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Effects of oxytocin and vasopressin on the neural response to unreciprocated cooperation within brain regions involved in stress and anxiety in men and women

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Abstract

Background—Anxiety disorders are characterized by hyperactivity in both the amygdala and the anterior insula. Interventions that normalize activity in these areas may therefore be effective in treating anxiety disorders. Recently, there has been significant interest in the potential use of oxytocin (OT), as well as vasopressin (AVP) antagonists, as treatments for anxiety disorders.

Methods—In this double-blind, placebo-controlled, pharmaco- fMRI study, 153 men and 151 women were randomized to treatment with either 24 IU intranasal OT , 20 IU intranasal AVP, or placebo and imaged with fMRI as they played the iterated Prisoner's Dilemma game with same-sex human and computer partners.

Results—In men, OT attenuated the fMRI response to unreciprocated cooperation (CD), a negative social interaction, within the amygdala and anterior insula. This effect was specific to interactions with human partners. In contrast, among women, OT unexpectedly attenuated the amygdala and anterior insula response to unreciprocated cooperation from computer but not

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Informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of the investigation. Informed consent was obtained from all patients for being included in the study.

human partners. Among women, AVP did not significantly modulate the response to unreciprocated cooperation in either the amygdala or the anterior insula. However, among men, AVP attenuated the BOLD response to CD outcomes with human partners across a relatively large cluster including the amygdala and the anterior insula, which was contrary to expectations.

Conclusions—Our results suggest that OT may decrease the stress of negative social interactions among men, whereas these effects were not found in women interacting with human partners. These findings support continued investigation into the possible efficacy of OT as a treatment for anxiety disorders.

Keywords

Amygdala; Anterior Insula; Anxiety; Oxytocin; Social Cooperation; functional Magnetic Resonance Imaging (fMRI)

1. Introduction

Anxiety disorders are characterized by hyperactivity in both the amygdala and the anterior insula (Etkin and Wager 2007). Interventions that normalize activity in these areas may be effective in treating anxiety disorders. For example, benzodiazepines, an effective pharmacological treatment for anxiety disorders, decrease the amygdala and anterior insula response to viewing fearful and angry faces (Del-Ben et al. 2012; Paulus et al. 2005). However, disadvantages of benzodiazepine treatment include the high abuse potential, lack of efficacy in treating co-morbid depression, and contribution to cognitive and coordination problems (Dell'osso and Lader 2013). This has motivated the search for alternative pharmacological treatments such as selective serotonin reuptake inhibitors, which also attenuate the amygdala response to fearful and angry faces (Anderson et al. 2008), but have several shortcomings of their own including short-term worsening of anxiety symptoms, sexual side effects, insomnia and potential weight gain - one or all of which restrict their use in patients with anxiety disorders (Koen and Stein 2011).

Oxytocin is a naturally occurring endogenous neuropeptide that is produced in the paraventricular and supraoptic nuclei of the hypothalamus and released into both the brain and the peripheral circulation (Meyer-Lindenberg et al. 2011). Recently, there has been significant interest in the potential use of intranasal oxytocin (OT) as a treatment for anxiety disorders (Macdonald and Feifel 2014; Meyer-Lindenberg et al. 2011). In rodents, acute or chronic central administration of OT exerts anxiolytic effects in both males and females (Neumann 2008). OT also inhibits hypothalamic-pituitary-adrenal (HPA) axis responses to a variety of stressors (Neumann 2008). These effects are at least partially mediated by OT actions at the central amygdala (CeA) and paraventricular nucleus of the hypothalamus (Neumann 2008). In fact, OT has been shown to decrease the firing frequency of CeA neurons that project to hypothalamic and brainstem regions that trigger fear responses (Huber et al. 2005; Viviani et al. 2011).

In healthy men, 24 IU intranasal OT has been shown to interact with social support to suppress the cortisol and subjective anxiety response to psychosocial stress (Heinrichs et al. 2003). These effects may be mediated by OT modulation of amygdala activity. Intranasal OT

attenuates the amygdala response to viewing emotional faces in healthy men (Domes et al. 2007; Kirsch et al. 2005), and also attenuates amygdala hyperactivity to fearful and angry faces in social anxiety disorder (Labuschagne et al. 2010) and borderline personality disorder (Bertsch et al. 2013), respectively. While face viewing paradigms have provided invaluable insights into the neural bases of human fear and anxiety, other studies have examined the neural response to actual negative social interactions based on the belief that these are more potent elicitors of stress and anxiety in real life. For example, social exclusion robustly activates bilateral anterior insula as well as the dorsal anterior cingulate cortex (Eisenberger et al. 2003). Similarly, we have previously shown that the anterior insula is robustly activated in response to two different types of negative social interaction: unreciprocated cooperation in the iterated Prisoner's Dilemma (PD) game (Rilling et al. 2008) and receiving an unfair offer in the Ultimatum game (Sanfey et al. 2003). Furthermore, we have shown that intranasal OT decreases negative relative to positive conflict behaviors among couples during conflict discussion (Ditzen et al. 2009). Finally, an important study by Baumgartner et. al. showed that intranasal OT attenuated amygdala activation when making decisions about whether to trust others in a trust game. OT also increased behavioral expressions of trust, suggesting that it may decrease the fear of betrayal during decision-making (Baumgartner et al. 2008). However, no study has yet demonstrated that intranasal OT treatment alters the neural response that immediately follows a negative social interaction in humans, an important issue since negative social interactions are a common cause of stress and anxiety (Lakey et al. 1994). Collectively, the existing literature leads to the prediction that intranasal OT will attenuate the neural response to unreciprocated cooperation within the amygdala and the anterior insula. Although most intranasal OT studies have been conducted with male subjects, there is accumulating evidence for sex differences in the effects of intranasal OT (Domes et al. 2010). Thus, it is important to examine the effects of OT on the response to negative social interactions in both men and women.

