A common allele in the oxytocin receptor gene (*OXTR*) impacts prosocial temperament and human hypothalamic-limbic structure and function

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The evolutionarily highly conserved neuropeptide oxytocin is a key mediator of social and emotional behavior in mammals, including humans. A common variant (rs53576) in the oxytocin receptor gene (*OXTR*) has been implicated in social-behavioral phenotypes, such as maternal sensitivity and empathy, and with neuropsychiatric disorders associated with social impairment, but the intermediate neural mechanisms are unknown. Here, we used multimodal neuroimaging in a large sample of healthy human subjects to identify structural and functional alterations in *OXTR* risk allele carriers and their link to temperament. Activation and interregional coupling of the amygdala during the processing of emotionally salient social cues was significantly affected by genotype. In addition, evidence for structural alterations in key oxytocinergic regions emerged, particularly in the hypothalamus. These neural characteristics predicted lower levels of reward dependence, specifically in male risk allele carriers. Our findings identify sex-dependent mechanisms impacting the structure and function of hypothalamic-limbic circuits that are of potential clinical and translational significance.

imaging genetics | neuroimaging | social behavior | prosocial neuropeptides | autism

In vertebrate species, oxytocin (OT) is a potent modulator of social and reproductive behaviors on multiple biological levels (1). Emerging from the paraventricular and supraoptic nuclei of the hypothalamus, OT regulates peripheral processes related to parturition and lactation as a pituitary hormone. Centrally released, OT facilitates offspring survival by initiating mother-infant bonding and the onset of maternal behavior (2). Studies in lower mammals have further revealed that OT is critical for the molding of complex behaviors related to the recognition of conspecifics, mating, the formation of partner preferences (3), and the emotional and somatic expression of fear and anxiety (4–6). The neural architecture of the oxytocinergic system is evolutionarily conserved and targets, among others, brain areas critical for emotion regulation (e.g., amygdala, lateral septum, and brainstem) (1, 7). In contrast, the regional expression of oxytocin receptors is highly variable and explains differences in social attachment within and between species (3).

Although invasive animal work suggested a role for OT in the human brain, this claim could not be subjected to direct experimental examination (8) until nasally administered neuropeptides were shown to impact the central nervous system (9). Subsequent pharmacological studies have provided behavioral evidence that OT improves memory for facial identity (10), enhances the ability to infer the mental state of others (11), and increases interpersonal trust (8) and generosity (12). Neuroimaging studies have implicated neural mechanisms driving the prosocial impact of OT in the human brain (13), and have highlighted activation decreases in areas involved in emotion processing and social danger monitoring, especially the amygdala (14, 15). Given the known heritability of social behavior in humans (16, 17), these data further suggest that genetic variation in the oxytocinergic system may modulate sociality (18) and confer risk for social dysfunction. Consequently, the association of common variants in the oxytocin receptor gene (*OXTR*) has been examined in the context of altruism (18), empathy (19), and risk for autism spectrum disorder (ASD) (20–23).

The human *OXTR* gene is located on chromosome 3p25, spans 17 kb, contains four exons and three introns (24), and encodes a 389-aa polypeptide with seven transmembrane domains belonging to the class I G-protein-coupled receptor family (25). A single nucleotide polymorphism (SNP) of unknown functionality in the third intron of *OXTR* has emerged as a particularly interesting candidate in the recent literature, rs53576 (G/A). Prior evidence suggests that rs53576A promotes deficits in sociobehavioral domains such as maternal sensitivity (26), empathy (19), attachment (27), and positive affect (28). Interest in the psychiatric relevance of this genetic variant was furthered by studies indicating that rs53576A is overtransmitted in some families to offspring with ASD (23, but see also ref. 21), and may form a central component in haplotypes related to high-functioning autism (29).

Despite this accumulating evidence in support of a relationship between *OXTR* rs53576 and human sociality, the intermediate neural mechanisms remain unknown (30). To address this issue, we pursued a stepwise multimodal neuroimaging genetics approach to identify neural alterations in healthy *OXTR* risk allele carriers. Based on the neuroanatomy of the oxytocinergic system (2), and prior evidence linking these structures to the pathophysiology of social dysfunction (31, 32), we were particularly interested in the effect of *OXTR* genotype in the hypothalamus and amygdala. We also studied the interaction of these structures with the dorsal anterior cingulate gyrus (dACG), a regulatory area of prefrontal cortex, because we have previously found that the functional and structural coupling of this region is susceptible to neurogenetic influences on limbic processing (33) and is abnormal in genetically prosocial individuals (34).

