpino, Pongwarin, Scott, & Matz, 2015; Nakagawa & Schielzeth, 2010; Vazquez et al., 2009), we estimated the narrow-sense heritability of each behavior j using the equation $\gamma_j^2/(\gamma_j^2 + \sigma_j^2 + \pi^2/3)$ where σ_j^2 is the sum of the variance components associated with permanent environmental and maternal effects. Note that heritability as estimated here refers to heritability of the latent continuous variables underlying logistic regression, rather than of the discrete behavioral data itself. Further, because fixed

effects are not taken into account, it is the heritability in a population of animals belonging to the same social group and sex, and of similar age and rank, and so on.

We estimated posteriors for the model parameters using Markov-Chain Monte-Carlo sampling sampling via the Stan software package (Stan Development Team, 2016). We ran three chains with 1000 samples each, discarding the first 200 iterations of each as burn-in, for a total of 2400 samples used for inference. Convergence was assessed using the Rhat metric reported by the rstan package.

We report the estimated effects of a SNV on behavior as the percent change in the odds of a behavior occurring associated with having one more copy of the minor allele. This corresponds to $\exp(\lambda) - 1$ rather than the raw λ values themselves. The reported point estimates of all parameters, effects, and quantities of interest are posterior means. We estimated the phenotypic variance contributed to behavior j by all the SNVs together as $\lambda_j' S S' \lambda_j$, where S is the matrix of mean-centered genotypes.

Results

OXTR and *AVPR* variants

We identified a total of 25 SNVs of interest (6 *OXTR*, 13 *AVPR1A*, and 6 *AVPR1B*). Table 1 shows genomic coordinates and descriptive information for these variants. Of these 25 SNVs, eight were missense variants (see Table 2). Two of the missense variants (one in *AVPR1A* and one in *AVPR1B*) were predicted to impact the structure of the receptor, according to assessments in SNAP2. Eleven SNVs had known analogues in the human genome, though none of those SNVs had known clinical significance (see Table 3). After pruning for high linkage disequilibrium among the 25 SNVs (see Methods), we retained 12 SNVs for phenotypic analysis (3 *OXTR*, 5 *AVPR1A*, and 4 *AVPR1B*).

Heritability and repeatability of rates of social behaviors

Before examining effects of specific single-nucleotide variants on social behaviors, we first assessed both genetic and non-genetic variability in social behaviors across individuals. Figure 1 shows the proportion of variance accounted for by additive genetic variance (heritability); the variance accounted for by permanent environmental effects; and the variance accounted for by maternal effects.

The three variance component estimates were modest for all behaviors. We estimated that the largest and smallest additive genetic effects accounted for 3.8% (passive contact) and 1.2% (noncontact aggression received) of total variance of their respective behaviors. Similarly the largest and smallest estimated permanent environment effects were 5.1% (contact aggression received) and 0.6% (approach given) of total variability, and the largest and smallest maternal effects were 3.1% (grooming given) and 0.5% (approach received). Posterior uncertainty for all three variance components were such that negligibly small variance contributions could not be ruled out for most behaviors. The explained variance had a greater than one-in-ten chance of being below 1% for all variance components and all phenotypes, with the exception that the additive genetic variance component explained <1% of the variability of approaches (given) with probability 0.06.

Some of this posterior uncertainty is due to the fact that additive genetic and permanent environment are effectively correlated because permanent environmental effects are independant per-animal effects, and animals are genetically identical to themselves. Similarly, the fact that many dams had only one offspring in the data set (201 macaques with 156 unique dams) resulted in a correlation between maternal and permanent environmental effects. This relationship made it difficult for the model to distinguish between one effect being large and the others small, or the reverse. However, the sum of the three variance components was better determined, as can be seen in Figure 1, from the fact that in several behaviors, the sum had smaller 95% credible intervals than any individual variance component. The sum of heritability, permanent environmental effects, and maternal effects is the “repeatability” of a trait within individuals; that is, variability that is consistent within animals across observations, but that is not explained by the demographic and environmental variables in the fixed effects or the observation period random effect. Repeatability contributed >1% of total variance with probabilities >0.95 for all nine behaviors.

