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Journal Title: Psychoneuroendocrinology
Volume: Volume 78
Publisher: Elsevier | 2017-04-01, Pages 22-30
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1016/j.psyneuen.2017.01.006
Permanent URL: <https://pid.emory.edu/ark:/25593/s8x3j>

Final published version: <http://dx.doi.org/10.1016/j.psyneuen.2017.01.006>

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Accessed November 14, 2019 9:47 PM EST



Published in final edited form as:

Psychoneuroendocrinology. 2017 April ; 78: 22–30. doi:10.1016/j.psyneuen.2017.01.006.

Within vs. between-subject effects of intranasal oxytocin on the neural response to cooperative and non-cooperative social interactions

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Abstract

The neuropeptide oxytocin (OT) plays a critical role in modulating social behavior across a wide range of vertebrate species. In humans, intranasal oxytocin (INOT) has been shown to modulate various aspects of social behavior, such as empathy, trust, in-group preference, and memory of socially relevant cues. Most INOT studies employ cross-sectional designs despite the enhanced statistical power and reduction in error variance associated with individual differences characteristic of within-subject designs. Using the Prisoner Dilemma task, which models a real-life dyadic social interaction, our group has systematically explored the effect of INOT on social cooperation and non-cooperation using a cross-sectional design. In the current study, we investigated if the main findings from our cross-sectional study could be replicated in a within-subject design using the same paradigm and whether new findings would emerge. We found OT to attenuate the ventral tegmental area response to reciprocated cooperation in women, an effect that is also present in our cross-sectional sample. However, other cross-sectional findings, especially those found in men, were not observed in this within-subject study. We hypothesize that the discrepancy can be explained by differing OT effects based on the degree of stimulus novelty/familiarity. Our within-subject study also revealed new effects not found previously in our cross-sectional study. Most importantly, OT treatment on scan 2 blocked amygdala habituation to unreciprocated cooperation found in a group that received placebo on both scans among men. Our results suggest that exogenous OT reduces the salience of positive social interactions among women and prevents habituation to negative social interactions among men. These findings may have implications for the potential clinical utility of OT as a treatment for psychiatric disorders.

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Keywords

Oxytocin; Ventral tegmental area (VTA); Within-subject design; Social cooperation; Functional magnetic resonance imaging (fMRI); Prisoner dilemma

1. Introduction

The neuropeptide oxytocin (OT) plays a critical role in modulating social behavior across a wide range of vertebrate species (Goodson and Thompson, 2010). In humans, oxytocin has been shown to modulate various aspects of social behavior, such as empathy (Shamay-Tsoory et al., 2013), trust (Baumgartner et al., 2008), in-group preference (De Dreu et al., 2010), and memory of socially relevant cues (Domes et al., 2007a; Gamer et al., 2010; Groppe et al., 2013).

Most intranasal oxytocin (INOT) studies employ cross-sectional designs despite the enhanced statistical power and reduction in error variance associated with individual differences characteristic of within-subject designs. However, a small number of within-subject, longitudinal studies have been published. In one study, INOT was found to attenuate amygdala responses to emotional faces regardless of valence (Domes et al., 2007a). In another, INOT improved “mind-reading” (Domes et al., 2007b). A third study found INOT to impair memory for social and non-social visual objects (Herzmann et al., 2012). A fourth study showed that INOT attenuated empathy-related activation within the neural circuitry of pain (Bos et al., 2015). Finally, INOT was found to enhance paternal sensitivity and decrease hostility in interactions with both typically developing children (Naber et al., 2010), as well as children with autism spectrum disorder (Naber et al., 2013).

Using the Prisoner Dilemma (PD) task, which models a real-life dyadic social interaction, our group has systematically explored the effect of INOT on social cooperation and non-cooperation. Relative to placebo, INOT increases the caudate nucleus BOLD response to reciprocated cooperation (a social interaction with positive valence) in men (Feng et al., 2015a). In women, however, INOT decreases the caudate nucleus response to the same stimulus, providing direct evidence for a sex difference in INOT effects on the neural response to positive social interactions. INOT was also shown to attenuate the amygdala and anterior insula response to unreciprocated cooperation (a social interaction with negative valence) in men, but not women (Chen et al., 2016). Finally, we found that the above mentioned INOT augmentation of the caudate response to reciprocated cooperation in men was restricted to those carrying the GG genotype at snp rs53576 of *OXTR* (Feng et al., 2015b). All of our above findings are based on cross-sectional, between-subject designs.

In the current study, we investigated if the main findings from our cross-sectional study could be replicated in a within-subject design using the same paradigm. This is, to our knowledge, the first double-blind, placebo-controlled, within-subject fMRI study that systematically explored the effect of INOT on social cooperation in the both men and women. A potential weakness of within-subject designs is that subjects may habituate to the task upon repeated exposure, attenuating brain activation at time 2 relative to time 1. This concern is usually addressed by counterbalancing the order of treatment and placebo (PBO)

administration so that half of the participants receive treatment on scan 1 and placebo on scan 2 (OT-PBO) and the other half receive the opposite (PBO-OT). Any significant effects of the treatment in the combined sample cannot be attributed to habituation. However, OT has well-known learning effects (Modi and Young, 2012; Sarnyai and Kovacs, 2014), raising the possibility that OT treatment at scan 1 could have effects on brain activation in the placebo group at time 2, rendering scan 2 an inappropriate baseline. To address this possibility, we include a third group that received placebo on both scans (PBO-PBO). PBO-PBO and OT-PBO groups can be compared at scan 2 to identify OT effects at scan 1 that persist to scan 2 (learning effects), controlling for habituation effects. If learning effects are not observed, then OT-PBO and PBO-OT groups can be combined for increased power to detect OT effects, controlling for habituation. If learning effects are found, then PBO-PBO can be compared with PBO-OT to isolate OT effects in the absence of learning effects and controlling for habituation. However, even without learning effects, this comparison could reveal any effects of OT on habituation responses.

We find no evidence of OT learning effects on brain activation in response to positive or negative social interactions, allowing us to pool OT-PBO and PBO-OT groups. This revealed OT attenuation of the ventral tegmental area (VTA) response to reciprocated cooperation in women, an effect that is also present in our cross-sectional sample. However, other cross-sectional findings, especially those found in men, did not replicate in this within-subject design. On the other hand, the within-subject design revealed some new effects not found previously in our cross-sectional study. Most importantly, OT treatment on scan 2 blocked amygdala habituation to CD outcomes found in the PBO-PBO group among men.

2. Methods

2.1. Subjects

Forty-five men and forty-five women from the Emory University community between the ages of 18 and 22 (mean (SD) = 20.5 (1.4) years for women, and 20.9 (1.6) for men), were randomized to three treatments: 14 men/15 women received 24 IU intranasal OT on session 1 and placebo on session 2 (OT-PBO), 15 men/15 women received placebo on session 1 and 24 IU intranasal OT on session 2 (PBO-OT), and 16 men/15 women received placebo on both sessions 1 and 2 (PBO-PBO). For each subject, we attempted to collect both sessions within a 2-week time period (mean (SD) = 9.4 (4.3) days, min = 1 day and max = 17 days). There was no difference in scan interval among the three treatment groups ($F(2,41) = 1.36$, $p = 0.27$ for men; $F(2,42) = 0.24$, $p = 0.79$ for women). Randomization was performed by the Emory University Investigational Drug Service (IDS) using Research Randomizer (<http://www.randomizer.org>).