First, we examined the impact of *OXTR* genotype on brain structure in 212 healthy subjects using voxel-based morphometry (VBM) (35). VBM is useful to detect regional alterations in gray matter (GM) volume with sufficient sensitivity to reveal psychiatric risk gene effects on the anatomy of the limbic system (33, 36). To study structural coupling between brain areas, we used

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structural covariance methods. Prior work has shown that this correlative measure mirrors the anatomical connectivity of brain regions (37, 38) and provides a powerful tool to map the impact of genotype effects on the structural architecture of neural networks (33, 39).

To relate these intermediate phenotypes to behavior, we examined the relationship between *OXTR* genotype and reward dependence in 309 subjects using the Tridimensional Personality Questionnaire (TPQ) (40) to quantify genotype effects on self-reported prosocial temperament. Via cross-correlation with other modalities, the ecological validity of our *OXTR*-related neuroimaging findings was examined.

Finally, to examine functional correlates of the observed structural alterations, we studied the blood oxygenation level-dependent (BOLD) response in fMRI data from 228 subjects during the processing of emotionally salient social stimuli [i.e., human facial expressions, face-matching task (FMT) (Fig. S1)]. This paradigm has been shown to elicit robust limbic activations, particularly in the amygdala, and is known to be sensitive to genotype effects on the activation and functional interaction of limbic areas (33, 41). Interregional coupling was studied using "functional connectivity," a correlative measure sensitive to genetic variation (33, 42).

Results

Regional Volume and Covariance of Gray Matter Structures. Based on the anatomy of the oxytocinergic system, and previous reports linking ASD to hypothalamic deficits (32) and alterations in amygdala volume (43, 44), we predicted that genetic variation in *OXTR* would impact GM volume in both structures. The outcome of our structural analysis provided evidence for a significant allele-load-dependent decrease in hypothalamus GM volume in rs53576 risk allele carriers [$t_{(208)} = 3.0$, $P_{FWE} = 0.012$; Table S1 and Fig. 1, all P values survive family-wise error correction (FWE), for multiple comparisons]. Notably, although there was no significant genotype by sex interaction in the hypothalamus, the observed effect was largely driven by males (Fig. S2*A*). In addition, we observed a significant genotype by sex interaction effect for rs53576, consistent with an allele-load-dependent increase in GM volume in male risk allele carriers in the right amygdala [$t_{(207)} = 2.9$, $P_{FWE} = 0.02$; Fig. S3]. Based on the known anatomical projections of the hypothalamus and higher-order limbic processing areas in the amygdala and the ACG, we also examined whether *OXTR* genotype impacts the structural coupling of GM structures in the limbic circuitry. Using the hypothalamus as a seed region, a significant allele-dependent increase in structural correlation with the dACG was shown for rs53576A [$t_{(206)} = 3.1$, $P_{FWE} = 0.049$; Table S1 and Fig. S4*A*]. In addition, an increase in the structural coupling of hypothalamus and amygdala was observed [$t_{(206)} = 2.6$, $P_{FWE} = 0.047$; Fig. S4*B*].

Temperamental Correlates. The TPQ (40) is a 100-item self-rating scale assessing four well-validated heritable temperamental traits. For the purposes of our study, we were particularly interested in the reward dependence (RD) subscale, an established measure that quantifies individual differences in human sociality in healthy subjects and psychiatric patient populations (45, 46). Subjects with a high RD score are characterized by heightened sociability, reliance on social approval, and enhanced learning from rewarding interpersonal feedback, whereas low-RD subjects display socially detached and cold interpersonal traits. Previous evidence has suggested a significant association between plasma OT levels and prosocial temperament (47). Based on this, we predicted that allelic variation in *OXTR* linked to a decrease in social behaviors such as empathy and attachment would be associated with lower prosocial temperament scores in healthy risk allele carriers.