Effect sizes and credible intervals for the fixed effects parameters are shown in Supplementary Figure 1.

Effects of SNVs on rates of social behaviors

The estimated effects on social behavior of oxytocin and vasopressin SNV minor alleles are shown in Fig. 2. All effects were small, with the absolute average effect size estimated as a 0.2% change in the odds of a social behavior occurring, and the largest absolute effect estimated as a 1.7% change in odds. The 95% credible intervals (CI) included zero for all loci and all behaviors. Collectively, all SNVs together contributed between 0.03% and 0.04% of total phenotypic variation for each behavior.

Beyond encompassing an effect size of zero, posterior distributions of the SNV effect sizes also indicated that the range of plausible effect sizes was small. Out of the 108 effects estimated (9 behaviors by 12 SNVs), 105 had 95% CIs that did not extend beyond an absolute effect size of a 6% change in odds. Of the remaining three effects whose CIs did exceed 6%, two were associated with *AVPR1A* missense variant chr11:62125302. The effects of this SNV were detected for approaches given (1.2% effect size, CI=[-1.7%, 8.7%]) and approaches received (1.6% effect size, CI=[-1.2%, 11.1%]).

Our analyses described above used both male and female rhesus macaques, but it is possible that genetic effects differ between sexes. We therefore re-analyzed our behavioral data using only female focal animals interacting with other adult females. Supplementary Figures 2 and 3 depict the results of this analysis, which were both qualitatively and quantitatively similar to the analysis that included both sexes.

Discussion

Our analysis of *OXTR* and *AVPR* genetic variants sought to measure the relationship between genetic variation in those genes and rates of spontaneous social interactions in a naturalistic setting in rhesus macaques, a highly social primate species. Our results are consistent with genetic variation in *OXTR* and *AVPR* having little to no influence on rates of social interaction. Though a number of previous studies found relationships between social

behaviors and genetic variants in these genes, including in NHPs, this result is not entirely surprising and has several potential explanations.

First, many genetic associations identified in the human literature entailed analyses of behavior from laboratory tasks (Johansson et al., 2012; Knafo et al., 2008; Rodrigues et al., 2009) or from clinical phenotypes such as autism and mood disorders (Dempster et al., 2007; Israel et al., 2008; Lerer et al., 2008). It may be that genetic influences become attenuated in the more naturalistic social situations and non-clinical phenotypes studied here. Previous studies in great apes were more similar to ours in that they involved natural, non-clinical social behaviors, but to our knowledge these examined only captive populations in zoos and research colonies (Hopkins et al., 2012; Latzman et al., 2014; Staes et al., 2015; V. A. D. Wilson et al., 2017). Such settings are more constrained than Cayo Santiago in that animals cannot easily self-sort into distinct social groups, and human researchers and caretakers often intervene in reproductive success, health, access to resources for members of the population, and in order to prevent aggression that could cause serious injury or death. It is possible that in a naturalistic, unconstrained environment such as Cayo Santiago, environmental variability is larger and effectively "drowns out" genetic contributions. Second, our results are consistent with recent findings that in general, complex behavioral and morphological phenotypes have a massively polygenic genetic architecture and individual variants have very small effects (Anney et al., 2012; Benjamin et al., 2012; Boyle, Li, & Pritchard, 2017; Chabris et al., 2013; G. Davies et al., 2011; Yang et al., 2010, 2015). Accordingly, it appears that sample sizes of tens of thousands or more may be required to reliably distinguish from zero the effects of common genetic variants (Lango Allen et al., 2010; McCarroll, Feng, & Hyman, 2014; Rietveld et al., 2013, 2014; Speliotes et al., 2010).

