HHS Public Access

Author manuscript

Biol Psychiatry. Author manuscript; available in PMC 2017 July 15.

Published in final edited form as:

Biol Psychiatry. 2016 July 15; 80(2): 160–169. doi:10.1016/j.biopsych.2015.12.008.

Variation in the Oxytocin Receptor Gene Predicts Brain Region Specific Expression and Social Attachment

Lanikea B. King, PhD, **Hasse Walum, PhD**, **Kiyoshi Inoue, PhD**, **Nicholas W. Eyrich**, and **Larry J. Young, PhD***

Silvio O. Conte Center for Oxytocin and Social Cognition, Center for Translational Social Neuroscience, Department of Psychiatry and Behavioral Sciences, Yerkes National Primate Research Center, Emory University, Atlanta GA

Abstract

Background—Oxytocin (OXT) modulates several aspects of social behavior. Intranasal OXT is a leading candidate for treating social deficits in autism spectrum disorder (ASD) and common genetic variants in the human oxytocin receptor (*OXTR*) are associated with emotion recognition, relationship quality and ASD. Animal models have revealed that individual differences in *Oxtr* expression in the brain drive social behavior variation. Our understanding of how genetic variation contributes to brain OXTR expression is very limited.

Methods—We investigated *Oxtr* expression in monogamous prairie voles, which have a well characterized OXT system. We quantified brain region-specific levels of *Oxtr* mRNA and OXTR protein with established neuroanatomical methods. We used pyrosequencing to investigate allelic imbalance of *Oxtr* mRNA, a molecular signature of polymorphic genetic regulatory elements. We performed next-generation sequencing to discover variants in and near the *Oxtr* gene. We investigated social attachment using the partner preference test.

Results—Our allelic imbalance data demonstrates that genetic variants contribute to individual differences in *Oxtr* expression, but only in particular brain regions, including the nucleus accumbens (NAcc), where OXTR signaling facilitates social attachment. Next-generation sequencing identified one polymorphism in the *Oxtr* intron, near a putative cis-regulatory element, explaining 74% of the variance in striatal *Oxtr* expression specifically. Males homozygous for the high expressing allele display enhanced social attachment.

Discussion—Taken together, these findings provide convincing evidence for robust genetic influence on *Oxtr* expression and provide novel insights into how non-coding polymorphisms in the *OXTR* might influence individual differences in human social cognition and behavior

*Address Correspondence to: Larry J. Young, 954 Gatewood Rd., Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329, USA, Phone: 404 727-8272, Fax: 404 727-8070, lyoun03@emory.edu.

Financial Disclosures

All authors report no biomedical financial interests or potential conflicts of interest.

Keywords

social behavior; autism; striatum; individual differences; allelic imbalance; prairie vole

INTRODUCTION

Oxytocin (OXT) is a neuromodulator that influences reproductive and social behavior through signaling via a single G-protein coupled oxytocin receptor (OXTR) in the brain. OXTR affects a range of social behaviors in animals including maternal nurturing and bonding (1, 2), social reward and gregariousness (3, 4), social recognition (5), and pair bonding in monogamous species (6–8). It has been proposed that OXT influences these complex social behaviors by increasing the salience and reinforcing value of social stimuli (9).

In humans, intranasal OXT reportedly enhances many aspects of social cognition, including trust, emotion recognition, and eye gaze (1, 10). However, individual reports of the effects of intranasal OXT should be interpreted cautiously (11). Nevertheless, the OXT system is a leading candidate target for improving social function in psychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia (12–17), and one report suggests that *OXTR* expression may be reduced in ASD (18). Single nucleotide polymorphisms (SNPs) in noncoding regions of the human *OXTR* have been associated with pair bonding behaviors (19), parenting (1), face recognition skills (20), and autism (21). Some individuals have heterogeneous responses to OXT (22, 23), which may involve interaction with *OXTR* genetic polymorphisms (24–27). Early life stress in humans can interact with *OXTR* variation to influence adult social behavior and emotional regulation (28–31). Despite these many associations with human social behavior and disorders, the neural mechanisms by which non-coding SNPs in the *OXTR* could influence behaviors has yet to be explored.

One potential mechanism is that typical expression of *OXTR* is disrupted when such SNPs occur in regulatory elements that primarily lie within non-coding portions of the DNA (*cis*-REs). OXTR is distributed throughout the brain of many vertebrates and, importantly, the pattern of OXTR distribution is diverse between species (32, 33). OXTR is often enriched within regions of crucial behavioral circuits such as the mesolimbic reward (MLR) network and social decision making network and appears to modulate these networks to generate species-specific social strategies such as monogamous social attachments (32) and social gregarity (7). Thus, the manner in which the *Oxtr* gene is regulated between species appears to have profound consequences for the manner in which neural networks activate in response to the social environment.

In a socially monogamous rodent, the prairie vole (*Microtus ochrogaster*), OXTR is enriched in important MLR regions such as the nucleus accumbens (NAcc) and prefrontal cortex (PFC) that constitute part of a neural network for pair bonding (6, 8, 34). OXTR density is much higher in the NAcc of prairie voles than promiscuous vole species and OXTR signaling in the NAcc is required for mating-induced partner preference formation, a laboratory proxy of pair bonding (6). Infusion of an OXTR antagonist into the NAcc or the PFC, but not the caudate putamen (CP) blocks mating-induced partner preferences in

females (35) and males (A.C. Keebaugh and LJY, unpublished data). OXTR density also varies between individual prairie voles, especially in the NAcc along with the CP (36). Increasing OXTR density in the NAcc using viral-vector mediated gene transfer facilitates partner preference formation, while decreasing OXTR density in the same region using RNA interference inhibits such bonding (37–39). Variation in NAcc OXTR density is correlated with individual differences in monogamy-related behavior in males in naturalistic settings (40). Furthermore, variation in prairie vole NAcc OXTR confers susceptibility or resilience to the effects of daily neonatal isolations, a model of neglect, in relation to the ability to form social attachments as adults (41). Mechanisms responsible for OXTR diversity in the prairie vole NAcc may be important determinants of individual differences in social behavior as well.

One likely causal explanation for OXTR diversity is that genetic polymorphisms in *cis*-REs regulating *Oxtr* generate variation in expression in a brain region-specific manner. Variation in gene expression mediated by cis-REs plays an important role in evolutionary phenotypic change (32, 42–46). In prairie voles, a microsatellite in the 5' flanking region of the vasopressin receptor gene (*Avpr1a*) containing cis-REs has been shown to have functional influence over species differences and individual variation in A*vpr1a* expression and is associated with variation in social behavior (47, 48). The influence of *cis*-REs can be detected by assaying for allelic imbalance, which is observed when two alleles of a gene in a heterozygous individual are expressed at different rates, creating an imbalance in the respective mRNAs (49–51). Any differences in mRNA levels between alleles occur in the same nuclear environment, where both alleles should be affected equally by environmental, hormonal or epigenetic factors, unless *cis*-REs proximal to the alleles are variable. Allelic imbalance is commonly observed in a tissue-specific manner (51, 52).

To determine whether prairie vole *Oxtr* gene expression is influenced by polymorphic *cis*-REs, we analyzed brain region-derived mRNA for allelic imbalance in animals heterozygous for a SNP in the *Oxtr* transcribed region. We found that robust allelic imbalance of *Oxtr* occurs within the striatum, but not in several other brain regions. Voles with alternative homozygous genotypes for this SNP had significant differences in NAcc OXTR density. Finally, to gain a more thorough understanding of the relationship between genetic polymorphisms in the prairie vole *Oxtr* and neural OXTR density, we sequenced 70 kilobases of DNA around the gene in 45 voles. We observed strong associations between several genetic markers and OXTR density that were particularly robust in the NAcc. A bioinformatics analysis using ENCODE data suggests that an intronic SNP is the most likely functional candidate for further investigation. This intronic SNP is strongly associated with OXTR density in the NAcc and was also found to associate with the propensity to form social attachments. Our results demonstrate for the first time that non-coding SNPs in the *Oxtr* can profoundly predict OXTR binding and *Oxtr* expression in a brain region-specific manner. These findings implicate that *cis*-regulation drives the remarkable variation in *Oxtr* transcription and has a more modest, but significant, influence on social behaviors. This is the first study to demonstrate that SNPs in the non-coding region of the oxytocin receptor gene robustly effect receptor density in the brain.

