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ARTICLE

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Medial frontal hyperactivity to sad faces in generalized social anxiety disorder and modulation by oxytocin

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Abstract

Generalized social anxiety disorder (GSAD) is associated with heightened limbic and prefrontal activation to negative social cues conveying threat (e.g. fearful faces), but less is known about brain response to negative non-threatening social stimuli. The neuropeptide oxytocin (Oxt) has been shown to attenuate (and normalize) fear-related brain activation and reactivity to emotionally negative cues. Here, we examined the effects of intranasal Oxt on cortical activation to non-threatening sad faces in patients with GSAD and matched controls (Con). In a double-blind placebo-controlled within-subjects design, the cortical activation to sad and happy (*vs.* neutral) faces was examined using functional magnetic resonance imaging following acute intranasal administration of 24 IU Oxt and placebo. Relative to the Con group, GSAD patients exhibited heightened activity to sad faces in the medial prefrontal cortex (mPFC/BA 10) extending into anterior cingulate cortex (ACC/BA 32). Oxt significantly reduced this heightened activation in the mPFC/ACC regions to levels similar to that of controls. These findings suggest that GSAD is associated with cortical hyperactivity to non-threatening negative but not positive social cues and that Oxt attenuates this exaggerated cortical activity. The modulation of cortical activity by Oxt highlights a broader mechanistic role of this neuropeptide in modulating socially negative cues in GSAD.

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Introduction

Generalized social anxiety disorder (GSAD), also called social phobia, is characterized by excessive fear in social and performance situations. Patients with GSAD have a negative bias and enhanced sensitivity to threat-related cues (Amir *et al.* 2003; Foa *et al.* 2000; Garner *et al.* 2009; Mogg & Bradley, 2002; Mogg *et al.* 2004). Functional imaging studies have shown that patients with GSAD exhibit heightened activation of the amygdala in response to social cues conveying threat and/or negative feedback such as fearful, angry, contemptuous and/or disgusted faces (Evans *et al.* 2008; Labuschagne *et al.* 2010; Phan *et al.* 2006; Stein *et al.* 2002).

In comparison, relatively little is known about the behavioural and brain responses to social cues that convey negative but non-threatening cues such as sad faces in GSAD, although we have previously shown evidence for exaggerated amygdala and insula responses to non-threatening emotional pictures (Shah *et al.* 2009). Behavioural studies suggest that patients

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with GSAD may be hypersensitive to cues conveying sadness. For example, patients with GSAD use more extensive visual scan paths when viewing sad faces (Horley *et al.* 2004) and recognize sad faces (in addition to fearful and happy faces) with greater sensitivity across varying facial intensities in comparison to healthy controls (Arrais *et al.* 2010). These findings suggest that GSAD may also be associated with abnormal brain responses to social cues conveying sadness.

Functional imaging studies in normal healthy subjects suggest that appraisal of sad visual cues is associated with activation of a number of prefrontal cortical regions within the ventral-rostral (BA 9, 10, 47) medial prefrontal cortex (mPFC) extending into the affective division of the anterior cingulate cortex (ACC) (BA 24, 25, 32, 33), in addition to limbic regions such as the amygdala. For example, studies have shown mPFC (e.g. BA 10) and ACC (e.g. BA 25) activation to sad facial cues (Britton *et al.* 2006; Lee *et al.* 2002; Lennox *et al.* 2004). Similar regions (e.g. BA 10, 24, 32, 33, 47) have also been shown to be activated following induced sadness using sad facial cues (George *et al.* 1995; Habel *et al.* 2005; Schneider *et al.* 1997). Studies have also reported activation of other prefrontal areas during processing of sad facial cues including the inferior frontal gyrus (BA 47; Suslow *et al.* 2010) and the superior frontal gyrus (BA 9; Lennox *et al.* 2004). While the majority of studies suggest that areas within the prefrontal cortex (PFC) are involved in processing sad facial cues, some studies have failed to find similar activation in these areas (Palm *et al.* 2010; Phillips *et al.* 1998).

Functional imaging studies in mood and anxiety disorders have been mixed with regard to frontal cortical activation during processing of sad facial cues. For example, patients with generalized anxiety disorder (GAD) have been shown to have reduced PFC activation to sad (and fearful, angry and happy) facial expressions in regions of the ACC (BA 32) and medial orbitofrontal cortex (BA 10) (Palm *et al.* 2010). Similarly, patients with mania show reduced subgenual ACC (BA 25) activity to sad faces (Lennox *et al.* 2004). In contrast, enhanced activation in ventral mPFC (BA 11, 47) has been reported in autism spectrum disorders (Weng *et al.* 2011). Similarly, in major depressive disorder (MDD), studies have reported enhanced ACC activity (extending into the mPFC) (BA 24, 32) to sad words (Elliott *et al.* 2002), as well as enhanced capacity of activation in the ACC (BA 23, 24, 32) and reduced dynamic range (i.e. intensity load response) in the ACC (BA 24, 32) and mFPC (BA 8, 9) to sad faces (Fu *et al.* 2004). Overall these findings suggest that cortical regions, in particular, the mPFC and ACC are involved in processing of sad facial cues and activation is these regions may be abnormal in patients with mood and anxiety disorders.

The frontal cortical response to social cues conveying sadness is yet to be examined in GSAD, although exaggerated mPFC and ACC activation have increasingly been reported in GSAD (Freitas-Ferrari *et al.* 2010). For example, in patients with GSAD, hyperactivity to faces conveying threat (i.e. angry, fear and disgust) have been reported in the mPFC (BA 9) (Stein *et al.* 2002) and ACC (BA 24, 32) (Amir *et al.* 2005; Phan *et al.* 2006). Similar increases in mPFC (BA 8, 9) activation have been reported during processing of negative emotional pictures (Brühl *et al.* 2011), with suggestions that this may reflect, increased self-focused thoughts, that is typical in GSAD (Bogels & Mansell, 2004), or efforts to control the emotions (John & Gross, 2004). Others have suggested that mPFC and ACC activations may be related to mentalization and self-referential processing (Mitchell *et al.* 2006; Northoff *et al.* 2006).

