Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Katherine Rubin April 8, 2019

Genetic predictors of OXTR expression in a genetically diverse group of monogamous prairie voles

by

Katherine Rubin

Dr. Larry Young
Adviser

Neuroscience and Behavioral Biology

Dr. Larry Young

Adviser

Dr. Robert Liu

Committee Member

Dr. Arri Eisen

Committee Member

2019

Genetic predictors of OXTR expression in a genetically diverse group of monogamous prairie voles

By

Katherine Rubin

Dr. Larry Young

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Neuroscience and Behavioral Biology

2019

Abstract

Genetic predictors of OXTR expression in a genetically diverse group of monogamous prairie voles

By Katherine Rubin

Oxytocin (OXT) modulates multiple elements of social behavior. Humans exhibit a large diversity of sociality, with social deficit disorders and other psychiatric illnesses existing at one extreme. It has been hypothesized that the OXT system plays a large role in influencing human social variation. Specifically, the OXT receptor (OXTR) has been implicated in impacting gradations of typical social behaviors. Previous work using the monogamous prairie vole model revealed that several perfectly-correlated single nucleotide polymorphisms (SNPs) in the oxytocin receptor gene (*Oxtr*) are robustly associated with differences in the concentration of OXTR in the brain, which may in turn affect behavior. However, it is still unclear which specific SNPs are the strongest predictors of OXTR density and whether or not associations with candidate SNPs generalize to the prairie vole species more broadly. This study investigated the association between genotype and OXTR concentration in the brains of 96 genetically diverse monogamous prairie voles. The sample included interbred colony voles with the addition of independent wild-caught animals. The voles were genotyped and OXTR concentration was quantified in five brain regions relevant to social behavior. After filtering, SNPs strongly predicting OXTR concentration in the nucleus accumbens (NAcc) were prioritized for subsequent analyses. This included 14 SNPs previously identified plus ten additional independent markers, the majority of which fall in the intron. Interestingly, these 24 SNPs did not have additional relationships to OXTR density in any other brain regions. When the sample

was broken up into wild-caught and colony groups, the strong associations were primarily driven by the colony animals. Additionally, the presence of a sex interaction was tested within each brain region, revealing a significant effect only in the anterior olfactory nucleus. Taken together, these findings confirm the region-specificity of the SNPs and highlight the importance of finding SNPs that are likely functional candidates in both colony and wild-caught groups. Further, it adds to the story of how non-coding polymorphisms in *OXTR* could influence individual social variation in humans.

Genetic predictors of OXTR expression in a genetically diverse group of monogamous prairie voles

By

Katherine Rubin

Dr. Larry Young

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Neuroscience and Behavioral Biology

2019

Acknowledgements

This work was supported by the National Institutes of Mental Health of the National Institutes of Health under award number 1P50MH100023. I would like to express my gratitude to Desiree De Leon for her guidance and support in performing the autoradiography and analyzing the data. Additionally, Desiree and Jamie LaPrairie were instrumental in reviewing and editing this paper. I would like to thank Soma Sanngrahi for her contribution in isolating the DNA and genotyping the animals. I would like to acknowledge the Ophir Lab at Cornell University for providing the wild-caught brain samples and voles for the interbreeding of the colony. Additionally, thank you to Lorra Julian for her assistance in sexing and managing the prairie voles used in this study.

Table of Contents

Table of Figures

Table of Tables

Introduction

Oxytocin (OXT) is a modulatory neuropeptide that enacts social effects by binding to G protein-coupled oxytocin receptors (OXTR) in the brain. In animals, OXT is directly involved in many social functions, including parental behavior (Yoshihara, Numan, & Kuroda, 2018), social cognition (Chang & Platt, 2014), and pair bonding (Kelly & Goodson, 2014; Walum & Young, 2018). It has been proposed that OXT enhances social behaviors by increasing the salience of social stimuli (Young, 2015). Specifically, in animal models, OXT intensifies social olfactory stimuli in brain areas important for social valence and reward. OXT may also modulate more complex behaviors, such as empathy (Demas & Jasnow, 2016; Stetzik, Sullivan, Patisaul, & Cushing, 2018).

It is well-documented that social behavior in humans is diverse. For example, typical humans vary in individual expression of empathy (Christov-Moore et al., 2014), cooperation (van den Berg, Molleman, Junikka, Puurtinen, & Weissing, 2015), and aggression (Cant, Llop, & Field, 2006). This variation plays important roles in forming relationships and familial bonds. While the exact mechanisms underlying individual variation in human social behavior remain unclear, there is mounting evidence that the OXT system is involved in many basic social behaviors for both typical and clinical populations.

In humans, OXT is thought to facilitate the ability to form social attachments and relationships (Carter, 2017). For example, increased baseline OXT levels are correlated with increased maternal care in humans (Kohlhoff et al., 2017). Moreover, the concentration of OXT in blood plasma explains variation in social perception (Lancaster et al., 2015). Specifically, increased plasma OXT is associated with increased perception of animacy and higher activation

in brain areas necessary for social understanding, such as superior temporal sulcus, inferior frontal gyrus, and medial prefrontal cortex (mPFC). Therefore, OXT is believed to impact individual variation in social behavior in humans (Love et al., 2012). It is important to study the underlying mechanisms of the OXT system's role in typical social variation in order to gain insight into how these mechanisms may be altered in disordered states.

At the extreme end of the social spectrum lies social dysfunction, including Autism Spectrum Disorder (ASD). ASD is a collection of neurodevelopmental disorders that are characterized by a disruption of social reciprocity and social cognition, language impairments, and restricted and repetitive interests and behavior (Volkmar, State, & Klin, 2009). ASD affects approximately 1 in 68 children in the US and results in a significant economic burden for family members and society (Centers for Disease Control). Because of its high incidence and consequences, it is important to explore mechanisms underlying the diverse symptomatology associated with ASD. While environmental effects play a role in incidence, ASD prevalence has been shown to be between 64-91% heritable (Tick, Bolton, Happé, Rutter, & Rijsdijk, 2016). This suggests that genes play a crucial role in the etiology of ASD. A primary gene of interest is the OXT receptor gene (*OXTR*). Multiple studies have noted that variation in *OXTR* is associated with core symptoms of ASD (Harrison, Gamsiz, Berkowitz, Nagpal, & Jerskey, 2015; Skuse et al., 2014; Walum et al., 2012). Therefore, the OXT system is being actively explored as a pharmacological target for enhancing social cognition in ASD (Andari et al., 2010; Guastella et al., 2015; Yatawara, Einfeld, Hickie, Davenport, & Guastella, 2016; Young & Barrett, 2015). Aside from ASD, the oxytocinergic system has been implicated in other psychiatric diseases, such as borderline personality disorder (Brüne, 2016), PTSD (Sippel et al., 2017), anxiety disorders

(Gottschalk & Domschke, 2018), and ADHD (Kalyoncu, Özbaran, Köse, & Onay, 2017). This demonstrates OXT's role in broad social dysfunction.

One current approach for manipulating the OXT pathway is using intranasal OXT. This treatment has been shown to safely alleviate the social, emotional, and cognitive symptoms of ASD (Bakermans-Kranenburg & van I Jzendoorn, 2013; Parker et al., 2017; Yatawara et al., 2016). Further, intranasal OXT administration significantly improves social functioning in children with ASD, as measured by the Social Responsiveness Scale (SRS) (Parker et al., 2017). Interestingly in a non-clinical population, intranasal OXT activates neural systems crucial for social and emotional behavior (Galbusera et al., 2017). Importantly, sensitivity to OXT treatments has been shown to be influenced by *OXTR* genotype (Chen et al., 2015; Feng et al., 2015).

However, it is important to view the aforementioned studies cautiously as many of them are statistically underpowered, leading to variable and inflated estimates of intranasal OXT's effects (Leng & Ludwig, 2016; Walum, Waldman, & Young, 2016). This may explain the amount of contrasting literature on intranasal OXT. Indeed, reports of the effectiveness of intranasal OXT have been inconsistent, with at least one study showing no effects at all (Guastella et al., 2015). Overall, it is difficult to draw any solid conclusions from the current literature on intranasal OXT as a potential treatment for individuals with ASD (Keech, Crowe, & Hocking, 2018). To better understand the underpinnings of social behavior and attempts at ASD intervention, it is beneficial to focus current efforts on the genetic variation of the OXT system.

Variations in typical human *OXTR* genotype are highly associated with variations in social behavior. For example, variations in *OXTR* impact the ability to form secure attachments

(Notzon et al., 2016), engage in facial recognition (Skuse et al., 2014; Westberg et al., 2016), and affect social cognition (Ebert & Brüne, 2018; Kalyoncu et al., 2017). *OXTR* may serve as a promising avenue for studying the genetic mechanisms of individual variability in sociality.

To properly explore the mechanisms by which genetic variation can influence downstream phenotypes, such as brain expression and subsequent behavior, it is necessary to have a robust animal model to allow for direct experimentation. Monogamous prairie voles (*Microtus ochrogaster*) have emerged as a model organism for studying OXT (McGraw & Young, 2010; Young & Wang, 2004). Unlike the meadow vole (*M. pennsylvanicus*) and the montane vole (*M. montanus*), which are relatively asocial, the prairie vole engages in monogamous relationships. This species disparity may be a result of differences in quantity and location of *Oxtr* binding in the brain (Insel & Shapiro, 1992). There is also evidence that this variation in OXTR density translates into variation in social behaviors. One study found that variation in accumbal OXT density modulates reactivity to early life stressors, such as neglect (Barrett, Arambula, & Young, 2015).The highly affiliative and socially monogamous behavior observed in prairie voles has provided remarkable insights into the neurobiology of social attachments and diversity in social behavior.

In addition to these between-species differences, previous within-species research has shown that among prairie voles, there is remarkable individual variation in the density of OXTR in brain areas important for social reward, with little variation in other regions (King, Walum, Inoue, Eyrich, & Young, 2016). A previous study conducted in our lab identified that this variation could be robustly explained by genetic markers within prairie vole oxytocin gene (*Oxtr*). A set of 14 single nucleotide polymorphisms (SNPs) in *Oxtr* were completely correlated

with one another, or in perfect linkage disequilibrium (LD). More so they all explained over 74% of the variation in the density of OXTR binding in the nucleus accumbens (NAcc) (King et al., 2016), a region which has been directly implicated in both drug and natural reward circuits (Kummer, El Rawas, Kress, Saria, & Zernig, 2015). Notably, these SNPs were predictive in a region-specific manner, such that brain regions unrelated to social reward such as the insula did not have a strong association with the SNPs.

One of the 14 SNPs, NT213739, was highlighted due to its putative location next to an enhancer. When mapped to mouse *Oxtr,* for which transcriptional and functional ENCODE data are available, the authors found that this SNP locus aligned with a binding site for the transcriptional regulator CCCTC-binding factor (CTCF), which modulates chromatin architecture allowing regulatory elements to interact.

In addition, NT213739 is close to sites showing signs of histone modification including mono-methylated histone H3 lysine 4 (H3K4me1) and acetylated histone H3 lysine 27 (H3K27ac), as well as a DNA hypersensitive (DNaseHS) site (King et al., 2016). H3K4me1 and H3K27ac marks are often associated with DNase hypersensitivity and transcription factor binding and are therefore markers for enhancers important for cell-type specific gene expression (Heintzman et al., 2009). CTCF binding is often found at enhancers and promotors and like the histone markers mentioned above, can show tissue dependent binding which is associated with tissue specific gene expression profiles (Ong & Corces, 2014; Shen et al., 2012). Thus, if a SNP falls next to an enhancer, it could influence properties and quantities of protein expression.

Interestingly, the NT213739 SNP was also indirectly associated with partner preference (Ahern, Modi, Burkett, & Young, 2009) in males through the SNP's relationship with OXTR density (King et al., 2016). This combination of evidence suggests that variation in a single SNP in *Oxtr* may have a large effect on brain phenotype, and consequently behavior.

It is important to note that the emphasis of King et al. (2016) on NT213739 SNP does not rule out the predictiveness of the other 13 SNPs. This is because they each are highly associated with OXTR density in the NAcc. The motivating factor for the current study is to determine which of the 14 SNPs have the strongest association with OXTR concentration in various brain regions.

Another modification in the current study is its use of both males and females. In the majority of studies involving OXT, males were used primarily. However, in humans, there are measurable differences in social behavior between sexes, with ASD being four times more prevalent in males (Coffman, Anderson, Naples, & McPartland, 2015). Further, studies have demonstrated clear sex differences in OXT modulation (Bethlehem et al., 2017; Ebner et al., 2016) and concentration (Kramer, Cushing, Carter, Wu, & Ottinger, 2004). Intranasal OXT differentially affects amygdala reactivity in woman and men (Domes et al., 2010). Alternatively, a previous study used the GTEx database to explore sex differences in the OXTR pathway in humans and no significant differences were noted (Quintana et al., 2019). It is possible that the previously revealed dimorphisms result from disparity in other areas of the OXT pathway, and not OXTR specifically. Therefore, it is important to study both sexes in experiments involving the OXT system.