With a Cronbach's alpha coefficient of 0.71, the RD subscale showed adequate internal consistency in our sample. The analysis of TPQ data confirmed a well-known main effect of gender on RD [$F_{(1,300)} = 22.1$, $P = 0.0001$; Fig. 2 *Inset*] and the expected distribution of prosocial temperament scores for rs53576 genotype groups (Fig. 2). Specifically, homozygotes for the risk allele showed the lowest RD values, whereas carriers and homozygotes of the G allele displayed intermediate and highest RD values, respectively [$F_{(2,300)} = 3.0$, $P = 0.05$]. Posthoc comparison of the two homozygote genotype groups (G/G vs. A/A) confirmed the presence of significantly lower RD scores in *OXTR* risk allele carriers [$t_{(167)} = 2.1$, $P = 0.02$]. Notably, though the exploratory plot of RD data in the gender-stratified subsamples (Fig. 2 *Inset*) suggested a more pronounced dose effect of rs53576A in male participants, the respective genotype by sex interaction analysis did not reach statistical significance ($P > 0.05$).

Relationship Between Temperament and Brain Structure. We reasoned that if the genotype-dependent differences in brain structure reported above reflect genetic risk for decreased sociality, then the derived neuroimaging measures should also predict individual differences in prosocial temperament. Therefore, we performed a correlation analysis between individual temperament ratings and local gray matter volumes in the hypothalamus and amygdala. Consistent with the amygdala gray matter increase in male carriers of *OXTR* rs53576A, we observed a significant negative correlation between local amygdala volume and individual RD scores in the total sample ($r = -0.190$, $P = 0.008$); the correlation did not reach statistical significance in the gender-stratified subsamples (males: $r = -0.084$, females: $r = -0.017$,

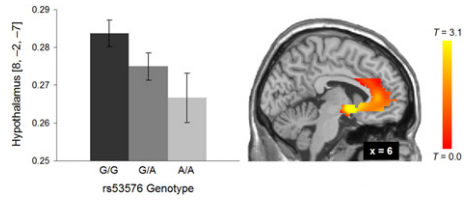

Fig. 1. Allelic variation in *OXTR* predicts differences in hypothalamus volume. The minor allele carriers of *OXTR* rs53576 show a significant decrease in hypothalamus gray matter ($P = 0.012$, FWE corrected). Bar plots depict the mean value for the parameter estimates of the peak voxels, stratified by genotype. Error bars illustrate the variance of parameter estimates (±SEM).

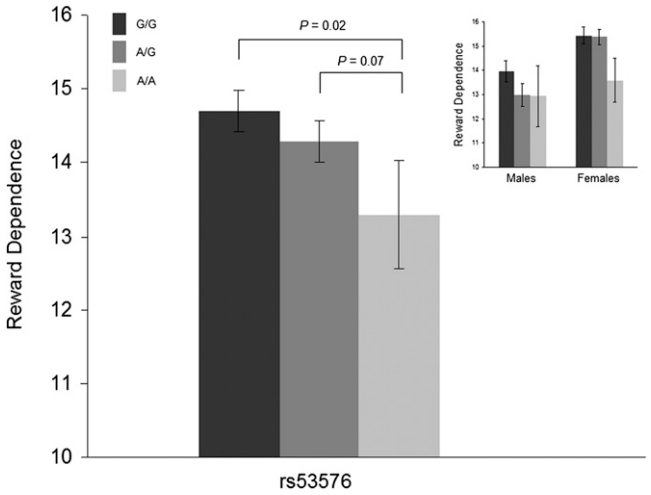

Fig. 2. Allelic variation in *OXTR* predicts differences in prosocial temperament. Distribution of TPQ reward dependence (RD) scores between rs53576 genotype groups. The comparison of the two homozygote groups (G/G vs. A/A) confirmed the presence of significantly lower RD values in *OXTR* risk allele carriers.

$P > 0.05$). Furthermore, we observed a significant sex by volume interaction effect in the hypothalamus [$F_{(1,191)} = 4.3, P = 0.039$]; specifically, decreased hypothalamus volumes predicted lower prosocial temperament scores in male ($r = 0.290, P = 0.005$; Fig. S2*B*) but not female ($r = –0.005, P = 0.96$; Fig. S2*C*) participants. This finding is consistent with the result described above, which showed an excessive decrease in hypothalamus GM in male risk allele carriers of the examined SNP (Fig. S2*A*).