Finally, it is worth noting that, historically, both genome-wide and candidate gene studies of complex phenotypes with small sample sizes have low replication rates and are prone to false-positives (Chabris et al., 2012; Hart, de Wit, & Palmer, 2013; Ho et al., 2010; Ioannidis, Tarone, & McLaughlin, 2011; Siontis, Patsopoulos, & Ioannidis, 2010). We know of two reported replication failures of *OXTR* gene effects (Apicella et al., 2010; Munk, Hermann, El Shazly, Grant, & Hennig, 2016). Furthermore, two recent meta-analyses

reported equivocal results regarding the influence of two heavily studied human *OXTR* SNVs on sociality, with (Bakermans-Kranenburg & van Ijzendoorn, 2014; J. Li et al., 2015). Similarly, though several studies have implicated a 5' *AVPR1A* polymorphism in chimpanzee social behavior, the identified effects have been inconsistent. Hopkins et al (2012) and Latzman et al (2014) reported sex-by-genotype interactions and no main effects, whereas Staes et al (2015) and Wilson et al (2017) found only main effects of genotype on personality traits and no interactions with sex. While Staes et al (2015) and Wilson et al (2017) both reported effects of genotype on personality traits relating to prosocial and affiliative behaviors, the effects were in opposite directions, with the same allele predicting higher prosociality in Staes et al (2015) and lower in Wilson et al (2017). While there may be unknown moderators that account for the differences between these studies, these inconsistent findings may also be the result of a lack of statistical power (Gelman & Carlin, 2014; Lemoine et al., 2016; Open Science Collaboration, 2015). Adding weight to this interpretation, we note that several of the papers listed above do not control for overall genetic relatedness within the population, do not adjust p-values for multiple comparisons or otherwise regularize effect estimates, or both, which can increase the likelihood of finding a false-positive genetic association. It may therefore be prudent to view *OXTR* and *AVPR* associations as preliminary, both in humans and in great apes, until they have been directly replicated.

Though we did not find evidence that *OXTR* and *AVPR* variants influenced social behavior, our results do suggest that social behaviors on Cayo Santiago have a modest additive genetic component. This is consistent with previous research on the same macaque population (Lauren J. N. Brent et al., 2013, 2014). This finding is also consistent with the theory that genetic influences on the social behaviors studied here are driven by small effects across large numbers of genetic polymorphisms (Fisher, 1918), however, it is worth noting that the magnitude of additive genetic effects relative to permanent environment and maternal effects was not well resolved in this study.

Our results do not indicate that all genotypic variability in the Cayo Santiago rhesus macaque population in *OXTR* and *AVPR* have small or no effects on rates of social interaction. Rare variants with very low MAF and *de novo* mutations are likely to have larger effects on complex phenotypes than common variants (Gratten,

Wray, Keller, & Visscher, 2014; Neale et al., 2012), and because common variants are imperfectly correlated with rare variants and not at all with *de novo* variants (Eberle, Rieder, Kruglyak, & Nickerson, 2006; Speed, Hemani, Johnson, & Balding, 2012), those sources of genetic variability are not well captured by the SNVs genotyped in this study. Future research may profitably target rare rather than common genetic variants. Alternatively, it may be fruitful to broaden the scope of the common variants examined to include not just the *OXTR* and *AVPR* genes themselves but also the broader gene networks that may impact OT and AVP function. Recent research suggests that aggregating information across large numbers of common variants may permit the identification of genetic contributions from specific genomic regions, even in sample sizes that are small relative to those used in traditional GWAS research (Benjamin et al., 2012; Yang, Manolio, et al., 2011), however, little is currently known regarding the sample sizes required to reliably estimate the contributions of a gene set or network.