MATERIALS AND METHODS

Animals

Prairie voles (*Microtus ochrogaster*) were housed in same-sex groups with 2–3 voles/cage from post-natal day (PND) 21. Housing consisted of a ventilated 36×18×19 cm Plexiglass cage filled with Bed-o-Cob Laboratory Animal Bedding under a 14:10 h light/dark cycle (lights on 7:00 AM–9:00 PM) at 22 °C with access to food (rabbit LabDiet) and water *ad libitum*. Our laboratory breeding colony was originally derived from field captured voles in Illinois. All procedures were approved by the Emory University Institutional Animal Care and Use Committee.

Sanger sequencing and polymorphism discovery for an allelic imbalance marker

DNA was isolated using a Qiagen DNeasy kit (Germantown, MD). We designed primers to amplify five loci spanning the two coding exons plus the 5' UTR and parts of the 3' UTR. For details see Supplemental Information. Nucleotide 204321 (NT204321), located in the 3'UTR, was polymorphic (minor allele frequency: 0.33) and was used for the detailed allelic imbalance study.

Allelic imbalance

Subjects were euthanized with CO_2. Brains were frozen in crushed dry ice and stored at −80° C. Nucleic acids for the allelic imbalance assay were isolated from microdissected brain tissues using the Qiagen mRNA/DNA Micro Kit. For details see Supplemental Information.

Long-range PCRs for target enrichment of 70kb surrounding *Oxtr*

DNA was isolated from previously sectioned brains stored at −80° C with the Qiagen DNeasy Kit. All PCRs were performed using the Qiagen LongRange PCR Kit. Ten loci between 6.6 – 10 kb were amplified. For details see Supplemental Information.

Amplicon Library Preparation

Sequencing library preparation and sequencing analyses were performed by the Yerkes Nonhuman Primate Genomics Core (Atlanta, GA). PCR amplicons from each animal were pooled and cleaned using Solid Phase Reversible Immobilization (SPRI) beads (Beckman Coulter). Libraries were generated using the Illumina Nextera XT DNA Library Prep Kit (San Diego, CA), dual barcoding and sequencing primers were added according to the manufacturer protocol. Libraries were validated by microelectrophoresis, quantified, pooled and clustered on Illumina TruSeq v3 flowcell. Clustered flowcell was sequenced on an Illumina HiSeq 1000 in 100-base single-read reactions.

Amplicon sequencing analysis

Sequencing reads were mapped to *Microtus ochrogaster* target 220 haplotype 2 genomic scaffold (DP001215.2) using the Burrows-Wheeler Aligner (bwa version 0.7.10) (53). The aligned reads were processed with the DNAseq Variant Analysis workflow of the Genome Analysis toolkit (GATK v3.2.2) (54), including marking duplicate reads and local

realignment around insertion/deletions. Variants were called on a per-sample basis and then combined to produce a joint variant call file.

Oxytocin receptor autoradiography

Oxytocin receptor autoradiography was performed as previously reported (37). For details see Supplementary Information.

In situ hybridization

Sense and antisense ^{35}S-UTP-labeled RNA probes for prairie vole *Oxtr* mRNA were generated as described previously (55). For details see Supplementary Information.

Partner preference test

The partner preference was performed as previously described (56). For details see Supplemental Information

Statistical analysis

All statistical analyses were performed in the R statistical software package version 3.1.1, unless stated otherwise. Associations between genetic information and brain data were examined using linear regression with Bonferroni corrections for multiple comparisons. Regarding the factor analyses, to determine how many factors to extract we used the "nFactors" package in R and the factor extraction decisions were based on the eigenvalues-greater-than-one rule, parallel analysis, the optimal coordinates method and acceleration factor. Exploratory factor analyses were performed with the factanal() function, using "varimax" as the rotation method. Processing of next-generation sequencing data was performed in VCFtools, a program package designed for working with VCF files from sequencing projects (57). For associations between individual markers in the *Oxtr* sequence and autoradiography expression data, linear regressions were performed using PLINK/SEQ, an open-source library for working with genetic variation data (URL: https://atgu.mgh.harvard.edu/plinkseq/). For further details see Supplemental Information.

RESULTS

As expected based on previous experiments, the NAcc and caudate putamen (CP) exhibited more individual variation in OXTR density than other brain regions (Figure 1a–c). Furthermore, OXTR binding density appeared to be correlated with *Oxtr* mRNA levels based on *in situ* hybridization signal (Figure 1a). To test the hypothesis that the high variability in OXTR density within the NAcc was due to the influence of putative *cis*-REs, we first assayed for allelic imbalance. We sequenced the transcribed region of the *Oxtr* in a small sample of voles to identify any SNP in our prairie vole colony with a relatively high minor allele frequency. One SNP in the 3' untranslated region (minor allele frequency 33%), heretofore referred to as NT204321 based on the position of this nucleotide on a sequenced prairie vole bacterial artificial clone (58) (DP001215.2), was identified using this method. In heterozygous animals for this SNP, we found significant allelic imbalance in NAcc (cDNA: 3.16, gDNA threshold: 1.11), caudate putamen (CP) (cDNA: 5.24, gDNA threshold: 1.13) and to a lesser degree, amygdala (Amyg) (cDNA: 1.19, gDNA threshold: 1.14). The allelic

imbalance was pronounced in the two striatal sub-regions, NAcc and CP, with the T-allele transcript being 3–5 times more prevalent than the C-allele in the same animals in these regions (Figure 1d). These data strongly suggest that *cis*-REs linked to NT204321 generate individual variation in expression of *Oxtr* in select brain tissues.

To test if NT204321 might serve as a marker to predict overall OXTR density in the NAcc, we collected expression data for 12 brain regions (N=31) using autoradiography. Visual inspection of OXTR density across brain regions suggested that density in some regions co-varied with NAcc density, while density in other regions did not. Therefore, we used factor analysis to determine the correlation structure between OXTR density data from the 12 brain regions investigated. We hypothesize that correlations between OXTR densities from different brain regions can be explained by unobserved variables, possibly reflecting transcriptional processes giving rise to the patterns of correlation. Exploratory factor analysis is a method to identify such unobserved, latent variables. Our analysis revealed two factors together explaining the majority of variance (58%). Factor 1 strongly reflects co-variability in a set of regions involved in reward processing (NAcc, CP, and olfactory tubercle) while the second factor reflects covariation between cortical and subcortical regions that have relatively uniform levels of OXTR density (Table 1a). We identified similar patterns when we investigated the associations between NT204321 and the OXTR expression in the 12 brain regions, with regions loading into Factor 1 being more related to genotype than those loading into Factor 2 (Figure 2). We performed a second factor analysis in an additional sample (N=85) (Table 1b) and this analysis, like the first one, revealed two factors explaining most of the variance. OXTR binding in the NAcc was almost perfectly correlated with the first factor and binding in the insular cortex (Ins) was close to perfectly correlated with Factor 2. Thus further analyses including brain data focused on these two regions as representatives of Factor 1 and Factor 2.

NT204321 was not chosen based on any assumption of functional importance and we suspected that other SNPs across and outside the *Oxtr* transcribed region might be more closely associated with the cis-RE and better predict *Oxtr* expression. We first characterized the suite of polymorphisms across the *Oxtr* gene by sequencing 70 kb of DNA including and surrounding the gene. Amongst the 45 voles we sequenced, we identified 967 SNPs with a read depth no lower than 100 reads, a quality score of at least 1000, and for which all were variable in our sample. Figure 3a shows a quantile-quantile plot for the association between the SNPs in the *Oxtr* sequence and NAcc OXTR binding density. Clearly, as can be seen in Figure 3a many of the SNPs in our set were strongly associated with NAcc OXTR density. We were primarily interested in identifying variants that could potentially be functional and therefore focused on the SNPs with the lowest *P*-values (and largest effect sizes). Due to linkage disequilibrium, 15 SNPs spanning a 30 kb region were associated with NAcc OXTR density with the same, minimal *P*-value ($P=1.06\times10^{-15}$, adjusted $R^2=0.78$), which is a remarkable effect size for a genotype-phenotype relationship.

In order to assess whether any of the 15 most associated SNPs are more likely than others to lie in a putative *cis*-RE, we investigated their homology with regions of the mouse *Oxtr* gene that overlap with signatures of functional activity occurring within neural tissues where OXTR is expressed in the mouse (59). We mapped vole sequence containing these SNPs and

surrounding sequence of approximately 500 bp per SNP to the mouse O*xtr* gene. We compared our vole sequences to ENCODE tracks for markers of general transcriptional activity such as DNase hypersensitivity, H3K4me1, and H3K27a as well as binding of the transcription factor CTCF, which can act as a canonical transcription factor or as an organizer of genomic architecture (60). Only one SNP overlapped with strong signatures of transcriptional function, a SNP occurring at nucleotide 213739 (NT213739, minor allele frequency: 0.32) (Figure 3b). The sequence containing NT213739 overlapped peaks of DNase hypersensitivity and CTCF binding within the large intron, a region proposed to contain *cis*-REs in humans (20, 61, 62). Based on this evidence we chose to further investigate the predictive power of NT213739 in two additional samples.