Another focus of the current study is to quantify whether the association is generalizable to independent groups of prairie voles. King et al. (2016) used animals which were bred from the same colony and were highly-related to one another. By selectively breeding individuals that highly expressed OXTR within this related group of animals, it is possible that the lab colony was unintentionally selectively-bred to have *Oxtr* genetic markers which co-occur with varying levels of expression, but which are not causally related to the observed expression. In addition to statistical concerns related to pseudoreplication (Lazic, 2010), this artificial selection process and use of related animals causes a lack of genetic diversity, which in turn creates uncertainty for whether the association between genotype and expression are specific to one colony, or can be applied to larger populations (Little & Colegrave, 2016). Knowing and enhancing the genetic diversity of a colony is especially important when studying genetic markers of complex phenotypes (Brekke, Steele, & Mulley, 2018; Charlesworth, 2003). This is because inbreeding and evolutionary bottlenecks increase LD (Gaut & Long, 2003). Therefore, the 14 SNPs that were previously found to be in perfect LD may be the result of artificial selection. The current study sought to break up this LD structure and narrow the pertinent SNPs, by outbreeding colony animals with wild-caught animals to improve diversity.

Recently, a preliminary study from the Young Lab attempted to confirm the King et al. (2016) results. Twenty-four animals from Brandon Aragona's laboratory colony at University of Michigan were used and the concentration of OXTR was measured in the NAcc as compared to the insula. All animals were genotyped at the NT213739 SNP and found to have the homozygous low-expressing genotype. Upon analysis, all 24 animals presented with lower-expressing OXTR as compared to a single high-expressing sample from the Young lab colony.

This preliminary data could neither confirm nor rule out the importance of NT213739 SNP in OXTR expression in the NAcc (Unpublished Data). This study involved a relatively small sample size (n=24) that lacked appropriate OXTR variation to complete a thorough analysis.

Among the 12 males and 12 females used in the aforementioned study, no sex differences were noted. Again, these results cannot be generalized to other genotypes because the animals used in the study were all from the same colony and all expressed the same genotype at the critical SNP. By exploring a larger number of animals, including genetically diverse animals from other labs and wild-caught animals, it provides the opportunity to better explore potential sex differences and advance previous insights from genetic associations.

The aim of the current study is to build upon the King et al. (2016) paper and explore potential sex and genetic predictors of OXTR concentration in the brains of monogamous prairie voles. A large sample size was used to incorporate genetic diversity and equal distribution of sex. OXTR was measured in the olfactory bulb, anterior olfactory nucleus, prefrontal cortex, NAcc, and insula. We hypothesize that the new samples will present with a different LD structure than that found in the King et al. (2016) paper, allowing us to narrow down the candidate SNPs for OXTR concentration. Additionally, we expect that the SNPs may predict OXTR expression levels in other brain regions relevant to social behavior, and that there may be sex differences in the association of SNPs with OXTR density in some brain regions.

Materials and Methods

Animals

Ninety-six adult monogamous prairie voles (*Microtus ochrogaster*) were used in this experiment. Eighty of the 96 animals were offspring from 13 breeder pairs composed of animals from our original colony (distantly derived from field captured voles in Illinois) paired with prairie voles from an outside colony. Animals were housed in same-sex groups with two or three voles per cage from postnatal day 21. Housing consisted of a ventilated 36 cm x 18 cm x 19 cm plexiglass cage filled with Bed-o'Cobs laboratory animal bedding (The Andersons Inc., Maumee, Ohio) under a 14/10-hour light/ dark cycle (lights on 7:00 AM–9:00 PM) at 22°C with access to food (rabbit diet; LabDiet, St. Louis, Missouri) and water ad libitum.

The remaining 16 animals were wild-caught prairie voles, which were provided from Alex Ophir's Lab at Cornell University. The brains of these subjects were received post-dissection and placed on dry ice. The brains were stored in a -80°C freezer until processing.

Subjects from the colony were decapitated following deep anesthetization with isoflurane. Brains were collected and frozen in crushed dry ice and then stored in a -80°C freezer. Both the interbred colony and wild-caught brains were sliced on a cryostat from olfactory bulb through amygdala in 6 series at 20μm and put on Fischer Frost-Plus slides. The slides were then stored again in a -80°C freezer until processing.

For the remainder of the paper, the interbred colony cohort will be referred to as "colony animals" and the independent wild-caught individuals will be referred to as "wild-caught".

Genotyping

Genotyping was conducted as previously described in King et al. (2016). Tail tissue was collected from the interbred prairie vole colony upon euthanasia. Tissue was then frozen on crushed dry ice. For the wild-caught animals, brain tissue was collected during slicing after being stored at -80°C. DNA was isolated from the tail and brain tissues with the Platinum SuperFi PCR Master Mix (Invitrogen). All polymerase chain reactions were performed using the Platinum SuperFi PCR Master Mix (Invitrogen). There were 5 loci amplified between 6.6–10 kb in size.

A 50 kb amplicon was amplified by PCR, using the Platinum SuperFi PCR Master Mix (Invitrogen). The thermocycler program used was, initial denature: 94˚C – 5 min 30s; 35x cycles: 1) denature: 94˚C – 30 s, 2) anneal: 53˚C – 30 s, 3) elongation: 72˚C – 30 s; final elongation: 72˚C – 10 min. Amplicons were digested for 1.5 hours at 37˚C with the SSP1 restriction enzyme (New England Biolabs, Ipswich, MA). SSP1 cuts the T-allele of NT204321 but not the C-allele. Thus, resultant banding patterns were used to identify genotypes. The primers used for this reaction were, forward: 5'-CTAGGCTTTGGTTGGGGAAATAAC-3', reverse: 5'-TTGGGTCTTGTTATGGTCCTGAC-3'.

Long-range PCRs for Target Enrichment of 70kb Surrounding *Oxtr*

Loci 3 through 7 were run with the same thermocycler program, initial denature: 93˚C – 3 min; 35x cycles: 1) denature: 93˚C – 15 s, 2) anneal: 62˚C – 30 s, 3) elongation: 68˚C – 10 min 20 s. 4 Primers for each loci are listed below: Locus 3 – initial, forward: 5'-CAATAAGCAGCTAGACAGGGCCCA-3', reverse: 5'-CCCTGGATCTACATGCTGTTCACG3', Locus 3 – nested, forward: 5'-CGCTGCAGTAGTGGGAAGACATTG-3', reverse: 5'-

ACGAACTTGTGCAGCGCTTTCTC-3', Locus 4, forward: 5'- GACCCTCTGATGGCTGAGTGACTG-3', reverse: 5'-CCCAGAGGGAACTGCATCTGAGTC3', Locus 5, forward: 5'-TCAGCCCTCAGAAACTTTTTCAAACAC-3', reverse: 5'- GAAGGGTGCCTGTCTTCTTTGGTC-3', Locus 6, forward: 5'- AAGGGGAGTGACTTTCAGGGGAAG-3', reverse: 5'-AGTGTGTGACAGCATTGGGACTTTG-3', Locus 7, forward: 5'- CCAAGGGATGACACAGCTTTGAGAG-3', reverse: 5'- CCAGCTTTGCTACAGAGGATCAGC-3'

Amplicon Library Preparation

Sequencing library preparation and sequencing analyses were performed by the Yerkes Nonhuman Primate Genomics Core (Atlanta, Georgia) as described in King et al. (2016). Polymerase chain reaction amplicons from each animal were pooled and cleaned using Solid Phase Reversible Immobilization beads (Beckman Coulter, Brea, California). Libraries were generated using the Illumina Nextera XT DNA Library Prep Kit (Illumina, Inc., San Diego, California), and dual barcoding and sequencing primers were added per the manufacturer's protocol. Libraries were validated by microelectrophoresis, quantified, pooled, and clustered on Illumina TruSeq Cluster Kit v3. The clustered flow cell was sequenced on an Illumina HiSeq 1000 in 100-base single- read reactions.

OXTR Autoradiography

OXTR autoradiography was performed to assess OXTR density at various brain regions in the prairie vole. Sections were removed from -80°C storage, allowed to air-dry, dipped in 0.1% paraformaldehyde in PBS, pH 7.4, and rinsed twice in .5 M Tris buffer, pH 7.4, to remove endogenous OXT. Next the tissue was incubated in 50 pM ^{125}I-OVTA for 1 hour. Unbound radioligand was removed by four washes in .5 M Tris plus 2% $MgCl_2$, pH 7.4, and then dipped

into ddH_2O and air dried under a stream of cool air. Once dry, the slides were exposed to BioMax MR film (Kodak) for 96 hours (Ross et al., 2009).

For evaluation, a semi-quantitative measure of OXTR binding density was calculated: disintegrations per minute per milligram of tissue (dpm/mg) was estimated by comparing raw optical density (ROD) values to a 125-I standard. Background binding was captured from the corpus callosum, a region of the brain with consistent lack of signal. Specific OXTR binding density was calculated by subtracting mean background binding from values of regions of interest (King et al., 2016).

Statistical Analysis

Statistical analysis was adapted from King et al. (2016). All statistical analyses were performed in the R statistical software package version 3.5.2 (R Project for Statistical Computing, Vienna, Austria), unless stated otherwise. For each brain region evaluated, associations between genetic information and brain expression data were examined using linear regression with Bonferroni corrections for multiple comparisons. A separate linear model was run for each SNP tested in each brain region. All analyses began with a full model, in which sex was included as an interaction term with genotype at the particular SNP site as the main effect. Within this model, sex interactions were included in further analyses only when the interaction term was significant ($p<0.05$), thereby improving the model fit. The MCMCglmm function within R was used to incorporate pedigree data into the linear model to control for relatedness among subjects, and p-values reported as “corrected p-values” reflect this. Results from the MCMCglmm model were also used to produce estimates of heritability in OXTR expression. In addition to running linear models on the total sample, associations were also

tested separately within the subsets of the colony animals and the wild-caught animals in order to explore disparities between the two groups.

Processing of next-generation sequencing data was performed in VCFtools, a program package designed for working with variant cell format files from sequencing projects (Danecek et al., 2011).

Results

Filtering SNPs

Results from the genotyping revealed a coverage of 4697 SNPs. Beginning with a full list of variants, we filtered down to 1628 SNPs using the following criteria. Minor Allelic Frequency (MAF) had to be at least 1%, no missing calls for any individuals, and a high threshold for quality score. To narrow the focus of this study, a linear model was run to identify the SNPs which robustly predicted expression in the nucleus accumbens (NAcc). NAcc was chosen given the relevance of this region in social reward and in the literature, which informed this study. This list was then ordered by strength of association and 24 SNPs were used for subsequent analyses. The 24 SNPs included the previously identified 14 SNPs (King et al., 2016) plus the 10 most predictive SNPs that were not in linkage disequilibrium (LD) (Figure 1). Bonferroni correction was then calculated by $\alpha = 0.05/19 = 0.003$, with 19 representing the number of SNPs out of the 24 that were not in perfect LD. Only p-value below this threshold were considered significant, unless otherwise stated.

The r-squared, beta, and p-value are displayed for each of the 24 SNPs included in the subsequent association studies (Table 1).

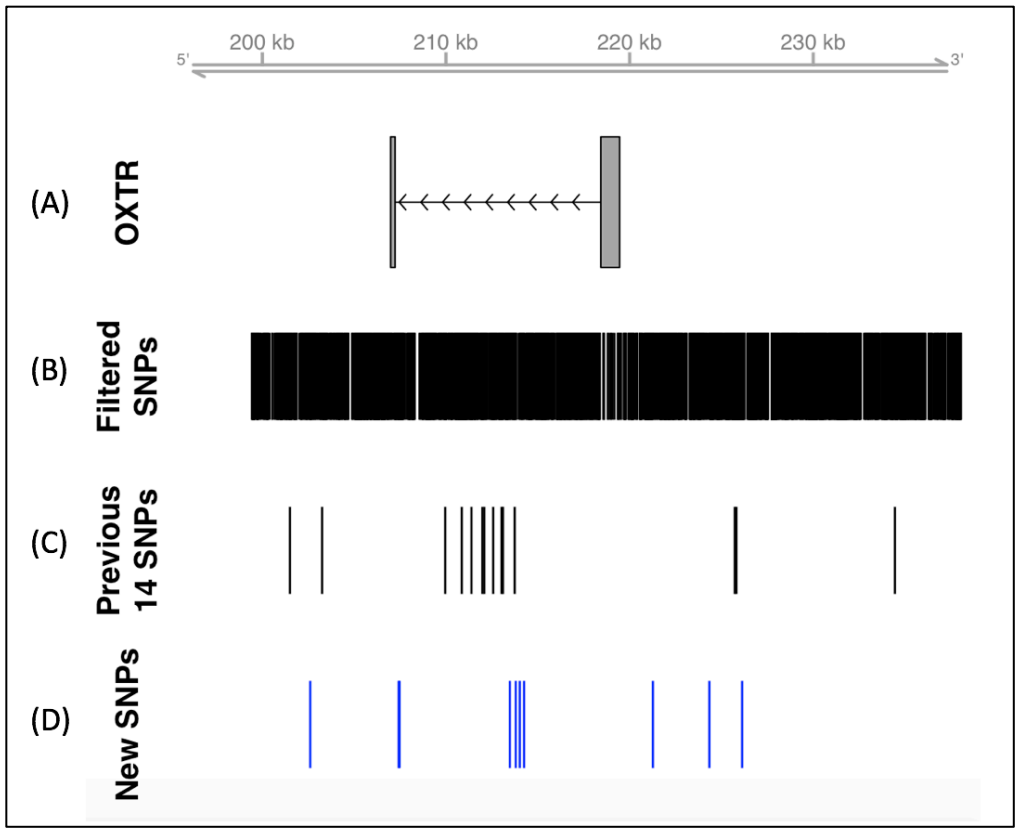


Figure 1. Pruning of genetic data. An illustration of the location of and distribution of SNPs mentioned in this study. (A) A simplified representation of the prairie vole OXTR gene. (B) The 1628 SNPs used after filtering down from the initial 4697 received from the genomics core. (C) The location of the previous 14 SNPs identified in King et al. (2016). (D) The location of the 10 new SNPs incorporated in this study.

