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Neuropsychopharmacology

Neuropsychopharmacology. 2010 Aug 18;35(12):2403–2413. doi: 10.1038/npp.2010.123

Oxytocin Attenuates Amygdala Reactivity to Fear in Generalized Social Anxiety Disorder

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PMCID: PMC3055328 PMID: 20720535

Abstract

Patients with generalized social anxiety disorder (GSAD) exhibit heightened activation of the amygdala in response to social cues conveying threat (eg, fearful/angry faces). The neuropeptide oxytocin (OXT) decreases anxiety and stress, facilitates social encounters, and attenuates amygdala reactivity to threatening faces in healthy subjects. The goal of this study was to examine the effects of OXT on fear-related amygdala reactivity in GSAD and matched healthy control (CON) subjects. In a functional magnetic resonance imaging study utilizing a double-blind placebo-controlled within-subjects design, we measured amygdala activation to an emotional face matching task of fearful, angry, and happy faces following acute intranasal administration of OXT (24 IU or 40.32 μg) and placebo in 18 GSAD and 18 CON subjects. Both the CON and GSAD groups activated bilateral amygdala to all emotional faces during placebo, with the GSAD group exhibiting hyperactivity specifically to fearful faces in bilateral amygdala compared with the CON group. OXT had no effect on amygdala activity to emotional faces in the CON group, but attenuated the heightened amygdala reactivity to fearful faces in the GSAD group, such that the hyperactivity observed during the

placebo session was no longer evident following OXT (ie, normalization). These findings suggest that OXT has a specific effect on fear-related amygdala activity, particularly when the amygdala is hyperactive, such as in GSAD, thereby providing a brain-based mechanism of the impact of OXT in modulating the exaggerated processing of social signals of threat in patients with pathological anxiety.

Keywords: oxytocin, fear, emotion, amygdala, social anxiety disorder, functional magnetic resonance imaging (fMRI)

INTRODUCTION

Generalized social anxiety disorder (GSAD), also known as social phobia, is characterized by an exaggerated fear of negative scrutiny in several types of social situations. Notably, patients with GSAD have deficits in emotion processing, and specifically exhibit attentional bias for critical/aversive faces and threatening social signals (Amir *et al*, 2003; Clark and McManus, 2002; Foa *et al*, 2000; Mogg *et al*, 2004), and tend to avert their gaze from salient facial features such as the eyes (Bogels and Mansell, 2004; Garner *et al*, 2006; Horley *et al*, 2004) to reduce excessive anxiety during social interactions. These deficits have been strongly linked to areas in the limbic system including the amygdala (Birbaumer *et al*, 1998; Blair *et al*, 2008; Etkin and Wager, 2007; Evans *et al*, 2008; Phan *et al*, 2006; Stein *et al*, 2002; Straube *et al*, 2004, 2005; Veit *et al*, 2002; Yoon *et al*, 2006), where threat-related hyperactivity is a cardinal pathophysiological feature in patients with GSAD (Etkin and Wager, 2007; Shin and Liberzon, 2010).

Several functional magnetic resonance imaging (fMRI) studies have shown that GSAD subjects exhibit heightened activation of the amygdala in response to social cues conveying threat (eg, fearful or angry faces) (Evans *et al*, 2008; Goldin *et al*, 2009; Phan *et al*, 2006; Stein *et al*, 2002; Straube *et al*, 2004). Selective-serotonin reuptake-inhibitors, often used to treat GSAD, can attenuate amygdala reactivity to fearful faces in healthy volunteers (Harmer *et al*, 2006) and in GSAD patients (Phan *et al*, unpublished data), and to public speaking in GSAD patients (Furmark *et al*, 2002), suggesting that the amygdala could serve as a brain target to test the underlying neural substrate of social behaviors and social anxiety.

Extensive research in diverse species has shown that oxytocin (OXT), a neuropeptide produced in the hypothalamus of the brain, decreases anxiety and stress and facilitates social encounters (Bartz and Hollander, 2006) potentially by exerting an inhibitory effect via activation of GABAergic interneurons within the amygdala (Ehrlich *et al*, 2009; Huber *et al*, 2005). In humans, intranasal OXT has been shown to increase trust and willingness to accept social risks arising through

interpersonal interactions (Kosfeld *et al*, 2005) and increase memory for positive social information (ie, happy faces; Rimmele *et al*, 2009), whereas suppressing physiological and subjective responses to stress (Heinrichs *et al*, 2003). Consistent with this evidence, functional imaging studies in healthy subjects have shown that equivalent doses of OXT attenuate amygdala reactivity to faces expressing fear and anger (Domes *et al*, 2007; Kirsch *et al*, 2005), as well as to fear-conditioned socially relevant faces (Petrovic *et al*, 2008) and in interpersonal interaction (Baumgartner *et al*, 2008), in the absence of overt changes in subjective mood or arousal (Domes *et al*, 2007; Kirsch *et al*, 2005; Petrovic *et al*, 2008). These findings are consistent with the distribution of oxytocin receptors (OXT-Rs) in areas associated with pro-social and anxiety behaviors such as the amygdala (Gimpl and Fahrenholz, 2001; Huber *et al*, 2005; Landgraf and Neumann, 2004; Vaccari *et al*, 1998; Yoshimura *et al*, 1993), and thus provide a putative brain-based mechanism to explore the underlying neural basis of OXT pharmacodynamic effects on the processing of social stimuli and related social behaviors, particularly as they relate to fear responding (Heinrichs *et al*, 2009).

The primary aim of this study was to use blood oxygenation level-dependent (BOLD) fMRI coupled with an emotional face matching task (EFMT) known to robustly and reliably activate the amygdala (Hariri *et al*, 2003) and which has been used in previous OXT studies in healthy controls (Kirsch *et al*, 2005), to examine the effects of OXT on amygdala reactivity to socially threatening faces in patients with GSAD and matched healthy control (CON) subjects. Given the evidence of heightened amygdala reactivity to social signals of threat (eg, fearful/angry faces) in GSAD (Phan *et al*, 2006; Stein *et al*, 2002), and of OXT's effects on amygdala response to aversive faces in previous fMRI studies (Domes *et al*, 2007; Kirsch *et al*, 2005), we employed an *a priori* region of interest (ROI) approach to test our primary hypothesis. Specifically, we hypothesized that OXT would (1) significantly attenuate threat related amygdala activity in both healthy controls and patients with GSAD, and (2) 'normalize' the threat-specific amygdala hyper-reactivity in GSAD subjects. In other words, we expected less amygdala reactivity to fearful/angry faces on OXT (*vs* placebo) in both groups, and the pattern of threat-related hyper-activity observed at baseline (placebo) in GSAD (*vs* CON) subjects would no longer be evident following acute administration of OXT.

