

Amygdala Subregions Tied to SSRI and Placebo Response in Patients with Social Anxiety Disorder

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The amygdala is a key structure in the pathophysiology of anxiety disorders, and a putative target for anxiolytic treatments. Selective serotonin reuptake inhibitors (SSRIs) and placebo seem to induce anxiolytic effects by attenuating amygdala responsiveness. However, conflicting amygdala findings have also been reported. Moreover, the neural profile of responders and nonresponders is insufficiently characterized and it remains unknown whether SSRIs and placebo engage common or distinct amygdala subregions or different modulatory cortical areas. We examined similarities and differences in the neural response to SSRIs and placebo in patients with social anxiety disorder (SAD). Positron emission tomography (PET) with oxygen-15-labeled water was used to assess regional cerebral blood flow (rCBF) in 72 patients with SAD during an anxiogenic public speaking task, before and after 6–8 weeks of treatment under double-blind conditions. Response rate was determined by the Clinical Global Impression-Improvement scale. Conjunction analysis revealed a common rCBF-attenuation from pre- to post-treatment in responders to SSRIs and placebo in the left basomedial/basolateral and right ventrolateral amygdala. This rCBF pattern correlated with behavioral measures of reduced anxiety and differentiated responders from nonresponders. However, nonanxiolytic treatment effects were also observed in the amygdala. All subgroups, including nonresponders, showed deactivation of the left lateral part of the amygdala. No rCBF differences were found between SSRI responders and placebo responders. This study provides new insights into the brain dynamics underlying anxiety relief by demonstrating common amygdala targets for pharmacologically and psychologically induced anxiety reduction, and by showing that the amygdala is functionally heterogeneous in anxiolysis.

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INTRODUCTION

Anxiety disorders, the most common mental disorders (Kessler *et al*, 2005), are associated with intense personal suffering (Kessler, 2003) and considerable societal costs (François *et al*, 2010). To be able to prevent and treat anxiety efficaciously, it is important to understand its neurobiological underpinnings. Subcortical regions such as the amygdala, hippocampus, brainstem, and hypothalamus, as well as cortical areas such as the insular, orbitofrontal (OFC), ventromedial prefrontal (vmPFC), and anterior cingulate (ACC) cortices have been suggested to be involved in the

neuropathology of anxiety disorders (Shin and Liberzon, 2010). The OFC, vmPFC, and ACC are thought to have a significant modulatory role in the treatment of anxiety (Mathew *et al*, 2008) because of their involvement in affective processing and emotional regulation (Ochsner and Gross, 2005; Rosen and Levenson, 2009). The amygdala, because of its key role in fear learning and processing (Phelps and LeDoux, 2005; Shin and Liberzon, 2010), is commonly viewed as the central target for anxiolytic treatments (Mathew *et al*, 2008).

Selective serotonin reuptake inhibitors (SSRIs) are generally accepted as the first-line pharmacological treatment for anxiety disorders (Davidson, 2006; Ravindran and Stein, 2010). Animal models provide evidence for a modulatory effect of serotonin on amygdala firing (Cheng *et al*, 1998; Stutzmann *et al*, 1998) and amygdala-related behavior (Inoue *et al*, 2004). Human imaging studies also suggest that SSRIs regulate amygdala hyper-responsivity to threat-relevant stimuli in healthy participants (Harmer *et al*, 2006; Murphy *et al*, 2009), and in patients with social

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anxiety disorder (SAD; Furmark *et al*, 2002, 2005). However, an inherent difficulty of pharmaco-imaging trials is to separate the biomarkers that are specifically associated with clinical response from the nonspecific pharmacodynamic effects that occur regardless of symptom improvement. Despite the extensive use of SSRIs, it is widely recognized that a considerable number of patients fails to achieve adequate therapeutic response with this medication (Fredman *et al*, 2000; Van Ameringen *et al*, 2000). Knowledge regarding brain perfusion in SSRI-nonresponders stems mainly from prediction studies (Evans *et al*, 2006) that do not explore the neural profile associated with insufficient response. As far as we know, there are no imaging activation study of anxiety patients that have compared SSRI responders and nonresponders.

In addition, the neural pathways that are specific for SSRI response relative to placebo response are not well characterized in anxiety treatment. Concerns regarding the clinical effectiveness of SSRIs over placebo have been raised by meta-analyses (Fournier *et al*, 2010; Khan *et al*, 2002; Kirsch *et al*, 2008), and it is well documented that the magnitude of the placebo response, ie, the therapeutic effect of inert substances, causes difficulties in establishing the efficacy of several pharmacological agents (Khan and Bhat, 2008; Uhlenhuth *et al*, 1997). Expectations of clinical improvement, elicited by placebo, are thought to modulate several clinical outcomes (Colloca and Miller, 2011; Fuente-Fernández *et al*, 2004; Lidstone *et al*, 2010; Scott *et al*, 2007) and neurofunctionally, this might correspond to a general top-down modulatory process (Benedetti *et al*, 2006; Krummenacher *et al*, 2010; Petrovic *et al*, 2005; Zubieta and Stohler, 2009). At a neurophysiological level placebo responses are disorder specific (Benedetti *et al*, 2005). Studies reporting reduced activity in single neurons in the subthalamic nucleus of placebo responsive Parkinsonian patients (Benedetti *et al*, 2004) and reduced activity in the dorsal horn of the spinal cord of participants undergoing placebo analgesia (Eippert *et al*, 2009) are intriguing examples of how expectancies of improvement can modulate specific processes within the central nervous system. Moreover, studies suggest that placebo-induced clinical benefits seem to target specific brain regions similar to the ones recruited by active drugs (Faria *et al*, 2008; Petrovic *et al*, 2002). However, with regard to SSRIs there is a lack of studies examining the neurofunctional communalities and differences between placebo and SSRI response. In depressed patients, Mayberg *et al* (2002) reported a common pattern of glucose metabolism in neocortical and limbic-paralimbic brain regions for both placebo and SSRI responders, whereas Leuchter *et al* (2002) findings on prefrontal cordance suggest a distinct pathway for SSRI and placebo responders. Thus, it remains generally unclear whether successful placebos and SSRIs engage common or distinct brain patterns.