Treatment studies have provided evidence that abnormal frontal cortical activation to emotional cues in mood disorders is predictive of clinical outcomes and can be restored (i.e. normalized) following periods of antidepressant or behavioural treatment. In MDD, the reduced ACC (BA 24) and insula activity to presentations of negative emotional pictures at baseline was increased (i.e. normalized) following 2 wk of venlafaxine treatment (Davidson *et al.* 2003). Similarly, enhanced capacity of activation in the ACC (BA 23, 24, 31, 32) and reduced dynamic range (intensity load response) in the ACC (BA 24, 32) and mFPC (BA 8, 9) to sad faces was reduced and enhanced respectively following 8 wk of fluoxetine treatment (Fu *et al.* 2004). These findings suggest that restoration or normalization of abnormal activity in cortical regions including the ACC and mPFC may be important for therapeutic efficacy.

Oxytocin (Oxt), a neuropeptide synthesized in the hypothalamus, has been shown to decrease anxiety and stress and to facilitate social encounters (for reviews see Bartz & Hollander, 2006; Heinrichs *et al.* 2009). Recent neuroimaging studies have provided proof of mechanism evidence in support of the use of Oxt as a potential treatment for GSAD. Specifically, Oxt has been shown to reduce amygdala response to threatening faces in healthy controls (Kirsch *et al.* 2005) and in patients with GSAD who showed exaggerated baseline amygdala response to fearful faces (Labuschagne *et al.* 2010). Oxt has also been shown to suppress amygdala response to non-threatening

happy faces (Domes *et al.* 2007) as well as negative non-social pictures (Kirsch *et al.* 2005), suggesting that is has broader pharmacological effects on brain responses to emotional cues. While these studies have suggested valence independent suppression of amygdala response to emotional cues, a recent study suggests that Oxt may have valence-dependent effects on subregions within the amygdala (Gamer *et al.* 2010).

In this study, we used functional magnetic resonance imaging (fMRI) to examine cortical reactivity to socially relevant sad faces in patients with GSAD and healthy controls, and their modulation by Oxt. To examine valence dependency and to examine cortical responses to sad faces in relation to an opposite emotional expression, we also examined cortical responses to happy faces and the effects of Oxt on these responses. Based on the evidence that the prefrontal cortical regions including the ACC and mFPC are; (1) commonly associated with processing sad faces in healthy subjects, (2) show abnormal activity in mood and anxiety disorders, and (3) are regions modulated by pharmacological treatments, we hypothesized that there would be an abnormal frontal cortical activity, specifically in the medial wall of the PFC (i.e. mPFC) extending into the ACC, to socially relevant sad faces in GSAD, and that Oxt would normalize this aberrant pattern of reactivity.

Methods

Subjects

Eighteen males with GSAD (values are mean $\pm$ S.D.) (age 29.4 $\pm$ 9.0 yr, education 14.7 $\pm$ 1.6 yr) and 18 age- and education-matched healthy control (Con) subjects (age 29.9 $\pm$ 10.2 yr, education 16.00 $\pm$ 2.5 yr) participated in the study. Diagnosis of GSAD was confirmed by the Clinical International Diagnostic Interview (CIDI) version 2.1 (WHO, 1997) with additional probes from the Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987), and a requirement of LSAS total score $>$70 and a score $>$30 on each subscale for performance and situational to satisfy the criteria for the generalized subtype. Additional screening measures were included to further assess the presence of psychiatric disorders: Beck Depression Inventory (BDI-II; Beck *et al.* 1996), Beck Anxiety Inventory (BAI; Beck & Steer, 1990), and the trait measure from the State-Trait Anxiety Inventory (STAI; Spielberger *et al.* 1983). None of the GSAD patients or controls had a current depressive episode (including within the last 6 months), or a lifetime history of bipolar disorder, schizophrenia, post-traumatic stress disorder, an organic syndrome, mental retardation or pervasive developmental disorder, a history of neurological trauma resulting in loss of consciousness, or a history of substance and/or alcohol abuse. One patient had comorbid obsessive compulsive disorder which was deemed less clinically significant than the GSAD diagnosis. All participants were right-handed, between the ages of 18 and 55 yr, non-smokers, free of psychotropic medication for at least 4 wk, free of ferrous-containing metals within the body, and did not suffer from claustrophobia or any (other) psychiatric or neurological disorder as assessed by the CIDI. Written informed consent was provided by all subjects and the study was approved by the Standing Committee on Ethics in Research involving Humans of Monash University.

Study design and drug administration

The study adopted a randomized, counterbalanced, double-blind, placebo-controlled within-subjects design in which all participants underwent fMRI scanning during an emotional face processing task following an acute drug administration of Oxt and placebo (Pbo) in two separate sessions, separated by a minimum of 1-wk washout period. At 45 min before each fMRI session, the subject self-administered an intranasal spray of either Oxt (24 IU or 40.32 μg; Syntocinon spray, Switzerland) or Pbo (containing all ingredients except for the peptide). This involved three puffs of 4 IU each (6.72 μg) per nostril in an alternating order with 45 s between each application. The dosages were comparable to that used in previous human studies (Domes *et al.* 2007; Heinrichs *et al.* 2003). The fMRI scanning was started 50-min post-drug administration to coincide with the expected peak pharmacokinetic effects (Born *et al.* 2002) and physiological effects (Domes *et al.* 2007; Heinrichs & Domes, 2008; Heinrichs *et al.* 2003; Labuschagne *et al.* 2010) of Oxt. Participants refrained from consuming alcohol and/or caffeine for 24 h before the scanning day and avoided food 1 h prior to their arrival.