Although OT often has anxiolytic effects, the closely related nonapeptide vasopressin (AVP) is often anxiogenic (Heinrichs et al. 2009). For example, V1a agonists increase anxiety in rats, whereas V1a receptor blockade reduces anxiety(Liebsch et al. 1996; Mak et al. 2012). Furthermore, rats that lack AVP due to a genetic mutation show low anxiety (Zelena et al. 2008), as do transgenic mice lacking the V1a receptor (Bielsky et al. 2004). Stress also triggers vasopressin release in rats(De Goeij et al. 1992; Engelmann et al. 2000). Finally, in humans, several anxiety disorders are associated with elevated plasma AVP levels (Surget and Belzung 2008). These findings suggest that V1a antagonists could have therapeutic value (Meyer-Lindenberg et al. 2011). Prior to evaluating the effect of a V1a antagonist, we sought to establish that AVP itself had the predicted anxiogenic effects. We predicted that intranasal AVP treatment would augment the neural response to unreciprocated cooperation within the amygdala and the anterior insula. Similar to OT, AVP can also have sexually differentiated effects in both humans and non-human animals (Thompson et al. 2006; Veenema et al. 2013), so it is important to examine AVP effects in both men and women. Finally, there is also evidence that AVP effects on men may be specific to male social stimuli(Uzefovsky et al. 2012). Thus, it is relevant that our study design involves men and women interacting with same-sex partners.

Previously, in a sample of 91 healthy, normal men (27 OT, 37 PBO, 27 AVP), we reported a trend for OT to attenuate the amygdala response to negative social interactions (unreciprocated cooperation in the PD game), but this result did not reach statistical significance. Nor did AVP modulate the amygdala response to CD outcomes in men (Rilling et al. 2012). In the current paper, we increase our sample size to 153 men (50 OT, 54 PBO, and 49 AVP) to assess whether significant modulatory effects of OT and AVP on the neural response to negative social interactions emerge with increased statistical power. We also add an equivalent sample of women (50 OT, 50 PBO, and 51 AVP) to determine if modulatory effects of OT and AVP differ for men and women. Finally, rather than subtracting away effects for computer partners as we did in our previous analysis, we analyze results for human and computer partners separately to account for the possibility that neuropeptide effects are similar for interactions with human and computer partners.

We have also previously reported OT and AVP modulation of the BOLD fMRI response to positive social interactions (reciprocated cooperation in the PD game), using a sub-set of the sample reported here (Rilling et al. 2014). This 2014 paper included a total of 91 men and 87 women, whereas the current paper includes a total of 153 men and 151 women. Thus, this new paper includes an additional 62 men and 64 women beyond what was included in our 2014 paper, and it is focused on negative rather than positive social interactions.

2. Methods

2.1. Subjects

In total, 153 men and 151 women from the Emory University community between the ages of 18 and 22 (mean (SD) = 20.7 (2.2) years for men, and 20.5 (1.3) years for women) were randomized(at a ratio of 1:1:1) to treatment with either intranasal oxytocin (n=50 for men, n=50 for women), placebo (PBO, n=54 for men ,n=50 for women) or intranasal vasopressin (AVP, n=49 for men , n=51 for women). Following previous studies that have reported social cognitive effects of intranasal OT and AVP administration (MacDonald et al. 2013; Thompson et al. 2006), the OT group self-administered 24 IU oxytocin (Syntocinon-Spray, Novartis), and the AVP group self-administered 20 IU of AVP (American Reagents Laboratory, Shirley, NY, USA). Randomization was performed by the Emory Investigational Drug Service (IDS) using Research Randomizer (http://www.randomizer.org), which randomizes each subject by using the method of randomly permuted blocks.

All potential subjects completed a full medical history questionnaire. Exclusion criteria are provided in the next section. 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. Eleven male subjects (AVP n = 3, OT n = 4, and PBO n = 4) were excluded from the neuroimaging analysis due to excessive motion (>1.5 mm) (n = 8), missing data (n = 2) or to abnormal brain anatomy (n=1). One male subject (PBO n = 1) was excluded from the behavioral analysis due to missing data. Two female subjects (OT n = 2) were excluded from the neuroimaging analysis due to excessive motion.

Preparation of study medication and details of randomization were maintained by the Emory IDS and all study personnel including the PI were blind to group assignment. Administration

of 24 IU oxytocin was generally safe. None of the subjects developed any major side effects of study medication, including anaphylaxis. One subject complained of mild nausea (PBO, n=1), three subjects experienced transient increases in vital signs (PBO n=2, AVP n=1), and one subject experienced claustrophobia severe enough for the scanning session to be terminated (PBO n=1).

2.2. 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 and isotretinoin topical (acne), synthroid and antihistamines for allergy.

