

An oxytocin receptor polymorphism predicts amygdala reactivity and antisocial behavior in men

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Abstract

Variability in oxytocin (OXT) signaling is associated with individual differences in sex-specific social behavior across species. The effects of OXT signaling on social behavior are, in part, mediated through its modulation of amygdala function. Here, we use imaging genetics to examine sex-specific effects of three single-nucleotide polymorphisms in the human oxytocin receptor gene (OXTR; rs1042778, rs53576 and rs2254298) on threat-related amygdala reactivity and social behavior in 406 Caucasians. Analyses revealed that among men but not women, OXTR rs1042778 TT genotype was associated with increased right amygdala reactivity to angry facial expressions, which was uniquely related to higher levels of antisocial behavior among men. Moderated meditation analysis suggested a trending indirect effect of OXTR rs1042778 TT genotype on higher antisocial behavior via increased right amygdala reactivity to angry facial expressions in men. Our results provide evidence linking genetic variation in OXT signaling to individual differences in amygdala function. The results further suggest that these pathways may be uniquely important in shaping antisocial behavior in men.

Key words: aggression; amygdala; neuroscience; neuroimaging; oxytocin; psychopathology; violence, OXTR, rs1042778

Introduction

The neuropeptide oxytocin (OXT) has been implicated in modulating social behavior using genetic, pharmacological and behavioral methods across species (Kumsta and Heinrichs, 2013). These effects appear to be mediated, in part, through OXT effects on amygdala function (Kanat et al., 2014), which plays a pivotal role in threat detection, stimulus reinforcement learning and fear conditioning (Phelps and LeDoux, 2005). An important determinant of neural OXT signaling is the availability of its target receptor, encoded by the oxytocin receptor gene (OXTR) (Gimpl and Fahrenholz, 2001). Imaging genetics studies have begun to show that OXTR polymorphisms predict variability in amygdala structure (Inoue et al., 2010) and function (Tost et al., 2010). In the single previous study investigating associations between OXTR

polymorphisms and amygdala function, Tost et al. found that rs53576 A allele carriers had relatively decreased threat-related amygdala reactivity when compared with G allele homozygotes. However, studies have yet to test a single model examining whether genetically mediated variability in OXT functioning affects amygdala reactivity and in turn, social behavior.

To address this gap in the literature, we examined whether OXTR polymorphisms previously linked to amygdala function and structure as well as antisocial behavior (i.e. rs1042778, rs2254298 and rs53576) were associated with threat-related amygdala reactivity among 406 Caucasians who completed the ongoing Duke Neurogenetics Study (DNS) (Swartz et al., 2015). We also examined the potential mediating role of genetically driven variability in amygdala reactivity in the expression of sex-specific antisocial behavior, which has been directly associated with OXTR

polymorphisms rs1042778 and rs2254298 in some but not all prior studies (Sakai et al., 2012; Dadds et al., 2014). Such inconsistency in mapping genetic variation directly onto behavior has been successfully addressed through the use of intermediate neural phenotypes, such as those targeted in imaging genetics (Hariri et al., 2006; Nikolova et al., 2013). Moreover, the use of genetics, an intermediate neural phenotype and self-reported social behavior in a single model allowed us to examine a full pathway from gene to brain to behavior—a mediated pathway that has been explicitly tested in very few previous studies (Hyde et al., 2011). On the basis of existing research linking antisocial behavior to greater threat-related amygdala reactivity (Hyde et al., 2013), we hypothesized that *OXTR* genotypes previously linked with increased antisocial behavior among male samples (i.e. rs1042778, rs53576 and rs2254298; Malik et al., 2012; Dadds et al., 2014) would be associated with relatively increased threat-related amygdala reactivity, which would, in turn, mediate the association between genotype and antisocial behavior. Given well-established sex differences in amygdala reactivity (Cahill, 2006), antisocial behavior (Archer, 2004) and *OXTR* expression (Lucht et al., 2009), as well as previous studies that have linked *OXTR* genotypes to antisocial behavior specifically among male samples (Malik et al., 2012; Dadds et al., 2014), we examined the moderating role of sex on observed associations. In particular, we hypothesized a sex-specific effect, such that *OXTR* genotype would be related to threat-related amygdala reactivity and, in turn to higher antisocial behavior, among men but not women.

Materials and methods

Participants

A total of 406 non-Hispanic Caucasian participants (213 women; age range 18–22 years) who completed the DNS as of September 2014 were included in analyses (see Supplementary Table S1 for demographic information). Participants provided informed written consent prior to participation in accord with the guidelines of the Duke University Medical Center Institutional Review Board. All participants were in good general physical health and free of DNS exclusion criteria, which include (i) medical diagnosis of cancer, stroke, diabetes requiring insulin treatment, chronic kidney or liver disease or lifetime psychotic symptoms; (ii) use of psychotropic, glucocorticoid or hypolipidemic medication and (iii) conditions affecting cerebral blood flow and metabolism (e.g. hypertension). Past or current DSM-IV Axis I and select Axis II disorders were assessed with the electronic Mini International Neuropsychiatric Interview (Sheehan et al., 1997) and Structured Clinical Interview for the DSM-IV Axis II (First et al., 1997). However, these disorders are not exclusionary as the DNS seeks to establish broad variability in multiple behavioral phenotypes related to psychopathology.