Conclusion

Though the relationship between social behavior, the molecules OT and AVP and their associated receptor genes, *OXTR* and *AVPR* has been studied extensively in laboratory settings in humans and captive animal populations, it is unknown to what extent those findings generalize to spontaneous behaviors in naturalistic environments. We examined this issue using an extensive behavioral and genomic data set from the free-ranging rhesus macaque population on Cayo Santiago, focusing on the relationship between *OXTR* and *AVPR* single nucleotide variants and social interactions related to the quality and kind of social relationships between animals. We found that the effects of SNVs in *OXTR* and *AVPR* on rates of social interactions were very small and possibly nonexistent, consistent with the idea that common genetic variants have generally weak effects on complex phenotypes.

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Figure 1: Proportion of residual variance attributed to additive genetic, permanent environmental, maternal effects, and the total proportion of residual variance explained by the three factors together. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

Figure 2: Effect sizes of *OXTR*, *AVPR1a*, and *AVPR1b* SNVs on social behaviors. Effect sizes are shown in terms of the additive effect of a minor allele on the percent change in the odds of a social behavior occurring during a focal observation. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

Figure S1: Effect sizes for fixed effects. Effect sizes are shown in terms of the percent change in the odds of a social behavior occurring during a focal observation associated with a unit increase in the dependant variable. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

Figure S2: Proportion of residual variance attributed to variance components and their sum for social behaviors between adult females only. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

Figure S3: Effect sizes of SNVs on social behaviors between adult females only. Effect sizes are shown in terms of the additive effect of a minor allele on the percent change in the odds of a social behavior occurring during a focal observation. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

Table 1: *OXTR*, *AVPR1A*, *AVPR1B* SNVs

Table 2: *OXTR*, *AVPR1A*, *AVPR1B* missense variants

Table 3: *OXTR*, *AVPR1A*, *AVPR1B* human genome analogs

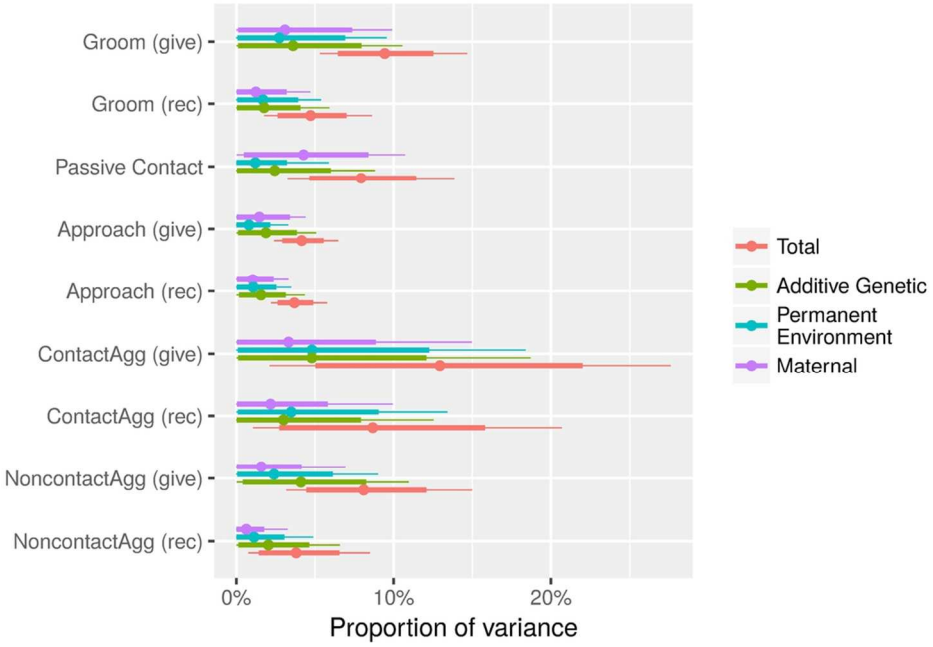

Figure 1: Proportion of residual variance attributed to additive genetic, permanent environmental, maternal effects, and the total proportion of residual variance explained by the three factors together. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