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. Four male and six female subjects were excluded from the neuroimaging analysis for either one or both sessions for a variety of reasons: 1) excessive motion (>1.5 mm) (1 female OT-PBO and 1 female PBO-PBO); 2) missing data (2 male PBO-PBOs and 1 female PBO-PBO had missing behavioral data, 1 male OT-PBO had missing neuroimaging data); and 3) lacking outcomes of interest (CChuman or CDhuman) (1 male PBO-PBO, 3 female PBO-PBOs).

The resulting sample size for analysis was CChuman (PBO-OT: 15 men, 15 women; PBO-PBO: 13 men, 10 women; OT-PBO: 13 men, 14 women) and CDhuman (PBO-OT: 15 men, 15 women; PBO-PBO: 13 men, 11 women; OT-PBO: 13 men, 14 women). Note that for 22 men and 35 women in the current within-subject cohort, scan 1 was analyzed in both the previous cross-sectional and the current within-subject study. Thus, the two samples are not completely independent.

Preparation of study medication and details of randomization were maintained by the Emory IDS and all study personnel were blind to group assignment. Administration of 24 IU oxytocin was generally safe. None of the subjects developed any major side effects of the study medication, including anaphylaxis.

2.2. Prisoner dilemma (PD) 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 we use here 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 own choice. Each of the four outcomes is associated with a different payoff. 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 (see Fig. S1A for the pay-off matrix). All subjects first completed a PD tutorial and two practice trials. We aimed to start both the task and fMRI scan at 40 min after drug administration. In actuality, this time period averaged 42 min across subjects. Prior to the start of each game, the visual display inside the scanner showed a picture of the partner the subject was about to play the game with. While being scanned with fMRI, subjects played 30 rounds of a sequential-choice, iterated PD game in four separate runs. For two runs, subjects were told they were playing with a human partner (a same sex confederate that was introduced to the subject prior to the experiment). For the other two runs, subjects were told they were playing with a computer partner. In actuality, subjects were always playing with a pre-programmed computer algorithm described in (Rilling et al., 2012). For both human and computer partners, in one of the two runs, subjects played in the role of first mover (player 1) and their partner played in the role of second mover (player 2). In the other run, roles were reversed. The order of human and computer runs was counterbalanced across subjects so that half of the subjects were scanned in the order: player 1 with human partner (H1), player 2 with human partner (H2), player 1 with computer partner (C1), player 2 with computer partner (C2), and the other half were scanned in the order: C1, C2, H1, H2. In this paper, we restrict our analyses to the player 1 data with human partners. After scanning, subjects were asked several questions about their experience during the PD game. Subjects were compensated with a total of approximately \$120; the exact amount was obtained by multiplying the total earnings across both runs by 2/3.

A timeline for a single PD trial is depicted in Fig. S1B. At the beginning of each round, the round number and partner's photo were displayed for 2 s. Player 1 then had 4 s to choose to cooperate or defect. Players were informed that if they did not decide within this 4 s interval, their response would default to defection. Player 1's choice was immediately revealed to player 2 and displayed for 1 s. A variable length fixation epoch of either 2, 4 or 6 s followed. Afterwards, player 2 had 4 s to cooperate or defect. Once player 2 decided, the outcome of the round was displayed for 4 s. Finally, the trial concluded with another variable length fixation epoch of either 2, 4 or 6 s. Trials were approximately 20 s long. Five null trials were interspersed among 30 PD trials in each run. Null trials consisted of 14 s of fixation. One run lasted approximately 12 min. All four runs lasted about 48 min.

2.3. Behavioral measures and emotion ratings

Given the aim of the current study was to examine OT effects on reciprocated cooperation and unreciprocated cooperation, three relevant behavioral measures were examined for each subject in each run: the total number of cooperate choices (#C), the probability of cooperating in the current round following reciprocated cooperation in the previous round (pC/CC), and the probability of cooperating in the current round following unreciprocated cooperation in the previous round (pC/CD). For each behavioral variable, three different analyses were conducted. (1) To examine a potential learning effect of OT on the behavior, the second scans of the PBO-PBO and OT-PBO groups were compared with a two-sample *t*-test; (2) In the absence of learning effects, paired *t*-tests were used to compare the OT and PBO treatment after combining the PBO-OT and OT-PBO groups; (3) If analysis 1 did reveal learning effects, then PBO-OT and PBO-PBO groups were analyzed with a two-way repeated-measures ANOVA to examine the within-subject effect of OT in the absence of OT learning effects and controlling for habituation effects. An OT effect is indicated by a significant interaction term. Even in the absence of learning effects as revealed by analysis 1, this model (analysis 3) was used to reveal potential OT effects on habituation responses. The above three analyses were done in IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY).

After each of the four 30-round runs, while still in the scanner, subjects rated their emotional reaction to the four PD game outcomes (CC, CD, DC, and DD). Seven-point Likert scales were used to rate the following emotions or feelings: afraid, angry, happy, guilty, disappointed and relieved. For reciprocated cooperation, a positive social interaction, we analyzed ratings of happy and relieved. For unreciprocated cooperation, a negative social interaction, we analyzed ratings of afraid, angry, and disappointed. Emotion ratings were analyzed as indicated above for the behavioral data.

2.4. Plasma estradiol assay

Estrogen is known to induce OT receptors (Champagne et al., 2001; Pedersen et al., 1994), which leads to the prediction that OT effects on brain activation should be more pronounced when estrogen levels are high. Plasma samples were assayed for estradiol in three separate batches by the Biomarkers Core Lab at Yerkes National Primate Research Center. Among all 45 women, thirty-four (34) subjects (9 OT-PBO, 12 PBO-PBO, and 13 PBO-OT) had valid estradiol measurements for both sessions. Fourteen subjects (9 OT-PBO, 2 PBO-PBO, and 3

PBO-OT) were assayed in batch 1, 11 subjects (5 PBO-PBO, 6 PBO-OT) were assayed in batch 2, and 9 subjects (5 PBO-PBO, 4 PBO-OT) were assayed in batch 3. Results from all 3 assay batches were pooled together for subsequent analysis. This is justified by strict calibration and quality control procedures across batches. Related samples Wilcoxon Signed Rank test was used to test for a difference in plasma estradiol level between scan 1 and scan 2 for each of the 3 batches separately and combined.

2.5. Neuroimaging data collection

A T1-weighted structural image and blood-oxygenation-level-dependent (BOLD) functional MRI images were collected from each subject using a 3 Tesla Siemens Trio MRI scanner (Siemens Medical System, Malvern, PA, USA).

High-resolution T1-weighted images were acquired using a 3D magnetization-prepared rapid gradient-echo (MPRAGE) sequence with a GRAPPA factor of 2. The T1 scan protocol, optimized for 3 Tesla, used the following imaging parameters: a repetition time/inversion time/echo time (TR/TI/TE) of 2600/900/3.02 ms, a flip angle of 8°, a volume of view of $256 \times 256 \times 176 \text{ mm}^3$, a matrix of $256 \times 256 \times 176$, and isotropic spatial resolution of $1.0 \times 1.0 \times 1.0 \text{ mm}^3$, one average. Total T1 scan time was approximately 5 min.