2.3. Prisoner Dilemma Game

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. All subjects first completed a PD tutorial and two practice trials (see Online Resource 1 for details). We aimed to start both the task and fMRI scan at 40 min after drug administration (see Online Resource 1 for the rationale). 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 (see Online Resource 1 for complete timeline of the PD game). 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. 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 other session, roles were reversed. The order of human and computer sessions 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 from each subject. 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.

2.4. Behavioral/Psychometric Measurement of Personality

For each sex, two behavioral measures were compared between the PBO group and the two drug groups (OT and AVP): the total number of cooperate choices, and the probability of cooperating in the current round following unreciprocated cooperation in the previous round (pC/CD). These measures were calculated for both human and computer partners. Measures were compared between drug and PBO groups using two-sample t-tests in IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY). Within each drug group, measures were compared between human and computer partners using paired t-tests.

The Revised NEO Personality Inventory (NEO PI-R) (Costa and McCrae 1992) was administered to all 304 subjects. The NEO PI-R is a detailed measure of personality traits with 240 items across five broad domains: Extraversion, Agreeableness, Conscientiousness, Neuroticism, and Openness to experience. In this study, we were specifically interested in the association between the neuroimaging data and the Anxiety facet of the Neuroticism domain.

After each of the four 30-round sessions, 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.

2.5. Neuroimaging Data Collection

A T1-weighted structural image and blood-oxygenation-level-dependent (BOLD) functional MRI images were collected for 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 256×256×176 mm^3, a matrix of 256×256×176, and isotropic spatial resolution of 1.0×1.0×1.0 mm^3, one average. Total T1 scan time was approximately 5 minutes.

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 X64, FOV=224mm, in-plane resolution 3.5mm, slice thickness=2.5mm, and 34 axial slices with a gap of 1.05mm in between. During fMRI scanning, subjects performed 30 rounds of a sequential-choice, iterated PD game with both putative human partners and computer partners.

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/). 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 5mm, 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 run (either with human or computer partner), 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 or a picture of the computer 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, three different contrasts (CC, CD and CC vs. CD) were specified to respectively compare BOLD response to CC with baseline, BOLD response to CD with baseline, and the difference in BOLD responses between CC and CD. The individual-level GLM was implemented using FILM (FMRIB's Improved Linear Model).

At the group level, we performed voxel-wise analyses within two a priori hypothesized structural ROIs (i.e., amygdala, and anterior insula, see next section for details) as well as across the whole brain. Random effect models were specified to investigate the effects of OT and AVP on the BOLD response to CD outcomes in both sexes. Controlling for both sex and drug effects, we first explored the random effect of CD vs. CC for all men and women to confirm that both the amygdala and the anterior insula were activated by CD outcomes (i.e. CD human>CC human). For each combination of sex (male, female) and partner (human, computer), we then used 2-sample unpaired t-tests to examine whether OT or AVP modulated the response to unreciprocated cooperation. 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 $Z > 1.96$ (voxel–wise 2-tailed $p<0.05$), and a family-wise error (FWE)–corrected cluster significance threshold of $p < 0.05$ was applied to the suprathreshold clusters. All illustrated results in this paper survived a FWE – correction of $p < 0.05$ unless otherwise specified.

Structural Region-of-Interests (sROI) analysis: All group effect models were applied to bilateral anterior insula and amygdala, two a priori regions of interest that are strongly related to anxiety in the literature. Both brain regions were defined based on the Harvard-Oxford cortical and subcortical structural atlases (http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html#ho). Anterior Insula was defined as the anterior half of the insular cortices based on the MNI y coordinate. For the structural ROI analyses, the abovementioned statistical threshold was applied, correcting only for the size of ROI volumes.

Functional ROI analysis: We investigated the association between BOLD fMRI responses in the anterior insula and amygdala and trait anxiety using a functional region-of-Interests (fROIs) analysis. These fROIs were defined as a 10mmX10mmX10mm cube centered at the peak of the activation within the structural ROIs defined above. The association between average BOLD percentage signal change and trait anxiety were explored using a regression model while controlling for the drug treatment effect. These fROIs were also used to statistically examine the possibility of sex differences in drug modulation using a 2 X 2 ANOVA.

All statistical tests were implemented in IBM SPSS Statistics 20.0 (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. Behavioral Results

3.1.1. Effect of Partner Type—The frequency of cooperate choices did not differ for human vs. computer partners, for either male or female subjects (all p>0.05). However, men in the placebo group were more likely to cooperate with computer vs. human partners following a CD outcome in the previous round (pC/CD, p=0.003). There was a non-significant trend in the same direction for women in the placebo group. In the AVP and OT groups, there were no differences in pC/CD between human and computer partners, for either male or female subjects.

3.1.2. Effect of Drug Treatment—OT had no effect on the number of cooperate choices or on the behavioral response to unreciprocated cooperation in the previous round (the probability of cooperating after a CD outcome) in either men or women, interacting with either assumed human or computer partners. AVP also had no effect on the number of cooperate choices or on the behavioral response to unreciprocated cooperation in the previous round in women, interacting with either assumed human or computer partners. In men, AVP decreased the probability of cooperating after a CD outcome with computer partners ($t_{97} = -2.48$, $p = 0.02$) but had no effect on the number of C choices with computer partners. There were also no behavioral effects of AVP among men interacting with assumed human partners (Table 1).

