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Abstract

Intranasal oxytocin (OT) can modulate social-emotional functioning and related brain activity in humans. Consequently, OT has been discussed as a potential treatment for psychiatric disorders involving social behavioral deficits. However, OT effects are often heterogeneous across individuals. Here we explore individual differences in OT effects on the neural response to social cooperation as a function of the rs53576 polymorphism of the oxytocin receptor gene (*OXTR*). Previously, we conducted a double-blind, placebo-controlled study in which healthy men and women were randomized to treatment with intranasal OT or placebo. Afterwards, they were imaged with fMRI while playing an iterated Prisoner's Dilemma Game with same-sex partners. Within the left ventral caudate nucleus, intranasal OT treatment increased activation to reciprocated cooperation in men, but tended to decrease activation in women. Here, we show that these sex differences in OT effects are specific to individuals with the rs53576 GG genotype, and are not found for other genotypes (rs53576 AA/AG). Thus, OT may increase the reward or salience of positive social interactions for male GG homozygotes, while decreasing those processes for female GG homozygotes. These results suggest that rs53576 genotype is an

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important variable to consider in future investigations of the clinical efficacy of intranasal OT treatment.

Keywords

Oxytocin; Oxytocin Receptor Gene Polymorphism; fMRI; rs53576; Cooperation

Introduction

In non-human animals, the neuropeptide oxytocin (OT) promotes the development and maintenance of social bonds (Lim & Young, 2006). Building on these findings, the past decade of research has revealed that OT also facilitates both non-reproductive and reproductive bonds in humans (Bartz *et al.*, 2011, Feldman, 2012). For instance, intranasal administration of OT increases trust (Kosfeld et al., 2005), altruism (Zak et al., 2007), and emotional empathy responses (Hurlemann et al., 2010) to unknown others, as well as paternal responsiveness during play with children (Naber et al., 2010). Recent neuroimaging studies suggest that OT may interact with the mesolimbic dopamine (DA) system to facilitate social bonding. OT treatment augments the ventral tegmental area response to socially relevant cues (Groppe et al., 2013) and enhances the ventral striatum response to viewing faces of romantic partners (Scheele et al., 2013) and to reciprocated cooperation from human partners (Rilling et al., 2012). Importantly, however, the effects of OT treatment are not ubiquitous, but are dependent on individual factors such as genotype and sex (Bartz *et al.*, 2011, De Dreu *et al.*, 2010, Olff *et al.*, 2013, Scheele *et al.*, 2014).

Genetic variation in the oxytocin receptor gene (*OXTR*), which may influence the number, organization, or functioning of OT receptors that mediate oxytocinergic function (Yamasue et al., 2012), may at least partially explain the heterogeneity of the OT effects. The human *OXTR* gene is located on chromosome 3p25, spans about 17 kb and consists of 3 introns and 4 exons (Inoue et al., 1994). A single-nucleotide polymorphism (SNP) of an adenine (A) or guanine (G) substitution within the third intron (rs53576) has been suggested as a particularly promising candidate to modulate oxytocinergic function (Tost *et al.*, 2010, Wu *et al.*, 2005), although the molecular functionality of this SNP is still unknown. Individuals homozygous for the G allele (GG), as compared with A allele carriers (AA/AG), are more likely to trust and help others (Krueger *et al.*, 2012, Verbeke *et al.*, 2013), show higher dispositional empathy (Rodrigues *et al.*, 2009, Smith *et al.*, 2014) and stronger empathic neural responses to the pain of in-group members (Luo et al., 2015), as well as display higher parental sensitivity (Bakermans-Kranenburg & Van Ijzendoorn, 2008, Riem *et al.*, 2011). Therefore, the rs53576 G allele is assumed to be associated with a more efficient oxytocinergic system (Marsh *et al.*, 2012, Moons *et al.*, 2014, Riem *et al.*, 2011). Preliminary evidence has provided support for this hypothesis by showing that GG homozygotes are more responsive to intranasal OT administration than A allele carriers. For example, OT administration increases preference for infants' faces among rs53576 GG homozygotes but not A allele carriers (Marsh *et al.*, 2012). The most plausible mechanism by which OXTR SNPs could influence OT effects is through altering expression of the OT receptor. Recent studies in prairie voles demonstrate the plausibility of this mechanism by

showing that a single OXTR SNP (SNP2) influences overall density of the OT receptor within the nucleus accumbens (King *et al.*, 2013). Specifically, T-allele genotypes of SNP2 (either T/T or C/T) had 100% higher OXTR density than C/C littermates within the nucleus accumbens, but not within other brain regions that were analyzed. Moreover T-allele pups emitted more ultrasonic vocalizations following maternal separation compared with C/C littermates.

Notably, there is also accumulating evidence for sex differences in effects of intranasal OT on social-emotional functioning and associated brain function (Campbell *et al.*, 2014, Ditzen *et al.*, 2012, Hoge *et al.*, 2014, Lynn *et al.*, 2014, Preckel *et al.*, 2014). For instance, while intranasal OT administration during couple conflict augments activity of the autonomic nervous system (ANS) in men, it attenuates ANS activity in women (Ditzen *et al.*, 2012). The authors interpreted these findings to support the proposal that OT might facilitate approach behavior in males, but warmth and calmness in females (Mccall & Singer, 2012). Another study (Hoge *et al.*, 2014) found that intranasal OT was associated with more negative ratings of unfamiliar faces in men but more positive ratings in women. The authors suggest that these findings could be attributed to sex differences in receptor density and distribution. Alternatively, they argued that OT might increase approach motivation in both genders, manifest as either positive (e.g., enthusiasm and trust) or negative (e.g., aggression and anger) approach behaviors among women and men, respectively (Kemp & Guastella, 2011). Finally, men treated with intranasal OT report less negative affect following social stress, whereas women treated with intranasal OT treatment report enhanced anger in the same situation (Kubzansky *et al.*, 2012). These findings raise the possibility that treatment with OT may benefit one sex but not the other in certain social contexts.