MATERIALS AND METHODS

Subjects

In all, 18 male GSAD subjects and 18 age- and gender-CON subjects, all right-handed and aged between 18–55 years, were included in the study and recruited via local newspaper and University advertisements (mean age±SD GSAD: 29.4±9.0 years; age range: 20–55 years) and CON: 29.9±10.2

years; age range: 19–54 years). Diagnosis of GSAD was established using the Clinical International Diagnostic Interview (CIDI Version 2.1; WHO, 1997) with additional probes from the Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987), and verified by a physician interview. A score of >70 on the LSAS (including >30 on the 'social situations' subscale) were required to be included as the 'generalized' subtype. No GSAD subject had a current depressive episode (evident ⩽6 months) or alcohol/substance abuse (within 12 months of study entry), or another anxiety disorder (eg, generalized anxiety disorder, specific phobia, and panic disorder) that was more clinically salient or preceded GSAD as assessed using the CIDI, which utilizes DSMIV and ICD-10 criteria. The following comorbid disorders were evident, but clinically less salient than GSAD: agoraphobia (five subjects), conversion disorder (two subjects), specific phobia for nature/environment (one subject), panic disorder (one subject), pain disorder (one subject), hypochondriasis (one subject), and obsessive-compulsive disorder (one subject). Subjects were excluded if they had a history of post-traumatic stress disorder, bipolar disorder, psychotic disorder, mental retardation, or developmental disorders. The CON subjects had no history of a psychiatric disorder as verified by the CIDI. All subjects were non-smokers, free of head injury, had no allergies, and no history of alcohol or substance abuse. None of the subjects were on medication at the time of the study or were previously medicated. As part of the clinical screening, all subjects went through a brief medical examination with the study physician to assess that they were otherwise fit to take part in the study. Other clinical screening measures involved the Primary Care Evaluation of Mental Disorders (PRIME-MD; Spitzer *et al*, 1994), the Beck Depression Inventory (BDI-II; Beck *et al*, 1996), the Beck Anxiety Inventory (BAI; Beck and Steer, 1990), and two measures from the State-Trait Anxiety Inventory (STAI; Spielberg *et al*, 1983). After complete description of the study to the subjects, written informed consent was obtained. The study was conducted in accordance with the guidelines of the Standing Committee on Ethics in Research Involving Humans of Monash University.

Study Design

The study utilized a randomized, double-blind, placebo-controlled within-subject design, in which each participant was tested under two acute treatment conditions separated by a 1 week wash-out period. Treatment conditions were randomized and counterbalanced (by a clinical trials pharmacist, using a computerized randomization program in Windows Excel) with subjects receiving an intranasal spray of either OXT (24 IU or 40.32 μg; Syntocinon-spray; Novartis, Switzerland) or placebo (PBO, containing all ingredients except for the peptide) in three puffs of 4 IU or 6.72 μg per nostril. OXT or placebo was administered 45 min before fMRI scanning, consistent with previous studies (Domes *et al*, 2007; Heinrichs *et al*, 2003; Kirsch *et al*, 2005). Subjects arrived for each treatment session ~1 h before the scheduled fMRI scanning session. No

caffeine or alcohol intake was allowed on the day of scanning and no food permitted 1 h before arrival. Following a period of rest, subjects self-administered either an OXT or a placebo nasal spray as instructed by the investigator. This involved inhaling a full spray per nostril in an alternating order with 45-s wait between each application until total amount (ie, three sprays per nostril) was reached. The fMRI scanning started 45-min post-treatment (lasting ~30 min). This time window was chosen to coincide with the time of oxytocin's predicted maximum pharmacokinetic (Born *et al*, 2002) and physiological effects (Domes *et al*, 2007; Heinrichs *et al*, 2003; Kirsch *et al*, 2005).

EFMT

The subjects were assessed using an EFMT (Hariri *et al*, 2002), which has been previously used in our pharmacological fMRI studies (Phan *et al*, 2008). This task involved photographs from a validated set of face stimuli (Gur *et al*, 2002), which has shown to reliably and robustly activate the amygdala and its use has been effective in other pharmacological fMRI studies (Hariri *et al*, 2000; Kirsch *et al*, 2005; Paulus *et al*, 2005; Tessitore *et al*, 2002). Using a block-design, participants viewed a trio of faces and selected one of two faces (bottom) that expressed the same emotion as the target face (top). The identity of all three faces was always different, and an equal number of male and female faces were presented. The target and congruent probe faces displayed one of three expressions (fearful, angry, or happy), and the other (incongruent) probe face always displayed a neutral/nonemotional expression. This design allowed us to isolate amygdala reactivity specifically to each emotional expression, which have similar perceptual characteristics except for the threat/nonthreat signal conveyed. To maintain attention and allow limbic (ie, amygdala) brain responses to return to baseline, the face matching tasks were interspersed with a 'baseline' task, in which subjects matched simple geometric shapes (circles, rectangles, or triangles) similar to instructions above. The paradigm consisted of 18 experimental blocks, each containing four sequential matching trials of 3-s presentation (ie, 12-s blocks). The 18 blocks consisted of 9 blocks of matching emotional faces (3 blocks of each target expression of fearful, angry, and happy), interleaved with 9 blocks of matching shapes, counterbalanced across two runs. Participants responded to the tasks by pressing the left or right response buttons with their right hand. The task was presented visually on a computer using Presentation software (http://www.neurobehavioralsystems.com) and deflected onto a screen. Participants' response accuracy and reaction times were analyzed in a 2 group (GSAD, CON) × 2 drug (OXT, PBO) × 3 emotion (fearful, angry, happy) repeated-measures ANOVA, Greenhouse-Geisser corrected where necessary. Significant main effects and interactions were followed by *post hoc* paired *t*-tests to examine the effect of OXT.

fMRI Acquisition and Statistical Analysis

Brain images were acquired with a 3T Siemens Tim Trio scanner using a 12-channel head coil. Functional gradient-echo echo-planar imaging (EPI) data depicting BOLD contrast were acquired during the experimental task (TE=40 ms, TR=3000 ms, flip-angle=90°, FoV=210 mm, 64 × 64 matrix, 44 contiguous 3 mm slices parallel to the hippocampus, and interleaved). Whole brain T1-weighted anatomical reference images were also acquired from all participants (TE=2.15 ms, TR=1900 ms, flip-angle=9°, FoV=256 mm, 176 sagittal slices, 1 mm slice thickness, perpendicular to the AC-PC line). Data processing and analyses were performed using Statistical Parametric Mapping (SPM5) software. Data from 2 participants (1 CON and 1 GSAD subject) was discarded because of poor data quality, resulting in a final analysis of 17 GSAD and 17 CON subjects. Images were spatially realigned to correct for head motion, warped to an EPI template in Montreal Neurological Institute space, and resampled to 2 mm^3 kernel. The general linear model was applied to the time series, convolved with the canonical hemodynamic response function (Friston *et al*, 1995) with a 128-s high-pass filter. Condition effects were modeled with box-car regressors representing the occurrence of each block type, and effects were estimated at each voxel, and for each subject before group analyses. Individual contrast maps (Statistical Parametric Maps) were then analyzed at the second level in a random effects statistical model (Holmes and Friston, 1998) for within-group, between-group, and between-session analyses.