3.2 Emotion Ratings

Among men, neither OT nor AVP treatment altered ratings of anger, disappointment or fear in response to CD outcomes relative to placebo. Among women, after multiple comparison correction, OT significantly increased self-reported fear (afraid) (p=0.006), and anger (p=0.009) in response to CD outcomes with human partners. However, OT treatment did not alter these emotion ratings in response to CD outcomes with computer partners. AVP had no effect with either human or computer partners in women.

3.3. Imaging Results

3.3.1. Main Effect of Unreciprocated Cooperation (CD > CC) with human partners—In both bilateral amygdala and bilateral anterior insula, the BOLD response to unreciprocated cooperation (CD) from human partners was stronger than that to reciprocated cooperation (CC) from human partners, after controlling for sex and drug effects. This activation pattern was evident in both sROI (Table 2, Fig. 1) and whole brain analyses (Fig. S2(Online Resource 2)).

3.3.2. OT effects

1) Men interacting with assumed same-sex human partners: OT treatment attenuated activation to CD outcomes within the right anterior insula and bilateral amygdala (Table 2, Fig.2a). Furthermore, in both brain regions, individual differences in trait anxiety were positively related to the BOLD response to unreciprocated cooperation (Fig.2b), after controlling for drug treatment in a regression model (for OT, AVP and Placebo groups combined: anterior insula p=0.002, amygdala p=0.01; for OT and Placebo groups only: anterior insula p=0.05, amygdala p=0.01) . Among all 6 facets in the Neuroticism domain, trait anxiety had the strongest relationship to the BOLD response in both regions (Tables S1 and S2 in Online Resource 1).

To evaluate the specificity of this effect, we also tested for OT effects on the response to CC outcomes and found none. Accordingly, OT did significantly decrease the value of the contrast CD-CC within both regions (Fig. S7 (Online Resource 7)).

A complementary whole brain analysis of OT effects on the BOLD response to CD outcomes with human partners is shown in Online Resource 3 (Fig.S3).

2) Men interacting with computer partners: OT treatment did not attenuate activation to CD outcomes within the anterior insula or the amygdala. Nor did OT treatment significantly augment activation to CD outcomes within either area.

3) Women interacting with assumed same-sex human partners: OT treatment did not significantly modulate the response to unreciprocated cooperation within the anterior insula or the amygdala. Importantly, within the areas where OT attenuated activation to CD outcomes with human partners among men (Fig.2), there was a significant sex (M vs. F) by drug treatment (OT vs. PBO) interaction ($F_{1, 184}$=8.41, p=0.004 for anterior insula; $F_{1,184}$=5.03, p=0.026 for amygdala). In both functional ROIs (amygdala and anterior insula),

OT attenuates the response to a greater degree in men than in women (see also Fig. S6a in Online Resource 6).

4) Women interacting with computer partners: OT treatment attenuated activation to CD outcomes within bilateral anterior insula and bilateral amygdala (Table 2, Fig.3a). However, trait anxiety was not associated with the BOLD response unreciprocated cooperation in either structure (for OT, AVP and Placebo groups combined: all p>0.05; for OT and Placebo groups only: all p>0.05) (Fig. 3b).). Importantly, only the amygdala functional ROI (not anterior insula) exhibited a significant sex (M vs. F) by drug treatment (OT vs. PBO) interaction ($F_{1, 185}$=7.66, p=0.006), in which OT attenuated the response to a greater degree in women than in men (see also Fig. S6b in Online Resource 6).

To evaluate the specificity of this effect, we also tested for OT effects on the response to CC outcomes and found none. Accordingly OT did significantly (uncorrected p<0.001) decrease the value of the contrast CD-CC within the right amygdala (Fig. S8 in Online Resource 8). However, this OT effect on CC-CD did not survive the multiple comparison correction.

A complementary whole brain analysis of OT effects on the BOLD response to CD outcomes with computer partners is shown in Online Resource 4 (Fig.S4).

Thus, in women, there were regions such as the anterior insula and the amygdala where OT selectively attenuated activation to CD outcomes with computer but not human partners, opposite the selectivity of OT modulatory effects observed in men.

3.3.3. AVP effects

1) Men interacting with assumed same-sex human partners: AVP treatment did not significantly modulate activation to CD outcomes within the anterior insula or the amygdala in our structural ROI analysis. Although a whole brain analysis showed that AVP attenuated activation to CD outcomes across a large cluster (1331 voxels) that spanned multiple brain regions including the right amygdala and anterior insula, the use of a spatial extent threshold meant that these activations could not be definitively localized to the amygdala and anterior insula (Woo et al. 2014) (Fig. S5 (Online Resource 5)).

2) Men interacting with computer partners: AVP treatment did not significantly modulate activation within the anterior insula or the amygdala.

3) Women interacting with assumed same-sex human partners: AVP treatment did not significantly modulate activation within the anterior insula or the amygdala.

4) Women interacting with computer partners: AVP treatment did not significantly modulate activation within the anterior insula or the amygdala.