In our previous study employing a subgroup of the current sample (64 males, OT=27, placebo=37; 58 females, OT=29, placebo=29), we demonstrated sexually differentiated effects of OT treatment on the left ventral caudate nucleus response to reciprocated cooperation with human partners in the context of the iterated Prisoner's Dilemma game (Rilling et al., 2014) such that OT augmented the male left ventral caudate nucleus response but there was a non-significant trend for OT to attenuate the female left ventral caudate nucleus response to reciprocated cooperation. Based on the evidence that women have higher baseline OT levels in cerebrospinal fluid (CSF) (Altemus *et al.*, 1999), we raised the possibility of an inverted-U shaped dose response function between brain OT levels and neural activity. According to this hypothesis, raising brain OT levels in men would augment the left ventral caudate response, moving them closer to the maximum level activity. On the other hand, raising OT levels in women might displace them to the right of the maximum, decreasing brain activity.

The current study builds on our prior report by examining how variation in rs53576 modulates the effects of OT on the neural response to social cooperation in both males and females. In light of recent findings on *OXTR* rs53576 variation, we predicted that GG homozygotes would have increased OXTR expression and would therefore be more sensitive to the effects of intranasal OT. More specifically, we hypothesized that the effects of OT treatment on the left ventral caudate nucleus response to reciprocated cooperation would be more pronounced among both males and females with the GG genotype, compared

with A allele carriers. In other words, it would be more likely that OT *enhances* the left ventral caudate nucleus response to reciprocated cooperation among male GG homozygotes than among male A allele carriers; and it would be more likely that OT *reduces* the response among female GG homozygotes than among female A allele carriers. This would result in augmented sexually differentiated OT effects among GG homozygotes as compared with A allele carriers. Importantly, this is the first pharmaco-fMRI study to explore individual differences in OT effects on social interactions and underlying brain function as a function of *OXTR* genetic variation and sex.

Materials and Methods

Subjects

104 men and 100 women from the Emory University community between the ages of 18 and 22 (mean age for men=19.97 years; mean age for women=20.21 years) were randomized to receive intranasal OT (50 men and 50 women) or intranasal placebo (54 men and 50 women). All subjects gave written informed consent, and the study was approved by the Emory University Institutional Review Board and the U.S. Food and Drug Administration. Seven men (OT n=2 and placebo n=5) and two women (OT n=2) were excluded from data analysis due to genotyping failure or missing data. Also, six men (OT n=3 and placebo n=3) and two women (OT n=2) were excluded from the neuroimaging analysis due to excessive motion (>1.5 mm, OT n=4 and placebo n=3) or abnormal brain anatomy (n=1, OT n=1). Finally, three women (OT n=1 and placebo n=2) had only data with computer partners due to excessive motion during playing with human partners, whereas two women (OT n=1 and placebo n=1) had only data with human partners due to excessive motion during playing with computer partners.

Exclusion Criteria

Subjects with a history of seizures or other neurological disorders, alcoholism or any other substance abuse, hypertension, cardiovascular disease, diabetes and other endocrine diseases or malignancy were excluded from the study. Subjects who reported a history of asthma or migraine headaches were excluded if their symptoms were persistent, disabling and required one or more medication adjustments within the past month. Subjects with a history of head trauma, psychiatric illness, or use of medications with known psychoactive effects over the past year were generally excluded. However, a post-hoc, secondary review of screening forms revealed inclusion of one subject who reported anti-depressant use and another who indicated a history of an unspecified psychiatric illness for which he did not receive treatment. We also included three subjects with a non-recent history of mild head trauma. Subjects with claustrophobia were excluded at the discretion of the Principal Investigator. Subjects were allowed to continue on their current medications if the agents in question were not reported to alter brain activity in regions of interest. Some of these medications included metformin (for polycystic ovarian disease), minocycline, isotretinoin topical (acne), and antihistamines for allergy.

Preparation of OT and placebo

Intranasal OT—3.6 ml of 40 IU/ml OT (Syntocinin-Spray, Novartis) was added to 2.4 ml OT placebo to make 6.0 ml of 24 IU/ml OT. 6 ml were transferred to a plastic bottle with a nasal applicator.

Intranasal AVP placebo—The AVP placebo consisted of the vasopressin vehicle only, and was prepared by adding 125 mg of 0.5% chlorobutanol to 50 ml saline, followed by acetic acid until the pH fell within the range of 2.5 to 4.5 as measured with a pH meter. The solution was then sterilized using a 0.22 micron filter. 6 ml were transferred to a plastic bottle with nasal applicator.

Intranasal OT placebo—The OT placebo consisted of the Syntocinon vehicle only. Each 5 ml of OT placebo consists of: Chlorobutanol hemihydrate 12.5 mg, Methyl-4-hydroxybenzoate 2.0 mg, Propyl-4-hydroxybenzoate 1.0 mg, 85% ethanol 125 µl, Sodium acetate 14.0 mg, purified water 4.8455 g. 6 ml were transferred to a plastic bottle with nasal applicator.

Administration of OT or placebo

Both experimenters and subjects were blind to the treatment subjects received. All solutions were administered intranasally. The OT group self-administered 24 IU oxytocin (Syntocinin-Spray, Novartis), which required 10 nasal puffs to administer 1 ml of solution. The placebo group self-administered 10 nasal puffs of either OT placebo or vasopressin placebo. Half of the placebo subjects received OT placebo and half received vasopressin placebo (the study also included a vasopressin arm that is beyond the scope of the current paper). Subjects were instructed to place the nasal applicator in one nostril and depress the lever until they felt a mist of spray in the nostril, to then breathe in deeply through the nose, and afterwards to place the applicator in the other nostril and repeat the process.

Genotyping

Saliva samples from were collected from all participants using Oragene-DNA kits (DNA Genotek Inc, Ontario, Canada). DNA was then extracted from the cells in the saliva using an automated system by Omega-Biotek (Omegabiotek.com). DNA samples were normalized in a 96-well plate to a final working concentration of 5 ng/µl and then seeded in a 384-well plate (10 ng/well). Each 96-well plate contained quality-control samples, including 2 positive control samples, 2 duplicate samples and 2 no-DNA blanks. rs53576 genotyping was performed by a TaqMan allelic discrimination assay, on an ViiA™7 Instrument (Applied Biosystems, Foster City, California), using a commercial TaqMan assay reagent kit (Applied Biosystems), as described in Bradley et al. (2008). Genotype calls were made using ViiA™7 software and visually checked (5 samples failed TaqMan Assay). Quality control (QC) included the analysis of positive and negative controls, duplicate samples, as well as the evaluation of Hardy Weinberg Equilibrium using an exact test (https://www.pharmgat.org/pharmgat.org/Tools/exacthwelongform).