Behavioural task and analysis

Inside the MRI scanner, each subject was presented with a computerized emotional face processing task consisting of 72 face images of black-and-white photographs (Ekman & Friesen, 1976). Single presentations of sad, happy and neutral faces were presented in 36-s blocks (12 images/block, two blocks of each emotion), which were alternating with 12-s baseline blocks of cross-hair images with different

greyscale backgrounds (four images/block, six blocks) in a single functional run. The cross-hair blocks interspersed between the emotional blocks, allowed for haemodynamic relaxation and minimized non-specific cognitive processing that can occur during long periods of blank fixation (Gusnard & Raichle, 2001). An additional three trials of cross-hairs were included at the start to prepare participants for the task, but which were discarded during analysis. Participants responded to each presentation of an emotional face stimulus using a three- button button-box to indicate whether the expression was negative (left button), neutral (middle button) or positive (right button) and to the presentation of the cross-hair stimulus by pressing the middle (neutral) button. Response accuracy and latency scores (i.e. reaction times) were statistically analysed in separate 2 (group) × 2 (drug) × 3 (emotion) repeated-measures analysis of variance (ANOVA), corrected using the Greenhouse–Geisser method.

fMRI acquisition and analysis

During the task, gradient echo echo-planar (EPI) blood oxygen level-dependent (BOLD) fMRI data were collected for each subject using a 3.0 T Trio Tim Siemens scanner and a 12-channel head coil (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). In addition, high-resolution T1-weighted images (TE = 2.15 ms, TR = 1900 ms, flip angle = 9°, FoV = 256 mm, 176 sagittal 1-mm slices, perpendicular to the AC–PC line) were acquired for anatomical reference. Images from all participants met the criteria for data quality. Conventional SPM5 data processing analyses were conducted, where images were spatially realigned (to correct for head motion), warped to an EPI template in Montreal Neurologic Institute (MNI) space, resampled to 2-mm^3 voxels and smoothed with an 8-mm^3 kernel. Using a 128-s high-pass filter, we applied a general linear model to the time-series, convolved with the canonical haemodynamic response function (Friston *et al.* 1995).

Face contrasts (sad > neutral and happy > neutral) were linearly generated and estimated at each voxel resulting in statistical parametric maps (SPMs) for each subject. These maps were then analysed at the second level in a random-effects statistical model (Holmes & Friston, 1998) for within-group, between-group, and between-session analyses. Because we had specific *a-priori* hypotheses about group differences in brain activation to sad faces at baseline (i.e. no drug), we first conducted a between-group (GSAD *vs.* Con) analysis within each drug session (Pbo and Oxt, separately) for each emotion-specific face contrast (sad > neutral and happy > neutral, separately) using a whole-brain voxel-wise independent-samples t test between the GSAD and Con groups. Second, to examine the effects of Oxt on brain activation to sad and happy faces, we conducted a between-session analysis (Pbo *vs.* Oxt) within each group (GSAD and Con, separately) for each emotion-specific face contrast (sad > neutral and happy > neutral, separately) using a whole-brain voxel-wise paired t test between the placebo and Oxt sessions. The significance level was set at $p < 0.005$ (uncorrected) with cluster (k = number of voxels) extent threshold of $k > 20$ contiguous voxels to balance between type I and type II errors (Lieberman & Cunningham, 2009), consistent with prior fMRI studies of GSAD.

We also pursued a more conservative analysis to further test our *a-priori* hypotheses about Oxt effects on brain responses to sad faces. An overall whole-brain voxel-wise ANOVA with group (GSAD, Con) as between-factor and drug (Pbo, Oxt) as within-subject factors was conducted for the sad > neutral face contrast. Here, we were specifically interested in the group × drug interaction effects of Oxt, and thus explicitly examined this interaction across the entire brain. From the peak clusters that show significant group × drug interactions, in order to clarify the effects driving this interaction, we created a 5-mm radius sphere to extract BOLD signal responses (or parameter estimates, β-weights in arbitrary units) to confirm direction of Oxt's effects. Data were analysed using independent t tests to compare between groups (GSAD and Con) for Pbo and Oxt sessions separately, and paired t tests to compare between Pbo and Oxt sessions in GSAD and Con groups separately. In addition a t test was performed to compare the GSAD group on Oxt *vs.* the Con group on Pbo to test for any normalization of activation. Given this more conservative test of an interaction, the significance level was set at $p < 0.05$ (uncorrected) with cluster (k = number of voxels extent threshold of $k > 20$ contiguous voxels.

Mood measures and analyses

Mood was assessed pre- and post-drug treatment using the Profile of Mood States (POMS; McNair *et al.* 1971) and the state measure of the STAI (Spielberger *et al.* 1983). Changes in mood (post- minus pre-drug administration) for each questionnaire were analysed within each group and drug condition using repeated-measures ANOVAs.

Results

Demographics

Con and GSAD groups did not differ on age (values are mean ± S.D.) [GSAD 28.9 ± 9.0, Con 28.4 ± 10.2 yr; $t(34) = 0.16$, $p = 0.88$] or education [GSAD 14.67 ± 1.6, Con 16.0 ± 2.5 yr; $t(34) = 1.93$, $p = 0.06$]. Compared to the Con group, the GSAD patients scored higher on depression (although below the cut-off of 13 for mild depression) [BDI-II: GSAD 10.8 ± 7.5, Con 1.28 ± 1.9; $t(34) = -5.27$, $p < 0.001$], were more anxious [BAI: GSAD 16.9 ± 8.2, Con 2.2 ± 5.0; $t(34) = -6.50$, $p < 0.001$] and had higher trait anxiety [STAI: GSAD 50.4 ± 11.5, Con 27.4 ± 8.2; $t(34) = -7.79$, $p < 0.01$].