After genotyping additional voles (Sample 2 n=35, Sample 3 n=31) for NT213739, we confirmed the SNP was robustly associated with OXTR density in the NAcc but not in the Ins (Figure 4a). Indeed, in both the second and third independent samples investigated, our findings from the sequenced sample were very closely replicated. In all of our relatively small samples, NT213739 was strongly associated with NAcc expression (all *P*-values$<4\times10^{-10}$). Similarly, the effect size of this association was very large in all samples (adjusted $R^2\geq74\%$), strongly suggesting that NT213739 explains at least 74% of the variance in NAcc expression of OXTR in prairie voles. The effect was marked, such that OXTR density values in the NAcc between homozygous animals of the two genotypes did not overlap at all while heterozygous animals displayed an intermediate phenotype.

To confirm that the association between NT213739 and receptor density is mediated through mRNA levels, we performed *in situ* hybridization on adjacent sections of brains from 31 individuals. We found that NT213739 is also significantly associated with *Oxtr* mRNA levels in the NAcc (Figure 4b) and that mRNA levels and OXTR density were significantly correlated (Figure 4c, 4d). Together these data suggest that NT213739 is strongly associated with transcriptional variation of the *Oxtr* gene and tightly linked to a *cis*-RE.

Since NT213739 robustly predicted NAcc OXTR density, and NAcc OXTR signaling is important for regulating partner preference formation in prairie voles, we sought to determine whether NT213739 would influence mating-induced partner preference formation, a measure of social attachment that involves social information processing and social reinforcement. Males of varying genotype were housed with a female for 6 h and then tested in the partner preference test. There was a significant effect of genotype on partner preference formation, with C/C voles spending significantly more time huddling with the partner than the stranger, while C/T and T/T males failed to display a partner preference (Figure 5).

DISCUSSION

We here demonstrate that genetic variation in the *Oxtr* exerts robust control over individual diversity in *Oxtr* expression and OXTR density in the prairie vole brain and that this influence on expression occurs in a region-specific manner. Allelic imbalance is strongest in the striatum and genotype-OXTR associations are most robust in this region. Exploratory factor analysis identified a cluster of striatal-olfactory regions with correlated OXTR density.

One intriguing interpretation of this finding is that the unobserved latent variable represented by Factor 1 may reflect a set of transcriptional regulators with maximal effect on *Oxtr* expression activity in prairie vole olfactory-reward processing regions. Whatever transcription factor or factors leads to the covariation appear to interact with the *cis*-REs associated with NT213739 to generate the high variation in expression observed in these regions, but not other regions. In this manner *cis*-REs appear to contribute exquisite control over OXTR and through this process, influence behavioral diversity in prairie voles.

Little is known about the molecular mechanisms regulating brain region-specific *Oxtr* expression in any species. Prairie voles and montane voles display species-specific patterns of OXTR expression. Comparisons of ~1500 bp of 5' flanking regions from the *Oxtr* gene reveal only a few SNPs and 99% homology between the species (63). Transgenic mice carrying a reporter gene driven by 5 kb of the prairie vole *Oxtr* 5' flanking sequence express the reporter in the brain in a pattern resembling prairie voles (64), suggesting the sequence may suffice for some aspects of brain region-specific expression. DNA methylation of *Oxtr* differs between brain regions in rodents (65, 66). Interestingly, DNA methylation of *OXTR* in humans may be mediated by an intronic SNP (67). In some rodent brain regions, OXTR expression is regulated by gonadal steroids in a species specific manner. For example, testosterone increases OXTR in the hypothalamus of rats but decreases OXTR in mice (68, 69). Clearly a better understanding of the molecular mechanisms underlying species differences and individual variation in *Oxtr* transcription in the brain is needed and may inform our understanding of how genetic variation in human OXTR relates to psychiatric phenotypes or responses to OXT based therapies.

We found that prairie vole *Oxtr* expression is strongly influenced by polymorphic *cis*-REs that include or are associated with NT213739, a SNP in the intron of the gene. We focused our attention on NT213739 because out of 15 SNPs in perfect LD with one another, only NT213739 mapped to a site in the mouse *Oxtr* intron with robust evidence of transcriptional activity. We note that such comparisons should be made with the understanding that transcription factor binding sites may differ between species (70). We were particularly interested in the proximity of the mapped prairie vole sequence to a putative CTCF binding site, as CTCF binding peaks are found in the intron as well as near the promoter of both the mouse *Oxtr* (Figure 3b) and human *OXTR* (20). One role of CTCF is to act as a spatial organizer, aiding DNA looping to permit *cis*-RE-promoter interactions required for gene transcription (60). Polymorphic *cis*-REs have been shown to influence CTCF binding (71, 72) and DNA looping (73). In the human *OXTR* third intron, the CTCF peak is found near a SNP, rs237887, that was predicted to be near a *cis*-RE, and associated with face recognition abilities (20) and ASD diagnosis (21). Additionally, the human intron may contain other *cis*-REs (61) and accumulates rare SNPs in cases of ASD (62). If the large intron of *Oxtr* contains *cis*-REs in multiple species, spatial organization of DNA by factors such as CTCF could offer a potentially general regulatory mechanism required for proper function of species or region-specific *cis*-REs. While this evidence provides a potential molecular mechanism by which NT213739 could lead to region-specific differential transcription, it is important to note that our current genotype-phenotype relationships do not implicate NT213739 above any of the other SNPs in perfect LD with it spanning the 30 kb. Future studies using large samples of more genetically diverse animals, including wild caught

specimens, may be needed to break this haplotype structure in order to identify which SNP is most likely functionally contributing to OXTR density variation. Furthermore, biochemical analyses, including chromatin immunoprecipitation followed by sequencing (ChIP-seq), chromosome conformation capture (3C) (74), and *in vitro* transcription assays could be used to investigate interactions between CTCF binding, DNA looping and *Oxtr* regulation.

One of the most remarkable findings of our study is the amount of OXTR density variance explained by genetic polymorphism. Our data suggests that NT213739, or any of the SNPs in perfect LD with it, explain 74% of the variation in NAcc OXTR. Behavioral genetic studies typically report that 1–10% of the variance in behavioral phenotype is explained by candidate gene polymorphisms. We presume that OXTR density is biologically more proximate to genotype than behavior, and in typical behavioral genetic studies many more variables are contributing to behavioral variation. Thus direct measures of brain phenotype are likely to yield stronger effect sizes than reported in behavioral studies.

Prairie voles strongly express OXTR in regions of the MLR, a conserved neural network (75) involved in reward processing and implicated in numerous human psychiatric disorders including depression (76), addiction (77), and schizophrenia (78). In a key region of the MLR, the NAcc, OXTR activation is necessary for prairie vole partner preference formation (35) and OXTR variation mediates individual differences in pair bonding behaviors (37–39). Here, we confirm a genetic role for naturally occurring OXTR density differences that contribute to individual variation in social behavior (36, 40). In addition to region-specific effects, OXTR signaling enhances functional connectivity within a network of regions during prairie vole pair bond formation (79). Recent research in humans found that intronic *OXTR* variants predicted individual differences in functional connectivity between brain regions during the processing of social information (80, 81). These results highlight a potentially conserved role for OXTR in social cognition between species despite likely differences in sites of expression. The prairie vole may prove a useful model to understand how individual differences in OXTR expression in key regions lead to variation in network connectivity.