Although we had *a priori* hypotheses about oxytocin's effects within the amygdala in response to threat (angry and/or fearful) faces, to obviate bias and generate exploratory findings for subsequent hypotheses, we conducted a whole-brain voxel-wise analysis of variance (ANOVA) with drug (OXT, PBO) and emotion (fear, angry, happy *vs* shapes) as within-subject factors, and group (GSAD, CON) as between-subject factor. The significance threshold was set at $p<0.001$ (uncorrected) with a cluster extend threshold of $k=10$. We were particularly interested in the main and/or interaction effects of OXT, and thus explicitly examined main effect of drug and drug-related interactions (drug × group, drug × emotion and group × drug × emotion) across the entire brain.

To test our *a priori* hypotheses, we also took a ROI analysis approach selectively for amygdala activity. Briefly, because the primary hypothesis was highly specific to amygdala and its response to threat (*vs* nonthreat/happy) faces, and to the direction of OXT effects (ie, attenuation), and was related to the often observed differences in amygdala reactivity in GSAD, we chose to approach the ROI analysis in a way that would minimize selection bias (Kriegeskorte *et al*, 2009) using a functional ROI localizer that is not particularly dependent on any one specific factor (threat, OXT, GSAD) (Poldrack, 2007; Poldrack and Mumford, 2009); this 'functional localizer' is the best representation of the activations exhibited by the cohort of subjects studied. Here, similar to

numerous pharmaco-fMRI studies that have used this task (Arce *et al*, 2008; Hariri *et al*, 2002; Kirsch *et al*, 2005; Paulus *et al*, 2005; Phan *et al*, 2008), we initially measured an overall amygdala response to the EFMT using the linear contrast estimate of BOLD signal difference between all faces (fearful, angry, and happy, combined) *vs* shapes thereby obviating bias toward one particular emotion expression; in addition to avoid bias toward a particular diagnosis or drug condition, we averaged amygdala response (all faces>shapes) across all subjects (GSAD, CON) and across scan session (OXT, PBO), totaling 68 individual contrast images. Focal activation of the amygdala was tested for significance at a stringent whole-brain voxel-wise threshold of $p<0.05$ family-wise error corrected for multiple comparisons across the entire brain. On the basis of strong *a priori* evidence across studies and from our own laboratory (Adolphs, 2003; Costafreda *et al*, 2008; Fusar-Poli *et al*, 2009; Phan *et al*, 2002, 2008; Zald, 2002), we expected robust bilateral amygdala activation to these emotional faces, compared with the nonsocial control 'shapes'. From these activation clusters, we created a functional ROI for the left and right amygdala composed of a 10 mm radius sphere surround the peak activation (all faces>shapes), representing 515 total voxels and a volume of 4120 mm^3 on each side. We then used these functional ROIs to extract BOLD signal responses (parameter estimates, β-weights in arbitrary units) of activation averaged across all 515 voxels (for left and right amygdala separately) from each GSAD and CON subject for each emotional condition separately (fearful faces>shapes, angry faces>shapes, happy faces>shapes) and from each session separately (OXT, PBO). We chose to use a relatively large 10 mm radius spherical ROI to maximize coverage and be inclusive of the functional activations of the 34 subjects in our sample, who show inter-individual variability in the exact location of the activation in the amygdala in response to the EFMT, as also demonstrated by two recent meta-analyses (Costafreda *et al*, 2008; Fusar-Poli *et al*, 2009); previous papers have utilized similar approaches and volumes for the amygdala ROI (Blasi *et al*, 2009; Williams *et al*, 2006). The functional ROI extractions were then used to test our *a priori* hypotheses by comparing the extent of left and right amygdala reactivity to each emotion by: (1) using a repeated measures ANOVA with emotion (fearful, angry, and happy) and drug (OXT, PBO) as within-subject factors, and group (GSAD, CON) as between-subject factor; and (2) using *post hoc* *t*-tests for follow-up analyses for (a) between GSAD and CON subjects during PBO and during OXT sessions separately, using independent samples *t*-tests; and (b) between PBO and OXT sessions in GSAD and CON groups separately, using paired *t*-tests. For these planned *t*-test comparisons, significance was set at $p<0.05$, two-tailed.

Subjective Mood and Statistical Analysis

Changes in subjective mood was assessed twice, once upon arrival (and before drug administration) and once following drug administration (succeeding the scan session) using the Visual Analogue Mood Scale (VAMS; Bond and Lader, 1974). The VAMS consists of 16 bipolar items

composed of two adjectives with opposite feelings, which are presented on a 100 mm line. These items were combined into three main factors to include: (1) alertness, (2) contentedness, and (3) calmness. The VAMS data were analyzed to examine change in scores over time following drug administration (post- minus pre-treatment), and were determined within each group and drug condition in a 2 group (GSAD, CON) × 2 drug (OXT, PBO) × 3 factor (alertness, contentedness, calmness) repeated-measures ANOVA.

RESULTS

Demographic and Clinical Characteristics

The characteristics of the groups are reported in Table 1. The CON and GSAD groups were matched on age and education with no significant difference between the groups on these variables. The GSAD group scored significantly higher on all clinical measures including the LSAS, BDI-II, BAI, and STAI. Of note, the GSAD subjects scored (mean±SD) 43.56±8.6 and 38.11±11.0 on the social situations and performance subscales, respectively, of the LSAS (total score: 81.67±17.5), suggesting broad and moderate-severe impairment.

Table 1. Demographic and Clinical Characteristics.

	CON	GSAD	$t_{(34)}$[a]	*p*
Age	29.89 (10.2)	29.39 (9.0)	0.156	0.877
Education	16.00 (2.5)	14.67 (1.6)	1.930	0.062
BDI-II	1.28 (1.9)	10.83 (7.5)	−5.265	<0.001
BAI	2.17 (5.0)	16.89 (8.2)	−6.503	<0.001
State anxiety (STAI)	23.59 (7.0)	38.81 (15.8)	−6.135	<0.001
Trait anxiety (STAI)[b]	27.35 (8.2)	50.44 (11.5)	−7.785	<0.001
LSAS: total	13.94 (8.3)	81.67 (17.5)	−14.828	<0.001
LSAS: performance	6.89 (5.1)	38.11 (11.0)	−10.916	<0.001
LSAS: social situations	7.06 (4.6)	43.56 (8.6)	−15.876	<0.001