Principal component analysis (PCA)

Genome wide genotypes from the Illumina PsychChip were used to calculate Principal Components using Principal Compinents Analysis (PCA) to account for population stratification. Briefly, 588K variants were genotyped using the Illumina PsycChip and QC was performed using Plink (http://pngu.mgh.harvard.edu/purcell/plink) (Purcell et al., 2007), to remove: samples with >=10% genotypes missing; related samples (1 sample); SNPs with minor allele frequency (MAF)   0.05; and SNPs with deviation from Hardy-Weinberg Equilibrium ($p< 10^{-6}$). Gender was then checked using the genetic clean data and no mismatches were found. After pruning for QC, ~ 26K autosomal independent SNPs (i.e., not in Linkage Disequilibrium) were extracted to calculate the PCA eigenvectors of the genetic relationship matrix (GRM) (http://gump.qimr.edu.au/gcta) (Yang et al., 2011). The first two PCA eigenvectors (PCA1 and PCA2), which discriminated individuals with European background from those with African and Asian background, were then included in the model as covariates to capture variance due to population structure.

Prisoner's Dilemma task

The iterated Prisoner's Dilemma (PD) game is a model for relationships based on reciprocal altruism. In the game, two players choose to either cooperate or defect and receive a payoff that depends upon the interaction of their respective choices. The game version used in the current study is a sequential-choice PD game, in which player 1 chooses and player 2 is then able to view player 1's choice before making his/her own choice. Each of the four outcomes is associated with a different payoff (Figure 1a). Player cooperation followed by partner cooperation (CC) pays $2 to both player and partner, player cooperation followed by partner defection (CD) pays $0 to the player and $3 to the partner, player defection followed by partner defection (DD) pays $1 to both player and partner, and player defection followed by partner cooperation (DC) pays $3 to the player and $0 to the partner. For instance, if player 1 chooses cooperation, player 2 can either choose cooperation, resulting in a $2 payoff to both players or defection, resulting in $3 to player 2 and $0 to player 1. If player 1 chooses defection, player 2 can either choose cooperation that results in $0 to player 2 and $3 to player 1 or choose defection that results in $1 to both players (Figure 1b). While being imaged with fMRI, subjects played 30 rounds of a sequential-choice, iterated Prisoner's Dilemma game in each of four sessions. For two sessions, subjects were told they were playing with the same-sex human partners they were introduced to. For the other two sessions, subjects were told that they were playing with a computer partner. In reality, subjects were always playing with a pre-programmed computer algorithm for all four sessions. For both human and computer partners, in one of the two sessions, subjects played in the role of first mover (player 1) and their partner played in the role of second mover (player 2). In the second session, roles were reversed. All four sessions were conducted in the same fMRI scan on the same day. Subjects were compensated with approximately $120; the exact amount was obtained by multiplying the total earnings across all four runs of the PD Game by 2/3.

Analysis

Given that rs53576 has been previously linked specifically with cooperative behavior in an economic game (the Trust game) (Krueger *et al.*, 2012) similar to the one we employ here (sequential choice PD game), and to mitigate loss of statistical power due to comparison across multiple SNPs, we elected to focus our analysis specifically on rs53576. Notably, rs53576 has been linked with other aspects of prosocial behavior beyond cooperation, such as empathy and parental sensitivity (Bakermans-Kranenburg & Van Ijzendoorn, 2008, Rodrigues *et al.*, 2009, Smith *et al.*, 2014).

Behavioral Analysis

In addition, our analyses are limited to player 1 data. Behavioral outcomes (Supporting information, Table S1) included CC, CD, DC and DD, as well as the probability of cooperating conditional on the outcome of the previous round. Therefore, there were four different transition probabilities: the probability of cooperating after a CC outcome (pC/CC), after a CD outcome (pC/CD), after a DC outcome (pC/DC) and after a DD outcome (pC/DD). Since this manuscript is focused on neural response to cooperative interaction, we only report results on the frequency of C choices and pC/CC. Effects of drug treatment (OT vs PBO), sex (male vs. female) and genotype (AA/AG vs. GG homozygotes – recessive model) and their interactions on these behavioral measures were evaluated using three-way ANOVA with the first 2 race principal components from genetic analysis as covariates in the model to correct for the possible effect of population stratification. AA and AG genotype were combined due to the limited number of AA individuals (Supporting information, Table S2 & S3) and because previous studies reported social cognitive differences between GG homozygotes and A allele carriers (AA/AG) (e.g., Bakermans-Kranenburg & Van Ijzendoorn, 2008, Rodrigues *et al.*, 2009). Post hoc comparisons were performed with a general linear model (GLM) in which drug treatment was the main independent variable and the first 2 race principal components from genetic analysis were included as covariates in the model.

Neuroimaging procedures

Anatomical image acquisition—Subjects were positioned head first in the supine position inside the scanner (Siemens Trio 3T), with padded head restraint to minimize head motion during scanning. Each scanning session began with a 15 s scout, followed by a 5 min T1-weighted MPRAGE 3d scan that was acquired in the sagittal plane and accelerated by generalized auto-calibrating partially parallel acquisitions (GRAPPA) with a factor of 2 (TR=2600 ms, TE=3.02 ms, matrix=256×256×176, FOV=256 mm×256 mm×176 mm, slice thickness=1.00 mm, gap=0 mm).

fMRI image acquisition—Subjects were imaged while playing the PD game. Functional scans used an EPI sequence with the following parameters: TR=2000 ms, TE=28 ms, matrix=64×64, FOV=224 mm, slice thickness=2.5 mm, 34 axial slices with a slice gap of 1.05 mm. TE employed in the current study (i.e., 28 ms) was minimally decreased from the typical value (i.e., 32 ms) in order to reduce magnetic susceptibility artifact in the

orbitofrontal region. The duration of each EPI scan was about 12 min (30 PD round×~20 s per round, plus five null trials×14 s per trial).

fMRI image analysis—Image processing was conducted with FEAT (FMRI Expert Analysis Tool) version 6.00, part of FSL (FMRIB's Software Library, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). Preprocessing involved motion correction using MCFLIRT, slice timing correction using Fourier-space time-series phase-shifting, non-brain removal using BET, spatial smoothing using a Gaussian kernel of FWHM 5 mm, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, and high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with cut-off=100.0 s). Registration to MNI space via corresponding extracted T1 brain was carried out using Boundary-Based-Registration. Time-series statistical analysis was carried out using FILM with local autocorrelation correction.