Self-report data were available in all 406 participants with overlapping amygdala reactivity data available for 377 participants. Participants were excluded from analyses because of scanner-related artifacts in fMRI data ($n=2$), incidental structural brain abnormalities ($n=3$), a large number of movement outliers in fMRI data ($n=7$), inadequate signal in our amygdala regions of interest (ROIs, $n=7$; <90% signal coverage in the bilateral AAL amygdala region of interest) and poor behavioral performance ($n=10$; accuracy lower than 75%). We had genetic data available for 325 participants, of which only two participants failed genotyping for at least one *OXTR* single-nucleotide polymorphisms (SNPs) (i.e. without a proxy of $r^2 > 0.90$). Overlapping genetic and amygdala reactivity data were available for 312 participants.

Behavioral measures

[H3]Antisocial behavior. Antisocial behavior was measured via self-report using an adapted version of the Self-Reported Antisocial Behavior Scale (Loeber et al., 1989). The Self-Reported Antisocial Behavior Scale assesses engagement in antisocial acts during the past year (0 = no; yes = 1) across six domains: vandalism (three items; e.g. 'Have you on purpose broken or damaged or destroyed things that did not belong to you?'), theft/robbery, (12 items; e.g. 'have you snatched someone's purse or wallet or picked someone's pocket?'), cheating/conning (three items; e.g. 'Have you tried to cheat someone by selling them something that was worthless or not what you said it was?'), physical violence (eight items; e.g. 'Have you attacked someone with a weapon or with the idea of seriously hurting or killing them?'), substance use (eight items; e.g. 'Have you tried methamphetamine or speed?') and contact with law enforcement (three items, e.g. 'Have you been placed in a police car or brought to the police station?'). To ensure robust measurement of antisocial behavior in our relatively low-risk community sample and avoid categorical data with very sparse endorsement, we created 'parcels' that were computed as mean scores across items within each of the six antisocial behavior domains (Supplementary Table S2). Each parcel was used as an indicator for a latent antisocial behavior factor using confirmatory factor analysis in Mplus v.7.0 (Muthén and Muthén, 2014) (Supplementary Figure S1). These types of 'variety' scored antisocial behavior factors are highly correlated with frequency scores and are more statistically stable (Bendixen et al., 2003). Moreover, use of a 'parceling' approach helps minimize potentially skewed item-specific variance, which then account for less overall variance in the newly created composite. In this way, variance in the true score (i.e. underlying 'antisocial behavior' construct) is increased (Little et al., 2002).

[H3]Callous-unemotional traits. Because research highlights the importance of stratifying antisocial behavior according to the presence or absence of callousness and lack of empathy, especially in relation to amygdala reactivity (Viding et al., 2012; Hyde et al., 2014), we also assessed callous unemotional (CU) traits via self-report using five of the six items (e.g. 'feel bad or guilty when you do something wrong') of the CU traits subscale of the Antisocial Process Screening Device (Frick and Hare, 2001), which have most consistently been shown to load together in prior studies examining the factor structure of the scale (i.e. excluding item 5, 'You hide your feelings or emotions from others'; see Frick and White, 2008 for a review). These five items were combined into a latent factor score using confirmatory factor analysis modeling in Mplus v.7.0 (Muthén and Muthén, 2014) that specified separate antisocial behavior vs CU traits factors (Supplementary Figure S1). A chi-square difference test demonstrated that a two correlated factor model (i.e. separate antisocial behavior vs CU traits factors) fits the data significantly better than a one factor model with all indicators loading onto a single factor ($\Delta\chi^2(df=1) = 29.41, P < 0.001$; overall model fit, Comparative Fit Index (CFI) = 0.95, Tucker-Lewis Index (TLI) = 0.94, root mean square error of approximation (RMSEA) = 0.04; see Supplementary Figure S1). The model and the significant chi-square difference test suggest that the items assessing antisocial behavior and CU traits index significantly separable, albeit related, constructs.

Genotyping

DNA was isolated from saliva derived from Oragene DNA self-collection kits (DNA Genotek) customized for 23andMe (www.23andme.com). DNA extraction and genotyping were performed

by the National Genetics Institute, a CLIA-certified clinical laboratory and subsidiary of Laboratory Corporation of America. Custom Illumina arrays were used to provide genome-wide data. Note that a genome-wide chip was used for reasons of cost effectiveness, the ability to examine ancestry markers and to facilitate potential future exploratory analyses but not for traditional genome-wide association purposes. We directly assessed the following OXTR genotypes rs1042778, rs53576 and rs2254298. On the basis of prior work (Inoue et al., 2010; Furman et al., 2011; Malik et al., 2012; Dadds et al., 2014), we grouped genotypes as follows: rs1042778 T homozygotes ($n = 49$) vs G carriers ($n = 308$), rs2254298 G homozygotes ($n = 277$) vs T carriers ($n = 81$) and rs53576 G homozygotes ($n = 163$) vs A carriers ($n = 192$). All SNPs were in Hardy–Weinberg equilibrium (Table 1) and showed low linkage disequilibrium (rs1042778 and rs2254298: $r^2 = 0.0301$; $D' = 0.5991$; rs1042778 and rs53576: $r^2 = 0.0487$; $D' = 0.4007$; rs53576 and rs2254298: $r^2 = 0.0101$; $D' = 0.3949$).