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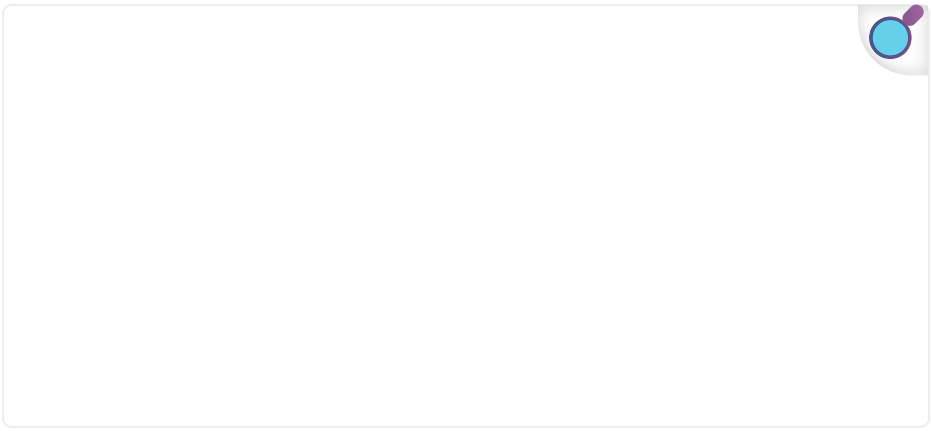
Abbreviations: CON, healthy control subjects; GSAD, generalized social anxiety disorder subjects; BDI-II, Beck Depression Inventory Version II; BAI, Beck Anxiety Inventory; STAI, State-Trait Anxiety Inventory; LSAS, Liebowitz Social Anxiety Scale.
Data are expressed as mean (±SD); *n*=18 per group.
[a]Unpaired *t*-tests, *two*-tailed.
[b]Data missing from two GSAD (*n*=16) and one CON (*n*=17) subjects.

Behavioral Performance (EFMT) During Scanning

Button-press response data from one GSAD subject was not successfully recorded because of technical failure, therefore, giving a final sample size of 17 for the GSAD group. Overall task performance was very high, with subjects making on average 92.6% (±SEM=0.8) correct responses during the entire paradigm. OXT relative to PBO did not affect emotion recognition or response times in either of the GSAD or CON groups. Figure 1 presents the means and standard deviations for accuracy (ie, percentage correct) and reaction times (milliseconds) for the individual emotions (fear, angry, and happy). For accuracy, a repeated-measures ANOVA revealed that there were no

significant performance differences for group ($F(1,66)=2.19$, $p=0.144$) or drug ($F(1,66)=0.618$, $p=0.434$) and no significant interactions among group, drug, or emotion (group × drug: $F(1,66)=0.005$, $p=0.942$; group × emotion: $F(2,132)=0.330$, $p=0.720$; drug × emotion: $F(2,132)=0.224$, $p=0.800$; group × drug × emotion: $F(2,132)=0.238$, $p=0.789$). A significant main effect was evident for emotion ($F(2,132)=28.22$, $p<0.0005$) with highest-to-lowest accuracy in the following pattern: happy>fear>angry (percentage mean±SEM: 97.63±1.0, 94.33±1.1, 86.28±1.9, respectively) across groups and drug conditions. For reaction times, repeated-measures ANOVA revealed that there were no difference in response times across all main effects (group: $F(1,66)=0.211$, $p=0.647$; drug: $F(1,66)=0.067$, $p=0.796$; emotion: $F(2,132)=2.489$, $p=0.106$), as well as no significant interactions (group × drug: $F(1,66)=3.164$, $p=0.080$; group × emotion: $F(2,132)=1.990$, $p=0.192$; drug × emotion: $F(2,132)=0.715$, $p=0.491$; group × drug × emotion: $F(2,132)=1.013$, $p=0.366$).

Figure 1.



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(a) Accuracy (percentage correct) and (b) reaction times (milliseconds) in the emotional face matching task following placebo/PBO and oxytocin/OXT treatment. Data are expressed as mean (±SEM).

Functional MRI Data

Whole-brain analyses

Whole-brain findings for the main and interaction effects of drug are summarized in Table 2. The whole-brain voxel-wise analysis did not detect significant main effects of drug, or interactions related to drug (drug × emotion, drug × group, drug × emotion × group) within the amygdala.

Table 2. Whole-Brain Voxel-Wise ANOVA for Main Effect of Drug and Drug-Related Interactions on the EFMT[a].

Region	Side	Coordinates			Cluster	Volume	Voxel	
		x	*y*	*z*	*k*	*V* (mm^3)	F	*Z*
Drug								
Brainstem	L	−4	−20	−48	55	440	14.21	3.5
Calcarine fissure	R	20	−64	8	24	192	14.25	3.51
Caudate	L	−14	−10	22	11	88	14.72	3.57
Cerebellum	L	−20	−36	−44	13	104	14.65	3.56
Cingulate, middle	R	18	−36	46	816	6528	26.33	4.79
	L	−18	−38	46	359	2872	23.4	4.52
Cuneus	L	0	−92	36	18	144	14.45	3.54
Lingual	R	6	−62	−2	34	272	14.17	3.5
Occipital, inferior	R	40	−94	−6	26	208	16.53	3.79
Occipital, middle	L	−24	−72	0	127	1016	23.87	4.57
Occipital, superior	L	−18	−100	16	46	368	17.11	3.86
Parahippocampal	R	36	−34	−14	52	416	18.05	3.97
	L	−12	−12	−24	53	424	22.77	4.46
Parietal, inferior	L	−24	−76	44	10	80	12.42	3.26
Postcentral	R	44	−18	30	27	216	16	3.73

Region	Side	Coordinates			Cluster	Volume	Voxel	
		x	*y*	*z*	*k*	*V* (mm^3)	F	*Z*
	L	−34	−24	40	10	80	12.64	3.29
Precentral	L	−28	−10	42	87	696	19.33	4.11
Supramarginal	R	64	−24	28	269	2152	16.17	3.75
	L	−60	−38	30	46	368	14.18	3.5
Temporal, middle	L	−66	−30	8	21	168	15.32	3.64
Temporal, superior	R	62	−30	10	23	184	15.37	3.65
	L	−40	0	−16	34	272	14.65	3.56
Thalamus	L	−8	−20	10	20	160	13.88	3.46
Drug × group								
Brainstem	L	−14	−20	−40	42	336	17.55	3.91
Caudate	L	−6	18	−8	21	168	14.92	3.6
Frontal, inferior	R	64	26	2	33	264	21.07	4.29
	L	−38	4	22	11	88	14.41	3.53
Frontal, superior	L	−20	22	−14	38	304	17.19	3.87
Fusiform	L	−24	−82	−12	14	112	14.88	3.59
Occipital, middle	L	−30	−92	4	60	480	14.92	3.59
Drug × emotion								
Fusiform	R	38	−18	−28	20	160	8.5	3.42
Temporal, middle	R	52	−52	−2	20	160	8.5	3.42

Region	Side	Coordinates			Cluster	Volume	Voxel	
		x	*y*	*z*	*k*	*V* (mm^3)	F	*Z*
Drug × group × emotion								
Cerebellum	L	−14	−58	−32	83	664	10.99	3.98