Behavioural results

Behavioural data from four patients (during Oxt sessions) were not recorded due to faulty equipment. Both GSAD and Con subjects performed equally well on the face recognition, and Oxt had no effect on task performance. Repeated-measures ANOVA showed no significant difference in accuracy for group [$F(1, 66) = 0.01$, $p = 0.913$] or drug [$F(1, 66) = 0.08$, $p = 0.784$], and no significant interaction between group, drug or emotion [group × drug: $F(1, 66) = 0.002$, $p = 0.965$; group × emotion: $F(2, 132) = 0.617$, $p = 0.541$; drug × emotion: $F(2, 132) = 1.400$, $p = 0.250$; group × drug × emotion: $F(2, 132) = 0.322$, $p = 0.725$]. There was a main effect for emotion [$F(2, 132) = 30.862$, $p = 0.0005$], with percentage (%) correct recognition of sad faces (79.5 ± 22.8%) being significantly less than happy (95.6 ± 12.9%) and neutral (94.5 ± 11.4%) faces (p's < 0.000).

Similar to the accuracy results, both participant groups (Con and GSAD) performed equally well on the latency measure on the behavioural task, and Oxt had no effect on task performance. In a repeated-measures ANOVA, no significant main effects were evident on latency scores for group [$F(1, 31) = 0.000$, $p = 0.991$], and drug [$F(1, 31) = 2.58$, $p = 0.118$], and no significant interaction between group, drug, or emotion (group × drug [$F(1, 31) = 0.21$, $p = 0.652$], group × emotion [$F(2, 62) = 0.565$, $p = 0.564$], drug × emotion [$F(2, 62) = 0.526$, $p = 0.557$] and group × drug × emotion [$F(2, 62) = 0.617$, $p = 0.511$]). The main effect of emotion on response to latency was significant [$F(2, 62) = 8.75$, $p = 0.001$]), with response latency to sad stimuli being significantly longer than that of happy or neutral. Means and standard deviations for latency as well accuracy scores for group (Con, GSAD), drug (Pbo, Oxt) and emotion (sad, neutral, and happy) are presented in Table 1.

Table 1. Performance during emotional face recognition task for accuracy (percentage correct, %) and latency (ms)

			Controls		GSAD	
Measure	Session	Condition	Mean	S.D.	Mean	S.D.
Accuracy (%)	Placebo	Sad	76.4	21.5	79.0	25.1
		Neutral	97.2	4.9	91.7	13.9
		Happy	96.3	6.4	97.3	6.1
	Oxytocin	Sad	80.3	24.1	81.2	20.6
		Neutral	94.4	13.1	92.9	11.3
		Happy	93.9	17.0	95.3	18.1
Reaction time (ms)	Placebo	Sad	1560.1	333.0	1497.6	363.0
		Neutral	1523.7	233.7	1497.4	349.2
		Happy	1530.1	343.5	1479.0	354.3
	Oxytocin	Sad	1414.6	204.2	1448.4	169.8
		Neutral	1405.8	174.2	1436.0	175.3
		Happy	1387.6	190.4	1415.4	139.5

GSAD, Generalized social anxiety disorder.

fMRI results

Tables 2 and 3 present the corresponding brain activation coordinates obtained during statistical analyses. This includes activation coordinates for sad and happy (>neutral) faces for group (GSAD *vs.* Con) during each session (Pbo alone and Oxt alone), and drug differences (Pbo *vs.* Oxt) for each group (GSAD alone and Con alone), at a threshold of $p < 0.005$ (uncorrected), k (number of contiguous voxels per cluster) >20.

Group differences at baseline/Pbo

In response to sad *vs.* neutral faces, the GSAD (compared to Con) group showed significantly enhanced activation in several clusters in bilateral mPFC (BA 10) extending into the left ACC (BA 32) (see Fig. 1). Other areas of enhanced activity included right primary postcentral gyrus (BA 1), right premotor (BA 6) and bilateral primary visual (BA 17) cortices, right superior temporal sulcus (BA 22) and left occipital lingual gyrus (BA 19) (see Table 2). Of note, the group differences in mPFC and ACC observed at baseline/Pbo were no longer evident after administration of Oxt. The Con group did not show any superior activation to the patients, and happy faces showed no group differences in regional activations under Pbo.

Oxt vs. *Pbo in GSAD*

In response to sad *vs.* neutral faces, Oxt attenuated regions hyperactive in the baseline (Pbo) condition,

Table 2. Group difference and effect of oxytocin on brain activation to sad (>neutral) faces

Contrast and activation foci		BA	MNI coordinates			Volume (mm^3)	Voxel	
			x	y	z		*T*	*Z*
Pbo								
GSAD > Con	Calcarine fissure	17	−16	−62	16	3168	3.94	3.51
	Precuneus	17	16	−56	14	1785	3.91	3.49
	Supplementary motor cortex	6	10	−16	62	504	3.67	3.31
	Anterior cingulate cortex	**32**	**−16**	**58**	**10**	**704**	**3.61**	**3.26**
	Medial prefrontal cortex	**10**	**−12**	**56**	**−4**	**976**	**3.59**	**3.25**
		10	**2**	**66**	**6**	**224**	**3.41**	**3.11**
		10	**14**	**56**	**−4**	**288**	**3.18**	**2.92**
	Lingual gyrus/cerebellum	19	−12	−46	−10	320	3.46	3.15
	Cuneus	17	−6	−98	16	640	3.44	3.13
	Superior temporal cortex	22	54	−30	10	272	3.24	2.98
	Postcentral gyrus	1	26	−40	74	520	3.20	2.95
Con > GSAD	No significant clusters							
Oxt								
GSAD > Con	Middle occipital/middle temporal gyrus	39	44	−74	24	824	3.91	3.49
	Mid-cingulate cortex	24	−6	8	24	1656	3.58	3.24
	Supplementary motor cortex	6	16	−20	54	440	3.30	3.03
	Precentral gyrus	3	−24	−30	54	256	3.17	2.92
Con > GSAD	No significant clusters							
Pbo > Oxt in GSAD								
	Superior parietal lobe	5	14	−58	66	1624	4.92	3.74
	Supplementary motor cortex	6	16	−18	62	504	4.44	3.49
		6	−14	−20	62	320	4.23	3.38
	Medial prefrontal cortex	**10**	**10**	**70**	**8**	**296**	**4.26**	**3.40**
	Medial prefrontal/anterior cingulate cortex	**10/32**	**−12**	**58**	**12**	**1056**	**4.00**	**3.25**
		10/32	**12**	**56**	**6**	**512**	**3.69**	**3.06**
	Lingual/fusiform gyrus	18	−22	−86	−12	192	3.64	3.03
	Primary visual cortex	17	−14	−96	8	200	3.45	2.92
Oxt > Pbo in GSAD	Inferior temporal gyrus/ fusiform gyrus	37	46	−52	−4	328	4.63	3.59
Pbo > Oxt in Con	**Anterior cingulate cortex**	**24**	**4**	**14**	**20**	**1072**	**4.70**	**3.63**
	Middle frontal gyrus	46	56	42	22	432	4.53	3.54
	Supplementary motor cortex	6	46	8	58	648	4.00	3.25
	Superior parietal cortex	7	−20	−56	44	216	3.82	3.14
Oxt > Pbo in Con								
	Thalamus	n.a.	−16	−14	−2	240	3.69	3.06