Neuroimaging

[H3]Paradigm. Our challenge paradigm has been used extensively to elicit robust amygdala reactivity across an array of experimental protocols and sample populations (Hariri et al., 2002, 2005). The paradigm consists of four task blocks requiring face-matching interleaved with five control blocks requiring shape matching (Ahs et al., 2013). In each face-matching trial within a block, participants view a trio of facial expressions derived from a standard set of facial affect pictures (expressing angry, fearful, surprised or neutral emotions) (Ekman and Friesen, 1976) and select which of two facial expressions presented on the bottom row of the display matches the target stimulus presented on the top row. Each emotion-specific block (e.g. fearful facial expressions only) consists of six individual trials, balanced for sex of the face. Block order is pseudo-randomized across participants. Each of the six facial expression trios is presented for 4 s with a variable inter-stimulus interval of 2–6 s; total block length is 48 s. In the shape-matching control blocks, participants view a trio of geometric shapes (i.e. circles, horizontal and vertical ellipses) and select which of two shapes displayed on the bottom matches the target shape presented on top. Each control block consists of six different shape trios presented for 4 s with a fixed inter-stimulus interval of 2 s, comprising a total block length of 36 s. The total paradigm is 390 s in duration. Reaction times and accuracy are recorded through an MR-compatible button box.

[H3]BOLD fMRI acquisition. Participants were scanned using one of two identical research-dedicated GE MR750 3T scanner equipped with high-power high-duty-cycle 50-mT/m gradients at 200 T/m/s slew rate and an eight-channel head coil for parallel imaging at high bandwidth up to 1 MHz at the Duke-UNC Brain Imaging and Analysis Center. A semi-automated high-order shimming program was used to ensure global field homogeneity. A series of 34 interleaved axial functional slices aligned with the anterior commissure–posterior commissure plane were acquired for full-brain coverage using an inverse-spiral pulse sequence to reduce susceptibility artifact [TR/TE/flip angle = 2000 ms/30 ms/

60; FOV = 240 mm; $3.75 \times 3.75 \times 4$ mm voxels (selected to provide whole brain coverage while maintaining adequate signal-to-noise and optimizing acquisition times); interslice skip = 0]. Four initial radio-frequency excitations were performed (and discarded) to achieve steady-state equilibrium. To allow for spatial registration of each participant's data to a standard coordinate system, high-resolution three-dimensional structural images were acquired in 34 axial slices co-planar with the functional scans (TR/TE/flip angle = 7.7 s/3.0 ms/12; voxel size = $0.9 \times 0.9 \times 4$ mm; FOV = 240 mm, interslice skip = 0).

[H3]BOLD fMRI data analysis. The general linear model of Statistical Parametric Mapping 8 (<http://www.fil.ion.ucl.ac.uk/spm>) was used for whole-brain image analysis. Individual subject data were realigned to the first volume in the time series to correct for head motion before being spatially normalized into the standard stereotaxic space of the Montreal Neurological Institute template using a 12-parameter affine model. Data were smoothed to minimize noise and residual differences in individual anatomy with a 6 mm full width at half maximum Gaussian filter. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. The ARTifact Detection Tool (Whitefield-Gabrieli, 2009) was used to generate regressors accounting for images due to large motion (i.e. > 2 mm relative to the previous time frame) or spikes (i.e. global mean intensity 4 s.d. from the entire time series).

Following preprocessing steps outlined above, linear contrasts employing canonical hemodynamic response functions were used to estimate expression-specific (e.g. 'Angry > Neutral') BOLD contrast images for each individual. We focused on 'Angry > Neutral' and 'Fearful > Neutral' contrasts for two reasons: First, to assay amygdala reactivity to cues that are conditioned social signals to threat in the environment (i.e. angry and fearful expressions) relative to signals that do not convey threat information about the environment (i.e. neutral expressions). Second, to be consistent with past research using similar tasks to examine antisocial behavior (e.g. Viding et al., 2012), which have focused on these expressions of threat (angry and fearful) contrasted with neutral facial expressions.

Individual contrast images were then used in second-level random-effects models to determine mean expression-specific amygdala reactivity using one-sample t-tests with a voxel-level statistical threshold of $P < 0.05$, FWE corrected for multiple comparisons within anatomically defined bilateral amygdala ROIs derived from the Montreal Neurological Institute-based automatic anatomic labeling atlas (Roosendaal et al., 2004) in the WFU PickAtlas v.2.4 (Maldjian et al., 2003).