4. Discussion

Reciprocal altruism is a core principal of human social life (Axelrod 1984; Trivers 1971), and human beings are highly sensitive to breaches of reciprocity (Cosmides and Tooby 2000). Unreciprocated cooperation can take many forms, from failing to reciprocate favors

or gifts, to sexual infidelity. Needless to say, these interactions may provoke stress and anxiety in the victim. The iterated PD game attempts to model real-life social interactions such as these, and there is evidence to suggest that it does so effectively. When a player cooperates and the partner fails to reciprocate that cooperation, the player experiences social rejection. In response to unreciprocated cooperation in the PD game, subjects report high levels of anger, irritation, and disappointment (Rilling et al. 2007). In addition, unreciprocated cooperation in the PD game robustly activated the anterior insula and the amygdala, both regions that have been linked with stress and anxiety and that are known to be hyperactive in patients with anxiety disorders (Etkin and Wager 2007). Anxiety disorders are associated with altered behavior in the PD game. For example, adolescents with anxiety or depressive disorders are more likely to reciprocate cooperation in the PD game than are healthy control subjects (McClure et al. 2007). In addition, after disruption of cooperation in the PD game, patients with borderline personality disorder are less likely to attempt to coax their partner back to cooperation compared with healthy controls (King-Casas et al. 2008). Despite these social behavioral correlates of anxiety, the psychological and neural response to negative social interactions is apt to be more important for understanding anxiety disorders. All human beings experience negative social interactions, but these take a greater mental and physical toll on some than others.

4.1. Main Findings

Here we investigate how intranasal OT and AVP modulate the neural response of men and women to same-sex negative social interactions with both human and computer partners. In men, we find that 24 IU intranasal OT attenuates the amygdala and anterior insula response to unreciprocated cooperation from same-sex human but not computer partners. On the other hand, OT had no effect on the response to CC outcomes within either of these regions, demonstrating the specificity of the effect for negative social interactions. In addition, the amygdala and anterior insula response is positively correlated with trait anxiety. Among all 6 facets in the Neuroticism domain, trait anxiety had the strongest relationship to the BOLD response in both regions. Thus, OT may render negative social interactions less stressful among men. These effects were not found in women, and a direct statistical comparison showed that OT attenuates the response to a greater degree in men than in women (Fig. S6a, c). On the other hand, in women, 24 IU intranasal OT attenuated the amygdala and anterior insula response to unreciprocated cooperation from computer but not human partners. These effects were not found in men, and a direct statistical comparison showed that OT attenuates the response to a greater degree in women than in men in amygdala (Fig.S6b, d). However, in this case activation in neither structure was correlated with trait anxiety.

Although the mechanism by which intranasal OT affects brain function and behavior is uncertain at this time (Churchland and Winkielman 2012), there is emerging evidence that intransal OT administration leads to elevations in central OT levels (Neumann et al. 2013; Striepens et al. 2013). Thus, the most obvious mechanism would involve OT binding to brain OT receptors. The modulatory effects of OT on amygdala function that we report here are consistent with the observation that the human amygdala has a high density of OT receptors (Boccia et al. 2013). On the other hand, there is no evidence for OT receptors in the human anterior insula, although it is unclear whether previous studies have specifically

looked there. If the anterior insula does not have a high density of OT receptors, then OT effects in the anterior insula may represent downstream effects of OT binding in other brain structures.

Our finding that OT attenuates the anterior insula response to CD outcomes with human partners initially seems in conflict with a recent meta-analysis that noted enhanced anterior insula activation as one of the most consistent effects of OT(Wigton et al. 2015). However, it is important to note that none of the studies included in the meta-analysis investigated the effect of OT on the BOLD response to negative social interactions as we do here. Moreover, only one of the studies showing OT augmentation of anterior insula activity utilized negatively valence stimuli as we do here. Thus, as argued previously, OT effects on brain function appear to be context-specific (Goodson and Thompson 2010).

Contrary to our hypothesis, 20 IU intranasal AVP did not significantly augment the amygdala and anterior insula response to unreciprocated cooperation. In fact, there was a trend for it to have the opposite effect, particularly among men playing with same-sex human but not computer partners, suggesting that AVP may render negative social interactions less stressful among men. With a larger sample size and additional statistical power, this result could possibly reach significance. However, given the large size of our existing sample, this effect could only be a weak one. Nevertheless, a significant effect in this direction would raise questions about the potential use of AVP antagonists as a treatment for anxiety and depression. It is possible that AVP is having weaker effects than OT because AVP effects are mediated by binding to the OT receptor, which AVP does with less affinity than OT(Derick et al. 2002). On the other hand, weak AVP binding has been reported in the dorsolateral part of the basal amygdaloid nucleus(Loup et al. 1991), raising the possibility that any AVP effects in the amygdala are due to its binding to its own receptor. Finally, these marginal effects of AVP could be related to dosage, since some published studies showing significant effects of AVP on brain activity use 40 IU (rather than 20 IU as used here) (Lee et al. 2013; Zink et al. 2011). In women, 20 IU intranasal AVP had no effect in either the anterior insula or the amygdala with either human or computer partners. In sum, OT significantly attenuates amygdala and anterior insula activation in response to negative social interactions when men interact with putative same-sex human but not computer partners. A similar trend was also found for AVP in men. On the other hand, while AVP had no effect in women, OT attenuated the amygdala and anterior insula response to unreciprocated cooperation from computer but not human partners in women.