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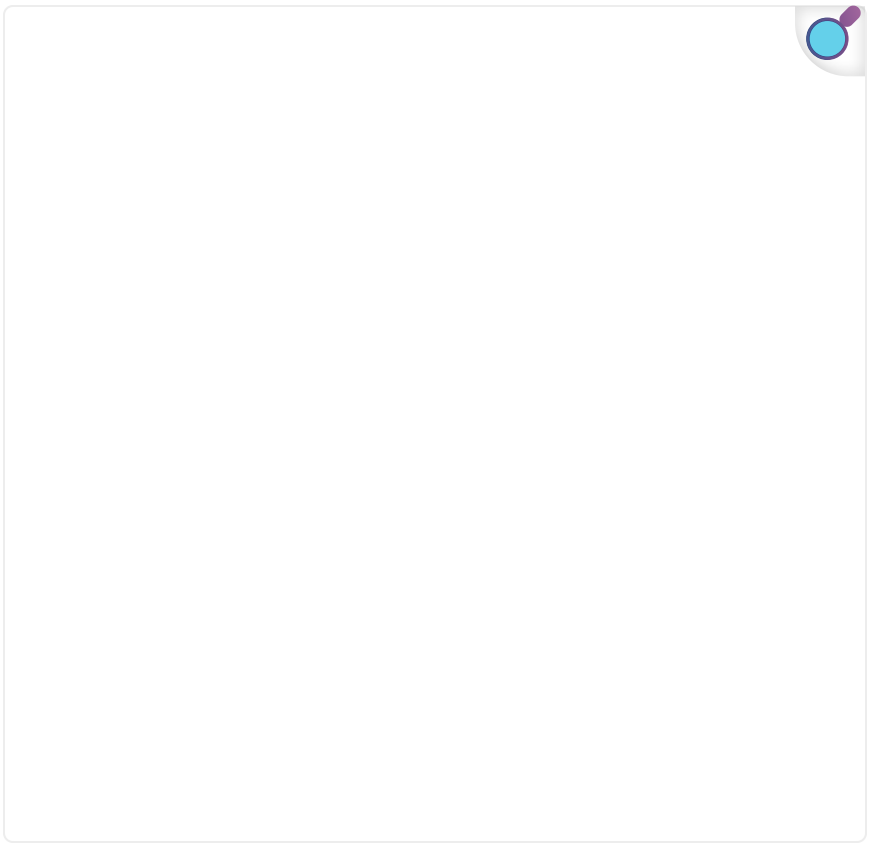
[a]Significance threshold at $p<0.001$(uncorrected) with a cluster extent threshold of *k* (number of contiguous voxels) ⩾10.

ROI Analyses

As expected, across all subjects and drug conditions, we observed robust bilateral amygdala activation to emotional (fearful, angry, and happy) faces, compared with the nonsocial control shapes: (right amygdala: (20, −4, −22), Z-score=6.08, $p_{corrected}<0.0005$, volume=904 mm^3; left amygdala: (−18, −6, −20), Z-score=7.28, $p_{corrected}<0.0005$, volume=1272 mm^3; Figure 2). Analysis of extracted BOLD signal from the bilateral amygdala spherical ROIs functionally derived from these activation peaks (Figure 3) revealed that both groups activated (positive β-weights) amygdala bilaterally in response to fearful, angry, and happy faces separately during PBO treatment (Table 3); this confirms the expected response to the EFMT (Adolphs, 2003; Costafreda *et al*, 2008; Fusar-Poli *et al*, 2009; Phan *et al*, 2002, 2008; Zald, 2002) and allows for inferences about oxytocin's effects on amygdala activation. Table 3 shows the magnitude (β-weights±SEM) of amygdala activation within each group from the OXT and PBO sessions separately. When we entered extracted BOLD signal (β-weights) from the functional amygdala ROIs into repeated-measures ANOVA for left and right amygdala separately, we observed a trend level significance for emotion × drug × group interaction for left and right amygdala ($F(2,64)=2.79$, $p=0.069$, and $F(2,64)=2.21$, $p=0.118$, respectively). The main effects of emotion, drug and group were non-significant ($p_s>0.2$). Subsequent *post hoc t*-test analyses revealed that the three-way interaction was driven by a hyperactivity and OXT effects in the patient group. That is, as predicted, GSAD subjects had greater amygdala reactivity to fearful faces in both left and right amygdala compared with CON subjects during PBO treatment (Figure 4). Following OXT treatment, the exaggerated amygdala response to fearful faces in GSAD subjects

(>CON) observed during the PBO session was no longer evident (Figure 4). As expected, in GSAD subjects, amygdala reactivity to fearful faces was greater during PBO than during OXT treatment, showing that OXT reduced fear-related amygdala activation (Figure 4). Unlike GSAD subjects, control participants did not exhibit differences in amygdala activation to fearful faces between OXT and PBO treatments (Figure 4). No between-group differences (GSAD *vs* CON) in the extent of amygdala activation were observed for angry and happy faces on OXT or PBO treatment (Table 3). Moreover, no between-session (OXT *vs* PBO) differences in the extent of amygdala activation were observed for angry and happy in either the GSAD or CON group (Table 3). As a final analysis, we tested for drug-order effects and found no drug-order effects on amygdala activation (p's>0.05).

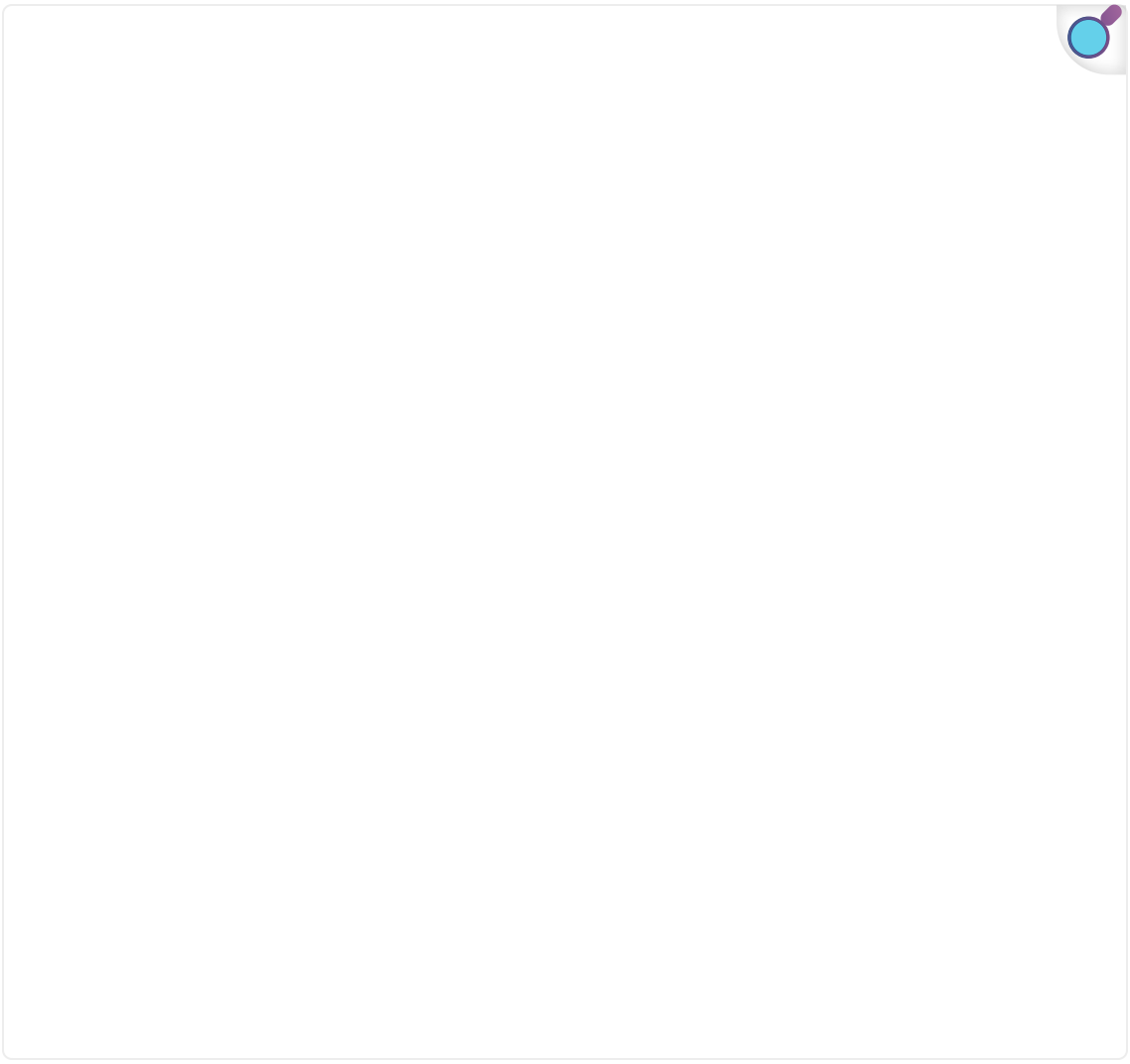
Figure 2.