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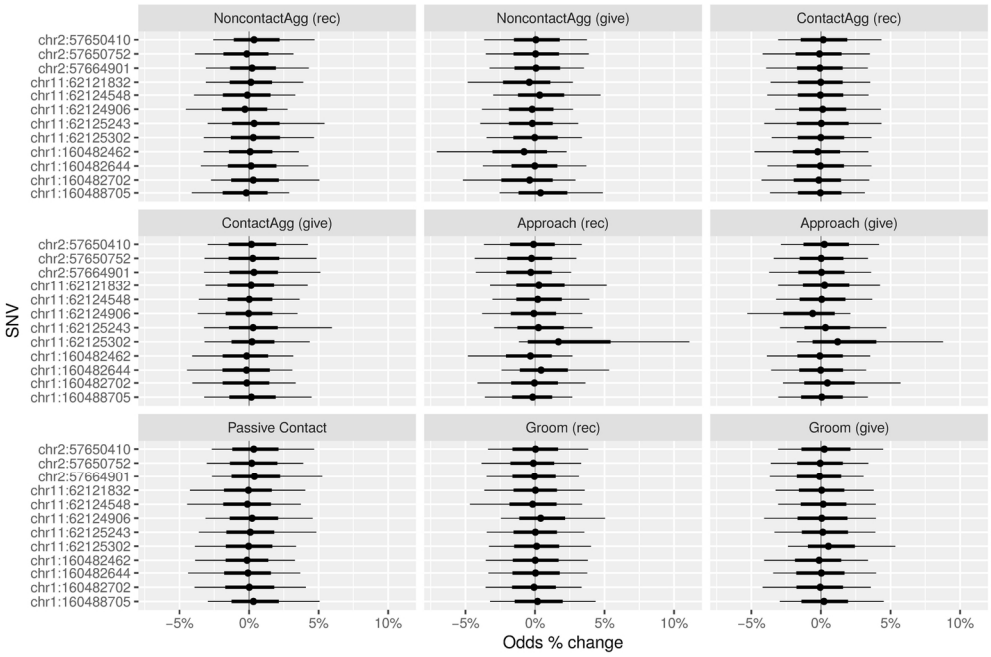

Figure 2: Effect sizes of OXTR, AVPR1a, and AVPR1b SNVs on social behaviors. Effect sizes are shown in terms of the additive effect of a minor allele on the percent change in the odds of a social behavior occurring during a focal observation. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

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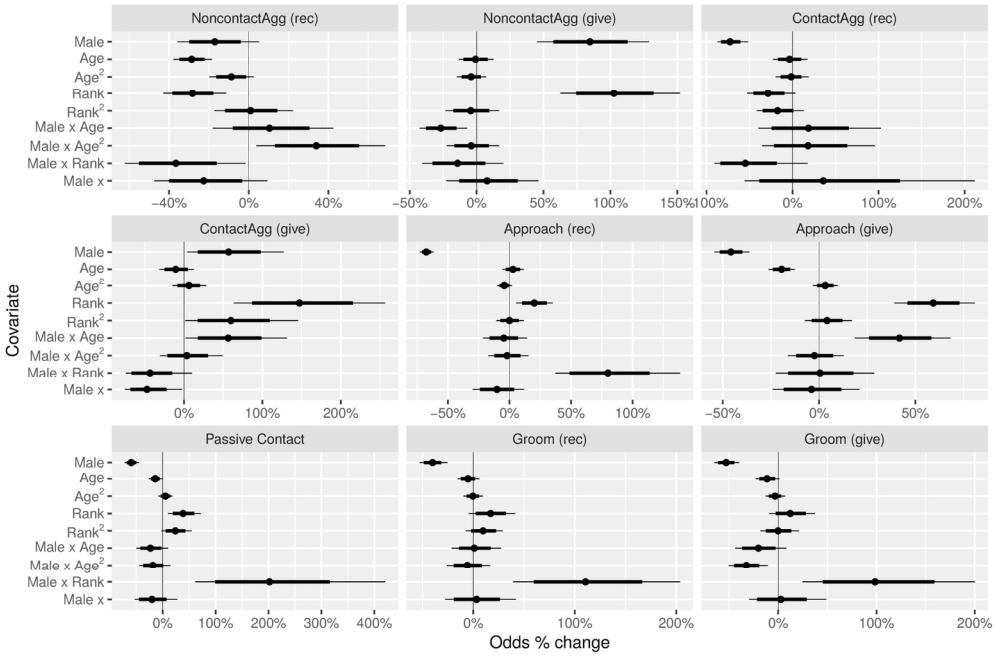