For the player 1 runs, a separate general linear model (GLM) was defined for each subject that examined the neural response to both the epoch in which the choice to cooperate or defect was made, as well as to the epoch in which the trial outcome was revealed. More specifically, the following regressors were defined for each subject in the role of player 1: (1) the beginning epoch when round number and the partner's face or a picture of computer was displayed, (2) the choice epoch when the subject chose to cooperate (choice C), (3) the choice epoch when the subject chose to defect (Choice D), (4) CC outcomes, (5) CD outcomes, (6) DC outcomes, and (7) DD outcomes. These regressors were specified separately for runs with human and computer partners, resulting in a total of 14 distinct regressors per subject. Parameter estimates for CC outcomes with human and computer partners were computed at every voxel within the brain. Five men (OT n=3 and placebo n=2) and two women (placebo n=2) were lack of CC outcomes with human partners, therefore these subjects were excluded from analysis of CC outcomes with human partners. In a similar vein, four men (OT n=2 and placebo n=2) and three women (placebo n=3) were excluded from analysis of CC outcomes with computer partners. Demographic information of males and females included in the final data analysis are provided in Supporting information, Table S2 & S3. In addition, genotypes for *OXTR* rs53576 did not deviate from the Hardy-Weinberg equilibrium (all $p>0.19$) for the sample as a whole or for each sub-group (Table 1).

We performed a region of interest analysis based on *a priori* hypotheses, supplemented with a whole brain analysis. The ROI analysis focused on the left ventral caudate nucleus, since this brain region is both activated by reciprocated cooperation with human partners and modulated by intranasal OT treatment (Rilling *et al.*, 2014, Rilling *et al.*, 2012). The ROI was defined as a 9 mm side cube centered on the activation maximum from our earlier study with an independent sample of subjects from those used here (Rilling et al, 2002). We focus our analysis on the left ventral caudate nucleus because: 1) we previously demonstrated sexually differentiated effects of OT on the neural response to CC outcomes within this ROI, using a sub-sample of the full sample we present here (Rilling *et al.*, 2014, Rilling *et al.*, 2012); 2) previous animal studies have shown that OT and DA systems interact in the ventral striatum to modulate affiliative behaviors (Liu & Wang, 2003, Young *et al.*, 2005);

and 3) we wanted to limit the number of ROIs to mitigate loss of power due to multiple comparisons (Poldrack, 2007).

For each subject, FSL's Featquery (http://fsl.fmrib.ox.ac.uk/fsl/fsl4.0/feat5/featquery.html) was used to extract mean percent signal change data from the predefined left ventral caudate nucleus ROI in response to CC outcomes. These data were then entered into an analysis of variance (ANOVA) to examine the effects of three factors on left ventral caudate nucleus activation separately for human and computer partners: drug treatment (OT vs. placebo), sex (male vs. female) and genotype (AA/AG vs. GG), as well as their interactions. Post-hoc comparisons were conducted using GLMs to test effects of drug treatment (i.e., comparing OT to placebo) in various groups, adding the first 2 race principal components from genetic analysis as covariates. Considering that female subjects were not all scanned at the same menstrual cycle phase, we evaluated the potential modulation by estradiol levels of OT effects on the left ventral caudate nucleus among females. Specifically, we calculated the correlation coefficient between the caudate response to reciprocated cooperation and plasma estradiol levels for each subgroup of females (i.e., female GG homozygotes and A allele carriers with OT treatment, female GG homozygotes and A allele carriers with placebo treatment).

The ROI analysis was supplemented with a whole brain analyses (group level), carried out using an Ordinary Least Square (OLS) model in FEAT. At each voxel, the three-way interaction of drug treatment (OT vs. placebo) × sex (male vs. female) × genotype (AA/AG vs. GG homozygotes) was tested separately for human and computer partners by calculating the contrast $1\ -1\ -1\ 1\ -1\ 1\ 1\ -1$ (male GG homozygotes with OT treatment, male GG homozygotes with placebo treatment, male A allele carriers with OT treatment, male A allele carriers with placebo treatment, female GG homozygotes with OT treatment, female GG homozygotes with placebo treatment, female A allele carriers with OT treatment, female A allele carriers with placebo treatment). T-statistics were converted to Z-statistics by equating probabilities under the tails of the T and Z distributions. The Z-statistic ("Gaussianised T") images were thresholded at $p<0.05$ (two-tailed) corrected for multiple comparisons based on the volume of clusters of contiguous voxels (voxel-level $p<0.05$). To visualize results from whole-brain analyses, functional regions of interest (ROIs) were defined as a 10 mm cube centered on the voxel of peak activation. Average percent signal changes of each ROI were extracted via FSL's Featquery (http://fsl.fmrib.ox.ac.uk/fsl/fsl4.0/feat5/featquery.html). These data were then plotted as a function of sex, drug treatment and genotype.

Other methodological details, such as PD tutorial and practice trials, pre-programmed computer algorithm for the PD game, monitoring of vital signs, *Positive and Negative Affect Schedule* (PANAS) ratings, counterbalancing of human and computer sessions, and confederate introductions are described in our recent publication (Rilling et al., 2012).

Results

Behavioral Results

For both frequency of choice C and the probability of C choices after a CC outcome, there were no main effects of drug, sex or genotype and no interaction effects for either human (Figure 2a) or computer partners (Figure 2b).