A recent meta-analysis supports the conclusion that genetic variation in *OXTR* is associated with ASD diagnosis (21), and others have reported associations with endophenotypes of ASD (20, 82, 83), including structural variability in brain regions relevant to social cognition (80, 84). Such findings in humans call for a better understanding of molecular mechanisms regulating OXTR variation (21, 85). The present study suggests that these genotype-phenotype relationships may be mediated by polymorphisms in *cis*-REs in the *OXTR* gene that influence OXTR density in a brain region-specific manner. Here we use the prairie vole model to demonstrate for the first time that a single SNP can predict the majority of variance in OXTR expression in specific brain regions. Further studies to identify functional mechanisms leading to this difference in *Oxtr* transcriptional activity may provide exciting insights into the precise genetic mechanism generating OXTR mediated diversity in social behavior, including human psychopathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to thank Lorra Mathews for her assistance in maintaining the prairie vole colony, Jamie LaPrairie for thoughtful discussions and comments on early drafts of this manuscript, Andy Kim, Lucky Khambouneheuang and Andrea S. Fernandez for technical assistance and Erin S. Keebaugh for methodological recommendations. This research was supported by NIH Grants R01MH096983, and 1P50MH100023 to LJY, 5T32GM008605 and 5T32DA015040 to LBK and the Swedish Brain Foundation to HW. Additional funding was provided by the NIH Office of Research Infrastructure Programs/OD P51OD11132 to YNPRC

References

1. Rilling JK, Young LJ. The biology of mammalian parenting and its effect on offspring social development. Science. 2014; 345(6198):771–776. [PubMed: 25124431]
2. Numan M, Young LJ. Neural mechanisms of mother-infant bonding and pair bonding: Similarities, differences, and broader implications. Hormones and behavior. 2015
3. Dolen G, Darvishzadeh A, Huang KW, Malenka RC. Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. Nature. 2013; 501(7466):179–184. [PubMed: 24025838]
4. Goodson JL, Schrock SE, Klatt JD, Kabelik D, Kingsbury MA. Mesotocin and nonapeptide receptors promote estrildid flocking behavior. Science. 2009; 325(5942):862–866. [PubMed: 19679811]
5. Ferguson JN, Aldag JM, Insel TR, Young LJ. Oxytocin in the medial amygdala is essential for social recognition in the mouse. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2001; 21(20):8278–8285. [PubMed: 11588199]
6. Young LJ, Wang Z. The neurobiology of pair bonding. Nature neuroscience. 2004; 7(10):1048–1054. [PubMed: 15452576]
7. Goodson JL, Kelly AM, Kingsbury MA. Evolving nonapeptide mechanisms of gregariousness and social diversity in birds. Hormones and behavior. 2012; 61(3):239–250. [PubMed: 22269661]
8. Johnson ZV, Young LJ. Neural networks involved in social attachment and pair bonding. Current Opinion in Behavioral Science. 2015; 3:38–44. [June 2015]
9. Young LJ. Oxytocin, social cognition and psychiatry. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2015; 40(1):243–244. [PubMed: 25482173]
10. Bakermans-Kranenburg MJ, van IJMH. Sniffing around oxytocin: review and meta-analyses of trials in healthy and clinical groups with implications for pharmacotherapy. Translational psychiatry. 2013; 3:e258. [PubMed: 23695233]
11. Walum H, Waldman ID, Young LJ. Statistical and Methodological Considerations for the Interpretation of Intranasal Oxytocin Studies. Biological psychiatry. 2015
12. Insel TR. The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. Neuron. 2010; 65(6):768–779. [PubMed: 20346754]
13. Young LJ, Barrett CE. Neuroscience. Can oxytocin treat autism? Science. 2015; 347(6224):825–826. [PubMed: 25700501]
14. Andari E, Duhamel JR, Zalla T, Herbrecht E, Leboyer M, Sirigu A. Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(9):4389–4394. [PubMed: 20160081]
15. Modi ME, Young LJ. The oxytocin system in drug discovery for autism: animal models and novel therapeutic strategies. Hormones and behavior. 2012; 61(3):340–350. [PubMed: 22206823]
16. Modi ME, Inoue K, Barrett CE, Kittelberger KA, Smith DG, Landgraf R, et al. Melanocortin Receptor Agonists Facilitate Oxytocin-Dependent Partner Preference Formation in the Prairie

Vole. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2015

17. Penagarikano O, Lazaro MT, Lu XH, Gordon A, Dong H, Lam HA, et al. Exogenous and evoked oxytocin restores social behavior in the Cntnap2 mouse model of autism. Science translational medicine. 2015; 7(271):271ra8.
18. Gregory SG, Connelly JJ, Towers AJ, Johnson J, Biscocho D, Markunas CA, et al. Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. BMC Med. 2009; 7:62. [PubMed: 19845972]
19. Walum H, Lichtenstein P, Neiderhiser JM, Reiss D, Ganiban JM, Spotts EL, et al. Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. Biological psychiatry. 2012; 71(5):419–426. [PubMed: 22015110]
20. Skuse DH, Lori A, Cubells JF, Lee I, Conneely KN, Puura K, et al. 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. 2014; 111(5):1987–1992. [PubMed: 24367110]
21. LoParo D, Waldman ID. The oxytocin receptor gene (OXTR) is associated with autism spectrum disorder: a meta-analysis. Molecular psychiatry. 2014
22. Bartz JA, Zaki J, Bolger N, Ochsner KN. Social effects of oxytocin in humans: context and person matter. Trends in cognitive sciences. 2011; 15(7):301–309. [PubMed: 21696997]
23. Olff M, Frijling JL, Kubzansky LD, Bradley B, Ellenbogen MA, Cardoso C, et al. The role of oxytocin in social bonding, stress regulation and mental health: an update on the moderating effects of context and interindividual differences. Psychoneuroendocrinology. 2013; 38(9):1883–1894. [PubMed: 23856187]
24. Marsh AA, Yu HH, Pine DS, Gorodetsky EK, Goldman D, Blair RJ. The influence of oxytocin administration on responses to infant faces and potential moderation by OXTR genotype. Psychopharmacology. 2012; 224(4):469–476. [PubMed: 22763666]
25. Montag C, Sauer C, Reuter M, Kirsch P. An interaction between oxytocin and a genetic variation of the oxytocin receptor modulates amygdala activity toward direct gaze: evidence from a pharmacological imaging genetics study. European archives of psychiatry and clinical neuroscience. 2013; 263(Suppl 2):S169–S175. [PubMed: 24071915]
26. Chen FS, Kumsta R, Dvorak F, Domes G, Yim OS, Ebstein RP, et al. Genetic modulation of oxytocin sensitivity: a pharmacogenetic approach. Translational psychiatry. 2015; 5:e664. [PubMed: 26506050]
27. Feng C, Lori A, Waldman ID, Binder EB, Haroon E, Rilling JK. A common oxytocin receptor gene (OXTR) polymorphism modulates intranasal oxytocin effects on the neural response to social cooperation in humans. Genes, brain, and behavior. 2015; 14(7):516–525.
28. Loth E, Poline JB, Thyreau B, Jia T, Tao C, Lourdusamy A, et al. Oxytocin receptor genotype modulates ventral striatal activity to social cues and response to stressful life events. Biological psychiatry. 2014; 76(5):367–376. [PubMed: 24120094]
29. Lucas-Thompson RG, Holman EA. Environmental stress, oxytocin receptor gene (OXTR) polymorphism, and mental health following collective stress. Hormones and behavior. 2013; 63(4): 615–624. [PubMed: 23470776]
30. Smearman EL, Winiarski DA, Brennan PA, Najman J, Johnson KC. Social stress and the oxytocin receptor gene interact to predict antisocial behavior in an at-risk cohort. Development and psychopathology. 2015; 27(1):309–318. [PubMed: 25003328]
31. Thompson RJ, Parker KJ, Hallmayer JF, Waugh CE, Gotlib IH. Oxytocin receptor gene polymorphism (rs2254298) interacts with familial risk for psychopathology to predict symptoms of depression and anxiety in adolescent girls. Psychoneuroendocrinology. 2011; 36(1):144–147. [PubMed: 20708845]
32. Donaldson ZR, Young LJ. Oxytocin, vasopressin, and the neurogenetics of sociality. Science. 2008; 322(5903):900–904. [PubMed: 18988842]
33. Freeman SM, Inoue K, Smith AL, Goodman MM, Young LJ. The neuroanatomical distribution of oxytocin receptor binding and mRNA in the male rhesus macaque (Macaca mulatta). Psychoneuroendocrinology. 2014; 45:128–141. [PubMed: 24845184]