SNP	R^2	β	p	corrected p
214019	0.6355	0.1158	3.26E-19	6.76E-04
202607	0.5741	0.0884	1.74E-16	6.76E-04
209958	0.5712	0.1045	2.28E-16	6.76E-04
210858	0.5712	0.1045	2.28E-16	6.76E-04
211980	0.5712	0.1045	2.28E-16	6.76E-04
212087	0.5712	0.1045	2.28E-16	6.76E-04
212570	0.5712	0.1045	2.28E-16	6.76E-04
213106	0.5712	0.1045	2.28E-16	6.76E-04
214253	0.5471	0.0804	2.09E-15	6.76E-04
213739	0.5235	0.0885	1.61E-14	6.76E-04
225727	0.5006	0.0675	1.08E-13	6.76E-04
213801	0.4882	0.0735	2.90E-13	6.76E-04
213026	0.4869	0.0921	3.20E-13	6.76E-04
213479	0.4545	0.0584	3.86E-12	6.76E-04
224345	0.4475	0.1165	6.45E-12	6.76E-04
207471	0.4180	0.1086	5.33E-11	6.76E-04
226128	0.4124	0.0591	7.89E-11	6.76E-04
207421	0.4107	0.0672	8.86E-11	6.76E-04
221265	0.4090	0.0757	9.99E-11	6.76E-04
201501	0.2339	-0.0434	4.18E-06	2.70E-03
203254	0.2229	0.0406	7.54E-06	4.05E-03
234447	0.1820	0.0525	6.43E-05	6.76E-04
225814	0.1744	0.0415	9.46E-05	1.35E-03
211385	0.1326	-0.0438	0.0008	5.41E-03

Table 1. Pertinent 24 SNPs. Table detailing the R^2, beta, p-value, and corrected p-value for the 24 SNPs focused on in the study. The list is ordered by significance in the NAcc (most significant at top). P-value is from a simple linear model, while corrected p-value reflects controlling for relatedness between animals. SNPs highlighted in gray are the 10 new SNPs; un-highlighted SNPs are the original 14 SNPs.

Animal Relatedness

It was important to quantify the relatedness of the colony animals for data interpretation. This allows validation of whether the interbreeding with outside voles increased genetic diversity sufficiently. When performing these tests, wild-caught animals were excluded

as there were all genetically independent animals. The calculated relatedness between individuals is displayed as a heatmap (Figure 2a). These values are then compiled into a graph to better visualize proportion (Figure 2b). The median relatedness of the colony is 0.056 and the mean is 0.1053.

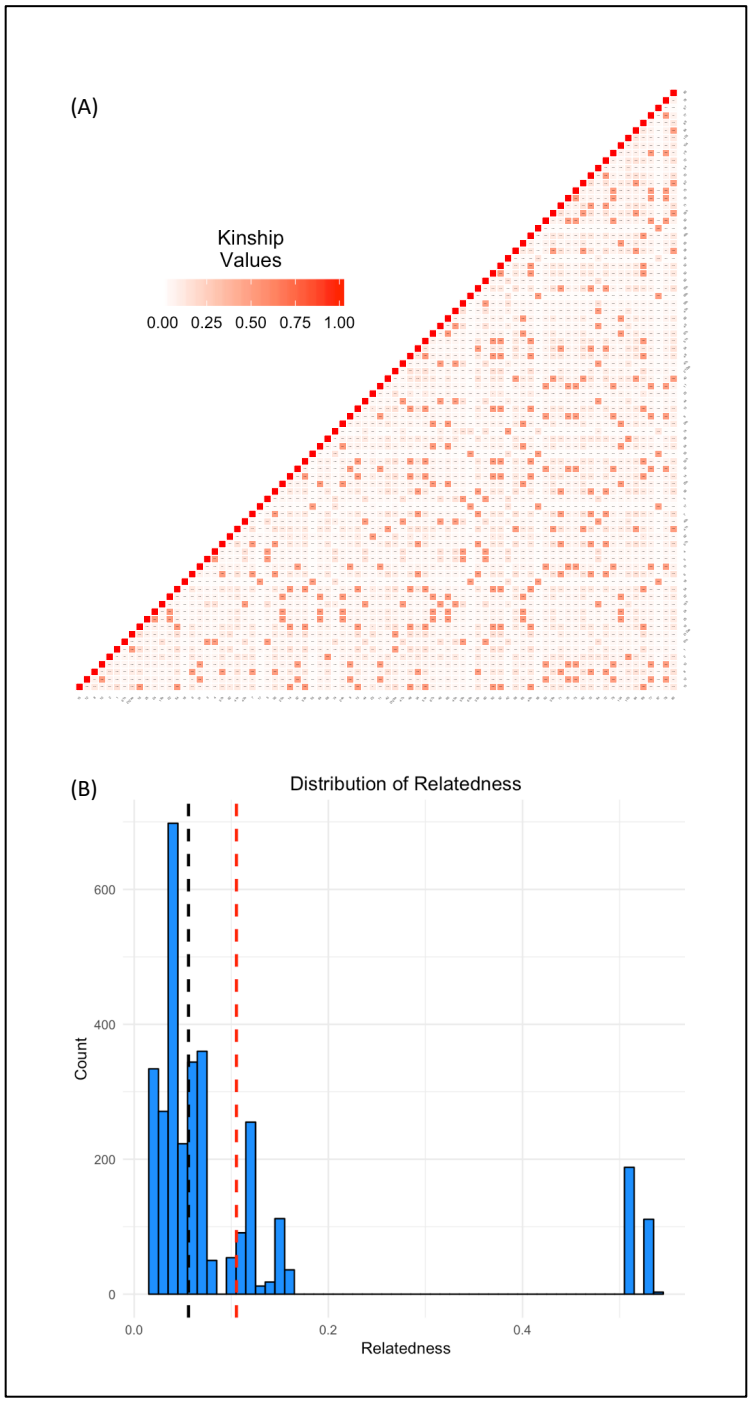


Figure 2. Relatedness of colony animals. (A) Heatmap of relatedness between individual animals. X and Y axes are the 80 colony animals used in the study. Relatedness is expressed on a 0.0 (white) to 1.0 (red) scale. (B). Histogram of the values from the heatmap to display overall distribution of relatedness. Black dashed line is the median (0.056) and dashed red line is the mean (0.1053). For reference, a coefficient of relatedness of 0.125 is equivalent to being first cousins, and a coefficient of 0.50 is equivalent to a full-sibling relationship.

Brain Regions Measured

Due to time constraints, only the first half of the brain was imaged and quantified for this study. Fewer slides also allowed the autoradiography to be completed in a single round, limiting variability of radioligand exposure. The following brain regions were measured: nucleus accumbens (NAcc), insula (IN), prefrontal cortex (PFC), olfactory bulb (OLF), and anterior olfactory nucleus (AON) (Figure 3). As a within-brain control, corpus callosum (CC) was also measured.

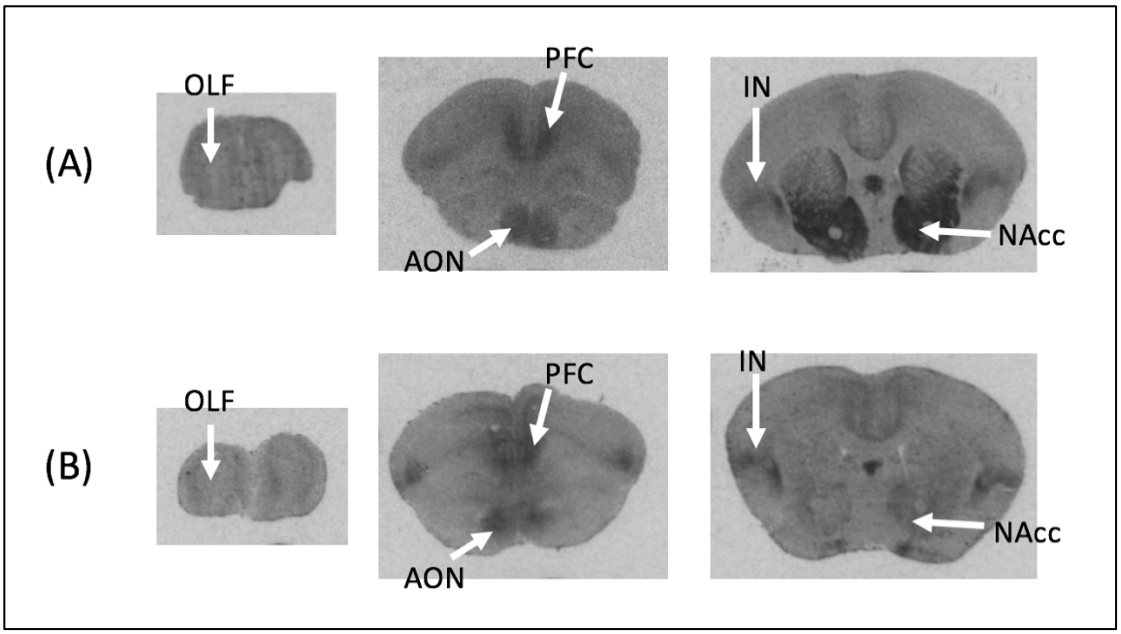


Figure 3. Location of brain regions measured. Arrows indicate the five regions quantified, as illustrated in both a (A) high-expressing and (B) low-expressing animal.

Heritability of OXTR Expression by Brain Region

Only brain regions included in the association study were used in this portion of the analysis. Expression within the NAcc was the most heritable, with a mean of 79.2%. The other brain regions were all found to be less heritable: IN (49.3%), OLF (46.1%), AON (46.6%), and PFC (48.9%) (Table 2).

Brain Region	Heritability	Lower Interval	Upper Interval
Nacc	0.7896	0.5852	0.9554
IN	0.4941	0.2664	0.7259
OLF	0.4552	0.2125	0.7098
AON	0.4681	0.2067	0.7668
PFC	0.4822	0.2289	0.7402

Table 2. Heritability of brain regions. The heritability value, lower confidence interval, and upper confidence interval for each brain region measured in the study.

Sex Interactions

It was necessary to determine whether sex had a significant effect on expression in each brain region. By starting with the most complex model, the sex variable could be removed if not significant. Using a MCMCglmm model, a calculation was used to determine whether there was a significant interaction between sex of the animal and OXTR expression in each brain region. Without correcting for multiple comparisons, the only brain region that had a statistically significant interaction was the AON (p-value= 0.0473) (Figure 4). Among the 24 SNPs highlighted in this study, nine had a significant sex interaction in the AON (Supplementary Figure 1). In all nine cases, the females were consistently higher expressing than the males. All

other brain regions were not significant (Figure 4). One example from each brain region from the same SNP was chosen as an indication of sex distribution. In all future analyses, sex was included as an interaction only in the AON and excluded in the other brain regions.