Functional Correlates of *OXTR*. Based on the structural imaging results in our sample and prior findings in autism (e.g., refs. 48 and 49), as well as own results on the impact of other neuropeptidergic variants on temperament (30), we investigated the effect of rs53576 on amygdala activation and interregional coupling during the processing of emotionally salient social cues. The analysis of FMT data revealed a significant allele-load-dependent decrease in amygdala activation related to risk for social dysfunction [$t_{(225)} = 2.7$, $P_{FWE} = 0.036$; Table S1]. Specifically, subjects homozygous for the risk allele rs53576A showed the lowest task-related amygdala activations, and homozygotes for the G allele showed the highest (Fig. 3*A*). In addition, the analysis provided evidence for significantly increased coupling of hypothalamus and amygdala in carriers of rs53576A [$t_{(225)} = 2.7$, $P_{FWE} = 0.036$]. Again, the observed connectivity increase mirrored the presumed dose of *OXTR* risk alleles (Fig. 3*B*). No significant sex-by-genotype interaction effects were observed for either amygdala activation or hypothalamus-amygdala coupling ($P > 0.05$). Notably, in behavior, all subjects had a >94% average of correct responses with no significant differences between genotype groups for both the control and faces conditions of the FMT (all P values > 0.30; Table S2).

Discussion

Using a multimodal imaging intermediate phenotype approach, the present study shows that a common genetic variant in *OXTR* linked to social function predicts individual differences in brain structure, brain function, and personality in healthy humans. Further, we provided evidence for a sex-dependent impact of *OXTR* genotype on limbic structures relating to prosocial temperament. Together, our findings indicate a neural mechanism for genetically increased risk of social impairments predominantly in males that is of potential relevance for psychiatric disorders.

We observed a significant difference between *OXTR* rs53576 genotype groups using Cloninger's TPQ reward dependence scale, a well-validated trait measure relating to human empathy, social communication, and the need for interpersonal contact (50). As expected, carriers of the *OXTR* risk allele were characterized by a decreased level of sociality, a finding that is well in line with the presumed role of OT in healthy human attachment behavior and social cognition (8, 11). In good agreement with our present findings, prior data relates *OXTR* rs53576A to deficits in empathy (19), attachment (27) and sensitive parenting (26), a behavioral phenotype characterized by reduced maternal awareness of the needs and subtle emotional signals of children. Also, though the precise role of *OXTR* in the pathophysiology of mental disease remains to be clarified, the present data are consistent with prior data linking rs53576A to risk for (high-functional) autism (23, 29). The finding is further complemented by evidence that variants in the arginine vasopressin receptor 1A gene (*AVPR1A*), which encodes for the central brain receptor for the related neuropeptide vasopressin (51), have been associated with decreased RD scores (52). Because there is currently a broad interest in oxytocin as a pharmacological agent targeted to social behavior (8, 53, 54), these gene-temperament associations also have potential translational relevance and may become useful as a source of interindividual variation to oxytocinergic stimulation.

At the level of brain structure, we provided evidence that the genetic risk for social dysfunction, as related to *OXTR*, is reflected in morphometric alterations of the hypothalamus and amygdala. Specifically, our analyses suggested a significant allele-load-dependent decrease in GM volume in the oxytocinergic "core" of the brain, the hypothalamus, a finding that predicted reduced reward dependence in males. Though this outcome is well in line with the presumed role of OT function in human sociality, its pathophysiological significance remains to be elucidated, as evidence for a hypothalamus dysfunction in social disorders such as autism is hitherto limited to single case studies (32, 55) and indirect markers of a potential OT deficit (53, 54, 56, 57).

In addition, a genetic effect on the structural connectivity of the hypothalamus was indicated by our analysis, which showed a strong increase in the correlation of the GM volume of the hypothalamus and that of the dorsal ACG and amygdala in *OXTR* risk allele carriers. Though it is important to bear in mind that the examined measure reflects covariation of regional volumes across subjects and does not directly quantify white-matter projections, we (33, 39) and others (38) have previously reported structural correlations that correspond well with the known anatomical connectivity of brain regions. Notably, the hypothalamus receives direct afferent inputs from amygdala and ACG, as demonstrated by tracing studies (58, 59) and MRI tractography (60). Moreover, both of these pathways have been implicated in the generation of autonomic responses to social and emotional stimuli (61, 62). Though prior work has suggested a hypofunction of this circuitry in sociopathy (63), our data provide evidence for an increase in functional interaction in *OXTR* risk allele carriers that might arise from deficits in top-down regulation of the hypothalamus (64). This supports the idea that genetic risk for social dysfunction translates into a neural mechanism that is prone to label social stimuli as somatically and emotionally undesirable (58).