T2*-weighted images were collected using an Echo-Planar Imaging (EPI) sequence for BOLD fMRI. EPI images were collected in an interleaved fashion with the following imaging parameters selected to minimize susceptibility and distortion artifacts in the orbitofrontal cortex: TR = 2000 ms, TE = 28 ms, matrix = 64×64 , FOV = 224 mm, in-plane resolution 3.5 mm, slice thickness = 2.5 mm, and 34 axial slices with a gap of 1.05 mm in between. During fMRI scanning, subjects performed 30 rounds of a sequential-choice, iterated PD game with putative same sex human partners. The total fMRI scan time was approximately 48 min (12 min per run).

2.6. Neuroimaging data analysis

The analysis was conducted with the Oxford Center for Functional Magnetic Resonance Imaging of the Brain's software library (FSL, <http://www.fmrib.ox.ac.uk/fsl/>).

2.6.1. Individual level analysis—The preprocessing pipeline of the fMRI data involves (1) motion correction using the MCFLIRT (Jenkinson et al., 2002), (2) non-brain tissue removal using the BET (Smith, 2002), (3) slice timing correction, (4) high-pass temporal filtering with a cut-off of 100s, (5) spatially smoothing with a Gaussian kernel of full-width at half maximum (FWHM) of 5 mm, and (6) normalizing to MNI space via corresponding extracted T1 brain using Boundary-Based-Registration (Greve and Fischl, 2009).

The preprocessed fMRI data were analyzed using the general linear model (GLM) for univariate statistical analysis. For each individual subject in each run, a separate GLM was constructed to examine 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 game outcome was revealed. More specifically, the following regressors were defined for each run of each subject: (1) the beginning epoch when round number and the partner's face were 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. For each individual GLM, contrasts of CC and CD were specified to compare BOLD response to CC and CD respectively with baseline. The individual-level GLM was implemented using FILM (fMRIB's Improved Linear Model).

2.6.2. Group level analysis—Both Region of Interest (ROI) and whole brain analyses were conducted at the group level for each sex and each condition (CC or CD).

2.6.2.1. Region of interest (ROI) analysis: For each condition, we defined ROIs based on our previous cross-sectional findings (Chen et al., 2016; Feng et al., 2015a) to determine if the current within-subject data showed similar effects. These ROIs were defined as a $10 \times 10 \times 10 \text{ mm}^3$ cube centered at the local maxima of the activation within a particular brain structure.

In men, a-priori ROIs were defined as the regions of the nucleus accumbens and caudate nucleus activated in the contrast CC (OT > PBO) (Family-wise error(FWE)-corrected cluster $p < 0.05$ with voxel-wise $p < 0.05$). For the response to CD, a priori ROIs for men were based on the male cross-sectional findings of CD (OT < PBO) (FWE corrected cluster $p < 0.05$ with voxel-wise $p < 0.05$) and include the anterior insula and the amygdala.

The a-priori ROIs for women are based on the female cross-sectional findings of CC (OT < PBO) (FWE corrected cluster $p < 0.05$ with voxel-wise $p < 0.005$) and include, left hippocampus, posterior cingulate cortex (PCC), right ventral lateral prefrontal cortex (VLPFC), posterior cingulate gyrus, rostral anterior cingulate cortex (ACC), postcentral cortex, supplementary motor area (SMA, precentral), thalamus, posterior insula, and superior temporal sulcus/middle temporal gyrus (STS/MTG). For CD outcomes, no a-priori ROIs were defined for women because no significant activation was found in the cross-sectional study.

For each ROI, the average BOLD signal was analyzed with 3 different models. (1) To examine a potential learning effect of OT on the neural response to reciprocated and unreciprocated cooperation (CC/CD), the 2nd scans of the PBO-PBO and OT-PBO groups were compared with a 2-sample t -test; (2) If no learning effects were detected in analysis 1, then paired t -tests were used to compare the OT and PBO groups after combining the PBO-OT and OT-PBO groups to examine the within-subject effect of OT under the assumption of no OT learning effects and counterbalancing habituation effects; (3) If analysis 1 did reveal learning effects, then PBO-OT and PBO-PBO groups were analyzed with a two-way repeated-measures ANOVA to examine the within-subject effect of OT in the absence of OT learning effects and controlling for habituation effects. An OT effect is indicated by a significant interaction term. Finally, even in the absence of learning effects as revealed by analysis 1, this model was used to reveal potential OT effects on habituation responses.

2.6.2.2. Whole brain analysis: The same three above models were also tested in whole brain analyses. An Ordinary Least Square (OLS) algorithm was used for the beta value (parameter) estimation in FEAT. The resultant Z statistic (*Gaussianized t*) images were thresholded using clusters determined by $Z > 3.1$ (voxel-wise 1-tailed $p < 0.001$), and a

family-wise error (FWE)–corrected cluster significance threshold of $p < 0.05$ was applied to the suprathreshold clusters. Functional ROIs were specified on the activation maps and applied to the larger cross-sectional cohort to see if the findings from the two studies replicated. There is some overlap in the sample between the cross-sectional and our current within-subject studies. To provide a completely independent test, we also re-ran this analysis after excluding subjects from the cross-sectional sample who were also in the within-subject sample.

To explore the potential modulation by estradiol of OT effects on the neural response to CC and CD in women, for the second group-level neuroimage analysis specified above, we examined the correlation between plasma estradiol levels (after OT administration) and the magnitude of OT-induced effects on brain activation. We also employed a repeated measure ANCOVA model (which is mathematically an expansion of the second group-level neuroimage analysis specified above by including estradiol for OT and PBO as separate covariates) to see if plasma estradiol level modulates OT effects in any brain region.

All statistical tests were implemented in IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY).

Other methodological details that are not directly related to the theme of this manuscript, such as the preparation and the administration of drugs, monitoring of vital signs, *Positive and Negative Affect Schedule* (PANAS) ratings, and confederate introductions, are described in (Rilling et al., 2012).

3. Results

3.1. Behavior

3.1.1. Between-subject learning effects of OT—Two sample *t*-tests revealed no significant group difference in any of the three behavioral measures between the 2nd scans in the PBO-PBO and OT-PBO groups in either men or women.

3.1.2. Within-subject effects of OT, assuming no learning effects and counterbalancing habituation effects (OT-PBO + PBO-OT)—For both men and women, OT had no effect on the number of cooperate choices, the behavioral response to reciprocated cooperation in the previous round (the probability of cooperating after a CC outcome), or the behavioral response to unreciprocated cooperation in the previous round (the probability of cooperating after a CD outcome). All $p > 0.05$.

3.1.3. Within-subject effects of OT, in the absence of OT learning effects and controlling for habituation effects (PBO-OT vs. PBO-PBO)—After multiple comparison correction, there was no significant interaction between scan number (1st vs. 2nd scan) and drug treatment (OT vs. PBO) for any of the three behavioral measures (# of C choices, p C/CC, p C/CD) in either men or women.

3.2. Emotion ratings

3.2.1. Between-subject learning effects of OT—After multiple comparison correction, there was no significant difference on any emotion ratings in response to either CC or CD outcomes between the 2nd scans in the PBO-PBO and OT-PBO groups, in either men or women.