34. Numan M, Young LJ. Neural mechanisms of mother-infant bonding and pair bonding: Similarities, differences, and broader implications. Hormones and behavior. 2015 In Press.
35. Young LJ, Lim MM, Gingrich B, Insel TR. Cellular mechanisms of social attachment. Hormones and behavior. 2001; 40(2):133–138. [PubMed: 11534973]
36. Olazabal DE, Young LJ. Species and individual differences in juvenile female alloparental care are associated with oxytocin receptor density in the striatum and the lateral septum. Hormones and behavior. 2006; 49(5):681–687. [PubMed: 16442534]
37. Ross HE, Freeman SM, Spiegel LL, Ren X, Terwilliger EF, Young LJ. Variation in oxytocin receptor density in the nucleus accumbens has differential effects on affiliative behaviors in monogamous and polygamous voles. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009; 29(5):1312–1318. [PubMed: 19193878]
38. Keebaugh AC, Young LJ. Increasing oxytocin receptor expression in the nucleus accumbens of pre-pubertal female prairie voles enhances alloparental responsiveness and partner preference formation as adults. Hormones and behavior. 2011; 60(5):498–504. [PubMed: 21851821]
39. Keebaugh AC, Barrett CE, LaPrairie JL, Jenkins JJ, Young LJ. RNAi knockdown of oxytocin receptor in the nucleus accumbens inhibits social attachment and parental care in monogamous female prairie voles. Social neuroscience. 2015
40. Ophir AG, Gessel A, Zheng DJ, Phelps SM. Oxytocin receptor density is associated with male mating tactics and social monogamy. Hormones and behavior. 2012; 61(3):445–453. [PubMed: 22285648]
41. Barrett CE, Arambula SE, Young LJ. The oxytocin system promotes resilience to the effects of neonatal isolation on adult social attachment in female prairie voles. Translational psychiatry. 2015; 5:e606. [PubMed: 26196439]
42. Wittkopp PJ, Kalay G. Cis-regulatory elements: molecular mechanisms and evolutionary processes underlying divergence. Nature reviews Genetics. 2012; 13(1):59–69.
43. Bendesky A, Bargmann CI. Genetic contributions to behavioural diversity at the gene-environment interface. Nature reviews Genetics. 2011; 12(12):809–820.
44. Linnen CR, Poh YP, Peterson BK, Barrett RD, Larson JG, Jensen JD, et al. Adaptive evolution of multiple traits through multiple mutations at a single gene. Science. 2013; 339(6125):1312–1316. [PubMed: 23493712]
45. Oleksiak MF, Churchill GA, Crawford DL. Variation in gene expression within and among natural populations. Nature genetics. 2002; 32(2):261–266. [PubMed: 12219088]
46. Cretekos CJ, Wang Y, Green ED, Martin JF, Rasweiler JJt, Behringer RR. Regulatory divergence modifies limb length between mammals. Genes & development. 2008; 22(2):141–151. [PubMed: 18198333]
47. Hammock EA, Young LJ. Microsatellite instability generates diversity in brain and sociobehavioral traits. Science. 2005; 308(5728):1630–1634. [PubMed: 15947188]
48. Donaldson ZR, Young LJ. The relative contribution of proximal 5' flanking sequence and microsatellite variation on brain vasopressin 1a receptor (Avpr1a) gene expression and behavior. PLoS genetics. 2013; 9(8):e1003729. [PubMed: 24009523]
49. Johnson AD, Wang D, Sadee W. Polymorphisms affecting gene regulation and mRNA processing: broad implications for pharmacogenetics. Pharmacology & therapeutics. 2005; 106(1):19–38. [PubMed: 15781120]
50. Gaur U, Li K, Mei S, Liu G. Research progress in allele-specific expression and its regulatory mechanisms. Journal of applied genetics. 2013; 54(3):271–283. [PubMed: 23609142]
51. Zhang K, Li JB, Gao Y, Egli D, Xie B, Deng J, et al. Digital RNA allelotyping reveals tissue-specific and allele-specific gene expression in human. Nature methods. 2009; 6(8):613–618. [PubMed: 19620972]
52. Wilkins JM, Southam L, Price AJ, Mustafa Z, Carr A, Loughlin J. Extreme context specificity in differential allelic expression. Human molecular genetics. 2007; 16(5):537–546. [PubMed: 17220169]
53. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009; 25(14):1754–1760. [PubMed: 19451168]

54. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research. 2010; 20(9):1297–1303. [PubMed: 20644199]
55. Inoue K, Terashima T, Nishikawa T, Takumi T. Fez1 is layer-specifically expressed in the adult mouse neocortex. The European journal of neuroscience. 2004; 20(11):2909–2916. [PubMed: 15579145]
56. Ahern TH, Modi ME, Burkett JP, Young LJ. Evaluation of two automated metrics for analyzing partner preference tests. Journal of neuroscience methods. 2009; 182(2):180–188. [PubMed: 19539647]
57. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant call format and VCFtools. Bioinformatics. 2011; 27(15):2156–2158. [PubMed: 21653522]
58. McGraw LA, Davis JK, Thomas PJ, Program NCS, Young LJ, Thomas JW. BAC-based sequencing of behaviorally-relevant genes in the prairie vole. PloS one. 2012; 7(1):e29345. [PubMed: 22238603]
59. Mouse EC, Stamatoyannopoulos JA, Snyder M, Hardison R, Ren B, Gingeras T, et al. An encyclopedia of mouse DNA elements (Mouse ENCODE). Genome biology. 2012; 13(8):418. [PubMed: 22889292]
60. Ong CT, Corces VG. CTCF: an architectural protein bridging genome topology and function. Nature reviews Genetics. 2014; 15(4):234–246.
61. Mizumoto Y, Kimura T, Ivell R. A genomic element within the third intron of the human oxytocin receptor gene may be involved in transcriptional suppression. Molecular and cellular endocrinology. 1997; 135(2):129–138. [PubMed: 9484908]
62. Liu X, Kawashima M, Miyagawa T, Otowa T, LK Z, Thiri M, et al. Novel rare variations of the oxytocin receptor (OXTR) gene in autism spectrum disorder individuals. Human Genome Variation. 2015
63. Young LJ, Huot B, Nilsen R, Wang Z, Insel TR. Species differences in central oxytocin receptor gene expression: comparative analysis of promoter sequences. Journal of neuroendocrinology. 1996; 8(10):777–783. [PubMed: 8910808]
64. Young LJ, Waymire KG, Nilsen R, Macgregor GR, Wang Z, Insel TR. The 5' flanking region of the monogamous prairie vole oxytocin receptor gene directs tissue-specific expression in transgenic mice. Annals of the New York Academy of Sciences. 1997; 807:514–517. [PubMed: 9071386]
65. Harony-Nicolas H, Mamrut S, Brodsky L, Shahar-Gold H, Barki-Harrington L, Wagner S. Brain region-specific methylation in the promoter of the murine oxytocin receptor gene is involved in its expression regulation. Psychoneuroendocrinology. 2014; 39:121–131. [PubMed: 24275011]
66. Beery AK, McEwen LM, MacIsaac JL, Francis DD, Kobor MS. Natural variation in maternal care and cross-tissue patterns of oxytocin receptor gene methylation in rats. Hormones and behavior. 2015
67. Reiner I, Van IMH, Bakermans-Kranenburg MJ, Bleich S, Beutel M, Frieling H. Methylation of the oxytocin receptor gene in clinically depressed patients compared to controls: The role of OXTR rs53576 genotype. Journal of psychiatric research. 2015; 65:9–15. [PubMed: 25890851]
68. Insel TR, Young L, Witt DM, Crews D. Gonadal steroids have paradoxical effects on brain oxytocin receptors. Journal of neuroendocrinology. 1993; 5(6):619–628. [PubMed: 8680433]
69. Bale TL, Dorsa DM. Regulation of oxytocin receptor messenger ribonucleic acid in the ventromedial hypothalamus by testosterone and its metabolites. Endocrinology. 1995; 136(11): 5135–5138. [PubMed: 7588251]
70. Odom DT, Dowell RD, Jacobsen ES, Gordon W, Danford TW, MacIsaac KD, et al. Tissue-specific transcriptional regulation has diverged significantly between human and mouse. Nature genetics. 2007; 39(6):730–732. [PubMed: 17529977]
71. Verlaan DJ, Berlivet S, Hunninghake GM, Madore AM, Lariviere M, Moussette S, et al. Allele-specific chromatin remodeling in the ZPBP2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease. American journal of human genetics. 2009; 85(3):377–393. [PubMed: 19732864]