Dissecting these findings further at the level of neural activation, we provided convergent evidence that allelic variation in *OXTR* linked to social impairment impacts not only the volume but also the functional response of the amygdala, a key neural structure in a regulatory circuitry mediating fear responses (65) and social information processing (66). The lateral and capsular divisions of the central amygdala (CeL) contain dense concen-

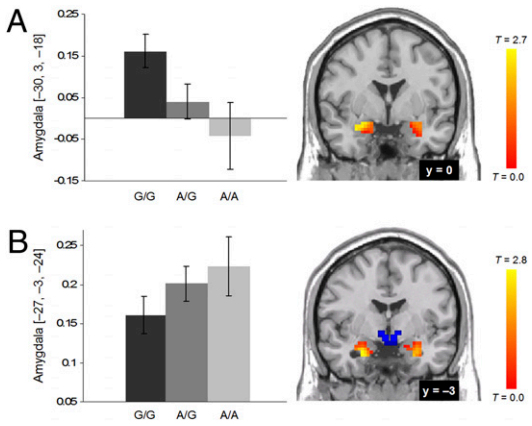

Fig. 3. Allelic variation in OXTR predicts differences in amygdala activation and connectivity during the perceptual processing of facial emotion. (*A*) Significant activation decrease of the amygdala in the minor allele carriers for rs53576 ($P = 0.036$, FWE corrected). (*B*) Significant increase in the functional correlation of the hypothalamus and amygdala in minor allele carriers for rs53576 ($P = 0.036$, FWE corrected). Bar plots depict the mean value for the parameter estimates of the peak voxels stratified by genotype. Error bars illustrate the variance of parameter estimates (±SEM). The blue region illustrates the hypothalamus seed region of the analysis.

trations of OT receptors that modulate the inhibitory input from the CeL to the medial part of the central amygdala (CeM) (67). Consequently, oxytocinergic stimulation inhibits the amygdaloid efferents to the hypothalamus and brainstem that generate aversive autonomic responses to social stimuli (68, 69), a mechanism that is thought to be dysfunctional in autism (70, 71). In line with this notion, we observed an increased functional coupling between hypothalamus and amygdala in *OXTR* risk allele carriers.

The potential pathophysiological implications of this data warrant further discussion. Though the anatomical localization of functional effects in the amygdala is consistent with previous OT challenge studies (14, 15) and our own prior imaging genetics work on neuropeptide receptor variants (30), no unitary directionality in neuropeptide-related susceptibility effects are apparent. Similarly, we have previously observed both hyper- and hypoactivations of the amygdala for different ASD risk variants mapping near the promoter region of *AVPR1A* (30). Similarly, both hyper- (72, 73) and hypoactivations (48, 74) of the amygdala during face processing have been demonstrated in social disorders such as autism. Consistent with these findings, complex interacting and mutually inhibiting neuropeptide effects have been identified on the level of cellular physiology in the amygdala (69, 75), where distinct populations of neurons encode for the emotional and social salience of positively and negatively valenced stimuli (76, 77). Thus, because the proximal neurogenetic mechanisms associated with risk for social dysfunction can be functionally localized to the amygdala, but may differ in directionality, we speculate that different genetic variants translate into different neural risk mechanisms relating to hyperactive social threat signaling (and possible avoidance behavior) and hypoactive limbic signaling (and possible impairment in social stimulus discrimination and processing). Though the present correlative data are consistent with, but do not prove, these speculations, we suggest that these phenotypes merit further study in clinical populations.

Finally, our structural neuroimaging analyses produced two distinct findings of sexual dimorphism, a result that is in agreement with our expectations. It is well known that in mammals, gender differences in social behavior relate to sexually dimorphic features of the social brain (78, 79). Furthermore, the strong impact of OT on female social behavior (3), and the disproportionately high risk of men for social disorders (80), suggest sex-dependent genotype effects at the neural systems level, particularly as emotional deficits have been previously associated with male, but not female, rs53576A carriers (28). First, our analysis suggested that the *OXTR*-related decrease in hypothalamus volume was, for the most part, driven by male risk allele carriers. The subsequent cross-correlation of VBM and TPQ measures confirmed that lower local hypothalamus volumes predicted lower sociality in male, but not female, subjects. Second, we observed a significant gene-by-sex interaction effect on GM volume in the right amygdala. Here, male carriers of rs53576A were characterized by an increase in amygdala volume that was consistent with the presumed dose of *OXTR* risk alleles. Again, the evidenced negative correlation between amygdala volume and prosocial temperament scores argued for the behavioral relevance of this finding, as do prior neuroimaging studies that have shown increased amygdala volume in autism (43, 81).