3.2.2. Within-subject effects of OT, assuming no learning effects and counterbalancing habituation effects (OT-PBO + PBO-OT)—OT treatment did not alter any emotion ratings in either men or women. All $p > 0.05$.

3.2.3. Within-subject effects of OT, in the absence of OT learning effects and controlling for habituation effects (PBO-OT vs. PBO-PBO)—After multiple comparison correction, there was no significant interaction between scan number (1st vs. 2nd scan) and treatment (OT vs. PBO) on any emotion ratings in response to either CC or CD outcomes, in either men or women.

3.3. Plasma estradiol concentration in females

Nine subjects from the OT-PBO group, 12 subjects from the PBO-PBO group, and 13 subjects from the PBO-OT group had valid plasma estradiol measurements for both sessions. There was no significant difference in plasma estradiol level between scan 1 and scan 2 for each of the three categories separately and for all three combined. All $p > 0.05$.

3.4. Neuroimaging

3.4.1. ROI analysis

3.4.1.1. Reciprocated cooperation (CC): Men: In our previous cross-sectional study, OT augmented the BOLD response to CC outcomes in both the caudate nucleus and nucleus accumbens (NAcc). However, OT did not augment the BOLD response to CC outcomes in these regions in the current, within-subject study. There was no significant learning effect of OT on the neural response to reciprocated cooperation (CC) in either the caudate or NAcc. Therefore, we combined the OT-PBO and PBO-OT groups, but found no significant effect of OT compared with PBO ($t(27) = -0.36$, $p = 0.72$ for caudate; $t(27) = -1.28$, $p = 0.21$ for NAcc). Finally, to test for effects of OT on habituation responses, we compared PBO-OT with PBO-PBO and found no significant interaction between scan number (1st vs. 2nd scan) and drug treatment (OT vs. PBO) ($F(1,26) = 0.76$, $p = 0.39$ for caudate; $F(1,26) = 0.05$, $p = 0.82$ for NAcc). Recently we found that OT effects on the neural response to social cooperation in the caudate was specific to the rs53576 GG genotype. Hence we also limited the ROI analysis to men with rs53576 GG, but again found no effects. (Fig. S2).

Women: In our previous cross-sectional study, OT attenuated the BOLD response to CC outcomes in left hippocampus, left STS/MTG, posterior cingulate cortex (PCC), post central cortex, supplementary motor cortex (SMA, precentral), thalamus, (posterior) insular, right ventral lateral prefrontal cortex (VLPFC), posterior cingulate cortex and rostral ACC (corrected $p < 0.05$ based on the cluster-extent with voxel-wise $p < 0.005$). These regions were defined as a-priori ROIs. There were no significant learning effects of OT on the neural response to reciprocated cooperation (CC) in any of the 10 ROIs. However, combining the

OT-PBO and PBO-OT groups, OT significantly attenuated the BOLD response to CC in VLPFC ($t(28) = 2.58, p < 0.05$), rostral ACC ($t(28) = 2.26, p = 0.03$), posterior insula ($t(28) = 2.06, p < 0.05$), and post central gyrus ($t(28) = 2.38, p = 0.02$). (Figs. 1 & S3). Finally, comparing PBO-OT with PBO-PBO revealed no significant interaction between scan number (1st vs. 2nd scan) and drug treatment (OT vs. PBO) in any of the 10 ROIs.

3.4.1.2. Unreciprocated cooperation (CD): Men: In our previous cross-sectional study, OT attenuated the BOLD response to CD outcomes in the amygdala and anterior insula. In the present study, OT did not have any significant learning effect on the neural response to unreciprocated cooperation (CD) in either the amygdala or the anterior insula. Combining the OT-PBO and PBO-OT groups, OT had no significant effect on the response to CD in either the amygdala or the anterior insula. Finally, comparing PBO-OT with PBO-PBO, there was a significant interaction between scan number (1st vs. 2nd scan) and drug treatment (OT vs. PBO) in the amygdala ($F(1,26) = 9.85, p = 0.004$) but not the anterior insula (Fig. 2). However, post-hoc comparisons did not find OT to decrease the amygdala response as expected based on our cross-sectional data. Instead, there was a significant amygdala habituation effect for the PBO-PBO group ($t(12) = -2.88, p = 0.01$) that was not present in the PBO-OT group ($t(14) = 1.36, p = 0.20$). Thus, OT prevented amygdala habituation to CD outcomes in men.

Women: No ROI was specified because no activation was found in our previous cross-sectional study.

3.4.2. Whole brain analysis

3.4.2.1. Between-subject learning effects of OT: For both CC and CD outcomes in both men and women, there was no significant group difference over the 2nd scans between the PBO-PBO and OT-PBO groups in any brain region.

3.4.2.2. Within-subject effects of OT, assuming no learning effects and counterbalancing habituation effects (OT-PBO + PBO-OT): In men, OT treatment had no effect on activation to CC outcomes in any brain region. In women, however, OT attenuated the BOLD response to reciprocated cooperation in the ventral tegmental area (VTA) (Fig. 3A) (peak: MNI $x = 6$ mm, $y = -26$ mm, $z = -18$ mm, $Z = 4.39$), and VTA was the only brain region affected by OT. Average BOLD signal change in VTA (mean \pm s.e.m.) is illustrated in Fig. 3B as a function of order of drug administration, which shows that the effect of OT was independent of the order of drug administration (Fig. 3B).

To determine if this effect of OT on VTA activation was also present in our cross-sectional sample at a statistical threshold below what we previously reported, the VTA activation was defined as a functional ROI and applied to our previous cross-sectional data (PBO, $n = 46$; OT, $n = 47$) (Rilling et al., 2014). Within the cross-sectional sample, the VTA BOLD signal of the OT group was significantly lower than that of the PBO group ($p < 0.005$) (Fig. 3C). However, there is overlap in the sample from the two studies. To provide a completely independent test of this effect, we also re-ran this analysis after excluding subjects from the cross-sectional sample who were also in the within-subject sample. When this is done, the

OT group ($n = 33$) still has significantly less VTA activation relative to the placebo group ($n = 36$) ($p < 0.05$) (Fig. 3D).

The magnitude of the OT effect in the VTA was significantly ($r = -0.49$, $p = 0.007$) negatively correlated with the VTA response to CC outcomes in the placebo condition (Fig. 4). Thus, subjects with a stronger VTA response to CC outcomes at baseline experienced the largest OT-induced decrease in VTA response to CC outcomes.

Finally, for both men and women, OT treatment did not significantly influence activation to CD outcomes in any brain region.

3.4.2.3. Within-subject effects of OT, in the absence of OT learning effects and controlling for habituation effects (PBO-OT vs. PBO-PBO): For both CC and CD outcomes in both men and women, there was no significant interaction between scan number (1st vs. 2nd scan) and drug treatment (OT vs. PBO) in any brain region.

3.4.2.4. Estradiol modulation of OT effect on brain activity: Within the VTA functional ROI, there was no significant correlation between plasma estradiol levels and the magnitude of OT-induced attenuation of VTA activation to CC outcomes ($r = -0.25$, $p = 0.27$). In addition, estradiol did not significantly modulate OT effects on the response to either CC or CD outcomes in a whole brain ANOVA analysis.