Figure S1: Effect sizes for fixed effects. Effect sizes are shown in terms of the percent change in the odds of a social behavior occurring during a focal observation associated with a unit increase in the dependant variable. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

153x102mm (300 x 300 DPI)

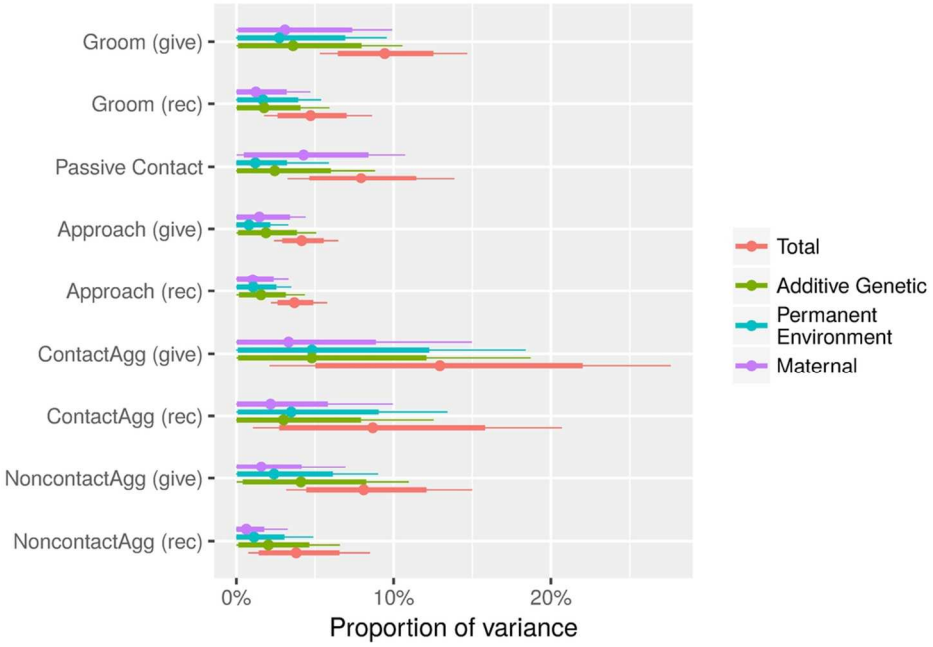

Figure S2: Proportion of residual variance attributed to variance components and their sum for social behaviors between adult females only. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

102x68mm (300 x 300 DPI)