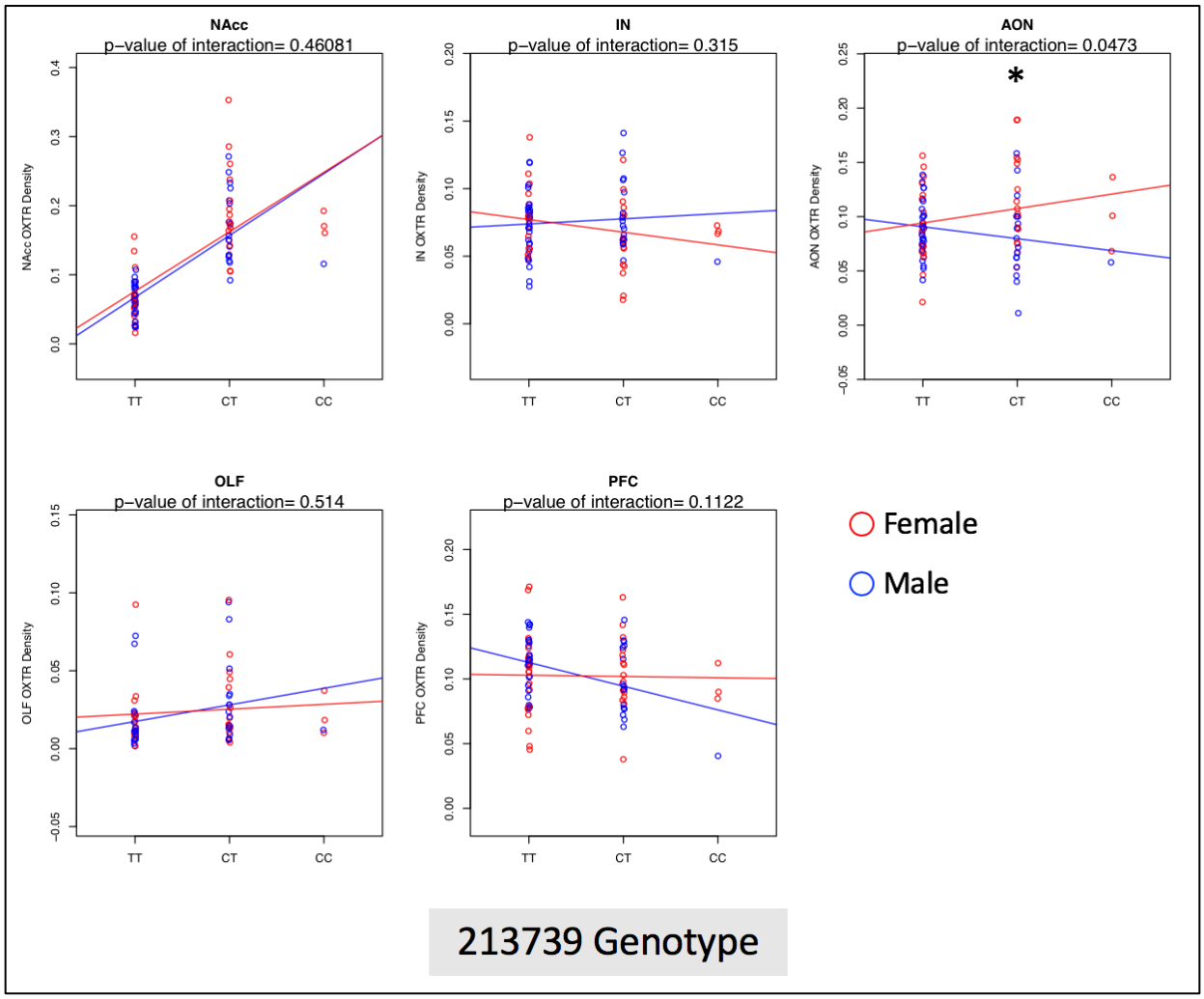


Figure 4. Sex interaction with OXTR expression. Linear models of sex differences in OXTR expression within each brain region: NAcc (p= 0.461), IN (p=0.315), PFC (p=0.112), OLF (p= 0.514), AON (p=0.047). Dots represent individual animals, with red being females and blue being males. * $p < 0.05$ (not corrected for multiple tests).

SNP and OXTR Expression Association in Brain Regions

The next step involved analysis of the association between OXTR expression and genotype in the 24 pertinent SNPs. In the NAcc, all 24 SNPs had a significant association between brain phenotype and genotype, as illustrated by the example in Figure 5a. This significance remained when controlling for relatedness among the colony animals, as indicated by the corrected p-value. This is not surprising as the 24 top SNPs involved in the deeper analysis were chosen based on their low p-values in the NAcc.

When controlling for relatedness, none of the 24 SNPs had a significant association in the IN, AON, or PFC (Figure 5b-d). In the OLF, only one SNPs had a significant association after controlling for multiple comparisons: 234447 (p-value= 0.0014) (Supplementary Table 1). One example from each brain region was chosen, but all other SNP information can be found in Supplementary Table 1. It is important to note that this does not necessarily reflect the larger group of 1628 SNPs because the 24 were chosen based on NAcc data. However, the SNPs that are highly correlative for OXTR density in the NAcc are not also predictive in the other brain regions measured.

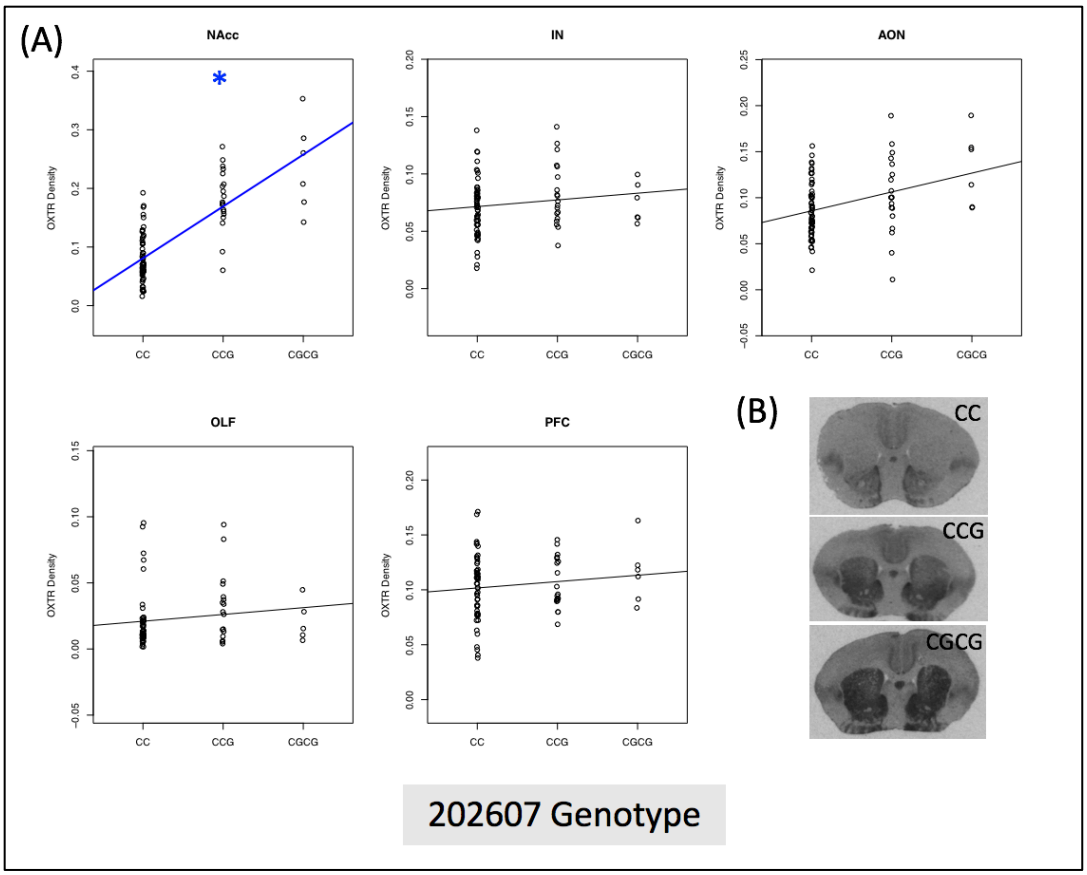


Figure 5. SNP and OXTR expression association. (A) Linear models showing the general association between OXTR concentration and genotype at the 202607 SNP. Areas include: NAcc (p= 1.74E-16, R^2= 0.5741, corrected p= 6.76E-04), IN (p= 0.193, R^2= 2.11E-02), AON (p= 0.2552, R^2= 0.2133), OLF (p= 0.2361, R^2= 0.0120), and PFC (p= 0.2421, R^2= 0.0171). P-value is derived from the linear model. When significant, the relationship was then controlled for relatedness, as shown by the corrected p-value. *significance at $\alpha < 0.05/19$ tests = 0.003 (B) Images of three different individual brains, exhibiting NAcc OXTR concentration associated with SNP 202607 polymorphism.

Colony and Wild-Caught

In order to better understand the strong association observed in the NAcc, the animals were divided based on colony or wild-caught. This also allowed for more insight into the

diversity of the colony animals and predictability of the data. For time purposes, these tests were run using linear models and did not control for relatedness. Within the NAcc, the trends between the two types of animals differ. Alone, the colony animals are all still strongly associated with OXTR expression in the NAcc (Figure 5). In fact, the p-values tend to decrease once the wild-caught animals are removed. On the other hand, the wild-caught animals overall have no significant association at these 24 SNPs when controlling for multiple comparisons (Figure 5). Without including Bonferroni corrections, only two showed a significant association with OXTR expression: SNP 203254 (p-value= 0.0296) and SNP 213026 (p-value= 0.0093).

Another area of interest is the OLF, in which the wild-caught animals on their own displayed strong associations between SNP and OXTR expression. The wild-caught animals had a significant association between all 24 SNPs and OXTR expression (Supplementary Table 3). Concurrently, the colony animals had no significance at any of the 24 SNPs (Supplementary Table 2). So, the overall lack of significance described earlier was due to the large sample size of the colony animals overpowering the smaller wild-caught sample.

A similar trend as observed in OLF was noted in the PFC, in which the overall lack of association is driven by the colony animals. None of the 24 SNPs were significantly correlated with OXTR expression when looking at colony animals (Supplementary Table 2). In the wild-caught animals, eleven of the SNPs were significant with not controlling for multiple comparisons (Supplementary Table 3). These include: SNP 214019 (p-value= 0.0496), SNP 202607 (p-value= 0.0063), SNP 214253 (p-value= 0.0215), SNP 207471 (p-value= 0.0496), SNP 209958 (p-value= 0.0496), SNP 210858 (p-value= 0.0496), SNP 211980 (p-value= 0.0496), SNP

212087 (p-value= 0.0496), SNP 212570 (p-value= 0.0496), SNP 213106 (p-value= 0.0496), and SNP 213739 (p-value= 0.0496).

On a smaller scale, the lack of significant association in the IN remained when excluding the wild-caught animals. Of the 24 SNPs in the colony animals, none had a significant association with OXTR expression in the IN (Supplementary Table 2). However, when looking at the wild-caught animals alone, three SNPs showed a significant association without Bonferroni corrections: SNP 213801 (p-value= 0.0229), SNP 207421 (p-value= 0.0104), and SNP 221265 (p-value= 0.0031) (Supplementary Table 3).

Within the AON, small variations can be seen when the voles are separated by type. When only looking at colony animals, three SNPs are significant without Bonferroni corrections: SNP 224345 (p-value= 0.0147), SNP 207471 (p-value= 0.0147), and SNP 225814 (p-value= 0.0289) (Supplementary Table 3). Within the wild-caught cohort, one SNP was significant without Bonferroni corrections: SNP 201501 (p-value= 0.0247) (Supplementary Table 3).

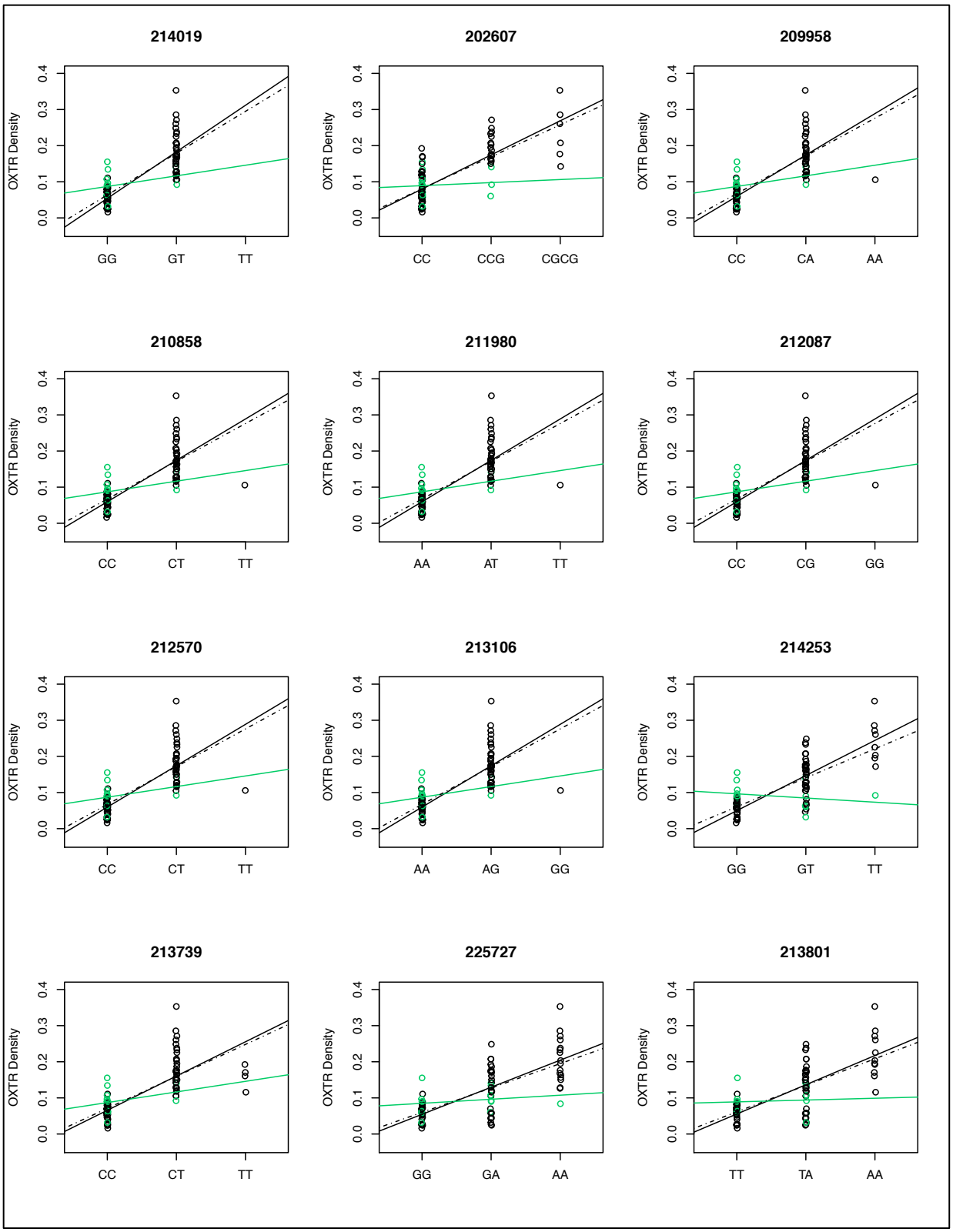

214019
OXTR Density
0.0
0.1
0.2
0.3
0.4
GG
GT
TT
202607
OXTR Density
0.0
0.1
0.2
0.3
0.4
CC
CCG
CGCG
209958
OXTR Density
0.0
0.1
0.2
0.3
0.4
CC
CA
AA
210858
OXTR Density
0.0
0.1
0.2
0.3
0.4
CC
CT
TT
211980
OXTR Density
0.0
0.1
0.2
0.3
0.4
AA
AT
TT
212087
OXTR Density
0.0
0.1
0.2
0.3
0.4
CC
CG
GG
212570
OXTR Density
0.0
0.1
0.2
0.3
0.4
CC
CT
TT
213106
OXTR Density
0.0
0.1
0.2
0.3
0.4
AA
AG
GG
214253
OXTR Density
0.0
0.1
0.2
0.3
0.4
GG
GT
TT
213739
OXTR Density
0.0
0.1
0.2
0.3
0.4
CC
CT
TT
225727
OXTR Density
0.0
0.1
0.2
0.3
0.4
GG
GA
AA
213801
OXTR Density
0.0
0.1
0.2
0.3
0.4
TT
TA
AA