Statistical analysis

To facilitate analyses, BOLD parameter estimates from amygdala clusters exhibiting main effects of task were extracted using the VOI tool in Statistical Parametric Mapping 8 and exported for analyses in Mplus 7.2 (Muthén and Muthén, 2014) and SPSS vs 22. Extracting parameter estimates from clusters activated by our fMRI paradigm, rather than those specifically

Table 1. OXTR SNPs used in this study

SNP	Genotype	mAF	Frequency	n	Total	P-HWE
OXTR rs1042778	GG/GT/TT	.37	.40/.47/.14	141/167/49	357	.97
OXTR rs2254298	GG/GA/AA	.12	.78/.21/.01	277/80/2	359	.14
OXTR rs53576	GG/GA/AA	.32	.46/.44/.10	163/157/35	355	.75

Note. mAF, minor allelic frequency; P-HWE, P value of Hardy–Weinberg equilibrium test.

correlated with our independent variables of interest following whole-brain analysis, precludes the possibility of any correlation coefficient inflation that may result when an explanatory covariate is used to select a region of interest. We have successfully used this more conservative analytic strategy in recent studies (Hyde et al., 2011; Carré et al., 2012), particularly when examining mediated pathways. No significant main effects for the 'Fearful > Neutral' contrast emerged. However, to examine specificity of effects for the 'Angry > Neutral' contrast, we modeled gene to brain and brain to behavior associations for all other contrasts that showed significant main effects to our task ('Fear > Shapes', 'Angry > Shapes', 'Neutral > Shapes' and 'Surprise > Shapes'). Consistent with other publications using this sample, to maintain variability but constrain the influence of extreme outliers, all imaging variables were winsorized in SPSS v. 22 prior to analyses (i.e. following data quality control procedures, five outliers more than ± 3 s.d. were set at ± 3 s.d. from the mean) (Nelson et al., 2015).

First, to test the effect of OXTR genotype on bilateral 'Angry > Neutral' reactivity, we modeled paths between all three OXTR SNPs to this amygdala contrast in Mplus in one model, accounting for the covariance between the three OXTR SNPs and between left and right amygdala to control for multiple comparisons (see Supplementary Figure S2). To test the specificity of the association between rs1042778 genotype on bilateral amygdala reactivity for the 'Angry > Neutral' contrast, we also modeled pathways to all other significant amygdala contrasts within a single multivariate model that controlled for covariance between contrasts across left and right amygdala to account for multiple comparisons (see Supplementary Figure S3). Second, to test for links between brain and behavior, we modeled pathways between amygdala reactivity, antisocial behavior and CU traits and examined potential moderating effects of sex (in Mplus 7.2; Muthén and Muthén, 2014). As before, we tested multivariate models that allowed us to account for overlap between antisocial behavior and CU traits, as well as between the different amygdala contrasts. Third, to test whether the link between OXTR genotype and antisocial behavior was mediated by amygdala reactivity, we used path modeling and specified an indirect effect between genotype and antisocial behavior via amygdala reactivity. To test whether the indirect pathway differed for men vs women, we examined a moderated mediation model (i.e. whether sex moderated the pathway from OXTR genotype to amygdala reactivity or from amygdala reactivity to antisocial behavior). Mplus v.7.0 provides two complementary methods of testing indirect effects: (i) a product coefficient test (also called the 'Sobel method') (MacKinnon et al., 2002) to quantify the magnitude of the indirect effect and (ii) unbiased confidence intervals using bootstrapping methods (i.e. 1000 draws of a Monte Carlo simulation), which do not assume normality of the distribution of indirect effects and thus represent more powerful tests of indirect, mediated pathways (Dearing and Hamilton, 2006).

To account for missing data, we used full information maximum likelihood (FIML) estimation in Mplus. Thus, we were able to include all participants in analyses, whether or not they were missing genetic ($n = 81$) or neuroimaging ($n = 29$) data. An FIML approach accommodates missing data and provides less biased estimates than listwise or pairwise deletion (Schafer and Graham, 2002). In one exception, the analysis examining the effect of OXTR genotype on amygdala reactivity only included 390 of the 406 participants because 16 participants were missing both genetic and imaging data. Note that the pattern of findings was broadly similar in all models when listwise deletion was used (results not presented for brevity). All analyses included

the following covariates of no-interest as relevant: any past or present DSM-IV disorder, sex and age. Controlling for the presence of DSM-IV disorder diagnosis in our analyses did not change the pattern of findings. Specifically, of the 406 Caucasian non-Hispanic participants included in this study, 79 met criteria for at least one psychiatric diagnosis. However, the majority of these diagnoses were for alcohol dependence or abuse ($n = 55$) or illicit drug dependence or abuse ($n = 14$). Given that alcohol and drug use overlapped with one domain within the antisocial behavior measure, inclusion of psychiatric diagnosis as a covariate is a conservative approach, suggesting that the effects found were specific to a narrower antisocial behavior phenotype.