BA, Brodmann area; MNI, Montreal Neurologic Institute; Pbo, placebo; GSAD, generalized social anxiety disorder; Con, healthy control; Oxt, oxytocin; n.a., not available.
Bold indicates the regions which showed pathologically abnormal activations as well as regions modulated by oxytocin.

including in several clusters in bilateral mPFC (BA 10) and ACC (BA 32) (see Fig. 1). This was expected given that the exaggerated mPFC/ACC response to sad faces in GSAD (*vs.* Con) was observed during the Pbo, but not Oxt, session. Furthermore, Oxt reduced activity in bilateral premotor (BA 6), right parietal (BA 5), and left visual (BA 17, 18) cortices. While the predominant effect of Oxt was suppression of cortical activity, there was enhanced activity in the right middle temporal (fusiform) cortex (BA 37)

Table 3. Group difference and effect of oxytocin on brain activation to happy (>neutral) faces

Contrast and activation foci		BA	MNI coordinates x	y	z	Volume (mm^3)	Voxel *t*	*Z*
Pbo								
GSAD>Con	No significant clusters							
Con>GSAD	No significant clusters							
Oxt								
GSAD>Con	Middle occipital gyrus/cuneus	18	−24	−100	−10	552	4.51	3.91
	Angular gyrus/middle temporal gyrus	39	54	−72	30	584	4.02	3.56
	Cerebellum	n.a.	50	−58	−30	208	3.18	2.93
	Inferior temporal cortex	37	−60	−58	−18	488	3.11	2.87
Con>GSAD	**Medial prefrontal/anterior cingulate cortex**	**24**	**2**	**26**	**−4**	**5800**	**5.25**	**4.38**
		32/10	**10**	**48**	**−14**	**608**	**3.50**	**3.18**
		9	**14**	**60**	**36**	**432**	**3.40**	**3.10**
		32	−10	52	30	160	3.01	2.79
	Operculum	43	−44	−6	16	264	3.68	3.32
	Inferior temporal gyrus	20	−44	−6	−34	2288	3.65	3.29
Pbo>Oxt in GSAD								
	Anterior cingulate cortex	**24**	**−2**	**28**	**−4**	**8304**	**5.04**	**3.80**
	Inferior temporal gyrus	20	68	−24	−22	808	4.99	3.77
		20	38	−18	−36	280	3.83	3.15
		20	−38	−18	−32	352	3.44	2.91
	Medial prefrontal/anterior cingulate cortex	**10/32**	**6**	**50**	**10**	**448**	**4.32**	**3.43**
	Medial prefrontal cortex	**9**	**12**	**66**	**30**	**520**	**3.99**	**3.25**
		11/10	**10**	**48**	**−14**	**344**	**3.64**	**3.03**
		9	**−16**	**52**	**34**	**288**	**3.28**	**2.80**
	Caudate head	n.a.	−6	14	2	424	3.98	3.24
	Cerebellum	n.a.	−8	−50	−32	296	3.71	3.08
	Middle temporal pole	20	42	18	−42	216	3.43	2.90
Oxt>Pbo in GSAD								
	No significant clusters							
Pbo>Oxt in Con								
	Cerebellum	n.a.	0	−58	−16	**400**	5.55	4.03
	Cerebellum/fusiform	37	−56	−62	−24	**592**	4.76	3.66
	Calcarine fissure/cerebellum	18/6	−2	−80	−8	5248	4.37	3.45
	Medial prefrontal cortex	**32**	**14**	**14**	**48**	**200**	**3.76**	**3.11**
		10	**10**	**60**	**−4**	**240**	**3.54**	**2.97**
	Middle frontal gyrus	46	−50	26	42	1200	4.04	3.27
	Precuneus	7	−10	−70	40	280	3.62	3.02
Oxt>Pbo in Con								
	Superior temporal gyrus	38	38	12	−22	**464**	4.86	3.71

BA, Brodmann area; MNI, Montreal Neurologic Institute; Pbo, placebo; GSAD, generalized social anxiety disorder; Con, healthy control; Oxt, oxytocin; n.a., not available.
Bold indicates the regions which showed pathologically abnormal activations as well as regions modulated by oxytocin.

following Oxt. For happy faces, Oxt suppressed bilateral mPFC (BA 9–11) along with bilateral ACC (BA 24, 32), bilateral temporal cortex (BA 20) and caudate and cerebellum.