Neuroimaging Results

ROI analysis—Three-way ANOVA was used to test for effects of drug, sex and genotype, as well as their interactions, on the left ventral caudate response (Figure 3a) to reciprocated cooperation (Table 2). For CC outcomes with human partners, there were no main effects of drug, sex or genotype, however, there was a significant drug × sex interaction ($F_{(1, 167)}=11.82$, $p=0.001$). Post-hoc comparisons revealed that OT decreased the left ventral caudate nucleus response to CC outcomes with human partners in females ($t_{87}=-3.08$, $p=0.003$), but not males ($t_{82}=0.41$, $p=0.69$). There were no drug × genotype or sex × genotype interactions. Notably however, the drug × sex × genotype interaction was significant ($F_{(1, 167)}=10.39$, $p=0.002$) such that among individuals with the GG genotype OT rendered the male left ventral caudate nucleus response similar to the female left ventral caudate nucleus response in the placebo condition and vice versa, whereas there were no such sex differences in OT effects among those with AA/AG genotypes (Figure 3b). Post-hoc contrasts revealed that OT augmented the left ventral caudate nucleus response to CC outcomes in males with the GG genotype ($t_{30}=2.39$, $p=0.023$), and OT attenuated the left ventral caudate nucleus response to CC outcomes in females with the GG genotype ($t_{28}=-2.30$, $p=0.029$). There were no effects of OT in males with AA/AG genotypes ($t_{48}=-1.76$, $p=0.084$) or females with AA/AG genotypes ($t_{55}=-1.77$, $p=0.083$). Furthermore, at baseline in the placebo condition, women with the GG genotype had a stronger left ventral caudate nucleus response to CC outcomes with human partners than did men with the GG genotype ($t_{35}=2.46$, $p=0.019$); whereas there were no such sex differences among A allele carriers ($t_{47}=-0.99$, $p=0.33$). In each subgroup of females (i.e., female GG homozygotes and A allele carriers with OT treatment, female GG homozygotes and A allele carriers with placebo treatment), there were no significant correlations between estradiol levels and the left ventral caudate nucleus response to CC outcomes with human partners (all $p>0.05$).

For CC outcomes with computer partners, only the three-way interaction was significant ($F_{(1, 168)}=5.09$, $p=0.025$). Among males, OT treatment had no effect in those with either the GG genotype ($t_{34}=0.92$, $p=0.36$) or the AA/AG genotypes ($t_{45}=-0.42$, $p=0.68$). Among females, OT attenuated the left ventral caudate nucleus response to CC outcomes with computer partners in GG homozygotes ($t_{29}=-2.29$, $p=0.029$), whereas there was no effect of OT treatment in those with AA/AG genotypes ($t_{54}=0.37$, $p=0.71$) (Figure 3c). Furthermore, at baseline in the placebo condition, women with the GG genotype had a stronger left ventral caudate nucleus response to CC outcomes with computer partners than did men with the GG genotype ($t_{36}=2.35$, $p=0.025$); whereas there were no such sex differences among A allele carriers ($t_{46}=-0.84$, $p=0.41$). In each subgroup of females (i.e., female GG homozygotes and A allele carriers with OT treatment, female GG homozygotes and A allele carriers with placebo treatment), there were no significant correlations between estradiol

levels and the left ventral caudate nucleus response to CC outcomes with computer partners (all *p*>0.05).

Whole brain analysis—A whole brain, three-way interaction analysis also revealed these effects in the ventral caudate nucleus that overlapped with the left ventral caudate nucleus ROI (Supporting information, Figure S1b). Three-way interactions were also found in the nucleus accumbens, the superior and middle temporal gyri, the anterior cingulate gyrus and prefrontal cortex for human partners (Supporting information, Figure S1b & Table S4). To reveal the nature of these three-way interactions, the percent signal change within each of these regions was also plotted as a function of sex, drug treatment and genotype (Supporting information, Figure S2). In most of these regions, similar to the caudate nucleus, OT significantly decreases activity relative to placebo in women and tends to increase activity relative to placebo in men among GG homozygotes. For computer partners, the three-way interaction whole-brain analysis also revealed activation in the ventral caudate nucleus that overlapped with the left ventral caudate nucleus ROI (Supporting information, Figure S1c). Three-way interactions were also found in the nucleus accumbens, putamen, and insula (Supporting information, Figure S1c & Table S5). To reveal the nature of the three-way interaction, the percent signal change within each of these regions were also plotted as a function of sex, drug treatment and genotype (Supporting information, Figure S3). Within each of these regions, OT attenuated the response to CC outcomes in female GG homozygotes but had no significant effect on male GG homozygotes.

Discussion

To our knowledge, this is the first study to report that a common *OXTR* gene polymorphism (rs53576) modulates the effect of OT administration on the ventral caudate nucleus response to positive social interactions in males and females. At baseline in the placebo condition, women with the GG genotype had a stronger left ventral caudate nucleus response to reciprocated cooperation with both human and computer partners than did men with the GG genotype; however, there were no such sex differences among A allele carriers. Among individuals with the GG genotype, OT increased the male left ventral caudate nucleus response to reciprocated cooperation from human partners to the level of the female left ventral caudate nucleus response in the placebo condition. Among the GG genotype, OT also decreased the female left ventral caudate response to CC outcomes to the level of the male left ventral caudate nucleus response in the placebo condition. In addition, OT treatment attenuated the left ventral caudate nucleus response to reciprocated cooperation with computer partners among female GG homozygotes. On the other hand, for both males and females with rs53576 AG/AA genotypes, OT treatment did not reveal any significant effects on the activity of left ventral caudate nucleus.

These results support the hypothesis that rs53576 G allele is associated with more efficient oxytocinergic function, perhaps due to upregulated receptor expression, and therefore leads to enhanced effects of OT treatment (Marsh *et al.*, 2012, Moons *et al.*, 2014). This interpretation fits with a growing body of evidence showing that the G allele is associated with improved social-emotional functioning (e.g., higher empathic and altruistic motivations) compared with A allele (Rodrigues *et al.*, 2009, Verbeke *et al.*, 2013). In

addition, Marsh and colleagues (2012) demonstrated effects of OT administration that were only evident among GG homozygotes but not A allele carriers, suggesting heightened responsiveness to OT treatment among individuals with the GG genotype. Therefore, our findings concur with previous observations by showing that the effects of OT on the left ventral caudate nucleus response to reciprocated cooperation among men and women, albeit in opposite directions, are specific to GG homozygotes.