4.2. Sex Differences

Given that negative social interactions are a common cause of stress and anxiety in everyday life (Lakey et al. 1994), our findings raise the possibility that intranasal OT will have utility in treating anxiety disorders. However, they also suggest that these treatments may be more effective for men than women. The sex differences in effects of OT that we observe here 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). Furthermore, plasma OT is negatively correlated with trait anxiety in men but not women (Weisman et al. 2013). At the neural level, fMRI studies have reported sex differences in OT effects on the neural response to negative social stimuli. OT decreased men's amygdala response to negative facial expressions (Kirsch et al. 2005), while increasing women's amygdala response to fearful faces and negative scenes (Domes et al. 2010). Thus, the sex differences in neural effects of intranasal OT we observe here are consistent with widespread evidence for sex differences in both humans and non-human animals.

4.3. Human vs. Computer Partners

In men, the OT effect on brain activity was specific to interactions with assumed human partners and was not found with computer partners. This is consistent with 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). Intranasal OT has also been found to improve socially reinforced learning but not non-socially reinforced learning (Hurlemann et al. 2010). Our findings are also consistent with evidence that OT effects on cooperative behavior in the PD game depend upon having prior contact with a partner (Declerck et al. 2014). In our paradigm, subjects meet a human confederate whom they are told they will be playing the PD game with, but of course never meet their computer partner. Importantly, all of these previous studies involve male subjects. Among women, we see the opposite interaction between oxytocin and partner type; OT effects on brain activity were specific to computer partners and were not found with human partners. While this was an unexpected result, women have higher endogenous CSF OT levels (Altemus et al. 1999), so one possibility is that OT effects with human partners emerge when central OT concentrations are moderate (i.e., female baseline levels and supplemented male levels) but then plateau at higher concentrations (i.e., supplemented female levels), whereas effects with computer partners only emerge at higher concentrations (i.e., supplemented female levels) that males never achieve in our study. Indeed, the data for the anterior insula fit this interpretation insofar as females have lower baseline levels of activity than males when interacting with human partners in the placebo group and OT treatment brings males down to female levels in the placebo group (Fig. S6a, c). Furthermore, when interacting with computer partners, OT-treated women showed lower activation than any of the other three groups (i.e., the greatest attenuation) (Fig. S6b, d). On the other hand, the amygdala data do not fit this interpretation since there is no difference between men and women at baseline (placebo group) when interacting with human partners (Fig. S6a, c). Furthermore, OT-treated women do not show less activation than either placebo or OT-treated men when interacting with computer partners. Rather, placebo-treated women start from a higher baseline and decrease to male levels upon treatment with OT (Fig. S6b, d).

4.4. Behavior

Men in the placebo group were more likely to cooperate with computer vs. human partners following a CD outcome in the previous round. This is consistent with evidence that reciprocal responses are substantially weaker when fairness attributions are removed from experimental designs (Falk et al. 2008). That is, subjects are less likely to reciprocate

defection from computer partners, who presumably lack unfair intentions. This result can also be interpreted as reflecting reduced betrayal aversion (Baumgartner et al. 2008) with computer partners that permits greater social risk-taking (i.e., cooperating with a potentially non-reciprocating partner).

Despite the observed effects of intranasal OT on the BOLD fMRI response to unreciprocated cooperation, intranasal OT had no effect on behavioral response to this outcome (pC/CD). Recent studies show that the effects of intranasal OT on cooperative behavior depend on the participant's attachment style (Bartz et al. 2011). For example, intranasal OT increases cooperation among men who are high rather than low in attachment avoidance (De Dreu 2012). 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. Another potential explanation for the lack of behavioral effects relates to the relative influence of emotion and cognition in decision-making. Intranasal OT modulated activity within limbic and paralimbic areas. It may be that decision-making in our iterated PD game was 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.

Intranasal OT also had no effect on self-reported ratings of negative emotion in response to CD outcomes among men. Emotion ratings are reported retrospectively after all 30 rounds of the game have been completed. Our findings therefore suggest that retrospective self-report may be less sensitive than fMRI. In women, on the other hand, OT increased self-reported fear and anger in response to CD outcomes with human partners, a result consistent with findings by (Kubzansky et al., 2012) that 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. In fact, though not statistically significant, these effects were paralleled by OT augmentation of the anterior insula response to CD outcomes among women (Fig. S6).

The modulatory effects of OT that we report here with a sample size of 104 men (50 OT, 54 PBO) did not reach statistical significance in our earlier publication based on 64 of these men (27 OT, 37 PBO). The fact that we needed such a large sample size to detect a significant effect implies that the effect is of only moderate magnitude, or that effectiveness is limited to subjects with specific genetic and epigenetic backgrounds (Bartz et al. 2011). Future research should explore the latter possibility.