Oxt vs. *Pbo in Con*

In response to sad *vs.* neutral faces, Oxt decreased activity in the right ACC (BA 24), right middle frontal

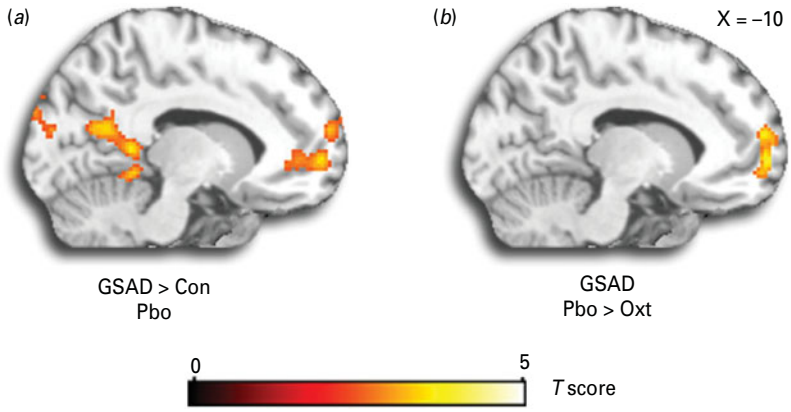

Fig. 1. (*a*) Increased medial prefrontal cortex (mPFC) (BA 10) and anterior cingulate cortex (ACC) (BA 32) activity to sad (*vs.* neutral) faces at baseline/placebo (Pbo) activation in generalized social anxiety disorder (GSAD) patients compared to healthy controls (Con) at $p < 0.005$. (*b*) Oxytocin (Oxt) suppression of heightened mPFC/ACC (BA 10, 32) in patients with GSAD at $p < 0.005$.

gyrus (BA 46), and right premotor (BA 6) and left superior parietal (BA 7) cortices, and enhanced activity in the thalamus. In response to happy faces, Oxt suppressed activity in the right mPFC (BA 10) extending into right ACC (BA 32), left middle frontal gyrus (BA 46), left precuneus (BA 7), left fusiform (BA 37), left calcarine fissure (BA 17) and cerebellum, whereas Oxt enhanced right superior temporal gyrus (BA 38) in the Con group.

Overall ANOVA and β-weight extractions

Results from the overall whole-brain ANOVA for Oxt effects on brain response to sad (>neutral) faces revealed a significant group × drug interaction ([12, 52, 4]; $F = 8.58$, $Z = 2.49$, 487 voxels/3892 mm³ in medial prefrontal-ACC region; BA 10/32). Analysis of the extracted BOLD signal (β-weights) from this mPFC/ACC cluster revealed an expected significant group difference on Pbo (greater activation in GSAD group compared to Con group) [$t(30) = 2.220$, $p = 0.034$]; attenuation by Oxt (greater activation in Pbo than Oxt) in the GSAD group [$t(15) = 3.165$, $p = 0.006$]; and no difference in activation between the GSAD group on Oxt and Con group on Pbo [$t(30) = -1.163$, $p = 0.254$]. Figure 2 presents the magnitude (β-weights ±S.E.M.) of the activations within each group from the Oxt and Pbo sessions separately, and significant *post-hoc t* tests.

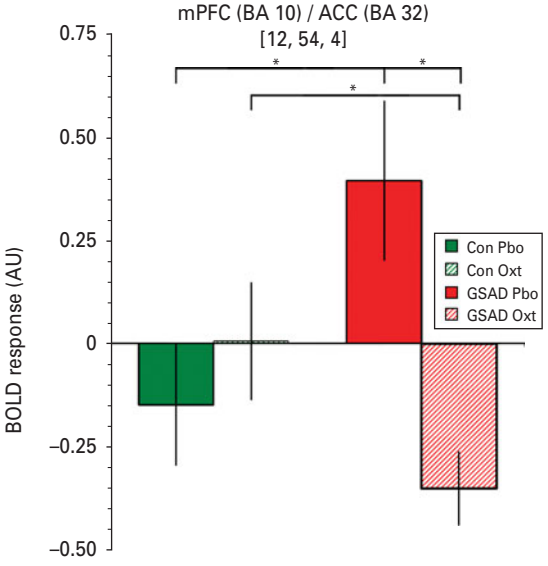

Fig. 2. Extracted blood oxygen level-dependent (BOLD) responses in mean β-weights (arbitrary units, AU) to sad (>neutral) faces extracted from the medial prefrontal cortex/anterior cingulate cortex (mPFC/ACC) cluster exhibiting a group × drug interaction, showing: (1) greater mPFC/ACC reactivity in the generalized social anxiety disorder (GSAD) group (>Con) during placebo (Pbo) treatment; (2) attenuation of mPFC/ACC reactivity in GSAD group by oxytocin (Oxt) treatment (Pbo > Oxt); and (3) extent of mPFC/ACC reactivity is similar in GSAD on Oxt and Con group on Pbo. Asterisks denote between-group and between-session differences (* $p < 0.05$, two-tailed).

Subjective data

No significant effects of Oxt (compared to Pbo) were evident on either the mood (i.e. POMS) or state-anxiety (STAI) measures in both the participant groups, with no drug or group main effects or drug × group interactions (all p's <0.05). For POMS, the repeated-measures ANOVA did not show significant

Table 4. Means (and standard error of the mean) of subjective mood and state-anxiety changes following drug administration

	Controls				GSAD			
	Placebo		Oxytocin		Placebo		Oxytocin	
	T1	T2	T1	T2	T1	T2	T1	T2
(*a*) POMS								
Tension (out of 36)	1.7 (0.4)	1.0 (0.2)	2.3 (0.5)	1.5 (0.4)	9.4 (1.3)	7.2 (1.1)	11.1 (1.8)	7.1 (1.1)
Depression (out of 60)	0.4 (0.2)	0.2 (0.2)	0.3 (0.2)	0.2 (0.2)	6.2 (1.6)	4.0 (1.2)	7.3 (1.9)	4.3 (1.2)
Anger (out of 48)	0.8 (0.3)	0.8 (0.4)	0.8 (0.4)	0.2 (0.1)	3.6 (1.2)	2.4 (1.1)	3.3 (0.9)	1.5 (0.5)
Vigour (out of 32)	18.2 (1.5)	19.3 (1.1)	18.1 (1.4)	18.0 (1.4)	12.7 (1.2)	10.7 (1.1)	12.4 (1.3)	11.0 (1.4)
Fatigue (out of 28)	4.3 (1.1)	3.3 (1.0)	4.6 (0.8)	3.4 (0.7)	7.8 (1.2)	9.7 (1.6)	8.7 (1.2)	9.2 (1.0)
Confusion (out of 28)	2.9 (0.7)	2.1 (0.4)	3.1 (0.5)	2.7 (0.6)	7.7 (1.0)	8.4 (1.2)	7.9 (0.9)	7.7 (0.8)
(*b*) STAI-Y1: state-anxiety (out of 80)	25.8 (1.4)	25.7 (1.4)	26.7 (1.4)	26.0 (1.3)	39.6 (1.9)	39.1 (1.6)	42.4 (2.6)	39.3 (2.0)