4. Discussion

The goal of the current study was to determine if our previous cross-sectional findings of OT effects on brain function during dyadic social interactions could be replicated and extended in a statistically more powerful within-subject design using the same paradigm. Two of our previous findings did not replicate. These include (1) INOT augmented the caudate nucleus response to reciprocated cooperation (CC) in men and attenuated the caudate nucleus response to reciprocated cooperation (CC) in women (Feng et al., 2015a), and (2) INOT attenuated the amygdala and anterior insula response to unreciprocated cooperation (CD) in men but not women (Chen et al., 2016). However, we did identify several regions in which OT attenuated activation to CC outcomes among women in both studies, including VLPFC, rostral ACC, post central gyrus, posterior insula, and most prominently the VTA. We also found that the baseline VTA response to reciprocated cooperation in the PBO condition predicted the strength of the OT attenuation effect in VTA. However, it is possible that this effect represents regression to the mean rather than an effect of OT. The current study also revealed an effect not previously detected in the cross-sectional study. OT prevented amygdala habituation to CD outcomes in men.

Consistent with our previous cross-sectional study (Feng et al., 2015a), our current within-subject study reveals no effect of OT on cooperative behavior after correction for multiple comparisons. Some previous studies have found INOT to increase cooperative behavior, however these effects depend on the participant's attachment style (Bartz et al., 2011). For example, intranasal OT increased cooperation among men who were high rather than low in attachment avoidance (De Dreu, 2012b). We did not characterize the attachment style of the

participants in our study, so we are unable to evaluate the potential influence of attachment style on neural and behavioral responsiveness to OT.

Our most robust neuroimaging finding in this study is that OT attenuates the BOLD response to CC outcomes in the VTA among women. Dopamine neurons in the VTA track both reward and reward prediction errors as well as motivational salience (Schultz, 2013). A human fMRI study similarly implicates the VTA in these functions (Bunzeck and Duzel, 2006). Thus, INOT may be decreasing the reward or salience of CC outcomes among women. These findings parallel results from hamsters, in which OT administration in the VTA has been shown to decrease social reward in females, while increasing social reward in males (Borland et al., 2016). In contrast to our findings, Groppe et al. (Groppe et al., 2013) found OT to significantly enhance VTA activation in response to cues signaling social reward or social punishment among women, and Gregory et al. (Gregory et al., 2015) found OT to increase the VTA response to infant and sexual stimuli in nulliparous and postpartum women. The discrepancy may be attributable to the use of different stimuli or to different experimental designs. While Groppe et al. focused on the response to cues that predicted friendly or angry faces, our study focuses on the reaction to positive and negative dyadic social interactions. Moreover, of the three studies, only ours utilized a within-subject design. Finally, it is worth noting that the statistical thresholds applied in the current study are more stringent (whole brain FWE-corrected cluster $p < 0.05$ with voxel-wise $p < 0.001$) than those used in the other two studies.

In men, the amygdala habituated to CD outcomes from scan 1 to scan 2 in the PBO-PBO group. However, OT treatment at scan 2 prevented this habituation, suggesting that INOT sustains the salience of negative social interactions among men. We previously reported that OT treatment attenuated amygdala activation at scan 1 in a cross-sectional design (Chen et al., 2016). This suggests that OT has opposing effects on amygdala activation at scan 1 vs. scan 2. One possible explanation for these opposing effects is that OT effects on amygdala activation depend on the degree of novelty/familiarity with the stimulus. Another possibility is that the partner is re-conceptualized from an ingroup to an outgroup member across the two scans and that OT elicits different effects depending on the ingroup-outgroup perception of the partner, as previously reported (De Dreu, 2012a; De Dreu et al., 2010). The putative human partner is actually a computer algorithm that fails to reciprocate the player's cooperation one-third of the time. After the game, participants rate that partner significantly lower on the question "how good of a friend would this partner be?" compared with a different partner they face as player 2 who always reciprocates cooperation (men scan 1: player 1 = 4.9, player 2 = 8.0, $t(44) = -9.7$, $p < 0.001$; men scan 2: player 1 = 4.8, player 2 = 7.9, $t(44) = -9.0$, $p < 0.001$). Thus, it is possible that the partner is initially conceptualized as an ingroup member but comes to be conceptualized as an outgroup member by virtue of their limited cooperation during scan 1. This would imply that OT decreases the salience of negative social interactions with ingroup members while increasing the salience of negative social interactions with outgroup members.

In addition to OT not attenuating amygdala activation to CD outcomes in men, other findings from our cross-sectional study did not replicate in this within-subject study. These include OT attenuation of anterior insula activation to CD outcomes in men, OT

augmentation of the caudate nucleus response to CC outcomes in men, and OT attenuation of the caudate nucleus response to CC outcomes in women. As mentioned above, one potential explanation for the discrepancy is that OT has different effects as stimuli become more familiar (i.e., at scan 2). Another possibility is that our within-subject design was not as well-powered as our cross-sectional study. However, further analysis would seem to rule out this possibility. For CC outcomes, in men, our cross-sectional sample size (42 OT, 48 PBO) provides 89.3% power to detect an effect of 0.13% BOLD signal change (Cohen's $d = 0.68$) within the caudate. For the same effect size, the within-subject study only requires a sample of 24 to obtain equal power. For CD outcomes, in men, our cross-sectional sample size (41 OT, 47 PBO) provides 97.5% power to detect an effect of 0.41% signal change (Cohen's $d = 0.85$) within the amygdala. It also provides 80% power to detect an effect of 0.16% signal change (Cohen's $d = 0.61$) within the anterior insula. For the same effect sizes, the within-subject study requires a sample of only 24 and 14 to achieve equivalent power. Because our current study employs an effective sample of 28 (13 OT-PBO, 15 PBO-OT), inadequate power cannot explain the unobserved findings. Another possible explanation for the lack of replication is that our initial results with the cross-sectional sample were false positives. This is more likely to be the case for OT attenuation of amygdala activation and OT augmentation of nucleus accumbens activation since these results are only present at a voxel-wise threshold of $p < 0.05$ along with a cluster threshold of $p < 0.05$ FWE corrected, which recent simulations suggest may not adequately control false positive rates (Eklund et al., 2016). On the other hand, we previously observed sex differences in OT modulation of caudate nucleus activation using a voxel-wise threshold of $p < 0.001$ along with a cluster threshold of $p < 0.05$ FWE corrected (Feng et al., 2015a) more consistent with recent recommendations (Eklund et al., 2016). Nevertheless, even the former results are consistent with known OT effects in rodents, in which OT decreases the firing frequency of central amygdala neurons (Huber et al., 2005) and synergizes with DA in the ventral striatum to promote social bonding (Numan, 2007; Ross and Young, 2009). Thus, we believe our initial cross-sectional findings are unlikely to be false positives, particularly for the caudate nucleus, and we therefore favor the explanation that OT effects vary as a function of stimulus familiarity.