Figure 6. Comparing wild-caught to colony animals in the NAcc. Linear models of the top 12 SNPs, as ranked by association with NAcc OXTR expression. Plots are divided by wild-caught (green) and colony (black) animals. Colony animals are always significant, while wild-caught are not. SNPs include: SNP 214019 (colony p= 2.38E-18, colony R^2= 0.694 , wild-caught p= 0.261, wild-caught R^2= 0.096), SNP 202607 (colony p= 1.27E-15, colony R^2= 0.629, wild-caught p= 0.710, wild-caught R^2= 0.011), SNP 209958 (colony p= 5.11E-15, colony R^2= 0.613, wild-caught p= 0.261, wild-caught R^2= 0.096), SNP 210858 (colony p= 5.11E-15, colony R^2=0.613 , wild-caught p= 0.261 , wild-caught R^2= 0.096), SNP 211980 (colony p= 5.11E-15, colony R^2= 0.613, wild-caught p= 0.261, wild-caught R^2= 0.096), SNP 212087 (colony p= 5.11E-15, colony R^2= 0.613, wild-caught p= 0.261, wild-caught R^2= 0.096), SNP 212570 (colony p= 5.11E-15, colony R^2= 0.613 , wild-caught p= 0.261, wild-caught R^2= 0.096), SNP 213016 (colony p= 5.11E-15, colony R^2= 0.613, wild-caught p= 0.261, wild-caught R^2= 0.096), SNP 214253 (colony p= 1.28E-18, colony R^2= 0.699, wild-caught p= 0.426, wild-caught R^2= 0.049), SNP 213739 (colony p= 7.63E-13, colony R^2= 0.549 , wild-caught p= 0.261, wild-caught R^2= 0.096), SNP 225727 (colony p= 3.98E-13, colony R^2= 0.558, wild-caught p= 0.440, wild-caught R^2= 0.047), and SNP 213801 (colony p= 1.35E-12, colony R^2= 0.541, wild-caught p= 0.786, wild-caught R^2= 0.006).

Discussion

The overarching goal of this project was to identify associations between genotype and OXTR brain density in a genetically diverse group of monogamous prairie voles. Additionally, sex interactions were explored within brain regions pertinent for social interaction (Ferris, 2008). This study directly built on a previously published paper that highlighted 14 SNPs in the

OXTR gene as being strongly correlated with OXTR expression in the NAcc (King et al., 2016). Specifically, the past study noted SNP 213739 as one of interest due to its putative location next to a transcription enhancer. To improve upon these findings, the current study included both sexes and increased the genetic diversity of the sample group. This involved the interbreeding of colony animals with outside voles and the addition of a small completely independent wild-caught cohort.

Brain region heritability was high for all tested regions, with NAcc being notably the highest. This confirms the valuable role of genetics in OXTR brain expression. While environmental factors have been shown to influence OXTR expression (Barrett et al., 2015; Perkeybile et al., 2019), the estimates from this sample imply that the majority of expression differences here can be explained by genetic factors. Thus, it is important to continue exploring these genetic relationships and processes.

When analyzing sex interactions within each brain region, the only one that was statistically significant was the AON. This is not surprising as the sexual dimorphism within the olfactory pathway is heavily documented. For example, spatiotemporal differences in primary sensory neurons may contribute to female rodents having a superior sense of smell (Kass, Czarnecki, Moberly, & McGann, 2017). More so, it is suggested that OXT strengthens maternal bonds by involving olfactory discrimination. Based on the current data it is unknown whether this is caused by a specific sex interaction within the SNPs or whether they are revealing a larger sexual dimorphism in the olfactory system.

From this research, it can be concluded that the 24 SNPs do not have prominent sex-interaction in the remaining regions we analyzed. A lack of sex-specific findings in other brain

areas also aligns with previous research. One study, in particular, found that while OXTR in mice are left lateralized in female auditory cortex (Mitre et al., 2017), no other sex-specific OXTR differences were detected in other brain regions. Additionally, in humans, a study using the GTEx database found no sex differences in OXTR gene expression (Quintana et al., 2019). Alternatively, it is possible that if sex differences were present in this study, the current sample may not have been large enough to detect them. When interaction terms are introduced into a statistical model, the total sample is effectively subdivided into smaller sized groups, making it more difficult to discern interactions with small effects (Cohen, 1983). Further, it is important to keep these findings in context, as 24 SNPs were chosen from a subset of over 1600. It is possible that other SNPs within the *Oxtr* gene not analyzed in here have a significant sex interaction for a brain region.

Looking at the association data, we first began with the combined sample group of both colony and wild-caught animals. It is clear that the NAcc is more strongly associated with genotype, while the same SNPs do not seem to correlate with expression in other brain regions. This solidifies previous conclusions that these SNPs are region-specific (King et al., 2016). While only associations within the NAcc were highlighted in this paper, other SNPs within the filtered 1628 are likely correlative with other brain regions.

In addition to breaking up some of the LD between the previously identified 14 SNPs, these data again support their high correlation with OXTR concentration in the NAcc. Notably, the 213739 SNP highlighted in the King et al. (2016) paper was still significantly associated with OXTR concentration when looking at the combined sample group. Further, additional SNPs were included in this study that are also strong candidates for influencing OXTR concentration

in the NAcc. The top eleven SNPs have R^2 values that explain more than 50% of individual variation in OXTR expression, with the top SNP exceeding 63%. Though this is lower than the 74% variance explained in King et al. (2016), these markers seem promising in influencing brain phenotype and potentially downstream behavior. Interestingly, the SNP with the strongest association in NAcc, SNP 214019, exists only 280 base pairs away from SNP 213739, which was previously chosen due to its alignment with a binding site for the transcriptional regulator CCCTC-binding factor (CTCF) in mice *Oxtr* (King et al., 2016). The fact that SNP 214019, and three other new SNPs, are all clustered around this locus is further support that this CTCF region may have functional value.

However, it is important to remember that a majority of this sample are colony animals interbred with a potentially artificially selected group. Thus, the sample is not entirely independent from the King et al. (2016) study. Given this, it is critical to see whether the associations detected here are generalizable to the subset of independent wild-caught animals. When the NAcc data is separated by colony and wild-caught animals, clear divisions are noted. All of the significant associations reported here are driven by colony animals. Among the wild-caught animals the 24 SNPs are non-significant, showing weaker or even opposite trends than in the colony animals. Though one may think that the lack of significance is solely due to the small sample size of wild-caught animals, a power calculation shows that this is not the case. For example, if the true effect size were to be what is reported for our strongest marker, SNP 214019 (i.e. $R^2 = 63.5\%$), we would expect that even a small sample of 16 wild-caught animals would be sufficient to detect a significant association for this SNP 84% of the time (Faul,

Erdfelder, Buchner, & Lang, 2009). As a result, we can conclude that the significant associations described here are most likely specific to the colony animals.

To answer the question of which SNPs may be the most unbiased markers for OXTR expression in prairie voles, a post-hoc analysis was carried out. This analysis examined the strongest associations of all 1628 SNPs in wild-caught animals and showed five SNPs that are significantly association to OXTR concentration in NAcc even after Bonferroni corrections. Most notably, SNP 221163 (R^2= 0.599) and SNP 213026 (R^2= 0.417) were discovered as being some of the strongest in wild-caught, while also being highly significant in colony animals (R^2= 0.363 and R^2= 0.487 respectively). The most predictive SNP in the wild-caught sample is SNP 221163, which was 45th most predictive SNP in colony animals for OXTR expression in the NAcc. Interestingly, this SNP is located in the promoter region of the *Oxtr* gene, which may affect transcription (Albert, 2011). Additionally, SNP 213026 was a top-ranking SNP in both groups, having been included in the 24 SNPs of this study. This highlights generalizable SNPs that would be strong candidates for exploring the evolutionary mechanisms of social behavior.

Beyond the NAcc, there are interesting differences between the colony and wild-caught animals in other brain regions as well. Most notably, in the olfactory bulb, the colony animals displayed no significant association between any of the 24 SNPs and OXTR expression. However, when looking at the wild-caught animals 11 of the SNPs were significantly associated with expression when not controlling for multiple comparisons. These opposing patterns between colony and wild-caught subsamples adds to the suggestion that the two groups are not completely analogous in regard to genetic predictors of OXTR concentration.

Two possible explanations to explain this group discrepancy between colony and wild-caught animals include environment and lack of adequate genetic representation. Laboratory animals experience a controlled and highly managed living experience, while wild-caught animals have variable exposures and experiences. For example, one's immune system may impact the oxytocin system. One study showed that administration of interleukin-1β (IL-1β), a lymphocyte activating factor, increased central and peripheral release of oxytocin in rats (Landgraf, Neumann, Holsboer, & Pittman, 1995). It is likely that the immune systems in wild-caught animals was activated more than the colony-bred animals. However, the high heritability estimates of brain region expression suggest that the variation of OXTR expression is strongly attributable to genetic factors, as opposed to all other factors (including environment and error). While heritability estimates will vary between random samples, such high estimates here indicate that the majority of expression is controlled by the genes that the voles inherit.

The second rationale is that the colony animals are not outbred enough to properly model the genetics governing social behavior in general. As previously described, it is possible that the past colony animals were unintentionally artificially selected for an association between genotype and OXTR expression. Although outside animals were introduced to create more genetic diversity, consistent outbreeding would ensure that the colony animals are a more representative cross-section of the species. The benefits are not only a matter of creating a generalizable group, but also to be able to form and test new hypotheses that are translatable to other mammals.

With these differences in animal groups it is important to keep in context the sample size. While 80 colony animals were included, only 16 wild-caught animals were included. To

better understand whether specific SNPs are generalizable to the species, more research will be needed on a larger sample of wild-caught animals.

Another limitation of this study was in the method of choosing SNPs to focus on. Due to time constraints, all 1628 SNPs could not be analyzed, and so a small fraction had to be chosen. Significance in the NAcc was selected as the criteria due to its role in social reward. Additionally, the King et al. (2016) paper, which motivated this study, focused on the NAcc. It is likely that other SNPs not included in this paper are valuable pieces of the story and may be predictive in other regions or strongly predictive in both groups of animals. Further analysis on the given data is needed to flesh out these possibilities.

Conclusion

Overall, this data provides more insight into how genetics impacts OXTR concentration within brain areas related to social function. The LD between the previous 14 SNPs was semi-broken up, meaning that some SNPs can now be excluded from the list of pertinent SNPs. In addition to verifying the importance of the 213739 SNP, new SNPs are also suggested from these data to better predict OXTR concentration in the NAcc. Specifically, SNP 213026 and SNP 221136, identified in a post-hoc analysis, are interesting in that they are both highly associated with OXTR density in the NAcc in both the colony and wild-caught populations. These data are consistent with past findings of region-specificity of the SNPs (King et al., 2016). While no one SNP can be selected from this data as being causal to expression differences, this study provides a solid foundation on which future functional genetic studies can be built.