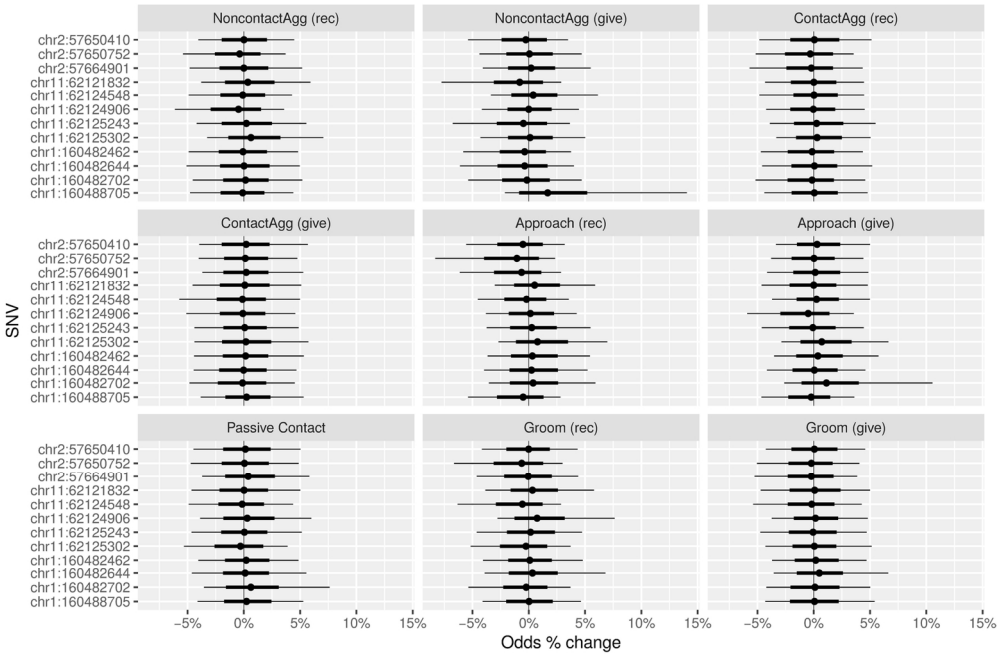

Figure S3: Effect sizes of SNVs on social behaviors between adult females only. Effect sizes are shown in terms of the additive effect of a minor allele on the percent change in the odds of a social behavior occurring during a focal observation. Points indicate posterior means, while thick lines and thin lines indicate 80% and 95% central credible intervals, respectively.

153x102mm (300 x 300 DPI)

Research highlights

- We examined whether genetic variation in the OT receptor gene *OXTR* and the AVP receptor genes *AVPR1A* and *AVPR1B* influence naturally-occurring social behavior in free-ranging rhesus macaques, gregarious primates that share many features of their biology and social behavior with humans.

- We measured rates of grooming, approaches, passive contact, contact aggression, and non-contact aggression, given and received, across 3,250 hours of observational behavioral data from 201 free-ranging rhesus macaques on Cayo Santiago island in Puerto Rico, and used genetic sequence data to identify 25 *OXTR*, *AVPR1A*, and *AVPR1B* single-nucleotide variants (SNVs) in the population.

- Though we found evidence for modest heritability of these behaviors, estimates of effect sizes of the selected SNVs were close to zero, indicating that common *OXTR* and *AVPR* variation contributed little to social behavior in these animals. Our results are consistent with recent findings in human genetics that the effects of individual common genetic variants on complex phenotypes are generally small.

Gene	Position	Consequence Type	Read Depth	Mapping Quality	Reference Allele	Alternate Allele	Alternate Allele Frequency	Included in Behavioral Analysis
OXTR	chr2:57649859	missense variant	893	59.95	G	T	0.72	
OXTR	chr2:57649912	synonymous variant	917	59.97	A	C	0.34	
OXTR	chr2:57650182	synonymous variant	1117	60	C	T	0.34	
OXTR	chr2:57650410	synonymous variant	1018	60	C	G	0.48	Yes
OXTR	chr2:57650752	synonymous variant	1693	59.96	C	T	0.34	Yes
OXTR	chr2:57664901	synonymous variant	2717	59.99	A	G	0.28	Yes
AVPR1A	chr11:62121832	missense variant	1767	60	A	C	0.09	Yes
AVPR1A	chr11:62124427	synonymous variant	1830	53.03	C	T	0.91	
AVPR1A	chr11:62124548	missense variant	1912	60.01	C	A	0.36	Yes
AVPR1A	chr11:62124701	missense variant	1650	60	A	C	0.48	
AVPR1A	chr11:62124871	synonymous variant	1046	60	C	G	0.89	
AVPR1A	chr11:62124901	synonymous variant	1049	60	G	A	0.11	
AVPR1A	chr11:62124906	missense variant	1069	60	A	T	0.72	Yes
AVPR1A	chr11:62125186	synonymous variant	822	60	T	C	0.1	
AVPR1A	chr11:62125214	missense variant	947	60	A	G	0.09	
AVPR1A	chr11:62125231	synonymous variant	970	60	C	T	0.46	
AVPR1A	chr11:62125240	synonymous variant	925	60	T	C	0.1	
AVPR1A	chr11:62125243	synonymous variant	911	60	A	G	0.1	Yes
AVPR1A	chr11:62125302	missense variant	1027	60	G	A	0.48	Yes
AVPR1B	chr1:160482462	synonymous variant	1353	60	G	A	0.83	Yes
AVPR1B	chr1:160482464	missense variant	1290	59.97	C	T	0.89	
AVPR1B	chr1:160482644	synonymous variant	1288	59.97	G	A	0.87	Yes
AVPR1B	chr1:160482660	synonymous variant	1282	59.96	G	A	0.88	
AVPR1B	chr1:160482702	synonymous variant	1274	60	C	G	0.88	Yes
AVPR1B	chr1:160488705	synonymous variant	1615	59.99	T	C	0.44	Yes