Our findings also have potential clinical implications. Recently, there has been interest in OT as a possible treatment for anxiety disorders (Hurlemann and Scheele, 2016; Macdonald and Feifel, 2014; Meyer-Lindenberg et al., 2011; Striepens et al., 2011). Anxiety disorders are associated with amygdala hyperactivity (Etkin and Wager, 2007), which INOT can normalize (Labuschagne et al., 2010). Moreover, OT has anxiolytic effects in both humans and non-human animals (Heinrichs et al., 2003; Neumann, 2008). However, our results show that the effect of OT on amygdala responses to a social stressor in the form of unreciprocated cooperation are not straightforward. While OT attenuates amygdala activation at initial exposure to the unreciprocated cooperation (scan 1), it actually augments amygdala activation in a subsequent negative encounter with the same individual. This raises the possibility that OT could actually exacerbate fear and anxiety in some circumstances. Specifically, the fact that OT prevented habituation to a social stressor raises the possibility that OT may prevent extinction of some conditioned fear stimuli, which might impair recovery from PTSD. While some studies have found INOT to accelerate extinction of

conditioned fear in humans (Eckstein et al., 2015), others have found it to interfere with extinction (Acheson et al., 2015). Future studies should attempt to identify variables that can explain these contrasting effects on extinction of conditioned fears. We also found that INOT decreased the VTA response to reciprocated cooperation among women, suggesting that it decreases the salience of positive social interactions among women. This was our most statistically robust finding. Given that positive social interactions signal social support that has known stress-buffering effects (Jaremka et al., 2013; Lakey et al., 1994), these data suggest that OT could negatively impact women with depression and other anxiety disorders. Indeed, although some INOT clinical trials have shown symptom improvement (Guastella et al., 2009; Hall et al., 2012), one study found that INOT actually increased anxiety (MacDonald et al., 2013). Further research is needed to evaluate whether treating anxiety-disordered patients with oxytocin is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was made possible through the support of a grant from the John Templeton Foundation. The opinions expressed in this publication are those of the author(s) and do not necessarily reflect the views of the John Templeton Foundation. This study was also partially supported by NIMH Grant R01 MH084068-01A1 and the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR000454. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We thank Susan Rogers, Jianguo Xu and Larry Young for assistance with various aspects of this study.

References

- Acheson DT, Feifel D, Kamenski M, McKinney R, Risbrough VB. Intranasal oxytocin administration prior to exposure therapy for arachnophobia impedes treatment response. *Depress Anxiety*. 2015; 32:400–407. [PubMed: 25826649]
- Bartz JA, Zaki J, Bolger N, Ochsner KN. Social effects of oxytocin in humans: context and person matter. *Trends Cogn Sci*. 2011; 15:301–309. [PubMed: 21696997]
- Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E. Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. *Neuron*. 2008; 58:639–650. [PubMed: 18498743]
- Borland, JM., Aiani, L., Grantham, KES., Albers, HE. Oxytocin (OT) in the Ventral Tegmental Area (VTA) Has Sex Specific Effect on Social Reward in Syrian Hamsters. *Society for Behavioral Neuroendocrinology*; Montreal, QC: 2016.
- Bos PA, Montoya ER, Hermans EJ, Keyzers C, van Honk J. Oxytocin reduces neural activity in the pain circuitry when seeing pain in others. *NeuroImage*. 2015; 113:217–224. [PubMed: 25818690]
- Bunzeck N, Duzel E. Absolute coding of stimulus novelty in the human substantia nigra/VTA. *Neuron*. 2006; 51:369–379. [PubMed: 16880131]
- Champagne F, Diorio J, Sharma S, Meaney MJ. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proc Natl Acad Sci U S A*. 2001; 98:12736–12741. [PubMed: 11606726]
- Chen X, Hackett PD, DeMarco AC, Feng C, Stair S, Haroon E, Ditzen B, Pagnoni G, Rilling JK. Effects of oxytocin and vasopressin on the neural response to unreciprocated cooperation within brain regions involved in stress and anxiety in men and women. *Brain Imaging Behav*. 2016; 10:581–593. [PubMed: 26040978]

- De Dreu CK, Greer LL, Handgraaf MJ, Shalvi S, Van Kleef GA, Baas M, Ten Velden FS, Van Dijk E, Feith SW. The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. *Science*. 2010; 328:1408–1411. [PubMed: 20538951]
- De Dreu CK. Oxytocin modulates cooperation within and competition between groups: an integrative review and research agenda. *Horm Behav*. 2012a; 61:419–428. [PubMed: 22227278]
- De Dreu CK. Oxytocin modulates the link between adult attachment and cooperation through reduced betrayal aversion. *Psychoneuroendocrinology*. 2012b; 37:871–880. [PubMed: 22055739]
- Domes G, Heinrichs M, Glascher J, Buchel C, Braus DF, Herpertz SC. Oxytocin attenuates amygdala responses to emotional faces regardless of valence. *Biol Psychiatry*. 2007a; 62:1187–1190. [PubMed: 17617382]
- Domes G, Heinrichs M, Michel A, Berger C, Herpertz SC. Oxytocin improves mind-reading in humans. *Biol Psychiatry*. 2007b; 61:731–733. [PubMed: 17137561]
- Eckstein M, Becker B, Scheele D, Scholz C, Preckel K, Schlaepfer TE, Grinevich V, Kendrick KM, Maier W, Hurlmann R. Oxytocin facilitates the extinction of conditioned fear in humans. *Biol Psychiatry*. 2015; 78:194–202. [PubMed: 25542304]
- Eklund A, Nichols TE, Knutsson H. Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. *Proc Natl Acad Sci U S A*. 2016; 113:7900–7905. [PubMed: 27357684]
- Etkin A, Wager TD. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry*. 2007; 164:1476–1488. [PubMed: 17898336]
- Feng C, Hackett PD, DeMarco AC, Chen X, Stair S, Haroon E, Ditzen B, Pagnoni G, Rilling JK. Oxytocin and vasopressin effects on the neural response to social cooperation are modulated by sex in humans. *Brain Imaging Behav*. 2015a; 9:754–764. [PubMed: 25416642]
- Feng C, Lori A, Waldman ID, Binder EB, Haroon E, Rilling JK. A common oxytocin receptor gene (OXTR) polymorphism modulates intranasal oxytocin effects on the neural response to social cooperation in humans. *Genes Brain Behav*. 2015b; 14:516–525. [PubMed: 26178189]
- Gamer M, Zurowski B, Buchel C. Different amygdala subregions mediate valence-related and attentional effects of oxytocin in humans. *Proc Natl Acad Sci U S A*. 2010; 107:9400–9405. [PubMed: 20421469]
- Goodson JL, Thompson RR. Nonapeptide mechanisms of social cognition, behavior and species-specific social systems. *Curr Opin Neurobiol*. 2010; 20:784–794. [PubMed: 20850965]
- Gregory R, Cheng H, Rupp HA, Sengelaub DR, Heiman JR. Oxytocin increases VTA activation to infant and sexual stimuli in nulliparous and postpartum women. *Horm Behav*. 2015; 69:82–88. [PubMed: 25562711]
- Greve DN, Fischl B. Accurate and robust brain image alignment using boundary-based registration. *NeuroImage*. 2009; 48:63–72. [PubMed: 19573611]
- Groppe SE, Gossen A, Rademacher L, Hahn A, Westphal L, Grunder G, Spreckelmeyer KN. Oxytocin influences processing of socially relevant cues in the ventral tegmental area of the human brain. *Biol Psychiatry*. 2013; 74:172–179. [PubMed: 23419544]
- Guastella AJ, Howard AL, Dadds MR, Mitchell P, Carson DS. A randomized controlled trial of intranasal oxytocin as an adjunct to exposure therapy for social anxiety disorder. *Psychoneuroendocrinology*. 2009; 34:917–923. [PubMed: 19246160]
- Hall SS, Lightbody AA, McCarthy BE, Parker KJ, Reiss AL. Effects of intranasal oxytocin on social anxiety in males with fragile X syndrome. *Psychoneuroendocrinology*. 2012; 37:509–518. [PubMed: 21862226]
- Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol Psychiatry*. 2003; 54:1389–1398. [PubMed: 14675803]
- Herzmann G, Young B, Bird CW, Curran T. Oxytocin can impair memory for social and non-social visual objects: a within-subject investigation of oxytocin's effects on human memory. *Brain Res*. 2012; 1451:65–73. [PubMed: 22424787]
- Huber D, Veinante P, Stoop R. Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. *Science*. 2005; 308:245–248. [PubMed: 15821089]