Results

OXTR genotype and amygdala reactivity

Consistent with prior sex-specific effects, analyses revealed a trend-level significant pathway between OXTR rs1042778 and right amygdala reactivity for 'Angry > Neutral' among men ($B = 0.17$, standard error (SE) = 0.09, $\beta = 0.13$, $P = 0.057$) but not women ($B = 0.06$, SE = 0.11, $\beta = 0.05$, $P > 0.61$) when we stringently accounted for the overlap of all three OXTR SNPs and the correlation between right and left amygdala reactivity. Neither of the other OXTR SNPs examined (i.e. OXTR rs2254298 and rs53576) were associated with amygdala reactivity for 'Angry > Neutral', neither across all participants nor within sex. Thus, only the OXTR rs1042778 genotype was included in subsequent analyses.

To check for specificity of the effects for the 'Angry > Neutral' contrast, we examined the effect of OXTR rs1042778 on all amygdala contrasts that showed significant main effects to our task (i.e. 'Angry > Neutral', 'Fear > Shapes', 'Angry > Shapes', 'Neutral > Shapes' and 'Surprise > Shapes') within a multivariate model that accounted for overlap between regions to control for multiple comparisons. Controlling for overlap with the activation to other facial expressions of emotion, the association between OXTR rs1042778 on right amygdala reactivity for 'Angry > Neutral' was significant in men ($B = 0.21$ SE = 0.10, $\beta = 0.15$, $P = 0.04$) but not women ($B = 0.05$, SE = 0.10, $\beta = 0.05$, $P > 0.60$) (Supplementary Table S3). A one-way analysis of variance test carried out in SPSS (i.e. listwise deletion) confirmed this same significant association between OXTR rs1042778 genotype on right amygdala reactivity to facial expressions specifically among men, with T homozygotes showing significantly higher right amygdala reactivity compared to G carriers (Figure 1).

Amygdala reactivity and behavior

We next determined whether amygdala reactivity was related to antisocial behavior and CU traits. Given previous studies showing that the direction of associations with amygdala reactivity appears to be in opposite directions for antisocial behavior vs CU traits, we examined path models while controlling for the overlap of antisocial behavior and CU traits as in previous studies (Hyde et al., 2014). We examined links between antisocial behavior and CU traits with our contrast of interest (i.e. 'Angry > Neutral'), as well as with all other amygdala contrasts that showed significant main effects to our task ('Angry > Neutral', 'Fear > Shapes', 'Angry > Shapes', 'Neutral > Shapes' and 'Surprise > Shapes') in a multivariate framework. We controlled for sex, age and psychiatric diagnosis (see Supplementary Table S3). For our primary contrast of interest, we found that sex moderated associations between amygdala reactivity to 'Angry > Neutral' and antisocial behavior, and between amygdala reactivity and CU traits (Model