Modulation of the left ventral caudate nucleus response by OT treatment may represent changes in the reward or salience of reciprocated cooperation (Bhanji & Delgado, 2014, Rilling *et al.*, 2002). That is, OT treatment might increase the reward or salience of positive social interactions for male GG homozygotes, but decrease those processes for female GG homozygotes. Although these sex differences in OT effects on the neural response to social cooperation may seem surprising; sexually differentiated effects of OT are not without precedent. In fact, many studies have reported sexually differentiated effects of OT on social cognition and behavior in both non-human animals and humans. For instance, men treated with intranasal OT report less negative affect following social stress, whereas women treated with intranasal OT treatment report enhanced anger in the same situation (Kubzansky *et al.*, 2012). In addition, OT administration increases ANS activity and emotional responses to couple conflict in males whereas decreases these physiological and subjective responses in females (Ditzen *et al.*, 2012). Therefore, our findings complement previous studies on sex differences in OT effects by demonstrating opposing effects of OT for men and women in response to positive social interactions (i.e., reciprocated cooperation).

One possible explanation for our observed sex differences in the effect of OT on brain activity could be that the dose-response function between brain OT levels and neural activity follows an inverted-U shape (Cardoso *et al.*, 2013, Cardoso *et al.*, 2014, Rilling *et al.*, 2014). There is evidence that women have higher cerebrospinal fluid (CSF) oxytocin levels than men do (Altemus *et al.*, 1999), and we find that at baseline in the placebo condition women with the GG genotype also have a stronger left ventral caudate nucleus response to reciprocated cooperation than do men with the GG genotype. Assuming an inverted-U shaped curve, raising brain OT levels in men would augment the ventral caudate response, moving them closer to the maximum level activity. On the other hand, raising OT levels in women might displace them to the right of the maximum, decreasing brain activity. This hypothesis is also supported by recent studies showing larger effects of 24 IU compared with 48 IU intranasal OT in men (Cardoso *et al.*, 2013, Cardoso *et al.*, 2014). However, this inverted-U shaped interpretation of OT effects is *very tentative* given that we did not measure baseline and post-drug levels of OT in CSF.

The ventral caudate nucleus is a known target of mesolimbic DA projections. Its modulation by intranasal OT treatment complements previous findings in both humans and non-human animals that OT may influence human social affiliation by interacting with the DA system to render those behaviors more rewarding (Groppe *et al.*, 2013, Rilling *et al.*, 2012, Scheele *et al.*, 2013, Young *et al.*, 2005). Unexpectedly, effects of OT were similar when playing with human and computer partners among female GG homozygotes, suggesting that OT effects are not limited to social cooperation but could extend to non-social cognition in this sub-group. Alternatively, there may be a tendency for female subjects to imbue computer

partners with human attributes. These findings seem contradictory with previous studies showing that intranasal OT increases trust, a form of social risk taking, but does not increase risk-taking in an analogous nonsocial task (Baumgartner *et al.*, 2008, Kosfeld *et al.*, 2005). However, it should be emphasized that these previous studies included only male subjects, among whom we do not find effects of OT on the left ventral caudate nucleus activation when interacting with computer partners.

Despite the effects of intranasal OT on the BOLD fMRI response to reciprocated cooperation, intranasal OT had no effect on the behavioral response to this outcome (p C/ CC). Several studies show that the effects of intranasal OT on cooperative behavior depend on the participant's attachment style (Bartz *et al.*, 2010, De Dreu, 2012) or cognitive style (Ma *et al.*, 2015). For example, intranasal OT increases cooperation in the PD game among men who are high rather than low in attachment avoidance (De Dreu, 2012). Further, it has been recently reported that OT has opposing effects on intergroup cooperation in the Public Goods Game among individuals with intuitive vs. reflective cognitive styles (Ma *et al.*, 2015). We did not characterize the attachment or cognitive style of the participants in our study, so we are unable to evaluate the potential influence of attachment style on cooperation-related neural and behavioral responsiveness to OT. Another potential explanation for the lack of behavioral effects relates to the relative influence of emotion and cognition in decision-making. Although intranasal OT modulated activity within the striatum, it may be that decision-making in our iterated PD game is more cognitively-based and cortically-mediated. That is, top-down cognitive processes may be overriding emotional influences in this sample of highly educated and high-functioning undergraduate students.

The modulation of intranasal OT effects by sex and *OXTR* gene polymorphism might have important implications for the potential clinical application of OT treatment. Indeed, intranasal administration of OT has been discussed as a promising candidate to improve social functioning in a variety of psychiatric disorders, including autism spectrum disorder, depression and schizophrenia (Macdonald & Feifel, 2013, Zink & Meyer-Lindenberg, 2012). However, an accumulating body of literature has reported individual differences in responses to OT treatment and therefore urged caution against uniform application of OT for potential clinical treatment (Bartz *et al.*, 2011, Olff *et al.*, 2013, Rilling *et al.*, 2014). In this regard, the current study is the first to reveal the contribution of sex and *OXTR* genetic variation to individual differences in OT effects on brain function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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(a)

Subject

	Cooperate	Defect
Cooperate	**$2**($2)	**$3**($0)
Defect	**$0**($3)	**$1**($1)

Partner

(b)

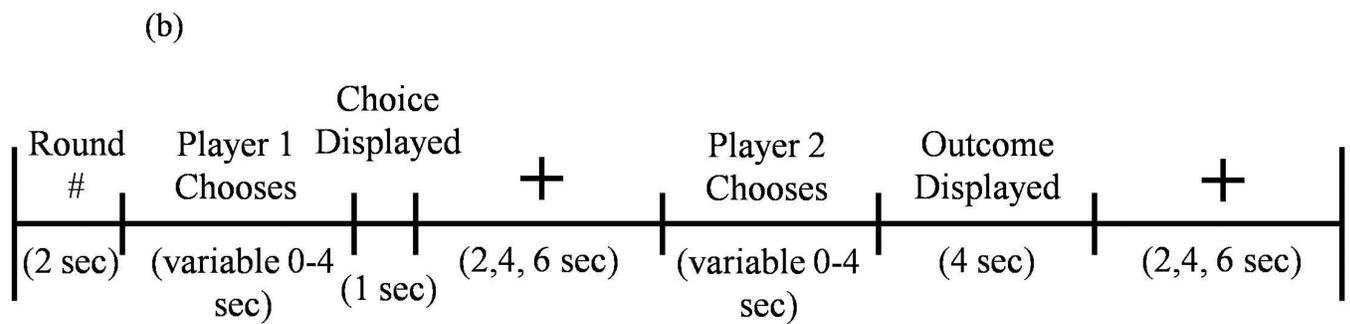| Round #	Player 1 Chooses	Choice Displayed	+	Player 2 Chooses	Outcome Displayed	+
(2 sec)	(variable 0-4 sec)	(1 sec)	(2,4, 6 sec)	(variable 0-4 sec)	(4 sec)	(2,4, 6 sec)

