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

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ABSTRACT

BACKGROUND: Oxytocin (OXT) modulates several aspects of social behavior. Intranasal OXT is a leading candidate for treating social deficits in patients with autism spectrum disorder, and common genetic variants in the human *OXTR* gene are associated with emotion recognition, relationship quality, and autism spectrum disorder. 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* messenger RNA and oxytocin receptor protein with established neuroanatomic 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 demonstrate that genetic variants contribute to individual differences in *Oxtr* expression, but only in particular brain regions, including the nucleus accumbens, where oxytocin receptor 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.

CONCLUSIONS: Taken together, these findings provide convincing evidence for robust genetic influence on *Oxtr* expression and provide novel insights into how noncoding polymorphisms in *OXTR* might influence individual differences in human social cognition and behavior.

Keywords: Allelic imbalance, Autism, Individual differences, Prairie vole, Social behavior, Striatum

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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. The 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). 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 patients with psychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia (12–17), and one report suggested that *OXTR* expression may be reduced in ASD (18). Single nucleotide polymorphisms (SNPs) in noncoding regions of the human *OXTR* gene 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 noncoding SNPs in *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 (REs) that primarily lie within noncoding portions of the DNA (*cis*-REs). The OXTR is distributed throughout the brain of many vertebrates, and the pattern of OXTR distribution is diverse among species (32,33). Within regions of crucial behavioral circuits such as the mesolimbic reward (MLR) network and social decision making network, OXTR is often enriched and appears to modulate these networks to generate species-specific social strategies, such as monogamous social attachments (32) and social gregariousness (7). Thus, the manner in

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which the *Oxtr* gene is regulated among 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 (NAc) and prefrontal cortex that constitute part of a neural network for pair bonding (2,6,8). The OXTR density is much higher in the NAc of prairie voles than promiscuous vole species, and OXTR signaling in the NAc is required for mating-induced partner preference formation, a laboratory proxy of pair bonding (6). Infusion of an OXTR antagonist into the NAc or the prefrontal cortex, but not the caudate putamen (CP), blocks mating-induced partner preferences in females (34) and males (A.C. Keebaugh, Ph.D., and L.J.Y., unpublished data, 2015). The OXTR density also varies among individual prairie voles, especially in the NAc and the CP (35). Increasing OXTR density in the NAc using viral vector–mediated gene transfer facilitates partner preference formation, whereas decreasing OXTR density in the same region using RNA interference inhibits such bonding (36–38). Variation in NAc OXTR density is correlated with individual differences in monogamy-related behavior in males in naturalistic settings (39). Furthermore, variation in prairie vole NAc 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 (40). Mechanisms responsible for OXTR diversity in the NAc in the prairie vole 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 (32,41–45). 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 *Avpr1a* expression and is associated with variation in social behavior (46,47). 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 messenger RNAs (mRNAs) (48–50). 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 (50,51).

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 OXTR density in NAc. 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 kb 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 NAc. A bioinformatics analysis using ENCODE (ENCyclopedia Of DNA Elements) 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 NAc and was found to be associated with the propensity to form social attachments. Our results demonstrate for the first time that noncoding SNPs in the *Oxtr* can profoundly predict OXTR density 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 noncoding region of the *OXTR* robustly affect receptor density in the brain.

METHODS AND MATERIALS

Animals

Prairie voles (*Microtus ochrogaster*) were housed in same-sex groups with two or three voles per cage from postnatal day 21. Housing consisted of a ventilated 36 cm × 18 cm × 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. 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, Maryland). We designed primers to amplify five loci spanning the two coding exons plus the 5′ untranslated region (UTR) and parts of the 3′ UTR. See the Supplement for details. Nucleotide 204321 (NT204321), located in the 3′ UTR, was polymorphic (minor allele frequency, .33) and was used for the detailed allelic imbalance study.

Allelic Imbalance

Subjects were euthanized with carbon dioxide. 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 the Supplement.

Long-Range Polymerase Chain Reactions for Target Enrichment of 70 kb Surrounding *Oxtr*

DNA was isolated from previously sectioned brains stored at −80°C with the QIAGEN DNeasy Kit. All polymerase chain reactions were performed using the QIAGEN LongRange PCR Kit. There were 10 loci 6.6–10 kb amplified. For details, see the Supplement.

Amplicon Library Preparation

Sequencing library preparation and sequencing analyses were performed by the Yerkes Nonhuman Primate Genomics Core (Atlanta, Georgia). Polymerase chain reaction amplicons from each animal were pooled and cleaned using Solid Phase