GSAD, Generalized social anxiety disorder; POMS, Profiles of Mood Scale; STAI-Y1, Spielberg Trait-State Anxiety Inventory, state measure.
Mean (±S.E.M.) scores, $n=18$; Time (T) where T1 = pre-drug administration and T2 = post-drug administration.

main effects for group [$F(1,68)=2.487$, $p=0.119$] or drug [$F(1,68)=1.404$, $p=0.240$] or interactions for group × drug [$F(1,68)=0.378$, $p=0.541$], drug × factor [$F(5,340)=0.10$, $p=0.960$] or group × drug × factor [$F(5,340)=0.433$, $p=0.722$]. For state anxiety (STAI) repeated-measures ANOVA showed no significant main effects for group [$F(1,68)=1.08$, $p=0.303$] or drug [$F(1,68)=1.25$, $p=0.267$], and no significant interaction for group × drug [$F(1,68)=0.51$, $p=0.479$]. Table 4 presents the means (±S.E.M.) for; (*a*) the POMS and (*b*) the state measure of the STAI across two time-points on each testing day (Pbo, Oxt) and between groups (Con and GSAD).

Discussion

In this study, we used fMRI to examine the cortical activation in response to sad and happy facial cues in patients with GSAD and healthy controls and their modulation by intranasal Oxt administration. GSAD patients compared to controls showed significantly greater prefrontal cortical activation to faces conveying sadness, predominantly in bilateral mPFC (BA 10) and left rostral ACC (BA 32) under Pbo treatment. In contrast, no hyperactivity to happy facial stimuli was observed. Oxt attenuated the sad-related hyperactivity in the mPFC/ACC in GSAD subjects, such that group differences in mPFC and ACC observed at baseline/Pbo were no longer evident after Oxt administration.

To our knowledge this is the first evidence of exaggerated mPFC/ACC response to non-threatening sad faces in patients with GSAD. Previous studies in patients with GSAD (Etkin & Wager, 2007) have shown amygdala and insula hyperactivity to social faces conveying social evaluative threat (i.e. fear, anger, and/or contempt/disgust) (Evans *et al.* 2008; Labuschagne *et al.* 2010; Phan *et al.* 2006; Stein *et al.* 2002), to negative non-threatening pictures (Shah *et al.* 2009) and more generally to anxiety-provocation (e.g. anticipation of public speaking) (Furmark *et al.* 2002; Lorberbaum *et al.* 2004). Other studies have reported hyperactivity in areas within the PFC including the mPFC (BA 9) and ACC (BA 24, 32) to faces conveying threat (i.e. angry, fear and disgust) (Amir *et al.* 2005; Phan *et al.* 2006; Stein *et al.* 2002). Our findings extend these observations showing cortical hyperactivity to non-threat-related sad facial cues in nearby or overlapping areas of the mPFC (BA 10) and ACC (BA 32). Together these findings suggest the GSAD is associated with both cortical and limbic hyperactivity to threat- and non-threat-related emotional cues.

Prefrontal cortical regions such as the mPFC (BA 9, 10) and ACC (BA 24, 32) are generally activated during) processing of sad facial stimuli in healthy subjects (Britton *et al.* 2006; Lee *et al.* 2002). While findings in mood and anxiety disorders have been inconsistent (Amir *et al.* 2005; Phan *et al.* 2006; Stein *et al.* 2002), these findings have highlighted that regions within the PFC including the ACC and mPFC have abnormal (hyper- or hypo-) activity to cues conveying sadness. Our findings are consistent with those reported in MDD where prefrontal cortical hyperactivity in similar regions of the mPFC and ACC has been reported during processing of emotional cues conveying

sadness (Elliott *et al.* 2002; Fu *et al.* 2004). This is thought to reflect an increased (compensatory) frontal cortical mediation of the relationship between mood (i.e. symptoms) and cognition (i.e. mPFC/attention). The reason for the hyperactivity to sad faces in patients with GSAD is unknown, but exaggerated cortical response to sad faces in the mPFC/ACC may reflect an impaired ability to disengage from negative self-referential emotional processing (Brühl *et al.* 2011; Lyubomirksy *et al.* 1998; Matthews *et al.* 2009; Wenzlaff & Bates, 1998). This is in accordance with findings showing abnormal visual scan paths in GSAD individuals when confronted with sad information (Horley *et al.* 2004). In support, a recent study reported heightened mPFC activity during negative self-referential processing in MDD (Yoshimura *et al.* 2010) and evidence in healthy controls, including a meta-analysis, suggesting that the PFC (i.e. mPFC/ACC) is involved in mentalization and self-referential processing (Mitchell *et al.* 2006; Northoff *et al.* 2006).