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Task-related activation (all faces >shapes) of bilateral amygdala. Statistical *t*-map overlaid on a canonical brain rendering (MNI coronal y-plane=−4) showing greater left and right amygdala reactivity to emotional faces (*vs* shapes) from all subjects (GSAD and CON) across both OXT and PBO sessions. Whole brain voxel-wise statistical map showing all significant activations at the threshold of $p<0.05$, family-wise error corrected for multiple voxel-wise comparisons across the entire brain. Activation details described in Results.

Figure 3.



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A two-dimensional view of the spherical ROI (10 mm radius) of the left and right amygdala, from which the parameter estimates were extracted.

Table 3. Within- and Between-Group Comparison of Extracted BOLD Signal (β-Weights) from the Functional Amygdala ROIs (Left and Right) in Response to Each Emotional (Fearful, Angry, and Happy) Face from Each Group (GSAD, CON) for Each Imaging and Treatment Session (OXT, PBO).

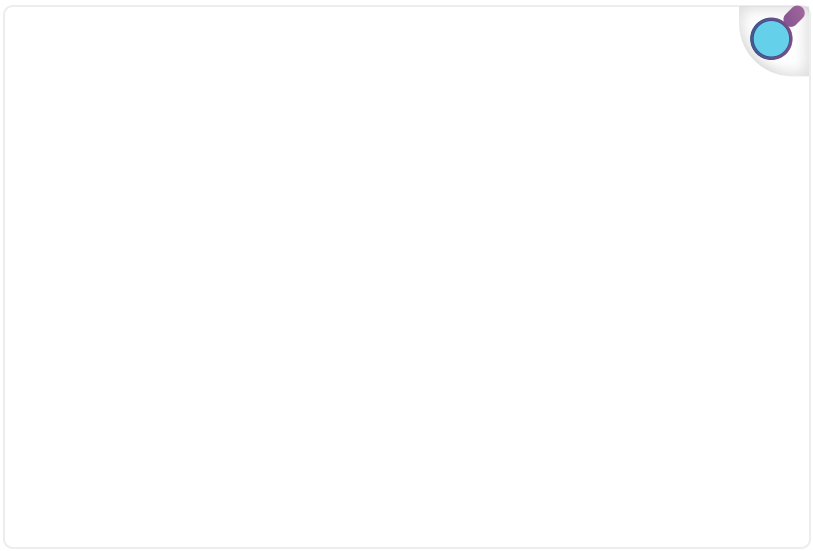
	CON		Paired *t*–test		GSAD		Paired *t*-test		G PBO *t*-
	PBO	**OXT**	$t_{(16)}$	***p***	**PBO**	**OXT**	$t_{(16)}$	***p***	$t_{(32)}$
Left									
Angry	0.174 (0.06)	0.136 (0.05)	0.606	0.55	0.034 (0.07)	0.121 (0.05)	1.035	0.32	1.484
Fearful	0.089 (0.04)	0.074 (0.05)	0.394	0.7	0.241 (0.06)	0.06 (0.04)	2.399**	0.03	2.177**
Happy	0.147 (0.06)	0.161 (0.03)	0.253	0.8	0.137 (0.04)	0.084 (0.04)	0.867	0.4	0.121
Right									
Angry	0.169 (0.05)	0.111 (0.07)	0.862	0.4	0.121 (0.07)	0.176 (0.04)	0.653	0.52	0.549
Fearful	0.076 (0.05)	0.051 (0.05)	0.538	0.6	0.297 (0.08)	0.137 (0.06)	1.865*	0.08	2.393**
Happy	0.18 (0.06)	0.168 (0.07)	0.17	0.87	0.167 (0.06)	0.148 (0.06)	0.245	0.8	0.159

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Abbreviations: CON, healthy control subjects; GSAD, generalized social anxiety disorder subjects; OXT, oxytocin; PBO, placebo.

Data are expressed as mean (±SEM); n=17 per group. $^{*}p<0.05$, *one*-tailed; $^{**}p<0.05$, *two*-tailed.

Figure 4.



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Extracted BOLD responses (mean β-weights, arbitrary units (a.u.)) to fearful faces extracted from the left and right amygdala within each group for each session, showing: (1) greater amygdala reactivity in GSAD group (>CON) during placebo/PBO treatment; (2) attenuation of amygdala reactivity in GSAD group by oxytocin/OXT treatment (PBO>OXT); and (3) extent of amygdala reactivity is similar in GSAD and CON groups on OXT. Asterisks denote between-group and between-session differences, $^{**}p<0.05$, two-tailed; $^{*}p<0.05$, one-tailed).

Subjective Mood

There were no effects of OXT (relative to PBO) on the VAMS factors in both the CON and GSAD group (means and SD are presented in Table 4). The repeated-measures ANOVA did not reveal significant main effects for group ($F(1,68)=0.283$, $p=0.596$) or drug ($F(1,68)=0.147$, $p=0.703$) or interactions for group × drug ($F(1,68)=0.145$, $p=0.705$), drug × factor ($F(2,136)=1.281$, $p=0.281$) or group × drug × factor ($F(2,136)=0.084$, $p=0.878$). A significant interaction was evident for group ×

factor ($F(2,136)=7.078$, $p=0.003$), such that regardless of drug administration, the GSAD patients showed significantly greater mood changes post-treatment compared with that of the CON group; patients showing significantly more calmness (mean±SEM: CON=−1.04±1.7, GSAD=7.56±2.3, $p=0.026$), and less alertness (CON=−1.74±1.8, GSAD=−6.88±2.0, $p=0.061$) following drug administration, and no group difference was evident for contentedness (CON=−0.66±1.3, GSAD=−0.81±1.3, $p=0.424$).