Bibliography

Ahern, T. H., Modi, M. E., Burkett, J. P., & Young, L. J. (2009). Evaluation of two automated metrics for analyzing partner preference tests. *Journal of Neuroscience Methods*, *182*(2), 180–188. doi:10.1016/j.jneumeth.2009.06.010

Albert, P. R. (2011). What is a functional genetic polymorphism? Defining classes of functionality. *Journal of Psychiatry & Neuroscience*, *36*(6), 363–365. doi:10.1503/jpn.110137

Andari, E., Duhamel, J.-R., Zalla, T., Herbrecht, E., Leboyer, M., & Sirigu, A. (2010). Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(9), 4389–4394. doi:10.1073/pnas.0910249107

Bakermans-Kranenburg, M. J., & van I Jzendoorn, M. H. (2013). Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. *Translational Psychiatry*, *3*, e258. doi:10.1038/tp.2013.34

Barrett, C. E., Arambula, S. E., & Young, L. J. (2015). The oxytocin system promotes resilience to the effects of neonatal isolation on adult social attachment in female prairie voles. *Translational Psychiatry*, *5*, e606. doi:10.1038/tp.2015.73

Bethlehem, R. A. I., Lombardo, M. V., Lai, M. C., Auyeung, B., Crockford, S. K., Deakin, J., … Baron-Cohen, S. (2017). Intranasal oxytocin enhances intrinsic corticostriatal functional connectivity in women. *Translational Psychiatry*, *7*(4), e1099. doi:10.1038/tp.2017.72

Brekke, T. D., Steele, K. A., & Mulley, J. F. (2018). Inbred or outbred? genetic diversity in laboratory rodent colonies. *G3 (Bethesda, Md.)*, *8*(2), 679–686.

doi:10.1534/g3.117.300495

Brüne, M. (2016). On the role of oxytocin in borderline personality disorder. *The British Journal of Clinical Psychology*, *55*(3), 287–304. doi:10.1111/bjc.12100

Cant, M. A., Llop, J. B., & Field, J. (2006). Individual variation in social aggression and the probability of inheritance: theory and a field test. *The American Naturalist*, *167*(6), 837–852. doi:10.1086/503445

Carter, C. S. (2017). The role of oxytocin and vasopressin in attachment. *Psychodynamic Psychiatry*, *45*(4), 499–517. doi:10.1521/pdps.2017.45.4.499

Chang, S. W. C., & Platt, M. L. (2014). Oxytocin and social cognition in rhesus macaques: implications for understanding and treating human psychopathology. *Brain Research*, *1580*, 57–68. doi:10.1016/j.brainres.2013.11.006

Charlesworth, D. (2003). Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *358*(1434), 1051–1070. doi:10.1098/rstb.2003.1296

Chen, F. S., Kumsta, R., Dvorak, F., Domes, G., Yim, O. S., Ebstein, R. P., & Heinrichs, M. (2015). Genetic modulation of oxytocin sensitivity: a pharmacogenetic approach. *Translational Psychiatry*, *5*, e664. doi:10.1038/tp.2015.163

Christov-Moore, L., Simpson, E. A., Coudé, G., Grigaityte, K., Iacoboni, M., & Ferrari, P. F. (2014). Empathy: gender effects in brain and behavior. *Neuroscience and Biobehavioral Reviews*, *46 Pt 4*, 604–627. doi:10.1016/j.neubiorev.2014.09.001

Coffman, M. C., Anderson, L. C., Naples, A. J., & McPartland, J. C. (2015). Sex differences in social perception in children with ASD. *Journal of Autism and Developmental Disorders*,

45(2), 589–599. doi:10.1007/s10803-013-2006-5

Cohen, J. (1983). The Cost of Dichotomization. *Applied Psychological Measurement*, *7*(3), 249–253. doi:10.1177/014662168300700301

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. doi:10.1093/bioinformatics/btr330

Demas, G. E., & Jasnow, A. M. (2016). Empathy in prairie voles: Is this the consolation prize? *Learning & Behavior*, *44*(4), 303–304. doi:10.3758/s13420-016-0232-3

Domes, G., Lischke, A., Berger, C., Grossmann, A., Hauenstein, K., Heinrichs, M., & Herpertz, S. C. (2010). Effects of intranasal oxytocin on emotional face processing in women. *Psychoneuroendocrinology*, *35*(1), 83–93. doi:10.1016/j.psyneuen.2009.06.016

Ebert, A., & Brüne, M. (2018). Oxytocin and social cognition. *Current Topics in Behavioral Neurosciences*, *35*, 375–388. doi:10.1007/7854_2017_21

Ebner, N. C., Chen, H., Porges, E., Lin, T., Fischer, H., Feifel, D., & Cohen, R. A. (2016). Oxytocin's effect on resting-state functional connectivity varies by age and sex. *Psychoneuroendocrinology*, *69*, 50–59. doi:10.1016/j.psyneuen.2016.03.013

Faul, F., Erdfelder, E., Buchner, A., & Lang, A.-G. (2009). Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behavior Research Methods*, *41*(4), 1149–1160. doi:10.3758/BRM.41.4.1149

Feng, C., Lori, A., Waldman, I. D., Binder, E. B., Haroon, E., & Rilling, J. K. (2015). A common oxytocin receptor gene (OXTR) polymorphism modulates intranasal oxytocin effects on the neural response to social cooperation in humans. *Genes, Brain, and Behavior*, *14*(7),

516–525. doi:10.1111/gbb.12234

Ferris, C. F. (2008). Functional magnetic resonance imaging and the neurobiology of vasopressin and oxytocin. *Progress in Brain Research*, *170*, 305–320. doi:10.1016/S0079-6123(08)00425-1

Galbusera, A., De Felice, A., Girardi, S., Bassetto, G., Maschietto, M., Nishimori, K., … Gozzi, A. (2017). Intranasal oxytocin and vasopressin modulate divergent brainwide functional substrates. *Neuropsychopharmacology*, *42*(7), 1420–1434. doi:10.1038/npp.2016.283

Gaut, B. S., & Long, A. D. (2003). The lowdown on linkage disequilibrium. *The Plant Cell*, *15*(7), 1502–1506.

Gottschalk, M. G., & Domschke, K. (2018). Oxytocin and anxiety disorders. *Current Topics in Behavioral Neurosciences*, *35*, 467–498. doi:10.1007/7854_2017_25

Guastella, A. J., Gray, K. M., Rinehart, N. J., Alvares, G. A., Tonge, B. J., Hickie, I. B., … Einfeld, S. L. (2015). The effects of a course of intranasal oxytocin on social behaviors in youth diagnosed with autism spectrum disorders: a randomized controlled trial. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *56*(4), 444–452. doi:10.1111/jcpp.12305

Harrison, A. J., Gamsiz, E. D., Berkowitz, I. C., Nagpal, S., & Jerskey, B. A. (2015). Genetic variation in the oxytocin receptor gene is associated with a social phenotype in autism spectrum disorders. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, *168*(8), 720–729. doi:10.1002/ajmg.b.32377

Heintzman, N. D., Hon, G. C., Hawkins, R. D., Kheradpour, P., Stark, A., Harp, L. F., … Ren, B. (2009). Histone modifications at human enhancers reflect global cell-type-specific gene

expression. *Nature*, *459*(7243), 108–112. doi:10.1038/nature07829

Insel, T. R., & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(13), 5981–5985. doi:10.1073/pnas.89.13.5981

Kalyoncu, T., Özbaran, B., Köse, S., & Onay, H. (2017). Variation in the oxytocin receptor gene is associated with social cognition and ADHD. *Journal of Attention Disorders*, 1087054717706757. doi:10.1177/1087054717706757

Kass, M. D., Czarnecki, L. A., Moberly, A. H., & McGann, J. P. (2017). Differences in peripheral sensory input to the olfactory bulb between male and female mice. *Scientific Reports*, *7*, 45851. doi:10.1038/srep45851

Keech, B., Crowe, S., & Hocking, D. R. (2018). Intranasal oxytocin, social cognition and neurodevelopmental disorders: A meta-analysis. *Psychoneuroendocrinology*, *87*, 9–19. doi:10.1016/j.psyneuen.2017.09.022

Kelly, A. M., & Goodson, J. L. (2014). Hypothalamic oxytocin and vasopressin neurons exert sex-specific effects on pair bonding, gregariousness, and aggression in finches. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(16), 6069–6074. doi:10.1073/pnas.1322554111

King, L. B., Walum, H., Inoue, K., Eyrich, N. W., & Young, L. J. (2016). Variation in the Oxytocin Receptor Gene Predicts Brain Region-Specific Expression and Social Attachment. *Biological Psychiatry*, *80*(2), 160–169. doi:10.1016/j.biopsych.2015.12.008

Kohlhoff, J., Eapen, V., Dadds, M., Khan, F., Silove, D., & Barnett, B. (2017). Oxytocin in the postnatal period: Associations with attachment and maternal caregiving. *Comprehensive*

Psychiatry, *76*, 56–68. doi:10.1016/j.comppsych.2017.03.010

Kramer, K. M., Cushing, B. S., Carter, C. S., Wu, J., & Ottinger, M. A. (2004). Sex and species differences in plasma oxytocin using an enzyme immunoassay. *Canadian Journal of Zoology*, *82*(8), 1194–1200. doi:10.1139/z04-098

Kummer, K. K., El Rawas, R., Kress, M., Saria, A., & Zernig, G. (2015). Social interaction and cocaine conditioning in mice increase spontaneous spike frequency in the nucleus accumbens or septal nuclei as revealed by multielectrode array recordings. *Pharmacology*, *95*(1–2), 42–49. doi:10.1159/000370314

Lancaster, K., Carter, C. S., Pournajafi-Nazarloo, H., Karaoli, T., Lillard, T. S., Jack, A., … Connelly, J. J. (2015). Plasma oxytocin explains individual differences in neural substrates of social perception. *Frontiers in Human Neuroscience*, *9*, 132. doi:10.3389/fnhum.2015.00132

Landgraf, R., Neumann, I., Holsboer, F., & Pittman, Q. J. (1995). Interleukin-1β Stimulates both Central and Peripheral Release of Vasopressin and Oxytocin in the Rat. *European Journal of Neuroscience*, *7*(4), 592–598. doi:10.1111/j.1460-9568.1995.tb00663.x

Lazic, S. E. (2010). The problem of pseudoreplication in neuroscientific studies: is it affecting your analysis? *BMC Neuroscience*, *11*, 5. doi:10.1186/1471-2202-11-5

Leng, G., & Ludwig, M. (2016). Intranasal oxytocin: myths and delusions. *Biological Psychiatry*, *79*(3), 243–250. doi:10.1016/j.biopsych.2015.05.003

Little, T. J., & Colegrave, N. (2016). Caging and uncaging genetics. *PLoS Biology*, *14*(7), e1002525. doi:10.1371/journal.pbio.1002525

Love, T. M., Enoch, M.-A., Hodgkinson, C. A., Peciña, M., Mickey, B., Koeppe, R. A., … Zubieta, J.-K. (2012). Oxytocin gene polymorphisms influence human dopaminergic function in a

sex-dependent manner. *Biological Psychiatry*, *72*(3), 198–206. doi:10.1016/j.biopsych.2012.01.033

McGraw, L. A., & Young, L. J. (2010). The prairie vole: an emerging model organism for understanding the social brain. *Trends in Neurosciences*, *33*(2), 103–109. doi:10.1016/j.tins.2009.11.006

Mitre, M., Kranz, T. M., Marlin, B. J., Schiavo, J. K., Erdjument-Bromage, H., Zhang, X., … Froemke, R. C. (2017). Sex-Specific Differences in Oxytocin Receptor Expression and Function for Parental Behavior. *Gender and the Genome*, *1*(4), 1–25. doi:10.1089/gg.2017.0017

Notzon, S., Domschke, K., Holitschke, K., Ziegler, C., Arolt, V., Pauli, P., … Zwanzger, P. (2016). Attachment style and oxytocin receptor gene variation interact in influencing social anxiety. *The World Journal of Biological Psychiatry*, *17*(1), 76–83. doi:10.3109/15622975.2015.1091502

Ong, C.-T., & Corces, V. G. (2014). CTCF: an architectural protein bridging genome topology and function. *Nature Reviews. Genetics*, *15*(4), 234–246. doi:10.1038/nrg3663

Parker, K. J., Oztan, O., Libove, R. A., Sumiyoshi, R. D., Jackson, L. P., Karhson, D. S., … Hardan, A. Y. (2017). Intranasal oxytocin treatment for social deficits and biomarkers of response in children with autism. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(30), 8119–8124. doi:10.1073/pnas.1705521114

Perkeybile, A. M., Carter, C. S., Wroblewski, K. L., Puglia, M. H., Kenkel, W. M., Lillard, T. S., … Connelly, J. J. (2019). Early nurture epigenetically tunes the oxytocin receptor. *Psychoneuroendocrinology*, *99*, 128–136. doi:10.1016/j.psyneuen.2018.08.037

Quintana, D. S., Rokicki, J., van der Meer, D., Alnæs, D., Kaufmann, T., Córdova-Palomera, A., … Westlye, L. T. (2019). Oxytocin pathway gene networks in the human brain. *Nature Communications*, *10*(1), 668. doi:10.1038/s41467-019-08503-8

Ross, H. E., Freeman, S. M., Spiegel, L. L., Ren, X., Terwilliger, E. F., & Young, L. J. (2009). Variation in oxytocin receptor density in the nucleus accumbens has differential effects on affiliative behaviors in monogamous and polygamous voles. *The Journal of Neuroscience*, *29*(5), 1312–1318. doi:10.1523/JNEUROSCI.5039-08.2009

Shen, Y., Yue, F., McCleary, D. F., Ye, Z., Edsall, L., Kuan, S., … Ren, B. (2012). A map of the cis-regulatory sequences in the mouse genome. *Nature*, *488*(7409), 116–120. doi:10.1038/nature11243

Sippel, L. M., Han, S., Watkins, L. E., Harpaz-Rotem, I., Southwick, S. M., Krystal, J. H., … Pietrzak, R. H. (2017). Oxytocin receptor gene polymorphisms, attachment, and PTSD: Results from the National Health and Resilience in Veterans Study. *Journal of Psychiatric Research*, *94*, 139–147. doi:10.1016/j.jpsychires.2017.07.008