72. Paredes UM, Quinn JP, D'Souza UM. Allele-specific transcriptional activity of the variable number of tandem repeats in 5' region of the DRD4 gene is stimulus specific in human neuronal cells. Genes, brain, and behavior. 2013; 12(2):282–287.
73. Visser M, Palstra RJ, Kayser M. Allele-specific transcriptional regulation of IRF4 in melanocytes is mediated by chromatin looping of the intronic rs12203592 enhancer to the IRF4 promoter. Human molecular genetics. 2015; 24(9):2649–2661. [PubMed: 25631878]
74. Dekker J, Marti-Renom MA, Mirny LA. Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. Nature reviews Genetics. 2013; 14(6):390–403.
75. O'Connell LA, Hofmann HA. The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. The Journal of comparative neurology. 2011; 519(18):3599–3639. [PubMed: 21800319]
76. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. Nature reviews Neuroscience. 2013; 14(9):609–625. [PubMed: 23942470]
77. Luscher C, Malenka RC. Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. Neuron. 2011; 69(4):650–663. [PubMed: 21338877]
78. Abi-Dargham A. Schizophrenia: overview and dopamine dysfunction. The Journal of clinical psychiatry. 2014; 75(11):e31. [PubMed: 25470107]
79. Johnson ZV, Walum H, Jamal YA, Xiao Y, Keebaugh AC, Inoue K, et al. Central oxytocin receptors mediate mating-induced partner preferences and enhance correlated activation across forebrain nuclei in male prairie voles. Hormones and behavior. 2015 (*in press*).
80. Tost H, Kolachana B, Hakimi S, Lemaitre H, Verchinski BA, Mattay VS, et al. A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic-limbic structure and function. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(31):13936–13941. [PubMed: 20647384]
81. Tost H, Kolachana B, Verchinski BA, Bilek E, Goldman AL, Mattay VS, et al. Neurogenetic effects of OXTR rs2254298 in the extended limbic system of healthy Caucasian adults. Biological psychiatry. 2011; 70(9):e37–e39. author reply e41–2. [PubMed: 21872215]
82. Parker KJ, Garner JP, Libove RA, Hyde SA, Hornbeak KB, Carson DS, et al. Plasma oxytocin concentrations and OXTR polymorphisms predict social impairments in children with and without autism spectrum disorder. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111(33):12258–12263. [PubMed: 25092315]
83. Lerer E, Levi S, Salomon S, Darvasi A, Yirmiya N, Ebstein RP. Association between the oxytocin receptor (OXTR) gene and autism: relationship to Vineland Adaptive Behavior Scales and cognition. Molecular psychiatry. 2008; 13(10):980–988. [PubMed: 17893705]
84. Inoue H, Yamasue H, Tochigi M, Abe O, Liu X, Kawamura Y, et al. Association between the oxytocin receptor gene and amygdalar volume in healthy adults. Biological psychiatry. 2010; 68(11):1066–1072. [PubMed: 20832055]
85. Kumsta R, Heinrichs M. Oxytocin, stress and social behavior: neurogenetics of the human oxytocin system. Current opinion in neurobiology. 2013; 23(1):11–16. [PubMed: 23040540]

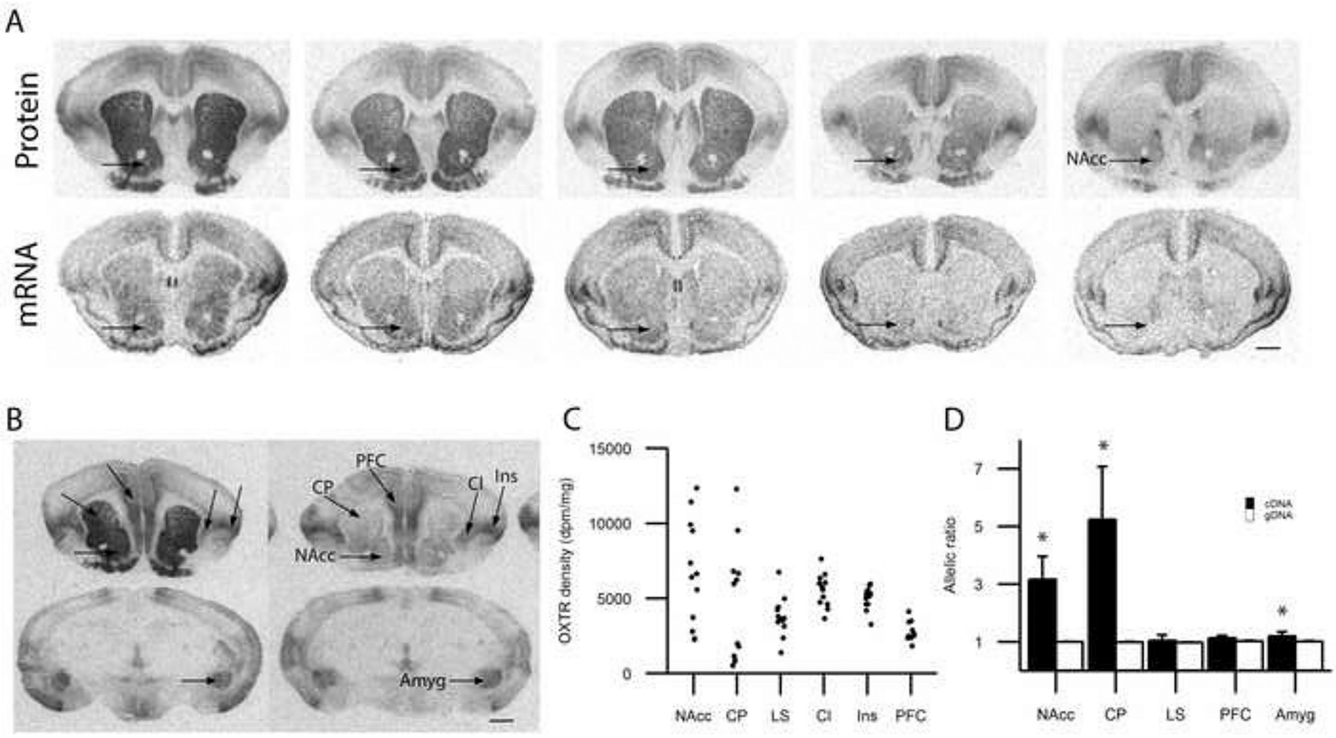

Figure 1.

Region-specific variation in neural expression of the prairie vole *Oxtr* gene is caused by *cis*-regulatory elements. (**a**) An illustration that OXTR protein density (visualized by receptor autoradiography) and *Oxtr* mRNA levels (visualized by *in situ* hybridization) vary in the striatum, including the nucleus accumbens (NAcc). (**b**) In contrast to the individual differences in NAcc and CP OXTR density, other brain regions show less variation. Black scale bar represents 100 μm. (**c**) Quantification of individual variation in OXTR density. Each dot represents OXTR density (dpm/mg) for an individual prairie vole (N=12, males). NAcc, nucleus accumbens; CP, caudate putamen; LS, dorsal lateral septum; Cl, claustrum; Ins, insular cortex; PFC, prefrontal cortex; Amyg, amygdala. (**d**) Allelic imbalance was calculated as the average allelic ratio (%T/%C) for cDNA and genomic DNA (gDNA) from animals heterozygous at NT204321 (N=8). A significant allelic imbalance was detected in the cDNA derived from the nucleus accumbens (NAcc), caudate putamen (CP) and amygdala (Amyg), but not in cDNA derived from the prefrontal cortex (PFC) or lateral septum (LS). The striatal regions had very high allelic imbalance, with 3–5 fold differences between alleles. gDNA T and C-alleles are amplified at equal levels in all tissues. * cDNA allelic ratio is significantly greater than a threshold calculated by the mean of the gDNA allelic ratio + 3 gDNA standard deviations. Data are expressed as mean ± standard error of the mean (SEM). Except for CP gDNA (n=6), n=7.