Table 4. Behavioral Changes in Visual Analogue Mood Scale (VAMS) Factors Pre- and Post Treatment (PBO, OXT) in Both Participant Groups (CON, GSAD).

Factor	Time	CON		GSAD	
		PBO	OXT	PBO	OXT
Alertness	Pretreatment	69.3 (3.0)	66.9 (3.3)	51.5 (3.1)	53.9 (2.9)
	Post-treatment	68.1 (2.7)	64.6 (3.8)	46.0 (3.6)	45.6 (3.0)
Contentedness	Pretreatment	76.3 (2.1)	75.5 (2.0)	56.9 (3.0)	56.4 (2.8)
	Post-treatment	74.6 (2.6)	75.8 (2.0)	57.8 (2.7)	57.0 (2.7)
Calmness	Pretreatment	69.1 (2.9)	71.0 (3.0)	55.6 (3.4)	50.2 (3.5)
	Post-treatment	68.6 (3.7)	73.6 (2.3)	61.7 (2.6)	59.3 (3.2)

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Abbreviations: CON, healthy control subjects; GSAD, generalized social anxiety disorder subjects; OXT, oxytocin; PBO, placebo.
Data are expressed as mean (±SEM); $n=18$ per group.

DISCUSSION

Modulation of the amygdala has been suggested to have a critical role in oxytocin's effects on pro-social- and anxiety-related behavior. Patients with GSAD show amygdala hyperactivity to threatening social cues and we reasoned that if OXT can attenuate this hyperactivity, then it may

provide a neural mechanism to facilitate processing of social stimuli and related social behaviors including social threat. Supporting our hypotheses, we observed that patients with GSAD showed bilateral amygdala hyperactivity specifically to fearful faces, relative to controls. OXT had no effect on amygdala activity to emotional faces in healthy controls, but attenuated the heightened amygdala reactivity to fearful faces in patients with GSAD, such that the hyperactivity observed in patients during the placebo session was no longer evident following OXT (ie, normalization).

In patients with GSAD, the amygdala hyperactivity was observed for fearful faces, but not happy faces, supporting previous suggestions that the hyperactivity is specific to aversive threatening social cues (Phan *et al*, 2006). The fear-related hyperactivity is consistent with a number of previous studies in GSAD where exaggerated amygdala reactivity has been reported to aversive social cues including fearful faces (Phan *et al*, 2006) and to 'harsh' (fearful, angry, and contemptuous) faces (Phan *et al*, 2006; Stein *et al*, 2002). However, we did not observe similar amygdala hyperactivity to angry faces, which has previously been reported in a number of studies (Evans *et al*, 2008; Phan *et al*, 2006; Stein *et al*, 2002; Straube *et al*, 2004). While we expected amygdala hyperactivity to 'harsh' (angry and fearful) faces, as each convey social signals of threat, the lack of hyperactivity to angry faces and the apparent inconsistency may be explained by a number of factors. For example, previous studies have shown amygdala activations to angry facial expressions are less intense compared with fearful faces (Fitzgerald *et al*, 2006; Whalen *et al*, 2001), whereas others have shown no amygdala activation to angry faces (Blair *et al*, 1999; Fusar-Poli *et al*, 2009), suggesting that activation to angry faces may not only be less intense, but also more variable. The latter findings may be a consequence of the relative differences in the recognition of fearful *vs* angry faces. Our behavioral findings showed that angry faces were overall less accurately recognized compared with fearful and happy expressions across groups, consistent with similar findings by others in healthy controls and patients with social phobia (Guastella *et al*, 2009; Joormann and Gotlib, 2006). Psychological models of emotional processing also suggest a more complex and divergent processing of facial cues conveying anger relative to fear. Anger, although commonly related to aversive contexts, is viewed as an approach-related behavior as it involves approaching particular desired outcomes, which may involve the creation of discomfort for someone else or of rectifying an injustice (Carver and Harmon-Jones, 2009). Hence, angry faces may have been viewed with some ambiguity resulting in both reduced amygdala activity, as well as behavioral face recognition accuracy.

In healthy human subjects, OXT has been shown to attenuate amygdala response to perception of threatening or aversive faces (ie, angry and fearful faces; Domes *et al*, 2007; Kirsch *et al*, 2005), as well as fear-conditioned socially relevant faces (Petrovic *et al*, 2008). Our findings advance this existing literature by showing for the first time that OXT can also attenuate clinically abnormal

fear-related hyperactivity of the amygdala in patients with GSAD, such that the extent of fear response was not different to that in healthy controls ('normalization'). Notably, the effects of OXT was specific to the fear-related hyperactivity observed in patients with GSAD, as OXT did not attenuate the amygdala activity to fear in the healthy control subjects, as well as the amygdala activity to angry and happy faces in both groups. However, these findings need to be interpreted with caution as these findings are in direct contrast to previous studies in healthy subjects described above, where OXT was shown to attenuate amygdala response to angry and fearful faces (Domes *et al*, 2007; Kirsch *et al*, 2005), as well as have a general effect on amygdala suppression regardless of valence (Domes *et al*, 2007). We provide the following interpretations to synthesize discrepant findings. First, it is possible that the effects of OXT on amygdala response to faces in healthy subjects are more variable than originally thought. The two previous fMRI studies in healthy subjects (Domes *et al*, 2007; Kirsch *et al*, 2005) reported small effects in the amygdala and the inconsistency of findings across studies, including the current investigation, may be due to the small sample sizes (13 subjects in both Domes *et al*, and Kirsch *et al*, and 17 controls in ours). Second, our EFMT task consisted of only three blocks per emotion, and therefore, the analysis of amygdala signal, particular in the healthy subjects who may have less sensitivity to these emotional faces, may be underpowered to detect OXT effects. Our null funding (in the largest fMRI study on OXT to date) and a recent study showing enhanced amygdala response to fearful faces in healthy women (Domes *et al*, 2010), suggests that at least in healthy subjects, the effects of OXT on amygdala response to facial cues may be variable and/or more subtle. Moreover, effects of OXT may also be moderated by individual differences in trait variables (eg, trait anxiety). A recent study has also shown that OXT may have differential effects on the activity of specific amygdala subregions during processing of emotional faces (Gamer *et al*, 2010). Future studies are needed to clarify the role of these important factors as they relate to oxytocin's effects on brain function in healthy humans.