Skuse, D. H., Lori, A., Cubells, J. F., Lee, I., Conneely, K. N., Puura, K., … Young, L. J. (2014). Common polymorphism in the oxytocin receptor gene (OXTR) is associated with human social recognition skills. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(5), 1987–1992. doi:10.1073/pnas.1302985111

Stetzik, L. A., Sullivan, A. W., Patisaul, H. B., & Cushing, B. S. (2018). Novel unconditioned prosocial behavior in prairie voles (Microtus ochrogaster) as a model for empathy. *BMC Research Notes*, *11*(1), 852. doi:10.1186/s13104-018-3934-0

Tick, B., Bolton, P., Happé, F., Rutter, M., & Rijsdijk, F. (2016). Heritability of autism spectrum

disorders: a meta-analysis of twin studies. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *57*(5), 585–595. doi:10.1111/jcpp.12499

van den Berg, P., Molleman, L., Junikka, J., Puurtinen, M., & Weissing, F. J. (2015). Human cooperation in groups: variation begets variation. *Scientific Reports*, *5*, 16144. doi:10.1038/srep16144

Volkmar, F. R., State, M., & Klin, A. (2009). Autism and autism spectrum disorders: diagnostic issues for the coming decade. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *50*(1–2), 108–115. doi:10.1111/j.1469-7610.2008.02010.x

Walum, H., Lichtenstein, P., Neiderhiser, J. M., Reiss, D., Ganiban, J. M., Spotts, E. L., … Westberg, L. (2012). Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. *Biological Psychiatry*, *71*(5), 419–426. doi:10.1016/j.biopsych.2011.09.002

Walum, H., Waldman, I. D., & Young, L. J. (2016). Statistical and methodological considerations for the interpretation of intranasal oxytocin studies. *Biological Psychiatry*, *79*(3), 251–257. doi:10.1016/j.biopsych.2015.06.016

Walum, H., & Young, L. J. (2018). The neural mechanisms and circuitry of the pair bond. *Nature Reviews. Neuroscience*, *19*(11), 643–654. doi:10.1038/s41583-018-0072-6

Westberg, L., Henningsson, S., Zettergren, A., Svärd, J., Hovey, D., Lin, T., … Fischer, H. (2016). Variation in the Oxytocin Receptor Gene Is Associated with Face Recognition and its Neural Correlates. *Frontiers in Behavioral Neuroscience*, *10*, 178. doi:10.3389/fnbeh.2016.00178

Yatawara, C. J., Einfeld, S. L., Hickie, I. B., Davenport, T. A., & Guastella, A. J. (2016). The effect of

oxytocin nasal spray on social interaction deficits observed in young children with autism: a randomized clinical crossover trial. *Molecular Psychiatry*, *21*(9), 1225–1231. doi:10.1038/mp.2015.162

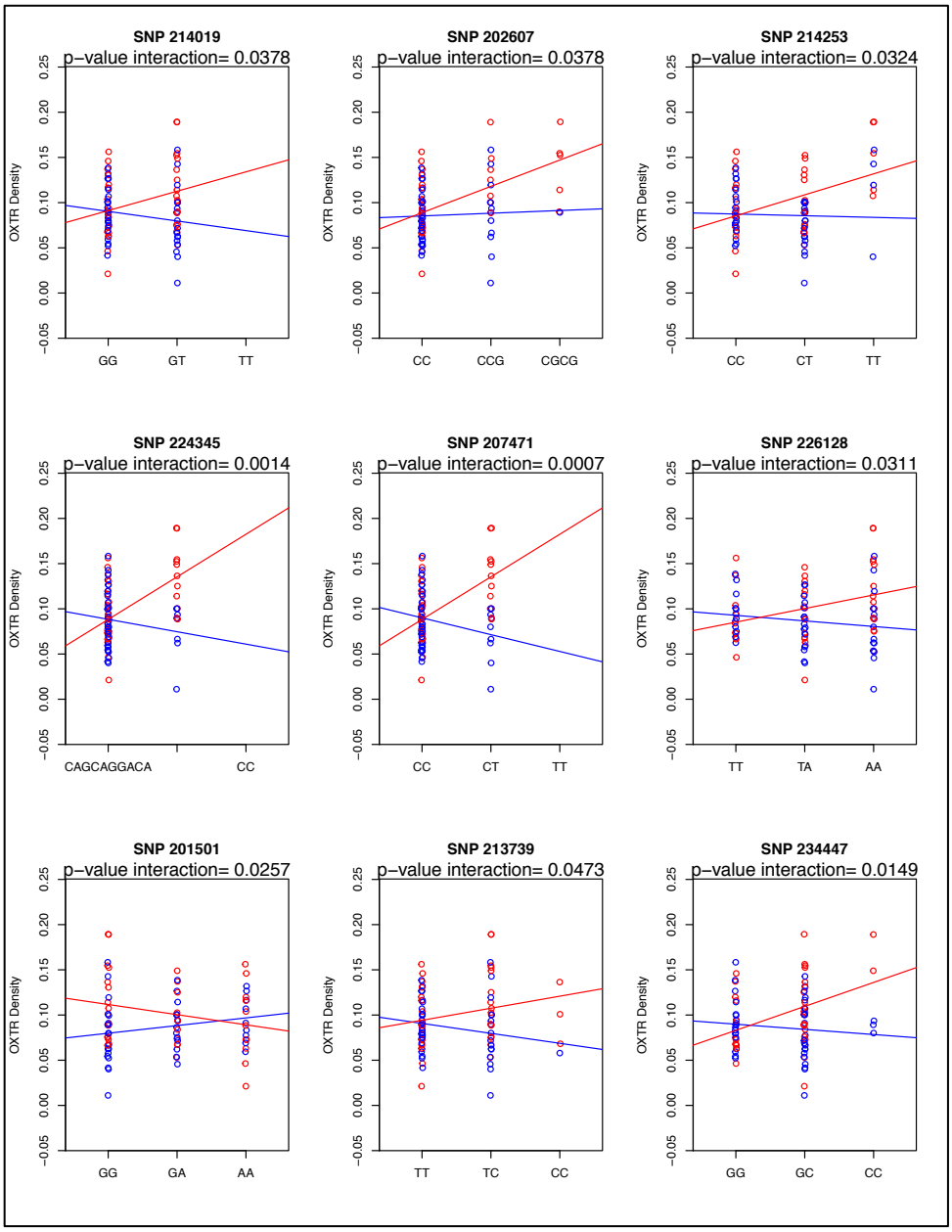
Yoshihara, C., Numan, M., & Kuroda, K. O. (2018). Oxytocin and parental behaviors. *Current Topics in Behavioral Neurosciences*, *35*, 119–153. doi:10.1007/7854_2017_11

Young, L. J. (2015). Oxytocin, social cognition and psychiatry. *Neuropsychopharmacology*, *40*(1), 243–244. doi:10.1038/npp.2014.186

Young, L. J., & Barrett, C. E. (2015). Neuroscience. Can oxytocin treat autism? *Science*, *347*(6224), 825–826. doi:10.1126/science.aaa8120

Young, L. J., & Wang, Z. (2004). The neurobiology of pair bonding. *Nature Neuroscience*, *7*(10), 1048–1054. doi:10.1038/nn1327

Supplementary Data



Supplementary Figure 1. Significant Sex Interaction in AON. Linear models of the nine SNPs (out of 24) that had a significant sex interaction in the AON. Females (red) are consistently higher expression than males (blue).

Overview of 24 SNPs: Combined (Colony and Wild-Caught Animals)																				
	Nacc				IN				PFC				OLF				AON			
SNP	R^2	β	p	corrected p	R^2	β	p	corrected p	R^2	β	p	corrected p	R^2	β	p	corrected p	R^2	β	p	corrected p
214019	0.6355	0.1158	3.26E-19	6.76E-04	4.35E-03	-0.0033	0.5562	0.7135	0.0228	-0.0083	0.1758	0.4878	0.0558	1.05E-02	0.0458	0.0514	0.1044	-0.0427	0.0771	0.1149
202607	0.5741	0.0884	1.74E-16	6.76E-04	2.11E-02	0.0058	0.1933	0.2541	0.0171	0.0058	0.2421	0.1946	0.0200	5.13E-03	0.2361	0.2297	0.2133	-0.0231	0.2552	0.3378
209958	0.5712	0.1045	2.28E-16	6.76E-04	1.27E-02	-0.0054	0.3134	0.4811	0.0400	-0.0105	0.0716	0.2689	0.0454	8.98E-03	0.0724	0.1230	0.0858	-0.0360	0.1318	0.1730
210858	0.5712	0.1045	2.28E-16	6.76E-04	1.27E-02	-0.0054	0.3134	0.4824	0.0400	-0.0105	0.0716	0.2500	0.0454	8.98E-03	0.0724	0.1270	0.0858	-0.0360	0.1318	0.1432
211980	0.5712	0.1045	2.28E-16	6.76E-04	1.27E-02	-0.0054	0.3134	0.4730	0.0400	-0.0105	0.0716	0.2149	0.0454	8.98E-03	0.0724	0.1149	0.0858	-0.0360	0.1318	0.1243
212087	0.5712	0.1045	2.28E-16	6.76E-04	1.27E-02	-0.0054	0.3134	0.4568	0.0400	0.0105	0.0716	0.2216	0.0454	8.98E-03	0.0724	0.1257	0.0858	-0.0360	0.1318	0.1459
212570	0.5712	0.1045	2.28E-16	6.76E-04	1.27E-02	-0.0054	0.3134	0.4838	0.0400	-0.0105	0.0716	0.2473	0.0454	8.98E-03	0.0724	0.1122	0.0858	-0.0360	0.1318	0.1446
213106	0.5712	0.1045	2.28E-16	6.76E-04	1.27E-02	-0.0054	0.3134	0.5054	0.0400	-0.0105	0.0716	0.2284	0.0454	8.98E-03	0.0724	0.1014	0.0858	-0.0360	0.1318	0.1311
214253	0.5471	0.0804	2.09E-15	6.76E-04	3.89E-03	0.0023	0.5776	0.5203	0.0006	0.0010	0.8250	0.6068	0.0259	5.38E-03	0.1771	0.1473	0.1452	-0.0269	0.1280	0.1514
213739	0.5235	0.0885	1.61E-14	6.76E-04	8.04E-03	-0.0038	0.4230	0.7378	0.0375	-0.0090	0.0813	0.2959	0.0329	6.70E-03	0.1270	0.1257	0.0916	-0.0353	0.0997	0.1176
225727	0.5006	0.0675	1.08E-13	6.76E-04	1.90E-02	0.0045	0.2169	0.6230	0.0012	-0.0013	0.7541	0.8459	0.0006	6.91E-04	0.8440	0.7649	0.1086	-0.0181	0.2351	0.3176
213801	0.4882	0.0735	2.90E-13	6.76E-04	7.39E-03	0.0031	0.4426	0.7514	0.0052	-0.0029	0.5185	0.4149	0.0006	7.88E-04	0.8378	0.6284	0.1194	-0.0135	0.4449	0.4514
213026	0.4869	0.0921	3.20E-13	6.76E-04	2.84E-03	-0.0024	0.6341	0.7027	0.0116	-0.0054	0.3354	0.6162	0.0165	5.16E-03	0.2828	0.3541	0.0948	-0.0225	0.3262	0.3297
213479	0.4545	0.0584	3.86E-12	6.76E-04	8.93E-04	0.0009	0.7898	0.8257	0.0061	-0.0026	0.4841	0.8757	0.0038	1.66E-03	0.6062	0.5919	0.0976	-0.0062	0.6543	0.8176
224345	0.4475	0.1165	6.45E-12	6.76E-04	1.18E-02	0.0065	0.3303	0.2459	0.0231	0.0101	0.1724	0.0595	0.0248	8.61E-03	0.1862	0.2351	0.2322	-0.0746	0.0103	0.0230
207471	0.418	0.1086	5.33E-11	6.76E-04	2.00E-02	0.0082	0.2055	0.1824	0.0100	0.0064	0.3703	0.1716	0.0791	1.47E-02	0.0167	0.0405	0.2457	-0.0843	0.0017	0.0081
226128	0.4124	0.0591	7.89E-11	6.76E-04	5.77E-03	0.0024	0.4976	0.8770	0.0011	0.0011	0.7719	0.7743	0.0455	5.95E-03	0.0720	0.0743	0.1126	-0.0274	0.0688	0.0797
207421	0.4107	0.0672	8.86E-11	6.76E-04	7.86E-04	0.0010	0.8026	0.9243	0.0050	-0.0028	0.5299	0.7919	0.0092	3.22E-03	0.4234	0.4054	0.0664	-0.0079	0.6465	0.6324
221265	0.409	0.0757	9.99E-11	6.76E-04	6.28E-02	0.0102	0.0232	0.1176	0.0247	0.0071	0.1581	0.1703	0.0044	2.37E-03	0.5789	0.3014	0.1090	-0.0190	0.3353	0.3797
201501	0.2339	-0.0434	4.18E-06	2.70E-03	7.96E-03	0.0028	0.4254	0.9405	0.0010	-0.0011	0.7819	0.4581	8.78E-06	8.42E-05	0.9803	0.9905	0.1011	0.0282	0.0621	0.0486
203254	0.2229	0.0406	7.54E-06	4.05E-03	2.57E-02	-0.0047	0.1501	0.5027	0.0240	-0.0051	0.1646	0.3432	0.0103	2.69E-03	0.3970	0.4486	0.0772	-0.0203	0.1577	0.1000
234447	0.182	0.0525	6.43E-05	6.76E-04	1.03E-02	0.0043	0.3646	0.5257	0.0030	0.0026	0.6232	0.6743	0.1463	1.47E-02	0.0009	0.0027	0.1369	-0.0379	0.0525	0.0784
225814	0.1744	0.0415	9.46E-05	1.35E-03	8.66E-05	0.0003	0.9339	0.8027	0.0142	-0.0045	0.2863	0.7257	0.0130	3.44E-03	0.3401	0.3459	0.0955	-0.0313	0.0502	0.0784
211385	0.1326	-0.0438	0.0008	5.41E-03	6.38E-02	0.0104	0.0220	0.3824	0.0232	0.0069	0.1723	0.8797	0.0152	-4.75E-03	0.3023	0.5527	0.0718	0.0184	0.3562	0.3635

Supplementary Table 1. 24 SNP data for combined animal group. R-squared, beta, p value, and corrected p value for the combine group of 96 animals. Corrected p value accounts for controlling for relatedness.