5. Conclusion

In this double-blind, placebo-controlled pharmaco-fMRI study, we find that pre-treatment with intranasal OT (24 IU) attenuates the BOLD fMRI response to unreciprocated cooperation from same-sex human partners, a negative social interaction, within both the amygdala and the anterior insula in men. Importantly, activation in these regions was also correlated with trait anxiety in men. Contrary to expectation, there was also a trend for AVP (20IU) treatment to attenuate activation in the amygdala and anterior insula in men. In women, on the other hand, OT attenuated the amygdala and anterior insula responses to

interactions with computer but not human partners, opposite the pattern observed in men. There were no effects of AVP on the amygdala or anterior insula response to unreciprocated cooperation with either human or computer partners in women. Our results suggest that OT may reduce the stress of negative social interactions among men, and support continued investigation into its possible efficacy as a treatment for anxiety disorders. However, it will be important to characterize the dose response function of OT on the BOLD fMRI response in both men and women, and to evaluate whether it has similar effects in patients with anxiety disorders before seriously considering it as a clinical treatment, especially since the small numbers of clinical trials that have investigated effects of intranasal OT in patients with anxiety disorders have yielded mixed results (Guastella et al. 2009; Hall et al. 2012; MacDonald et al. 2013; Pitman et al. 1993).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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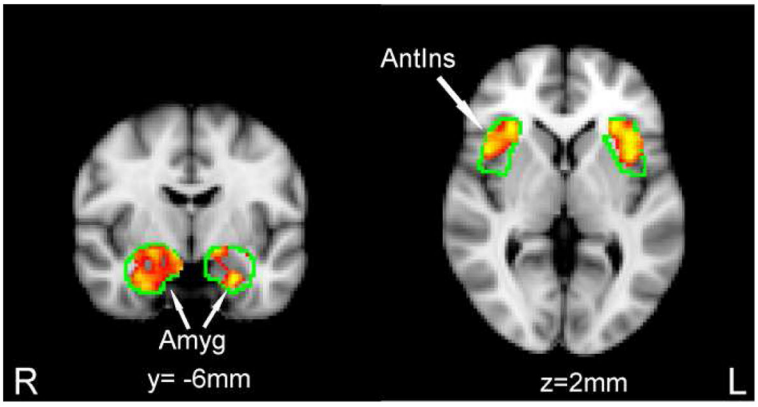

Fig 1.

BOLD response to unreciprocated cooperation (CD outcomes) relative to reciprocated cooperation (CC outcomes) from human partners. Analysis was restricted to the anterior insula and the amygdala (delimited with green line). Both the amygdala and anterior insula respond more strongly to CD from human partners compared with CC from human partners, after controlling for sex and drug effects. Results were thresholded at $p<0.05$, corrected for multiple comparison based on cluster size. Amyg = Amygdala, AntIns=Anterior Insula.

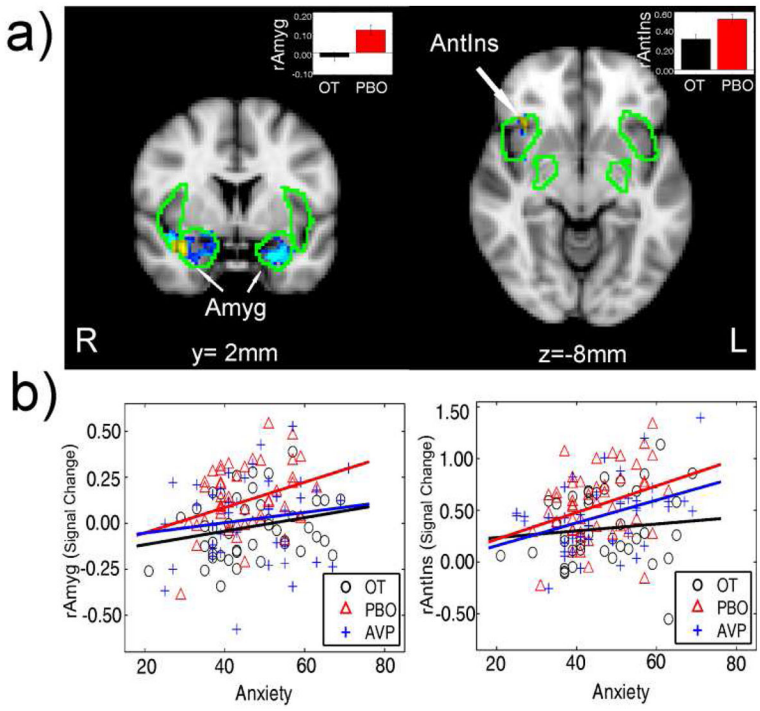

Fig 2.

OT effects on the neural response to unreciprocated cooperation (CD) from human partners in men. Analysis was restricted to the anterior insula and the amygdala (delimited with green line). (a) OT treatment attenuated activation to CD outcomes with human partners relative to PBO in both anterior insula and amygdala. Inset compares BOLD signal (mean and s.e.m.) between OT and PBO groups in the anterior insula and amygdala respectively. (b) Scatter plot of trait-anxiety against average BOLD response in the right amygdala and right anterior insula functional ROIs (fROIs, yellow in Fig 2a). These correlations are significant overall when controlling for drug treatment in a regression model. For the PBO group, average BOLD response and trait anxiety are significantly correlated in both fROIs (r=0.33, p=0.02 for the amygdala; r=0.32, p=0.03 for the anterior insula.). For the OT group, average BOLD response and trait anxiety are not significantly correlated in either fROI (r=0.24, p=0.11 for the amygdala; r=0.11, p=0.49, for the anterior insula.). For the AVP group, average BOLD response and trait anxiety are significantly correlated in the anterior insula only (r=0.40, p=0.01) (r=0.15, p=0.35 for the amygdala).