The mechanisms underlying the observed sex-predominant findings are currently unknown. However, prior work has shown that the expression of *OXTR* in the limbic system is highly plastic, and exceptionally sensitive to gonadal steroids (79). In particular, estrogens have been shown to promote sex-dependent alterations in the oxytocinergic system by up-regulation of *OXTR* expression (82), stimulation of OT release from hypothalamic neurons (83), and induction of OT receptor binding in the amygdala (84). Notably, hormone-dependent neural plasticity can be reflected by local GM changes on the neural systems level (85). It is thus tempting to speculate that, in the context of social disorders, estrogens may prime the neuroarchitecture of females toward increased sociality, whereas the relative lack of the hormone may promote an opposite state in the male brain through relative OT inefficiency. In the context of allelic variation in *OXTR*, systematic differences in steroid levels might resemble a sex-specific epigenetic factor conferring risk and protection for social dysfunction, a neural predisposition that is likely to be mirrored by sex- and genotype-dependent differences in GM volume. Further work is necessary to verify this assumption. Importantly, because our imaging genetics findings might indicate an increased susceptibility of male risk allele carriers for decreased sociality, the results may also be relevant for male parenting behavior, because accumulating evidence indicates a role for OT in human fathers (86–88). This suggests that the impact of the variant studied here, and the neural circuits impacted by genetic variation in *OXTR*, should be examined in relation to male parenting behavior in future studies.

Several limitations of this study deserve consideration. First, because the functionality on the level of gene expression and receptor physiology is unknown, our data do not rule out the possibility that the observed effects reflect the impact of genetic variants outside *OXTR* in linkage disequilibrium (LD) with our typed SNP. However, the fact that our findings mapped onto brain regions with abundant *OXTR* expression, and the convergence of our data with findings from prior neuropeptide studies both point toward a functional effect in the OT system. Second, to comprehensively assess the impact of the studied variants on brain and behavior, we studied a variety of phenotypes, raising the issue of potential false-positive findings. To address this concern, we used conservative statistical methods that have been previously found to control for false positives in imaging genetics (89) in conjunction with a stepwise neuroimaging approach, where our structural findings, together with the existing OT literature, guided our functional analysis. Third, our participant count, though the highest of any imaging genetics study we are aware of, is still low compared with population-based studies in behavioral genetics. However, convergent evidence (90, 91) indicates that the higher penetrance of genetic variation at the level of intermediate neural phenotypes allows detection of genetic effects at sample sizes as low as 80 participants, a sample size well below the one studied here. Fourth, in operationalizing the construct of human sociality, the current study relied on a self-report questionnaire rather than observer-dependent data, a limitation that deserves further consideration in future studies. Finally, though the evidenced effects of *OXTR* on prosocial temperament and neurobiology support, but cannot prove, a clinically relevant genetic risk mechanism for social dysfunction, these data may serve to generate hypotheses about the pathophysiology of social impairments in psychiatric disease.

In summary, we present multimodal imaging data implicating *OXTR* in hypothalamic-limbic circuits critical for emotion regulation and sociality in humans. We provide evidence for a neural mechanism linking both structural and neural signaling alterations in the oxytocinergic system to individual differences in emotional reactivity and prosocial temperament. Our findings support the idea that the disproportionate risk for social dysfunction in males has a sex-related neural basis. This study extends prior knowledge on neuropeptide function in the human brain and provides further insight into the neural mechanisms that shape our capacity to develop successful social relationships.