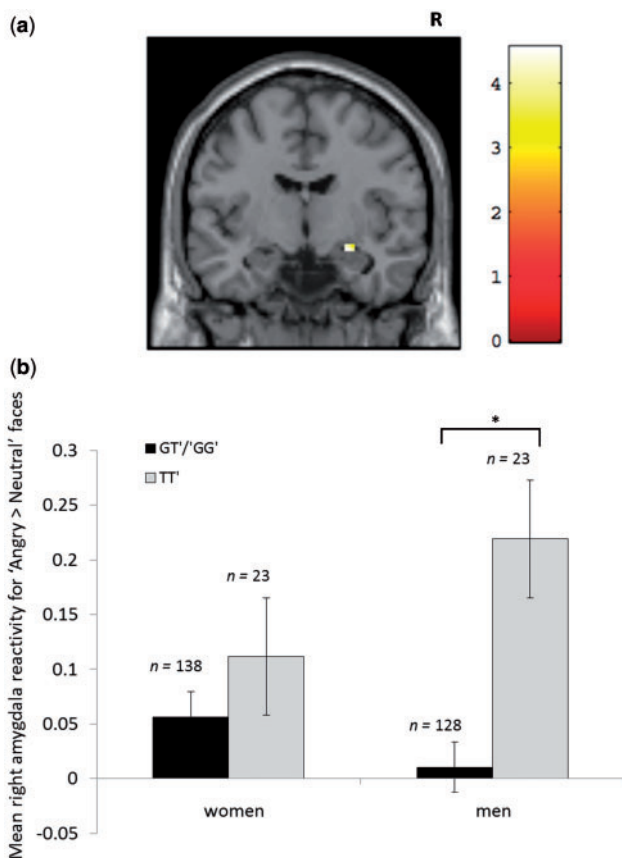


Fig. 1. Effect of OXTR rs1042778 genotype on right amygdala reactivity to 'Angry > Neutral' facial expressions among women and men. * $P < 0.05$. (a) Statistical parametric map illustrating mean right amygdala reactivity to 'Angry > Neutral' facial expressions: $[x\ y\ z]$ [24-8-14], $t = 2.89$, $P = 0.01$, $k_E = 42$. Activation clusters are overlaid onto canonical structural brain images and the color bar represents t -statistics. (b) Consistent with the multivariate analysis run in Mplus that controlled for all three SNPs tested (Supplementary Table S3), one-way analysis of variance in SPSS vs 22 using $n = 312$ participants with overlapping genetic and imaging data showed that OXTR rs1042778 TT genotype showed a trend-level association with higher right amygdala reactivity to 'Angry > Neutral' facial expressions ($F(1,310) = 3.55$, $P = 0.06$) when examining the whole sample. When separated by sex, we found that this effect was evident only among men, where T homozygotes showed significantly higher right amygdala reactivity compared to G carriers ($F(1,150) = 4.04$, $P = 0.046$), whereas women who were G carriers did not differ significantly from women who were T homozygotes.

2, Supplementary Table S3). Accordingly, we examined associations with amygdala reactivity separately for men and women. We found that among men, antisocial behavior was significantly related to greater bilateral amygdala reactivity for 'Angry > Neutral', (left, $B = 3.82$, $SE = 1.22$, $\beta = 0.47$, $P < 0.01$; right, $B = 1.09$, $SE = 0.43$, $\beta = 0.33$, $P < 0.05$), whereas there were no significant associations between antisocial behavior and amygdala reactivity among women (Model 3, Supplementary Table S3). In contrast, CU traits were associated with reduced bilateral amygdala reactivity to 'Angry > Neutral' among men, highlighting the divergent associations that these related constructs show with threat-related amygdala functioning (Hyde et al., 2014) (see Supplementary Table S3).

Indirect prediction of behavior by OXTR rs1042778 genotype via amygdala reactivity

In a final analysis, we examined whether OXTR rs1042778-driven variability in amygdala reactivity to 'Angry > Neutral'

was indirectly related to antisocial behavior. Given the sex differences noted for associations between OXTR rs1042778 genotype and amygdala reactivity, as well as between amygdala reactivity and antisocial behavior, we tested moderation of the indirect effect using moderated mediation analysis (i.e. whether sex moderated the links from OXTR rs1042778 genotype to amygdala reactivity or from amygdala reactivity to antisocial behavior). Consistent with past studies examining this type of mediated/indirect pathway (Fakra et al., 2009), analyses revealed no significant direct path between OXTR rs1042778 genotype and antisocial behavior in the model and this path was dropped. Moderated mediation analyses examining associations across the whole sample revealed that sex significantly moderated pathways between OXTR rs1042778 genotype, amygdala reactivity to 'Angry > Neutral' and antisocial behavior ($\Delta\chi^2(df = 6) = 21.49$, $P = 0.001$; Figure 2). In men, this model revealed a significant direct path from OXTR rs1042778 genotype to relatively increased right amygdala reactivity ($B = 0.20$, $SE = 0.09$, $P = 0.03$) and from right amygdala reactivity to increased antisocial behavior ($B = 0.03$, $SE = 0.01$, $P = 0.007$). Moreover, in men, the indirect path from OXTR rs1042778 genotype to antisocial behavior through amygdala reactivity was marginally significant ($B = 0.004$, $SE = 0.003$, $P = 0.09$; 90% confidence intervals = 0.00–0.01; Figure 2). In contrast, among women, we found no significant association between OXTR rs1042778 genotype and amygdala reactivity to 'Angry > Neutral' ($B = 0.06$, $SE = 0.11$, $P > 0.60$) or between amygdala reactivity and antisocial behavior ($B = -0.003$, $SE = 0.01$, $P > 0.50$).

Discussion

We found that OXTR rs1042778 T allele homozygotes had greater right amygdala reactivity to angry facial expressions, which in turn predicted higher antisocial behavior in men but not women. The sex-specific behavioral expression of variable amygdala reactivity is consistent with previous studies that have reported heightened amygdala reactivity to angry facial expressions among men with impulsive traits (Carré et al., 2012) or with intermittent explosive disorder (Coccaro et al., 2007). Consistent with these prior findings, and even in our relatively low-risk cohort of young adult university students, antisocial behavior among men was underpinned by amygdala hyperreactivity to explicit interpersonal threat as represented by angry facial expressions (Davis and Whalen, 2001). In contrast, CU traits were related to relatively decreased amygdala reactivity to angry facial expressions among men in our sample, which is consistent with the literature suggesting amygdala hyporeactivity to threat in those high on CU traits (Hyde et al., 2014). However, previous studies have reported this negative relationship between CU traits and amygdala reactivity to fearful facial expressions (Marsh et al., 2008; Viding et al., 2012), which we did not find in our sample. It is noteworthy that these prior studies were carried out among youth making it harder to compare the results directly. Additionally, among women, we found that CU traits were related to relatively increased amygdala reactivity to angry facial expressions. One explanation for this finding centers on our relatively high functioning sample, which contrasts with the forensic (Marsh et al., 2008) and clinic-referred (Viding et al., 2012) samples of previous studies. Thus, in the current sample, we may not have been able to extract 'unique' aspects of the CU traits construct, which could have simply indexed more severe antisocial behavior among women. Nevertheless, the divergent pattern of associations we found between amygdala reactivity and antisocial behavior vs CU traits among men,