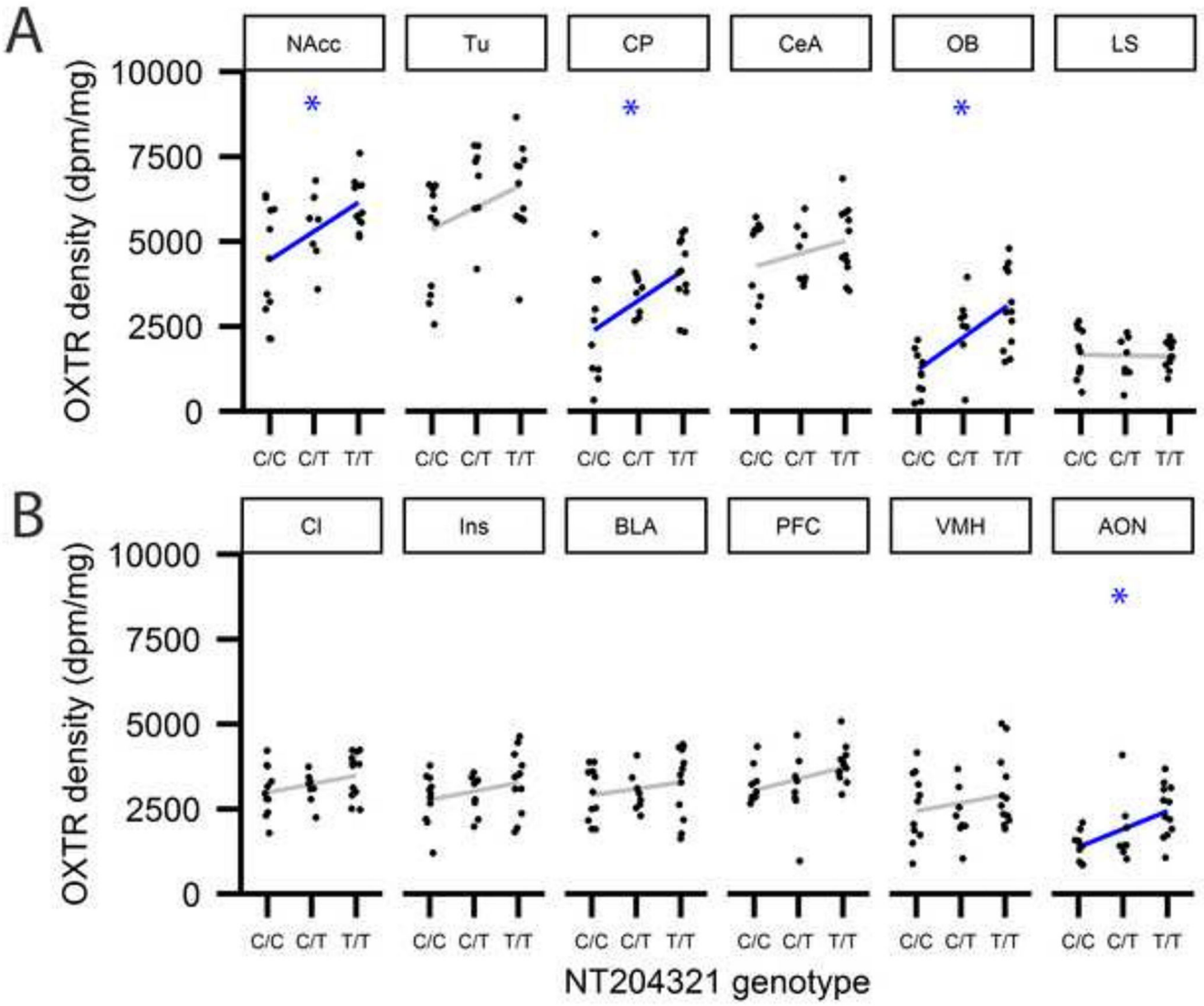

Figure 2.

OXTR binding density in striatal and olfactory regions is associated with NT204321. Samples sizes for each genotype are C/C=11, C/T=8 and T/T=12. (**a**) Brain regions are sorted based on the how strongly the regions loaded on Factor 1. Within Factor 1 regions, OXTR binding is significantly related to genotype in NAcc, CP and OB. (**b**) Brain regions are sorted based on the how strongly the regions loaded on Factor 2. In the Factor 2 grouping, only the AON was significantly associated with genotype. Associations were investigated using simple linear regression. * $P < 0.004$ (α corrected for 12 comparisons). NAcc, nucleus accumbens; Tu, olfactory tubercle; CP, caudate putamen; CeA, central amygdala; OB, olfactory bulb; LS, dorsal lateral septum; Cl, claustrum; Ins, insular cortex; BLA, basal lateral amygdala; PFC, prefrontal cortex; VMH, ventral medial hypothalamus; AON, anterior olfactory nucleus. Data is shown as individual OXTR density (dpm/mg) with trend line for the linear regression.

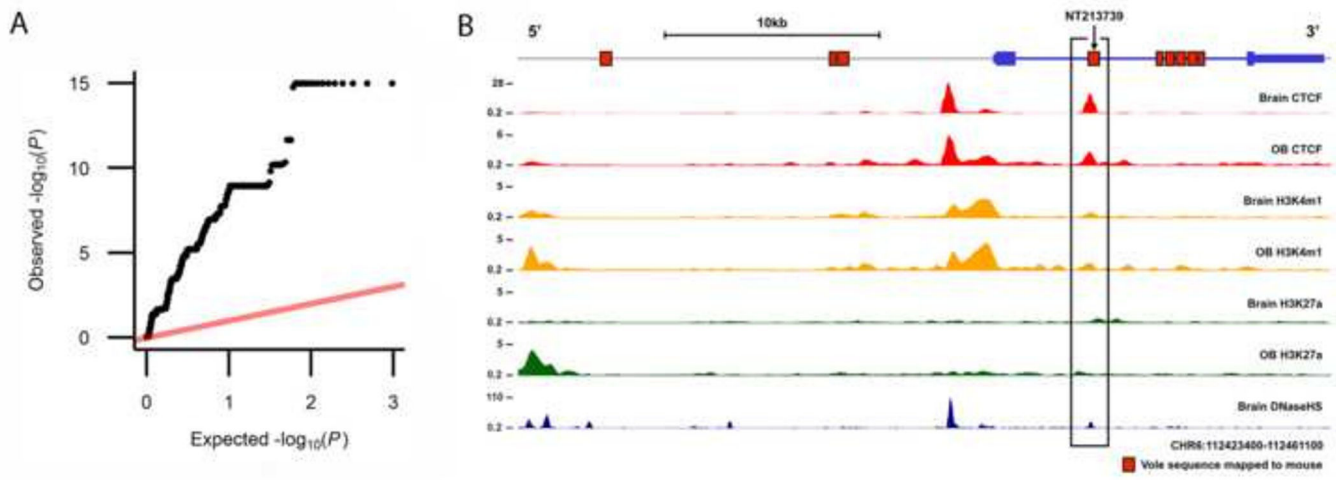

Figure 3.

Identification of NT213739 as a marker of NAcc OXTR density. (**a**) Quantile-quantile plot of the associations between the 967 *Oxtr* markers and NAcc OXTR density. Each dot represents the –log P of the association between a particular SNP with OXTR density placed in ascending order. (**b**) A schematic of the mouse *Oxtr* gene with accompanying functional data from the ENCODE project. The prairie vole sequences containing the SNPs showing the strongest association with NAcc OXTR density that also map to mouse *Oxtr* sequence. Approximately 500 bp per SNP of prairie vole sequence surrounding each SNP was aligned to the mouse genome as indicated by red rectangles in the figure. ChIP-seq signal or DNaseHS signal is shown. OB, olfactory bulb; Brain, whole brain; CTCF, CCCTC-binding factor (red) ; H3K4m1, a single methyl modification of lysine 4 of the H3 histone (orange); H3K27a, acetylation modification of lysine 27 on the H3 histone (green); DNaseHS, deoxyribonuclease I hypersensitive sites (dark blue).