Materials and Methods

Subjects. All participating subjects were recruited as part of the Clinical Brain Disorders Branch "Sibling Study" as previously described (see ref. 92 for details on the implemented recruiting and screening procedures). Briefly, participants gave written informed consent for a protocol approved by the National Institute of Mental Health Institutional Review Board (Protocol 95-M-0150, Principal Investigator Daniel R. Weinberger). All subjects were carefully screened by a psychiatrist to ensure they were free of any lifetime

history of psychiatric or neurological illness, psychiatric treatment, or drug or alcohol abuse. Only Caucasians of European ancestry were included to avoid population stratification artifacts. No siblings were included in this study. A total of 345 healthy adults were studied: 212 participants (103 males, 109 females, mean age = 29.9 ± 9.0 y) in the structural MRI study and 228 participants (102 males, 126 females, mean age = 31.9 ± 9.9 y) in the functional MRI study. Ninety-eight subjects (28.4%) from the functional analyses were also a part of our morphometric analyses. Personality assessment was performed in 309 (89.6%) of the 345 participants. Subject demographics of the samples stratified by *OXTR* genotype are reported in Table S2. Further information on the demographic comparison of our study populations is provided in Table S3. Apart from a nominally significant age difference between our structural and functional MRI samples ($P < 0.02$), no demographic differences were observed. Notably, the observed age difference does not constrain the validity of the reported neuroimaging results, which have been corrected for the effects of age.

Genotyping Procedures. Details on the collection and analysis of DNA are provided in *SI Materials and Methods*.

Structural MRI. ***Acquisition and processing of structural images.*** Details on the acquisition and processing of structural images are provided in *SI Materials and Methods*.

Statistical inference. The effects of *OXTR* genotypes on regional GM volume were examined within the framework of the general linear model with random-effects group statistics at the second level as previously described (33, 39). Following standard practice in neuroimaging (93), we used a strongly hypothesis-driven ROI to investigate genotype-dependent alteration in the limbic circuitry, i.e., the bilateral hypothalamus, amygdala, and dorsal ACG using the Wake Forest University (WFU) PickAtlas utility (http://www.fmri.wfubmc.edu/). Though whole-brain analyses are susceptible to type II errors, neuroanatomically informed ROI analyses over multiple network components provide a good balance between sensitivity and specificity, while allowing for a rigorous control of false positive findings in imaging genetics (89). We included all brain regions known to express *OXTR* in humans that are accessible with noninvasive in vivo neuroimaging; due to the limited spatial resolution of the method, very small structures such as the septal nuclei and the basal nucleus of Meynert were not examined.

Genotype effects were examined using a multiple-regression model in SPM5 with genotype as covariate of interest, coding for the number of risk alleles (0, 1, or 2), and the following nuisance covariates: total gray-matter volume, orthogonalized first- and second-order polynomials of age and sex. Interactions with sex were investigated using genotype-by-sex interaction covariates. Pearson's correlation coefficient analyses (two-tailed, $P < 0.05$) between the reward dependence subscale of the TPQ, our measure of prosocial temperament, and parameter estimates from significant peak voxels in the hypothalamus and amygdala were used to examine whether regional GM volumes related to individual differences in prosocial temperament.

Structural covariance analysis. Details on the structural covariance analysis are provided in *SI Materials and Methods*.

Functional MRI. ***Emotional face processing task.*** Details on the emotional face processing task are provided in *SI Materials and Methods*.

fMRI data acquisition, processing, and analysis. Details on the acquisition, processing, and analysis of fMRI data are provided in *SI Materials and Methods*.

Statistical Assessment of Neuroimages. For all analyses, the significance threshold was set to $P < 0.05$, family-wise error (FWE) corrected for multiple comparisons at the voxel level in limbic ROIs defined using the WFU PickAtlas utility. We have recently provided empirical evidence that this statistical procedure provides thorough protection against false-positive findings in imaging genetics analyses (89).

Assessment of Prosocial Personality Traits. The Tridimensional Personality Questionnaire (40) is a 100-item self-rating scale assessing four well-validated heritable temperamental traits. For the purposes of our study, we had particular interest in the reward dependence (RD) subscale, which quantifies prosocial interpersonal traits. The TPQ was administered to a total of 309 subjects; 195 of these subjects (62.7%) participated in the morphometric study and 199 (64.0%) participated in the functional study (see Table S2 for demographic details). In the same session, global intelligence was evaluated with the revised Wechsler Adult Intelligence Scale (WAIS-R) (94). Analysis of psychological data was performed using SPSS 11.0.1 for Windows. χ^2 tests were used to examine group differences in categorical variables. Univariate analyses of variance were used to examine group differences in continuous demographic variables. ANCOVA models were used to test for the effect of genotype on TPQ measures (covariates: age, gender, IQ).

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