The mPFC and ACC are among several cortical and limbic regions modulated by antidepressant drugs. For example, selective serotonin reuptake inhibitors (SSRIs) have been shown to modulate regional brain glucose metabolism in regions including the mPFC and ACC as measured using positron emission tomography (PET) imaging in MDD (Kennedy *et al.* 2001; Mayberg *et al.* 1997). More recent studies suggest that abnormal activity during the processing of visual cues conveying sadness in these areas is also normalized by antidepressant drugs including SSRIs. In MDD, the reduced ACC (BA 24) and insula activity to presentations of negative emotional pictures at baseline was increased (i.e. normalized) following 2 wk of venlafaxine treatment (Davidson *et al.* 2003). Similarly, enhanced capacity of activation in the ACC (BA 23, 24, 31, 32) and reduced dynamic range (intensity load response) in the ACC (BA 24, 32) and mFPC (BA 8, 9) to sad faces was reduced and enhanced, respectively, following 8 wk of fluoxetine treatment (Fu *et al.* 2004). In GSAD, similar reduction or normalization regional brain glucose response was observed following SSRI treatment during a symptom provocation study (Furmark *et al.* 2002). It can therefore possible that suppression or restoration of mPFC/ACC activity by Oxt may represent an important mechanism leading to normalization of dysfunctional processing of social emotional cues. In support, a recent study showed that Oxt modulated ACC activity during a mentalizing-related task (i.e. reading the mind in the eyes) in unmedicated depressed subjects (Pincus *et al.* 2010) suggesting that Oxt may attenuate cortical activity associated with mentalizing sad facial cues in GSAD.

Contrary to our findings and those discussed above, other studies using PET imaging have observed reduced regional cerebral blood flow (rCBF) in the mPFC/ACC during resting-state and symptom-provocation (public speaking) paradigms in patients with GSAD compared to controls (Tillfors *et al.* 2002; Van Ameringen *et al.* 2004). Similarly, reduced pre-treatment regional brain glucose metabolism in the mPFC/ACC at baseline in GSAD, was found to be increased following treatment with the GABAergic modulator, tiagabine (Evans *et al.* 2009). The discrepancy between these findings is probably explained by differences in the methodology including study design (i.e. public-speaking *vs.* resting-state *vs.* emotion recognition), neurochemical systems (oxytocinergic *vs.* serotonergic *vs.* GABAergic), and clinical population [generalized subtype *vs.* heterogeneous (generalized, and public speaking-limited) social anxiety disorder].

We did not observe altered brain response to happy faces in patients with GSAD. This suggests that the hyperactivity in the frontal cortex (mPFC/ACC) may be specific to social cues conveying sadness in GSAD. Oxt also attenuated frontal cortex activity to happy faces in GSAD individuals, suggesting a more general effect across both positive and negative facial emotional cues; however, the functional significance of these effects are unknown as the groups did not differ in response to happy faces at baseline. It is possible that general suppression of cortical activity to sad and happy cues may be related to a general effect of Oxt in reducing emotionally driven arousal; however, a recent study showed no evidence for modulation of sympathetic arousal during emotional face processing following Oxt (Gamer & Büchel, 2011). The attenuation of cortical activity to both positive and negative facial cues by Oxt is consistent with a previous study showing similar valence independent attenuation of amygdala activity (Domes *et al.* 2007), although valence-dependent modulation has been observed in subregions within the amygdala (Gamer *et al.* 2010). Moreover, Oxt generally attenuated brain response to both sad and happy faces in a number of other cortical areas such as in parietal, temporal and occipital cortices, supplementary motor area and cerebellum. The attenuation of activity in the parietal, temporal and occipital cortices may be related to attenuation of activity related to face (Clark *et al.* 1996; Kanwisher *et al.* 1997; Pitcher *et al.* 2011) and attentional (Clark *et al.* 1997; Narumoto *et al.* 2001; Wojciulik *et al.* 1998) processing, respectively. Moreover, previous studies have shown that the SSRI, citalopram, modulates occipital cortex response

to negative and positive emotional cues (Kemp *et al.* 2004). However, the clinical significance of these effects is unknown since there was no hyperactivity in these regions in patients with GSAD. Additional studies are needed to further delineate these effects about which we did not have *a-priori* hypotheses.

There are some limitations of the current study that warrant discussion. First, it should be highlighted that in contrast to many previous neuroimaging studies in GSAD which employed a threat-related or anxiety-provoking paradigm, the current study did not observe amygdala or insula hyperactivity in patients with GSAD. However, the current study employed facial stimuli with sad expressions, which may not have directly conveyed threat or signal danger in our GSAD group. In addition, the current thresholds and contrasts (i.e. sad and happy faces relative neutral faces) may have precluded the detection of amygdala responses, as neutral faces have also been shown to robustly activate the amygdala (Fitzgerald *et al.* 2006). Furthermore, the sample size of the current study was small and it is likely the there was insufficient power to detect activations in the amygdala or insula. However, it should be highlighted that the findings on amygdala/insula activations in response to emotional stimuli are mixed, with some studies showing activations (e.g. Britton *et al.* 2006; Evans *et al.* 2008; Phillips *et al.* 2003; Straube *et al.* 2004, 2005) and others showing no activation (e.g. Bishop *et al.* 2004; Goldin *et al.* 2009). Second, it is possible that the attenuation of cortical activation following Oxt may have been the result of potential effects of on cerebrovasculature coupling. However, this is unlikely to be the case as we observed valence-specific effects, and if there were any effects on cerebrovasculature coupling, one would have expected a general attenuation of cortical response to both negative and positive facial cues. Finally, the findings of the current study are specific to patients with GSAD and hence cannot be generalized to other psychiatric populations or to healthy controls.

In summary, we report that patients with GSAD show frontal cortex (mPFC/ACC) hyperactivity to non-threatening sad faces, and this hyperactivity is attenuated by Oxt to levels resembling that of controls. These findings suggest that Oxt may normalize aberrant brain response to social cues that convey self-relevant negative information in GSAD, which may not be related to negative social feedback/scrutiny or to fear/threat signals, and this may be an added mechanism underlying Oxt's broad, pro-social actions in GSAD.

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Statement of Interest

Pradeep J. Nathan is an employee of GlaxoSmithKline Pharmaceuticals. He is also an editorial board member of the *International Journal of Neuropsychopharmacology*, but was not involved in the review of this paper.

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