Overview of 24 SNPs: Colony Animals																				
	Nacc				IN				PFC				OLF				AON			
SNP	R^2	β	p	corrected p	R^2	β	p	corrected p	R^2	β	p	corrected p	R^2	β	p	corrected p	R^2	β	p	corrected p
214019	0.6936	0.1288	2.38E-18	6.76E-04	2.22E-03	-0.0025	0.7048	0.7797	0.0087	-0.0053	0.4522	0.9149	0.0096	4.44E-03	0.4695	0.3770	0.1056	-0.0331	0.2381	0.3095
202607	0.6288	0.0944	1.27E-15	6.76E-04	3.69E-02	0.0077	0.1195	0.2243	0.0498	0.0098	0.0695	0.0811	0.0017	1.42E-03	0.7614	0.6000	0.2503	-0.0092	0.6870	0.7514
209958	0.6127	0.1145	5.11E-15	6.76E-04	1.01E-02	-0.0049	0.4195	0.5054	0.0233	-0.0083	0.2180	0.5351	0.0059	3.24E-03	0.5696	0.6095	0.0847	-0.0259	0.3520	0.3905
210858	0.6127	0.1145	5.11E-15	6.76E-04	1.01E-02	-0.0049	0.4195	0.5270	0.0233	-0.0083	0.2180	0.5135	0.0059	3.24E-03	0.5696	0.5811	0.0847	-0.0259	0.3520	0.4095
211980	0.6127	0.1145	5.11E-15	6.76E-04	1.01E-02	-0.0049	0.4195	0.4568	0.0233	-0.0083	0.2180	0.5419	0.0059	3.24E-03	0.5696	0.5676	0.0847	-0.0259	0.3520	0.3784
212087	0.6127	0.1145	5.11E-15	6.76E-04	1.01E-02	-0.0049	0.4195	0.4946	0.0233	-0.0083	0.2180	0.5243	0.0059	3.24E-03	0.5696	0.5959	0.0847	-0.0259	0.3520	0.3770
212570	0.6127	0.1145	5.11E-15	6.76E-04	1.01E-02	-0.0049	0.4195	0.4865	0.0233	-0.0083	0.2180	0.5514	0.0059	3.24E-03	0.5696	0.6270	0.0847	-0.0259	0.3520	0.3486
213106	0.6127	0.1145	5.11E-15	6.76E-04	1.01E-02	-0.0049	0.4195	0.5041	0.0233	-0.0083	0.2180	0.5189	0.0059	3.24E-03	0.5696	0.6041	0.0847	-0.0259	0.3520	0.3622
214253	0.6993	0.0973	1.28E-18	6.76E-04	1.52E-02	0.0048	0.3207	0.3351	0.0167	0.0056	0.2967	0.1514	0.0041	2.17E-03	0.6348	0.4554	0.1806	-0.0138	0.5049	0.5527
213739	0.5489	0.0949	7.63E-13	6.76E-04	5.11E-03	-0.0031	0.5653	0.8149	0.0225	-0.0071	0.2261	0.6514	0.0031	2.02E-03	0.6816	0.5041	0.0904	-0.0277	0.2615	0.3095
225727	0.5577	0.0750	3.98E-13	6.76E-04	2.67E-02	0.0055	0.1867	0.6946	0.0004	-0.0007	0.8713	0.9041	0.0026	-1.48E-03	0.7043	0.8324	0.1350	-0.0197	0.2535	0.4135
213801	0.5410	0.0810	1.35E-12	6.76E-04	5.90E-03	0.0028	0.5365	0.9932	0.0006	-0.0010	0.3249	0.6757	0.0071	-2.70E-03	0.5327	0.8595	0.1566	-0.0058	0.7703	0.6757
213026	0.5707	0.1105	1.49E-13	6.76E-04	9.29E-03	-0.0047	0.4377	0.4689	0.0181	-0.0073	0.2776	0.6054	0.0094	4.08E-03	0.4721	0.4851	0.0968	-0.0290	0.2945	0.3459
213479	0.5194	0.0652	6.13E-12	6.76E-04	3.35E-05	0.0002	0.9629	0.9351	0.0149	-0.0041	0.3249	0.9419	0.0045	1.80E-03	0.6210	0.5716	0.0926	-0.0049	0.7592	0.9203
224345	0.4515	0.1172	4.81E-10	6.76E-04	2.49E-02	0.0093	0.2025	0.1959	0.0352	0.0121	0.1285	0.0608	0.0225	7.60E-03	0.2657	0.2932	0.2617	-0.0750	0.0147	0.0541
207471	0.4515	0.1172	4.81E-10	6.76E-04	2.49E-02	0.0093	0.2025	0.2297	0.0352	0.0121	0.1285	0.0541	0.0225	7.60E-03	0.2657	0.3054	0.0846	-0.0750	0.0147	0.0405
226128	0.4879	0.0719	4.99E-11	6.76E-04	2.06E-02	0.0050	0.2461	0.6932	0.0211	0.0055	0.2409	0.2919	0.0161	3.70E-03	0.3476	0.2473	0.1397	-0.0183	0.3306	0.4243
207421	0.4587	0.0739	3.12E-10	6.76E-04	9.68E-04	-0.0011	0.8026	0.6378	0.0058	-0.0031	0.5411	0.8351	0.0039	2.10E-03	0.6431	0.5662	0.0846	-0.0073	0.7063	0.6608
221265	0.5340	0.0905	2.22E-12	6.76E-04	3.25E-02	0.0075	0.1440	0.4176	0.0151	0.0056	0.3225	0.3162	0.0085	3.20E-03	0.4965	0.3068	0.1397	-0.0194	0.3860	0.4716
201501	0.3384	-0.0544	2.44E-07	6.76E-04	2.52E-03	0.0016	0.6865	0.6189	0.0050	-0.0024	0.5693	0.2554	3.00E-04	-4.50E-04	0.9049	0.8635	0.0900	0.0183	0.2912	0.1649
203254	0.3232	0.0525	5.19E-07	6.76E-04	1.23E-02	-0.0034	0.3717	0.8689	0.0096	-0.0033	0.4301	0.7000	0.0055	1.98E-03	0.5851	0.6081	0.0759	-0.0112	0.5139	0.3432
234447	0.1851	0.0606	2.80E-04	6.76E-04	2.34E-02	0.0073	0.2170	0.4568	0.0325	0.0094	0.1440	0.2446	0.0498	2.37E-03	0.0953	0.1297	0.1523	-0.0374	0.1293	0.1905
225814	0.2032	0.0482	1.29E-04	6.76E-04	6.81E-04	0.0009	0.8340	0.9284	0.0109	-0.0041	0.3995	0.9041	0.0061	9.41E-03	0.5649	0.5757	0.1266	-0.0404	0.0289	0.0851
211385	0.1188	-0.0440	4.28E-03	4.05E-03	5.97E-02	0.0105	0.0463	0.5432	0.0141	0.0056	0.3390	0.7919	0.0021	-1.78E-03	0.7359	0.9878	0.1053	0.0290	0.2091	0.2000

Supplementary Table 2. 24 SNP data for colony animals. R-squared, beta, p value, and corrected p value for the 80 colony animals alone. Corrected p value accounts for controlling for relatedness.

	Overview of 24 SNPs: Wild-Caught Animals														
	Nacc			IN			PFC			OLF			AON		
SNP	R^2	β	p	R^2	β	p	R^2	β	p	R^2	β	p	R^2	β	p
214019	0.0962	0.0294	0.2606	0.0404	0.0097	0.4723	0.2650	-0.0310	0.0496	0.8464	0.0564	1.20E-06	0.1557	-0.0339	0.1648
202607	0.0110	0.0084	0.7099	0.0122	-0.0045	0.6946	0.4490	-0.0343	0.0063	0.5057	0.0370	0.0030	0.1124	-0.0247	0.2447
209958	0.0962	0.0294	0.2606	0.0404	0.0097	0.4723	0.2650	-0.0310	0.0496	0.8464	0.0564	1.20E-06	0.1557	-0.0339	0.1648
210858	0.0962	0.0294	0.2606	0.0404	0.0097	0.4723	0.2650	-0.0310	0.0496	0.8464	0.0564	1.20E-06	0.1557	-0.0339	0.1648
211980	0.0962	0.0294	0.2606	0.0404	0.0097	0.4723	0.2650	-0.0310	0.0496	0.8464	0.0564	1.20E-06	0.1557	-0.0339	0.1648
212087	0.0962	0.0294	0.2606	0.0404	0.0097	0.4723	0.2650	-0.0310	0.0496	0.8464	0.0564	1.20E-06	0.1557	-0.0339	0.1648
212570	0.0962	0.0294	0.2606	0.0404	0.0097	0.4723	0.2650	-0.0310	0.0496	0.8464	0.0564	1.20E-06	0.1557	-0.0339	0.1648
213106	0.0962	0.0294	0.2606	0.0404	0.0097	0.4723	0.2650	-0.0310	0.0496	0.8464	0.0564	1.20E-06	0.1557	-0.0339	0.1648
214253	0.0493	-0.0116	0.4264	0.0405	-0.0053	0.4721	0.3440	-0.0194	0.0215	0.2544	0.0170	0.0552	0.2032	-0.0140	0.7644
213739	0.0962	0.0294	0.2606	0.0404	0.0097	0.4723	0.2650	-0.0310	0.0496	0.8464	0.0564	1.20E-06	0.1557	-0.0339	0.1648
225727	0.0466	0.0112	0.4397	0.0390	0.0052	0.4803	0.0016	-0.0013	0.8873	0.0830	0.0097	0.2978	0.0977	0.0428	0.3972
213801	0.0059	0.0050	0.7860	0.3383	0.0194	0.0229	0.1207	-0.0145	0.2046	0.2450	0.0211	0.0606	0.1761	0.0058	0.9133
213026	0.4170	0.0336	0.0093	0.0312	0.0047	0.5286	0.0002	0.0004	0.9620	0.0575	0.0081	0.3895	0.2405	-0.0193	0.6394
213479	0.0602	0.0110	0.3780	0.0991	0.0072	0.2530	0.0780	0.0080	0.3133	0.0007	-0.0008	0.9268	0.2384	-0.0251	0.4529
224345	0.0000	0.0086	NA	0.0000	0.0044	NA	0.0000	0.0055	NA	0.0000	0.0056	NA	0.0017	0.0030	NA
207471	0.0962	0.0294	0.2606	0.0404	0.0097	0.4723	0.2650	-0.0310	0.0496	0.8464	0.0564	1.20E-06	0.1557	-0.0339	0.1648
226128	0.0414	0.0083	0.4670	0.0024	-0.0010	0.8625	0.2369	-0.0126	0.0658	0.2066	0.0120	0.0887	0.2972	0.0076	0.8510
207421	0.0823	0.0161	0.3000	0.4078	0.0182	0.0104	0.0001	0.0004	0.9715	0.0384	0.0071	0.4841	0.1022	0.0350	0.4960
221265	0.1637	0.0326	0.1347	0.5019	0.0289	0.0031	0.1162	0.0174	0.2138	0.0170	0.0068	0.6430	0.0169	-0.0092	0.6741
201501	0.2593	0.0241	0.0526	0.2493	0.0120	0.0581	0.0887	0.0090	0.2810	0.0052	0.0022	0.7994	0.4301	0.1003	0.0248
203254	0.3147	-0.0224	0.0296	0.1757	-0.0085	0.1198	0.2245	-0.0120	0.0744	0.0215	0.0038	0.6024	0.2804	-0.0123	0.7659
234447	0.0853	0.0164	0.2909	0.0463	0.0061	0.4410	0.2297	-0.0171	0.0707	0.8705	0.0339	3.90E-07	0.0249	-0.0079	0.6027
225814	0.0097	0.0044	0.7274	0.0002	0.0003	0.9623	0.0274	-0.0048	0.5556	0.0443	0.0062	0.4514	0.0978	0.0429	0.3736
211385	0.1630	-0.0265	0.1356	0.0122	0.0037	0.6947	0.0912	0.0126	0.2740	0.1342	-0.0156	0.1794	0.0839	-0.0012	0.9825

Supplementary Table 3. 24 SNP data for wild-caught animals. R-squared, beta, p value, and corrected p value for the 16 wild-caught animals alone. Corrected p values are not included because all 16 animals were completely independent and thus did not require controlling for relatedness.