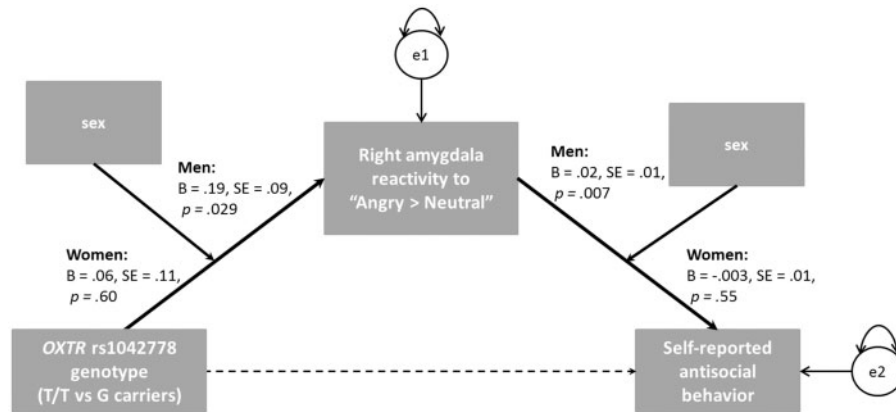


Fig. 2. Moderated mediation model testing sex-specific effects of OXTR rs1042778 genotype on antisocial behavior mediated by right amygdala reactivity to angry facial expressions. Note. Analyses conducted on full sample ($N = 406$) using FIML to account for any missing data. Overall model fit was good: $\chi^2(df = 14) = 16.75; P = 0.04; CFI = 0.99; TLI = 0.99; RMSEA = 0.03; confidence intervals: 0.00-0.08; SRMR = 0.045$. Moderated mediation analysis revealed that amygdala reactivity mediated the relationship between OXTR rs1042778 genotype and higher antisocial behavior significantly differently among men and women ($\Delta\chi^2(df = 6) = 21.49, P = 0.001$). Among men, OXTR rs1042778 T homozygotes had significantly higher right amygdala reactivity to 'Angry > Neutral' facial expressions, which in turn showed trend-level indirect prediction of higher antisocial behavior ($B = 0.004, SE = 0.003, P = 0.09; 90\%$ confidence intervals = 0.00-0.01). Numbers represent unstandardized parameter estimates (B) and bootstrapped standard errors (SE). The circles labelled e1 and e2 represent variance in amygdala reactivity and antisocial behavior unaccounted for by the model. The following covariates were included in the model: age, callous unemotional traits and psychiatric diagnosis. Antisocial behavior and CU traits were moderately related among men ($B = 0.54, SE = 0.12, P < 0.001$) and women ($B = 0.39, SE = 0.09, P < 0.001$) (Supplementary Figure S1). Among men, psychiatric diagnosis (majority were alcohol or illicit drug misuse diagnoses) was related to higher antisocial behavior ($B = 0.03, SE = 0.01, P = 0.01$).

despite the moderate overlap of these behavioral phenotypes, provides support for their potentially unique neural underpinnings and etiology (also see Hyde et al., 2014). Finally, it should be noted that there was no main effect of a task contrast comparing amygdala reactivity for 'angry vs fearful' facial expressions. Thus it was not possible to fully test the specificity of our results to angry facial expressions. Future studies are needed to investigate the differential effects of OXTR genotype on angry vs fearful facial expressions, given the differing nature of their implied threat (Davis et al., 2011).

Although we found no significant effect of OXTR genotype on amygdala reactivity across the whole sample, the sex-specific nature of the findings is consistent with the established role of gonadal steroids in the early morphogenesis of the OXTR system (Choleris et al., 2003; de Vries, 2008; Insel, 2010). In particular, estradiol levels differentially increase OXTR responsiveness among women (Champagne et al., 2001). Not surprisingly, previous studies have found sex differences in the influence of intranasally administered OXT on neural reactivity and behavior. For example, OXT administration was associated with decreased amygdala reactivity to fear-inducing stimuli among men (Kirsch et al., 2005; Domes et al., 2007) but increased reactivity women (Domes et al., 2010; Lischke et al., 2012). Considered alongside our findings, genetically mediated variability in OXTR functioning appears to modulate amygdala reactivity to threat cues differently among men and women.