Figure 1.
Task design. (a) Payoff matrix used for the four outcomes in the Prisoner's Dilemma Game. Subjects' choices (C or D) are listed atop columns and partner's choices (C or D) are listed aside rows. Dollar amounts in bold are awarded to subjects. Amounts in parentheses are awarded to partners. (b) Time course of a single round of the Prisoner's Dilemma Game.
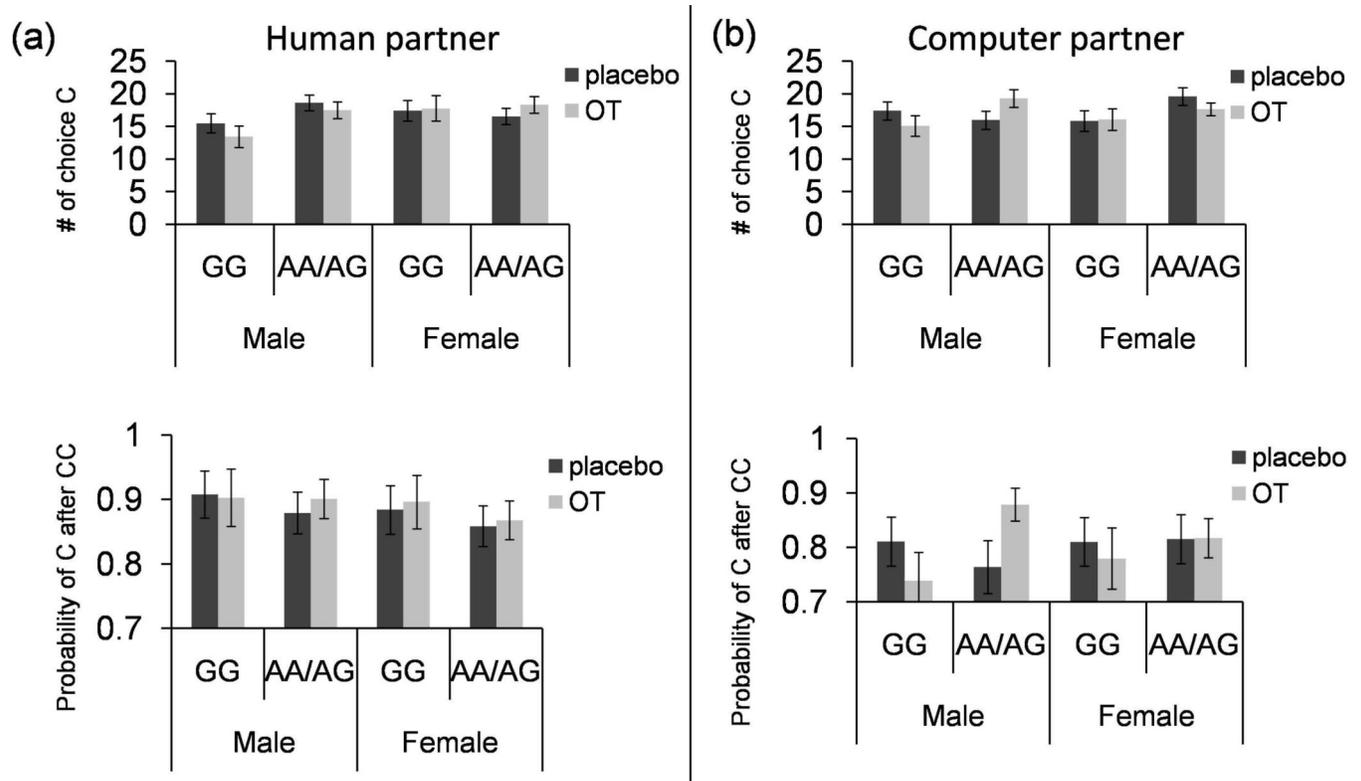
Figure 2.
Behavioral results. (a) Frequency of cooperation choice and probability of cooperating after an outcome of reciprocated cooperation (CC outcome) with human partners as player 1, as a function of drug treatment, sex and genotype (male: GG homozygotes with placebo treatment, n=21; GG homozygotes with oxytocin treatment, n=14; A carriers with placebo treatment, n=26; A carriers with oxytocin treatment, n=30; female: GG homozygotes with placebo treatment, n=20; GG homozygotes with oxytocin treatment, n=16; A carriers with placebo treatment, n=28; A carriers with oxytocin treatment, n=32). (b) Frequency of cooperation choice and probability of cooperating after an outcome of reciprocated cooperation with computer partners as player 1, as a function of drug treatment, sex and genotype (male: GG homozygotes with placebo treatment, n=22; GG homozygotes with oxytocin treatment, n=17; A carriers with placebo treatment, n=25; A carriers with oxytocin treatment, n=28; female: GG homozygotes with placebo treatment, n=19; GG homozygotes with oxytocin treatment, n=16; A carriers with placebo treatment, n=28; A carriers with oxytocin treatment, n=32). OT=oxytocin. Error bars indicate one standard error.
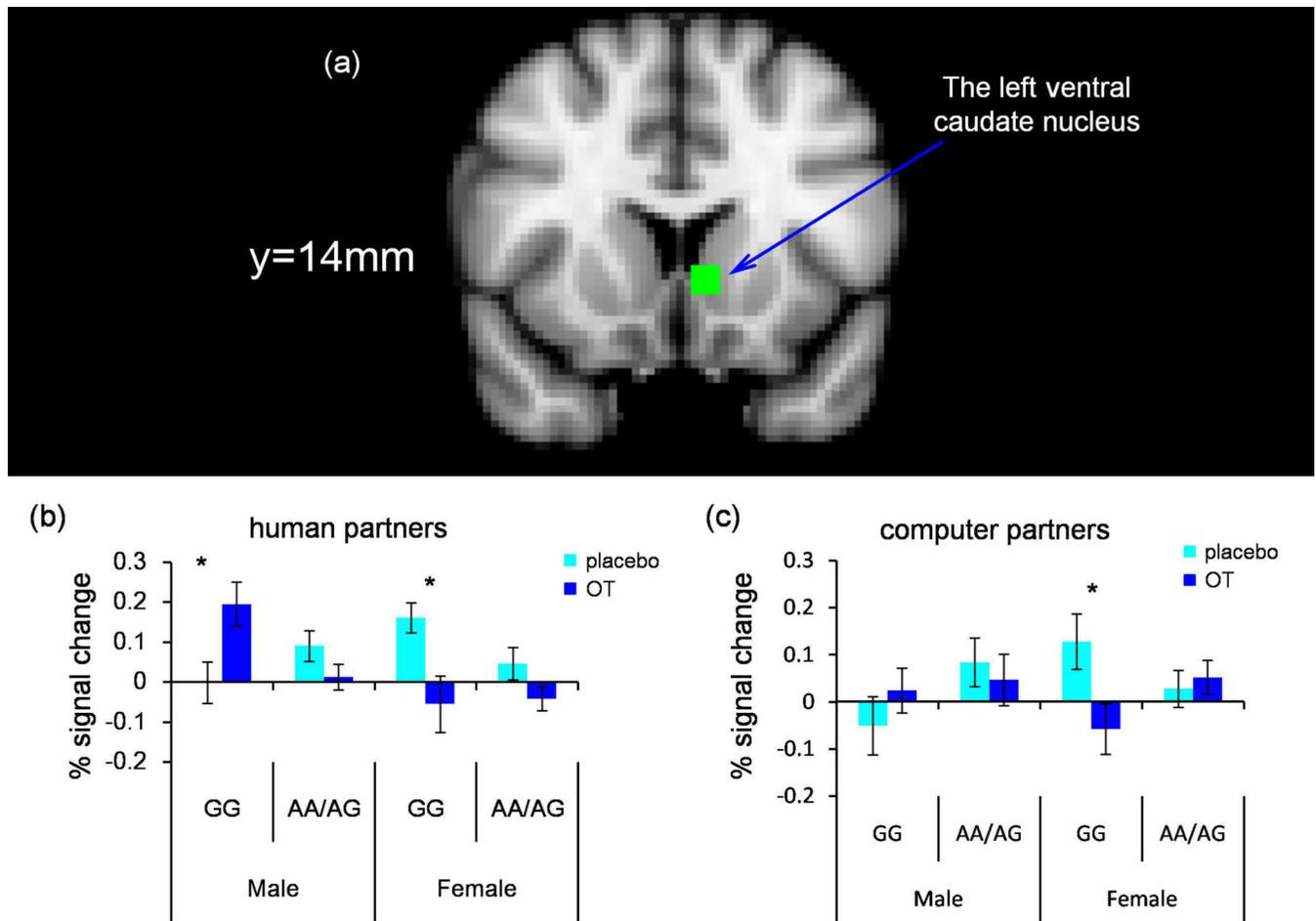
Figure 3.
Left ventral caudate nucleus response to reciprocated cooperation (CC outcomes). (a) ROI in the left ventral caudate nucleus, (b) left ventral caudate nucleus % signal change in response to CC outcomes with human partners as a function of drug treatment, sex and genotype (male: GG homozygotes with placebo treatment, n=21; GG homozygotes with oxytocin treatment, n=13; A carriers with placebo treatment, n=23; A carriers with oxytocin treatment, n=30; female: GG homozygotes with placebo treatment, n=18; GG homozygotes with oxytocin treatment, n=14; A carriers with placebo treatment, n=28; A carriers with oxytocin treatment, n=31), (c) left ventral caudate nucleus % signal change in response to CC outcomes with computer partners as a function of drug treatment, sex and genotype (male: GG homozygotes with placebo treatment, n=22; GG homozygotes with oxytocin treatment, n=16; A carriers with placebo treatment, n=22; A carriers with oxytocin treatment, n=27; female: GG homozygotes with placebo treatment, n=18; GG homozygotes with oxytocin treatment, n=15; A carriers with placebo treatment, n=28; A carriers with oxytocin treatment, n=30). OT=oxytocin, *p<0.05. Error bars indicate one standard error.