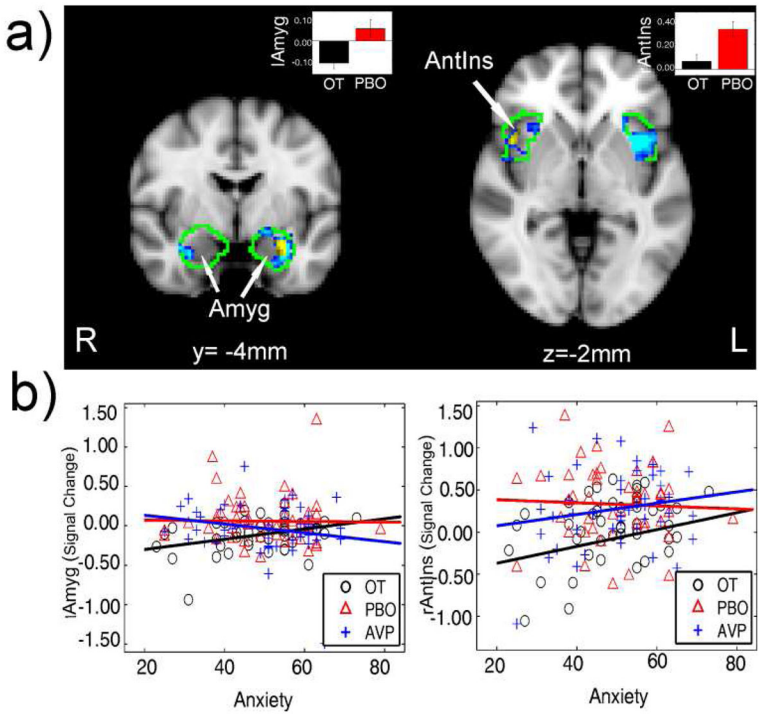

Fig 3.

OT effects on the neural response to unreciprocated cooperation (CD) from computer partners in women. Analysis was restricted to the anterior insula and the amygdala (delimited with green line). (a) OT treatment attenuated activation to CD outcomes with computer partners relative to PBO in both anterior insula and amygdala. Inset compares BOLD signal (mean and s.e.m.) between OT and PBO groups in the anterior insula and amygdala respectively. (b) Scatter plot of trait-anxiety against average BOLD response in the left amygdala and right anterior insula fROIs (yellow in Fig.3a). For the PBO group, average BOLD response and trait anxiety are not significantly correlated in either fROI (r= −0.02, p=0.92 for the amygdala; r=−0.05, p=0.76 for the anterior insula.). For the OT group, average BOLD response and trait anxiety are significantly correlated in both fROIs (r=0.35, p=0.02 for the amygdala; r=0.39, p=0.01 for the anterior insula.). For the AVP group, average BOLD response and trait anxiety are not significantly correlated in either fROI (r= −0.20, p=0.18 for the amygdala; r=0.18, p=0.24, for the anterior insula.)

Table 1

Comparison of average behavioral measures between the placebo group and the two drug groups.

Drug	Sex	Partner			
		Human		Computer	
		#C	pC/CD	#C	pC/CD
Placebo	Male (n=53)	17.68 (0.93)	0.28 (0.04)	17.34 (0.98)	0.41 (0.04)
	Female (n=50)	16.92 (0.98)	0.29 (0.04)	18.02 (1.04)	0.34 (0.04)
Oxytocin	Male (n=50)	16.18 (1.01)	0.32 (0.04)	17.50 (1.04)	0.33 (0.04)
	Female (n=50)	18.14 (1.03)	0.31 (0.05)	17.00 (0.82)	0.29 (0.04)
Vasopressin	Male (n=49)	15.71 (0.98)	0.23 (0.04)	15.73 (1.05)	**0.27 (0.04)** *
	Female (n=51)	15.84 (0.76)	0.25 (0.03)	17.18 (0.81)	0.30 (0.04)

Note: #C is the number of cooperate choices; pC/CD is the probability of cooperating after a CD outcome; standard error of the mean in parentheses.

* $p<0.05$ for comparison with placebo group.

Table 2

Activated brain regions for contrasts with significant findings

Brain Regions	MNI Coordination of Local Maxima (mm)			Local Maxima Z	Cluster Size (voxel)
	x	y	z		
Main effect (CD>CC)					
R Anterior Insula and Amygdala	48	20	−10	5.22	2231
R Anterior Insula	48	20	−10	5.22	
R Amygdala	26	−10	−10	4.31	
L Anterior Insula and Amygdala	−36	14	−6	5.71	1578
L Anterior Insula	−36	14	−6	5.71	
L Amygdala	−24	−6	−26	4.10	
OT effect of CD on men with human partner					
R Anterior Insula and Amygdala	14	−4	−24	3.82	798
R Amygdala	14	−4	−24	3.82	
R Anterior Insula	36	24	−10	3.21	
L Amygdala	−16	−4	−22	3.74	476
OT effect of CD on women with computer partner					
L Anterior Insula and Amygdala	−36	0	6	4.0	1255
L Anterior Insula	−36	0	6	4.0	
L Amygdala	−30	−4	−18	3.97	
R Anterior Insula and Amygdala	36	4	−10	3.1	410
R Anterior Insula	36	4	−10	3.1	
R Amygdala	−42	−34	36	2.9	