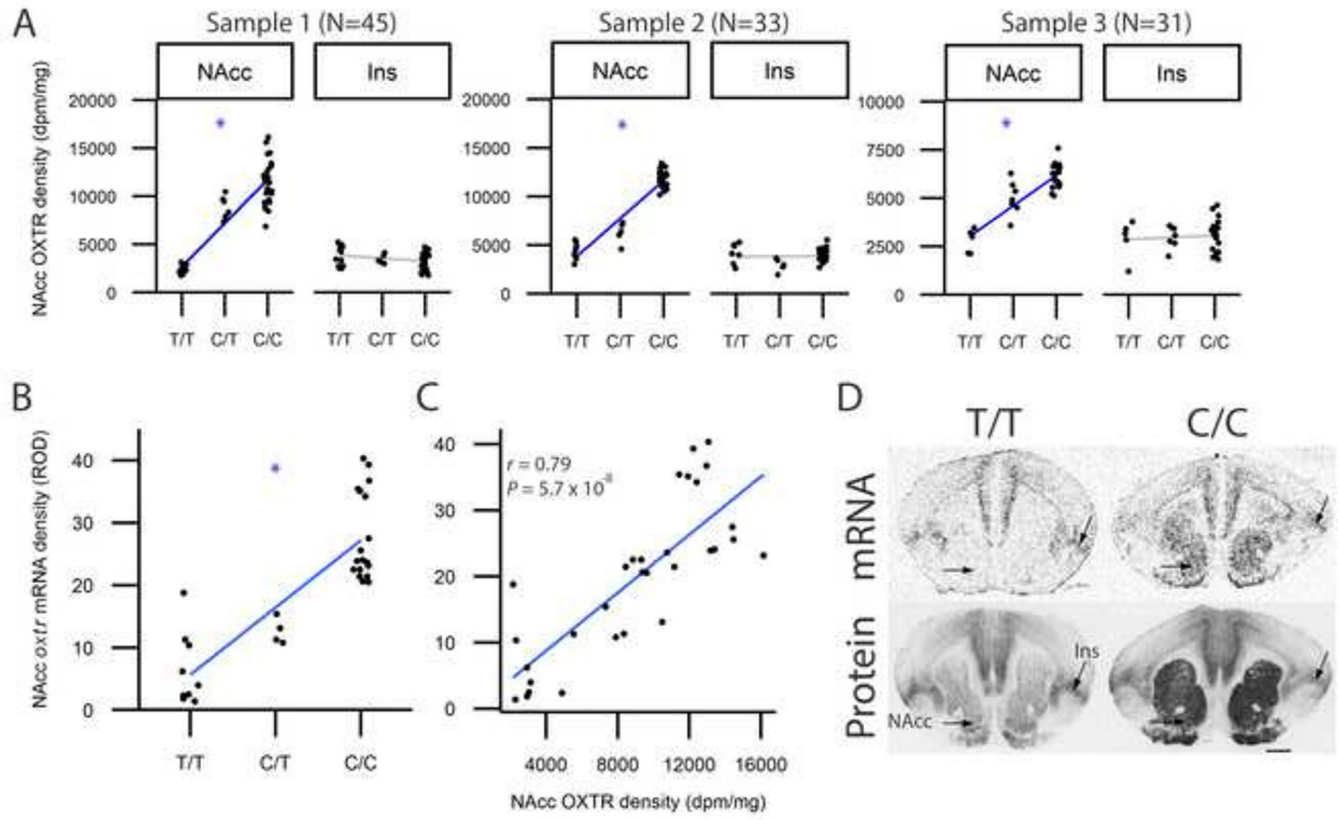

Figure 4.

NT213739 genotype robustly predicts *Oxtr* expression. (**a**) NAcc OXTR density was associated with NT213739 genotype across three independent samples (Sample 1, adjusted R^2=0.81; Sample 2, adjusted R^2=0.90; Sample 3, adjusted R^2=0.74). OXTR density in the insula (Ins) was not associated with NT213739. (**b**) (**c**) NAcc *Oxtr* mRNA density was significantly associated with NT213739 genotype (N=31, adjusted R^2=0.69). (**c**) NAcc Oxtr mRNA density is significantly correlated with OXTR protein binding density (N=31). (**d**) Representative images highlighting the differences between NT213739 genotypes in *Oxtr* expression within the striatum, particulary the nucleus accumbens (NAcc). Black scale bar represents 100 μm.* $P < 1\times10^{-8}$. Data is shown as individual OXTR density (dpm/mg) or individual mRNA density (ROD) with trend line for the linear regressions.

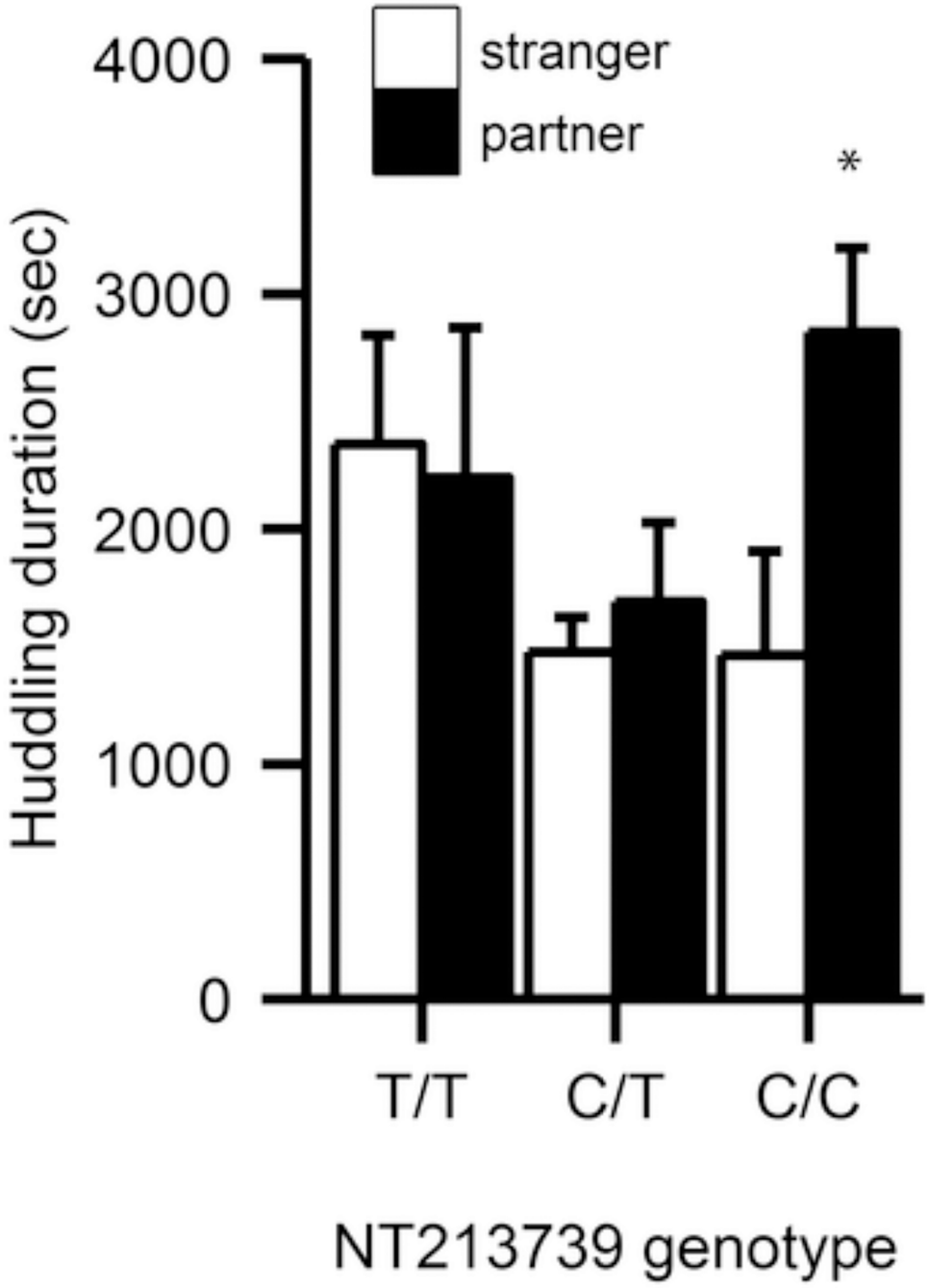

Figure 5.

NT213739 genotype influences partner preference formation in male prairie voles. The effect of genotype on behavior was investigated using two-way ANOVA. The interaction of genotype×stimulus on huddling duration was significant ($F_{(1,136)}$=4.45, P=0.037). Males with a C/C genotype (N=39) spent significantly more time huddling with the partner than the stranger while animals with a C/T (N=13) or T/T (N=18) genotype did not. * indicates a

partner preference, mean partner huddling time is significantly greater than mean stranger huddling time (t-test, $P < 0.01$). Data are expressed as mean ± SEM.

Table 1

Two factors encompass the covariation in OXTR density amongst brain regions

a	Factor 1	Factor 2
NAcc	0.99	0.14
Tu	0.82	0.09
CP	0.82	0.18
CeA	0.69	0.3
OB	0.56	0.5
LS	0.56	0.15
Cl	0.17	0.88
Ins	0.12	0.85
BLA	0.04	0.71
PFC	0.5	0.51
VMH	0.3	0.47
AON	0.28	0.41

b	Factor 1	Factor 2
NAcc	0.99	0.04
CP	0.92	0.1
Tu	0.91	−0.01
LS	0.56	0.14
Ins	−0.06	0.99
Cl	0.07	0.75
PFC	0.51	0.6

Values in the table represent factor correlations. NAcc, nucleus accumbens; Tu, olfactory tubercle; CP, caudate putamen; CeA, central amygdala; OB, olfactory bulb; LS, dorsal lateral septum. Cl, claustrum; Ins, insular cortex; BLA, basal lateral amygdala; PFC, prefrontal cortex; VMH, ventral medial hypothalamus; AON, anterior olfactory nucleus.