- Hurlemann R, Scheele D. Dissecting the role of oxytocin in the formation and loss of social relationships. *Biol Psychiatry*. 2016; 79:185–193. [PubMed: 26122876]
- Jaremka LM, Lindgren ME, Kiecolt-Glaser JK. Synergistic relationships among stress, depression, and troubled relationships: insights from psychoneuroimmunology. *Depress Anxiety*. 2013; 30:288–296. [PubMed: 23412999]
- Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage*. 2002; 17:825–841. [PubMed: 12377157]
- Labuschagne I, Phan KL, Wood A, Angstadt M, Chua P, Heinrichs M, Stout JC, Nathan PJ. Oxytocin attenuates amygdala reactivity to fear in generalized social anxiety disorder. *Neuropsychopharmacology*. 2010; 35:2403–2413. [PubMed: 20720535]
- Lahey B, Tardiff TA, Drew JB. Negative social interactions—assessment and relations to social support, cognition, and psychological distress. *J Soc Clin Psychol*. 1994; 13:42–62.
- MacDonald K, MacDonald TM, Brune M, Lamb K, Wilson MP, Golshan S, Feifel D. Oxytocin and psychotherapy: a pilot study of its physiological, behavioral and subjective effects in males with depression. *Psychoneuroendocrinology*. 2013; 38:2831–2843. [PubMed: 23810433]
- Macdonald K, Feifel D. Oxytocins role in anxiety: a critical appraisal. *Brain Res*. 2014; 1580:22–56. [PubMed: 24468203]
- Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat Rev Neurosci*. 2011; 12:524–538. [PubMed: 21852800]
- Modi ME, Young LJ. The oxytocin system in drug discovery for autism: animal models and novel therapeutic strategies. *Horm Behav*. 2012; 61:340–350. [PubMed: 22206823]
- Naber F, van Ijzendoorn MH, Deschamps P, van Engeland H, Bakermans-Kranenburg MJ. Intranasal oxytocin increases fathers' observed responsiveness during play with their children: a double-blind within-subject experiment. *Psychoneuroendocrinology*. 2010; 35:1583–1586. [PubMed: 20457491]
- Naber FB, Poslawsky IE, van Ijzendoorn MH, van Engeland H, Bakermans-Kranenburg MJ. Brief report: oxytocin enhances paternal sensitivity to a child with autism: a double-blind within-subject experiment with intranasally administered oxytocin. *J Autism Dev Disord*. 2013; 43:224–229. [PubMed: 22544470]
- Neumann ID. Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. *J Neuroendocrinol*. 2008; 20:858–865. [PubMed: 18601710]
- Numan M. Motivational systems and the neural circuitry of maternal behavior in the rat. *Dev Psychobiol*. 2007; 49:12–21. [PubMed: 17186513]
- Pedersen CA, Caldwell JD, Walker C, Ayers G, Mason GA. Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. *Behav Neurosci*. 1994; 108:1163–1171. [PubMed: 7893408]
- Rilling JK, DeMarco AC, Hackett PD, Thompson R, Ditzen B, Patel R, Pagnoni G. Effects of intranasal oxytocin and vasopressin on cooperative behavior and associated brain activity in men. *Psychoneuroendocrinology*. 2012; 37:447–461. [PubMed: 21840129]
- Rilling JK, Demarco AC, Hackett PD, Chen X, Gautam P, Stair S, Haroon E, Thompson R, Ditzen B, Patel R, Pagnoni G. Sex differences in the neural and behavioral response to intranasal oxytocin and vasopressin during human social interaction. *Psychoneuroendocrinology*. 2014; 39:237–248. [PubMed: 24157401]
- Ross HE, Young LJ. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front Neuroendocrinol*. 2009; 30:534–547. [PubMed: 19481567]
- Sarnyai Z, Kovacs GL. Oxytocin in learning and addiction: from early discoveries to the present. *Pharmacol Biochem Behav*. 2014; 119:3–9. [PubMed: 24280016]
- Schultz W. Updating dopamine reward signals. *Curr Opin Neurobiol*. 2013; 23:229–238. [PubMed: 23267662]
- Shamay-Tsoory SG, Abu-Akel A, Palgi S, Sulieman R, Fischer-Shofty M, Levkovitz Y, Decety J. Giving peace a chance: oxytocin increases empathy to pain in the context of the Israeli-Palestinian conflict. *Psychoneuroendocrinology*. 2013; 38:3139–3144. [PubMed: 24099859]

Smith SM. Fast robust automated brain extraction. *Hum Brain Mapp.* 2002; 17:143–155. [PubMed: 12391568]

Striepens N, Kendrick KM, Maier W, Hurlmann R. Prosocial effects of oxytocin and clinical evidence for its therapeutic potential. *Front Neuroendocrinol.* 2011; 32:426–450. [PubMed: 21802441]

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.psyneuen.2017.01.006>.

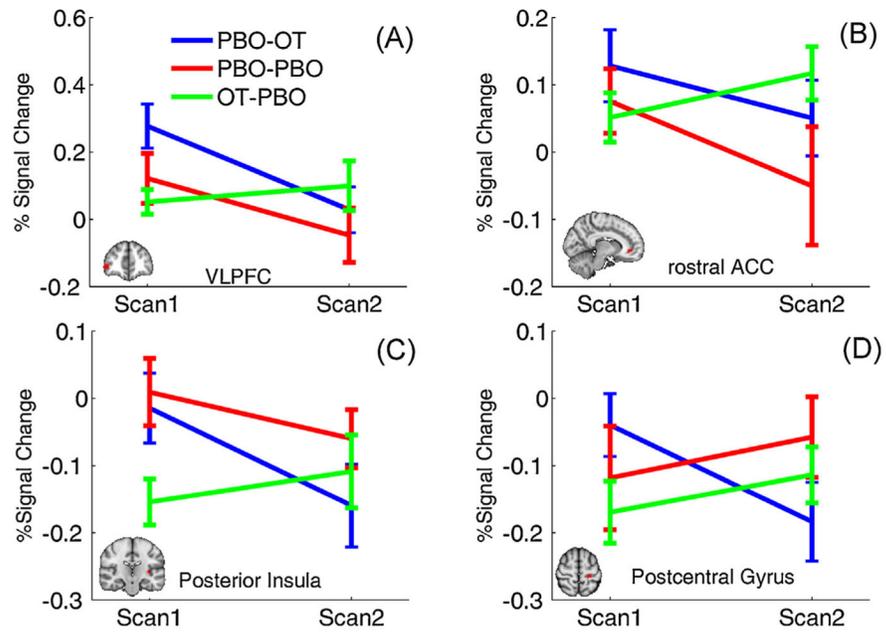


Fig. 1. ROI-specific BOLD response to CC in women within VLPFC (A), rostral ACC (B), posterior insula (C), and post central gyrus (D) for the complete sample of PBO-OT, PBO-PBO, and OT-PBO.