The molecular mechanisms through which common OXTR polymorphisms affect amygdala reactivity are unclear. Nevertheless, we hypothesize that increased right amygdala reactivity in rs1042778 T allele homozygotes may reflect reduced OXT signaling or reduced peripheral OXT activity (Malik et al., 2014). OXTR rs1042778 is located in the 3'-untranslated region of exon 4 in the OXTR gene, which encodes a 389 aa polypeptide with seven transmembrane domains of the class I G-protein-coupled receptor family (Gimpl and Fahrenholz, 2001). Prior work has reported lower plasma OXT in T allele carriers of OXTR rs1042778, suggesting that this genotype may influence regulation of OXT signaling pathways and/or peripheral OXT activity (Feldman et al., 2012). Electrophysiological studies suggest that activation of OXT

receptors influences neuronal excitability by closing inward rectifying potassium channels and activating sodium currents (e.g. Gravati et al., 2010). Via both mechanisms, OXT has both excitatory and inhibitory effects on synaptic transmission (Raggenbass, 2001), which could underpin differential effects of stimulus type on amygdala reactivity, including varying effects reported due to context (Bartz et al., 2011) or emotional valence of stimuli (Kemp and Guastella, 2011). Future studies are needed to examine plasma OXT as a mediator between OXTR genotype and amygdala reactivity or to examine whether OXTR genotype moderates the influence of OXT administration on amygdala reactivity.

Despite the strengths of this study, including the use of a well-established task for eliciting amygdala reactivity, a relatively large sample size, a sophisticated quantitative approach and the first examination of an indirect gene-brain-behavior pathway between OXTR genotype, amygdala reactivity and antisocial behavior, there are limitations worth noting. First, because the functionality of OXTR rs1042778 is unknown, our results do not rule out the possibility that the associations found reflect other genetic variants in linkage disequilibrium with the OXTR SNPs that were the focus of this study. Second, we examined associations in a high-functioning university sample, which contains a very small number of individuals meeting criteria for clinically diagnosable antisocial behavior (i.e. Antisocial Personality Disorder), which reduces the generalizability of our data to psychiatric/forensic populations. However, studies in large, healthy samples, such as ours, are essential to demonstrate that associations are present dimensionally across a full range of functioning. Third, our results are based on a total of 24 s of exposure to angry facial expressions across six blocks, which is somewhat less time than other recent studies in this area (e.g. Viding et al., 2012), and may have resulted in a more conservative estimation of effect sizes for associations between OXTR genotype and amygdala reactivity and subsequent pathways to antisocial behavior. Use of whole brain analysis, rather than extracted fMRI data from a specific ROI is an important future step to identify other brain regions that may be related to variation in OXTR genotype (see Supplementary Table S5). Fourth, we did not replicate the findings of Tost et al., who

reported lower amygdala reactivity to emotional facial expressions for OXTR rs53576 A carriers among men, but not women, whereas we found no significant effect of OXTR rs53576 genotype on amygdala reactivity among men or women. An explanation for this lack of replication centers on differences in the fMRI contrasts of interest. Specifically, we analyzed amygdala reactivity to angry vs neutral facial expressions, whereas Tost et al. identified greater reactivity during the processing of both angry and fearful facial expressions contrasted with a control sensorimotor task. Thus, the divergent observations between our study and that of Tost et al. may reflect differential OXTR genotype modulation of amygdala reactivity to broadly salient social stimuli (i.e. facial expression stimuli > non-facial expression stimuli) vs specific reactivity to interpersonal threat (i.e. angry > neutral expressions). Alternatively, it is noteworthy that Tost et al. assessed a sample comprising siblings of patients with a history of schizophrenia. Although these siblings were screened by a psychiatrist to ensure they were free of any lifetime history of psychiatric illness, they had been shown to exhibit small differences in a test of attention relative to healthy controls (Egan et al., 2000). Thus, there may be important neurobiological differences between our sample and that of Tost et al., which could have contributed to divergent effects of OXTR genotype.

Finally, although we found complex sex-specific patterns of association, a larger sample may have revealed more robust sex-dependent associations, particularly for the indirect pathway from OXTR rs1042778 genotype to antisocial behavior via amygdala reactivity among men. Moderated mediation may require samples as large as 500–1000 for sufficient power to detect pathways (Preacher et al., 2007), emphasizing that even among our relatively large sample, we may still have been unable to find these complex pathways with sufficient power. Moreover, while our sample size was large for an imaging genetics study, it is relatively small in relation to population-based studies in behavioral genetics, and several pathways showed only trend-level significance when we accounted for multiple comparisons. Thus, it is critical that our observations are replicated in independent samples, as well as samples with greater variation in racial/ethnicity group composition, before their true inferential value can be established for understanding the biological pathways through which variability in antisocial behavior may emerge. Nevertheless, an improved understanding of the effects of risk genes in non-clinical samples is of utmost importance for parsing neurobiological heterogeneity in complex phenotypes, such as antisocial behavior and overlapping callous and psychopathic traits (Hyde et al., 2014). Our findings demonstrate that a multimodal imaging genetics approach can provide empirical documentation for the basic premise that genetic variation indirectly influences behavioral outcomes by biasing the response of underlying neural circuitries (Hariri et al., 2006).

Supplementary data

Supplementary data are available at SCAN online.

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