Table 1

OXTR SNP (rs53576) genotype frequency in each sub-group of subjects.

Group	With human partner			With computer partner		
	mAF	Frequency (GG/AG/AA)	p-HWE	mAF	Frequency (GG/AG/AA)	p-HWE
Male with PBO treatment	0.307	21/19/4	1.00	0.284	22/19/3	1.00
Male with OT treatment	0.476	13/18/11	0.36	0.430	16/17/10	0.22
Female with PBO treatment	0.369	18/22/6	1.00	0.369	18/22/6	1.00
Female with OT treatment	0.489	14/18/13	0.23	0.467	15/18/12	0.23
Total	0.410	66/77/34	0.21	0.388	71/76/31	0.20

OXTR=oxytocin receptor gene; SNP=single nucleotide polymorphism; PBO=placebo; OT=oxytocin; mAF=minor allele frequency; p-HWE=p-value of Hardy-Weinberg equilibrium.

Table 2

Modulation of sex and genotype on effects of drug treatment in ventral caudate nucleus response to reciprocated cooperation with human and computer partners.

Effect	Ventral Caudate ROI with human partner			Ventral Caudate ROI with computer partner		
	DF	F	P	DF	F	P
Drug	1,167	2.47	0.12	1,168	0.58	0.45
Sex	1,167	2.54	0.11	1,168	0.15	0.70
Genotype	1,167	3.17	0.08	1,168	1.70	0.19
Drug × Sex	1,167	**11.82**	**0.001**	1,168	1.76	0.19
Drug × Genotype	1,167	1.49	0.22	1,168	0.55	0.46
Sex × Genotype	1,167	0.02	0.89	1,168	0.85	0.36
Drug × Sex × Genotype	1,167	**10.39**	**0.002**	1,168	**5.09**	**0.025**