The specific effects of OXT on fear in patients with GSAD also suggest an intriguing possibility that OXT may have the greatest and most consistent effects on amygdala activity when the amygdala is dysfunctional such as the hyperactivity observed in patients with GSAD. Under these conditions, OXT may be able to reduce activity to 'normal' levels of function. Therefore, from an evolutionary prospective, OXT's role could be to specifically modulate amygdala under pathological rather than normal physiological states (eg, to reduce the processing of threatening social cues after the initial threat has been detected), as it would be undesirable to have a general dampening of the amygdala to all emotional cues. Such a mechanism could also explain why OXT specifically attenuated the amygdala response to fear rather than a general suppression of response to all faces regardless of valence. Alternatively, the apparent specificity for fearful faces may be due to OXT having differential effects on processing of fear relative to anger and happy faces. Indeed, OXT has shown improved recognition memory for angry faces (Savaskan *et al*, 2008) and increased encoding, and

subsequent retrieval of happy faces (relative to threatening faces; Guastella *et al*, 2008), suggesting that consolidation of these faces in memory (via amygdala-hippocampal interactions) may explain the lack of attenuation of angry- and happy-related amygdala activity following OXT. However, the apparent fear specific attenuation of amygdala activity by OXT in GSAD and our explanations of the findings require confirmation and warrant further investigation.

The effect of OXT in normalizing fear-related amygdala hyperactivity in patients with GSAD suggests that the amygdala may be critical target for OXT's pharmacodynamic effects on the processing of social stimuli and related social behaviors. In support, increased release of OXT from local OXTergic fibers has been noted in the central nucleus of the amygdala during stressful or anxiety-provoking situations (De Vries and Buijs, 1983; Landgraf and Neumann, 2004), and behavioral changes in fear and anxiety correlate with differences in the levels of OXT-R expression and the potency of OXT-R antagonism (Bale *et al*, 2001; Champagne and Meaney, 2001; Ebner *et al*, 2005; Landgraf and Neumann, 2004; Lubin *et al*, 2003). Furthermore, there is evidence that a sub-population of GABAergic interneurons in the amygdala are activated by OXT-R stimulation (Huber *et al*, 2005). These GABAergic interneurons are part of the intra-amygdala system of inhibitory GABAergic connections, which are thought to integrate the output activity of the central nucleus of the amygdala (Cassell *et al*, 1999; Savander *et al*, 1996). OXT's physiological effects may indeed be mediated by the inhibition of amygdala output pathways that are critical for the behavioral and physiological expression of fear.

OXT's effect on fear-related amygdala activity was observed in the absence of overt subjective changes in mood or anxiety. These findings are entirely consistent with previous neuroimaging (Kirsch *et al*, 2005) and behavioral (Di Simplicio *et al*, 2008; Fischer-Shofty *et al*, 2010; Rimmele *et al*, 2009; Unkelbach *et al*, 2008) and clinical (Guastella *et al*, 2009) studies, which have shown similar insensitivity of behavioral and clinical (ie, LSAS) measures of mood and anxiety following single doses or short-term administration of OXT. OXT also failed to affect behavioral task performance (ie, accuracy and reaction times for face matching) consistent with previous studies that have shown no effects of OXT on an identical face matching task (Kirsch *et al*, 2005) and face recognition using facial cues of similar intensity (ie, 100% Di Simplicio *et al*, 2008; Marsh *et al*, 2010). However, OXT has been shown to enhance speed of recognition of moderately intense fear (Di Simplicio *et al*, 2008) and happy faces (Marsh *et al*, 2010), as well as dynamic fearful faces (Fischer-Shofty *et al*, 2010), suggesting that behavioral effects of OXT may be greater when requiring greater interpersonal processing. These data suggest that fMRI may be more sensitive in detecting changes in neural processing that lead up to social behavioral changes. Hence, it is possible that by modulating amygdala response to threat-related cues, prolonged treatment with OXT may reveal clinical and behavioral improvements in patients with GSAD, as OXT in humans

appears to enhance the ability to interact socially (Kosfeld *et al*, 2005) and improve control over stress and anxiety in social interactions (Heinrichs *et al*, 2003). Recent studies, have also shown a relationship between social anxiety symptoms and plasma OXT levels in GSAD patients (Hoge *et al*, 2008), and that administering OXT improves patients' self-evaluation of appearance and speech performance, albeit without additive effect on exposure treatment outcomes (Guastella *et al*, 2009). The current acute findings provide a proof of mechanism in support of further studies to examine the potential anxiolytic and pro-social effects of chronic OXT or OXT-R agonists in patients with GSAD. Future studies are needed to clarify the differential effects, if any, between acute and more chronic administration of OXT on behavior, anxiety symptomatology, and brain function.

Several limitations of the current study should be noted. First, our whole-brain analysis did not yield a significant drug × group × emotion interaction for the amygdala. This may have been due to the small sample studied and there was insufficient power to test this three-way interaction. Second, our main contrast of interest used the shapes condition as the 'control', to maximize amygdala activation (Hariri *et al*, 2000); moreover, because the neutral face stimuli was present within each trial of face trio, we could not isolate out the neutral faces as a separate condition. Therefore, we cannot ascertain if these findings would hold had we been able to use neutral faces as an alternate control condition. Also, given the lack on an additional 'control'/baseline condition (eg, fixation/rest), we cannot determine group or drug effects in brain response to the matching shapes condition. Third, it is possible that our laboratory/fMRI study environment (ie, entering the laboratory and going through MRI scanning) may have impacted on subjective mood and this may have potentially confounded any drug effects. However, our behavioral findings on mood are consistent with previous OXT studies where similar assessments and procedures were performed. Finally, our findings in males cannot be extended to females, particularly as gender differences could have a role in modulating the neural effects of OXT (Domes *et al*, 2010), as well as the amygdala reactivity to emotional faces (Killgore and Yurgelun-Todd, 2001; Lee *et al*, 2002). Future studies are needed to address these important issues.

In conclusion, data from this study showed that OXT has a specific effect on fear-related amygdala activity, particularly, when the amygdala is hyperactive, such as in GSAD. By showing that OXT can 'normalize' this hyperactivity in socially anxious individuals who exhibit aberrant fear responses, the current data provides initial evidence for a brain-based mechanism involving OXT-Rs within the amygdala, previously posited to involve inhibitory effects via GABAergic interneurons, thereby, suppressing the behavioral and physiological expression of fear and facilitating social behavior.

Acknowledgments

This work was supported by a NARSAD Independent Investigator Award and a grant from Monash University to Pradeep J Nathan. K Luan Phan and Mike Angstadt were supported by NIH Grant MH076198. Costs for oxytocin nasal sprays were supported by a Grant from the Swiss National Science Foundation (SNSF PP001-114788) to Markus Heinrichs. Amanda Wood was supported by an Australian Rotary Health fellowship.

The authors IL, KLP, AW, MA, PC, MH, JS, and PJN declare that no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest. PJN is also an employee at GlaxoSmithKline Pharmaceuticals, but has no conflict of interest with this study, which was performed at Monash University where he holds an academic appointment.

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