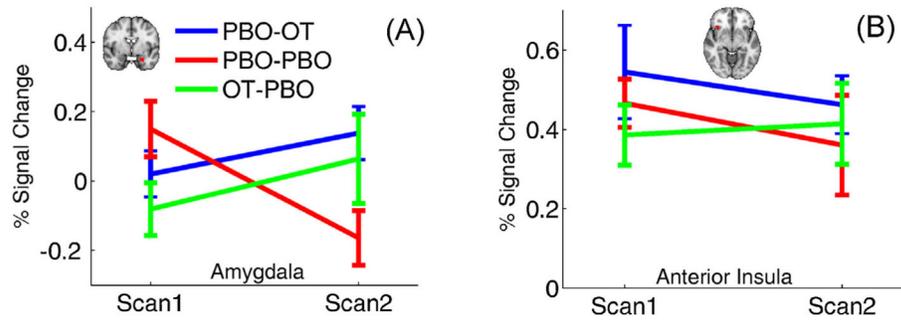


Fig. 2. ROI-specific BOLD response to CD in men within Amygdala (A) and Anterior Insula (B) for the complete sample of PBO-OT, PBO-PBO, and OT-PBO.

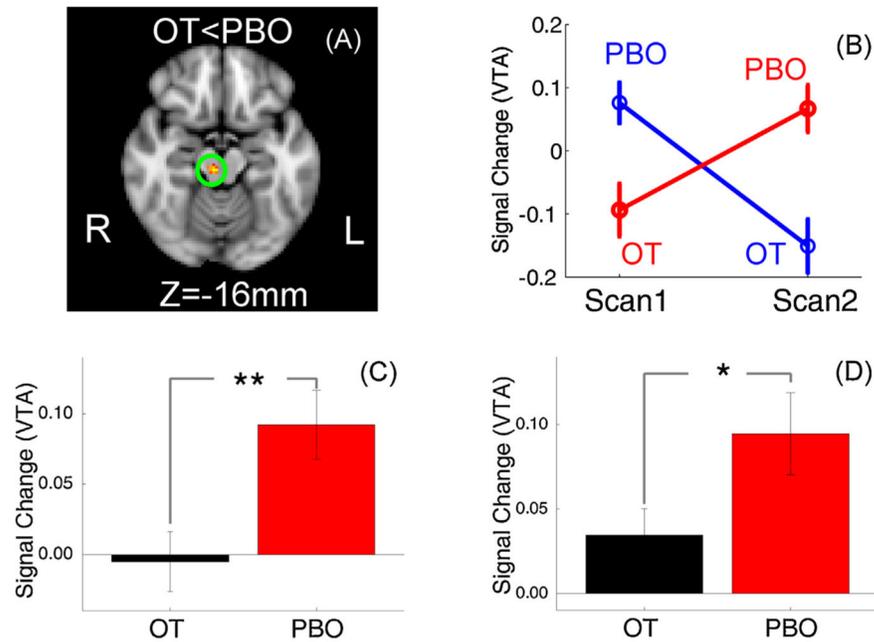


Fig. 3. OT effects on the VTA response to reciprocated cooperation among women. OT attenuated the BOLD response to reciprocated cooperation in the VTA of female participants (FWE-corrected cluster $p < 0.05$ with voxel $p < 0.001$) (A). (B) Average BOLD signal change in VTA (mean \pm s.e.m.) as a function of scan number for each drug administration order. This OT effect also replicates in cross-sectional data using the same task in a much larger cohort (C). The OT group still has significantly less VTA activation relative to the placebo after excluding subjects from the cross-sectional sample who were also in the within-subject sample (D). **: $p < 0.005$ (1-tail); *: $p < 0.05$ (1-tail).

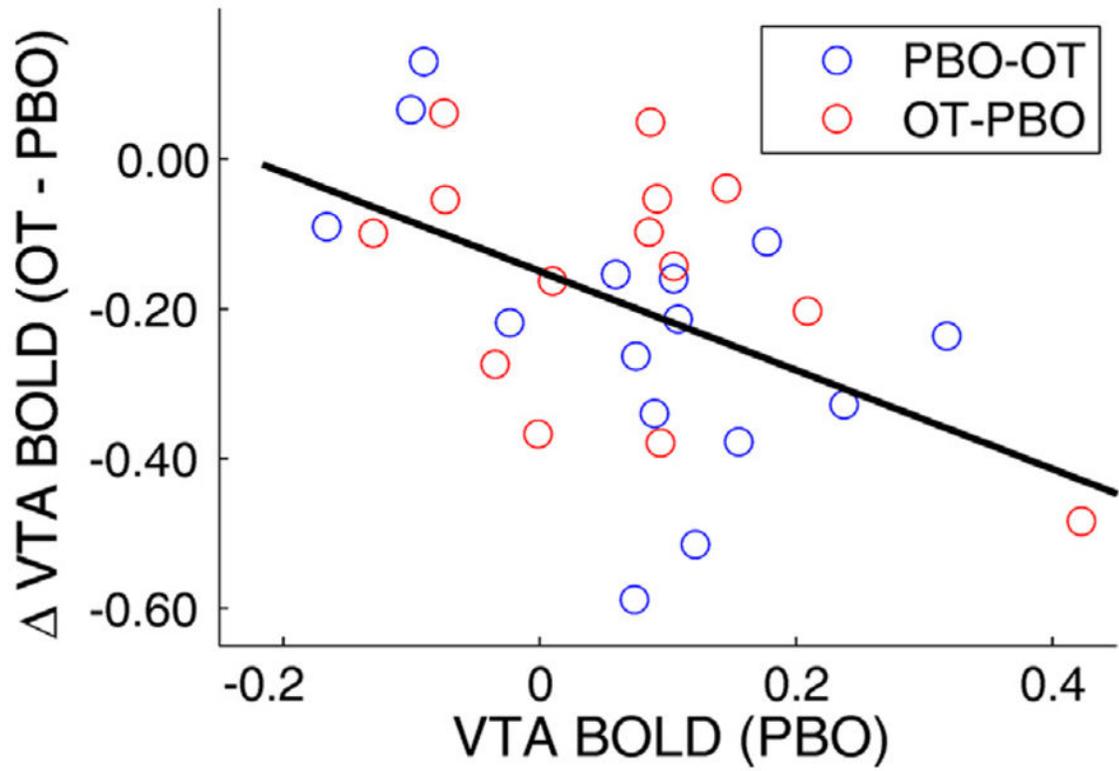


Fig. 4.
BOLD response to CC under PBO predicts the strength of OT effect in VTA.