Table 1. *OXTR*, *AVPR1A*, and *AVPR1B* SNVs

Gene	Position	Amino Acid Substitution	Amino Acid Position	Functional Effect	SNAP2 Score	Expected Accuracy
OXTR	chr2:57649859	Ala>Ser	6	no effect	-88	93%
AVPR1A	chr11:62121832	Ile>Val	419	no effect	-98	97%
AVPR1A	chr11:62124548	Ala>Glu	263	no effect	-70	82%
AVPR1A	chr11:62124701	Gln>Pro	212	no effect	-25	61%
AVPR1A	chr11:62124906	Met>Leu	144	effect	44	71%
AVPR1A	chr11:62125214	Asp>Gly	41	no effect	-38	66%
AVPR1A	chr11:62125302	Ala>Thr	12	no effect	-91	97%
AVPR1B	chr1:160482464	Ala>Val	84	effect	59	75%

Table 2. OXTR, AVPR1A, AVPR1B missense variants

Gene	Macaque Position	Human Genome Liftover Position	Human Genome Liftover rsID	Clinical Significance in Humans
OXTR	chr2:57649859	chr3:8768172		
OXTR	chr2:57649912	chr3:8768119	rs780323772	none known
OXTR	chr2:57650182	chr3:8767849	rs775129787	none known
OXTR	chr2:57650410	chr3:8767621	rs762128258	none known
OXTR	chr2:57650752	chr3:8767279	rs769535684	none known
OXTR	chr2:57664901	chr3:8752980	rs146441685	none known
AVPR1A	chr11:62121832	chr12:63147370		
AVPR1A	chr11:62124427	chr12:63149937		
AVPR1A	chr11:62124548	chr12:63150058	rs776846916	none known
AVPR1A	chr11:62124701	chr12:63150211	rs190242785	none known
AVPR1A	chr11:62124871	chr12:63150381	rs553995625	none known
AVPR1A	chr11:62124901	chr12:63150411		
AVPR1A	chr11:62124906	chr12:63150416		
AVPR1A	chr11:62125186	chr12:63150696		
AVPR1A	chr11:62125214	chr12:63150724		
AVPR1A	chr11:62125231	chr12:63150741		
AVPR1A	chr11:62125240	chr12:63150750		
AVPR1A	chr11:62125243	chr12:63150753		
AVPR1A	chr11:62125302	chr12:63150812		
AVPR1B	chr1:160482462	chr1:206116822		
AVPR1B	chr1:160482464	chr1:206116640	rs138075414	none known
AVPR1B	chr1:160482465	chr1:206116639		
AVPR1B	chr1:160482660	chr1:206116624		
AVPR1B	chr1:160482702	chr1:206116582	rs781803425	none known
AVPR1B	chr1:160488705	chr1:206110210	rs781813621	none known

Table 3. OXTR, AVPR1A, AVPR1B SNV human genome analogs