In patients with SAD, we previously reported that placebo response was accompanied by reduced anxiety-related activity in the amygdala (Furmark *et al*, 2008), resembling the neural effect of citalopram (Furmark *et al*, 2002, 2005) and cognitive-behavior therapy (CBT; Furmark *et al*, 2002). Exaggerated amygdala responsivity in SAD and other anxiety disorders may thus be normalized with successful treatment. However, even though amygdala hyper-responsiveness has been a consistent finding in neuroimaging

activation studies of anxiety disorders (Domschke and Dannlowski, 2009), some counterintuitive results have been reported, such as increased amygdala activity in anxiety patients following successful CBT (Maslowsky *et al*, 2010), higher amygdala responsivity to aversive stimuli in healthy participants than in anxiety patients (Britton *et al*, 2005; Phan *et al*, 2006; Straube *et al*, 2006), and higher amygdala responsivity to emotionally neutral than to anxiogenic tasks (Britton *et al*, 2005; Kilts *et al*, 2006). Mixed amygdala findings have also been reported after acute administration of citalopram in healthy participants (Bigos *et al*, 2008; Murphy *et al*, 2009). Genetic variation and heterogeneity of the investigated samples, imaging methods, and experimental tasks might underlie these inconsistencies (Furmark *et al*, 2009). Mixed findings may also be related to the fact that the amygdala, which is usually treated as a single structure in neuroimaging studies, is functionally and anatomically heterogeneous as extensively demonstrated in animal studies (LeDoux, 2007). Hence, it is unlikely that the amygdala is a functionally homogeneous region in human anxiety. It is possible that SSRIs and placebo engage different subregions of the amygdala and/or different modulatory cortical activity patterns.

Impelled by the limited knowledge regarding the neuro-functional mechanisms underlying effective and ineffective treatments of clinical anxiety, the present study reanalyzed previously published data on placebo anxiolysis (Furmark *et al*, 2008) together with newly merged unpublished data on SSRIs, to examine the neural correlates of pharmacologically and psychologically induced anxiety reduction in patients with SAD. Neuroimaging data were extracted from three randomized double-blind placebo-controlled trials. Regional cerebral blood flow (rCBF) was assessed with oxygen-15-labeled water positron emission tomography (PET) during an anxiogenic public speaking task, before and after 6–8 weeks of treatment with either SSRIs or placebo. We used conjunction analysis and interaction contrasts to examine overlapping and unique neural changes in responders and nonresponders of both treatments. The general aim was to identify neural alterations specifically underlying symptom improvement with SSRIs and placebo. In addition, we sought to describe nonspecific neural effects of intervention such as non-anxiolytic pharmacodynamic influences and effects of repeated testing.

MATERIALS AND METHODS

Participants

A total of 72 patients who fulfilled the DSM-IV (American Psychiatric Association, 1994) criteria for SAD were included (see Table 1 for demographics). Participants were recruited through newspaper advertising. All the participants gave written informed consent, and the study was approved by the Swedish Medical Products Agency as well as the local ethics and radiation safety committees. A DSM-IV (American Psychiatric Association, 1994) diagnosis of SAD was established through the Structured Clinical Interview for DSM Disorders (First *et al*, 1998). Additional disorders were screened for, by use of the structured diagnostic MINI-interview (Sheehan *et al*, 1998). All patients had marked public speaking anxiety and SAD as

Table 1 Demographic and Clinical Characteristics of Responders and Nonresponders to SSRIs or Placebo

Variable	SSRI responders (n = 20)	SSRI nonresponders (n = 15)	Placebo responders (n = 11)	Placebo nonresponders (n = 26)
Sex				
Female, n(%)	12 (60)	10 (67)	9 (82)	14 (54)
Age (years)				
Mean (SD)	36.45	34.67	37.18	33.42
Range (min–max)	19–50	19–53	23–55	22–49
Subtype				
Generalized, n(%)	12 (60)	11 (73)	6 (55)	20 (77)
LSAS pre				
Mean (SD)	71.15 (25.56)	64.07 (21.53)	61.45 (25.44)	67.85 (21.01)
LSAS post				
Mean (SD)	34.85 (21.84)	59.60 (27.23)	31.54 (22.26)	59.23 (17.33)
STAI-S pre				
Mean (SD)	63.15 (7.00)	62.27 (10.09)	56.73 (10.39)	61.23 (10.30)
STAI-S post				
Mean (SD)	46.60 (9.24)	53.20 (12.16)	47.18 (13.99)	58.23 (10.59)
Fear pre				
Mean (SD)	66.75 (20.47)	64.13 (29.55)	54.73 (26.55)	59.85 (29.24)
Fear post				
Mean (SD)	35.60 (22.01)	58.27 (25.57)	35.00 (24.70)	51.85 (32.41)

Abbreviations: LSAS, Liebowitz Social Anxiety Scale; SSRI, selective serotonin reuptake inhibitor; STAI-S, Spielberger State-Trait Anxiety Inventory (state portion).

the primary diagnosis. Forty-nine patients (68%) were diagnosed with the generalized subtype and 14 (19%) qualified for a comorbid anxiety disorder: specific phobia ($n = 9$), generalized anxiety disorder (GAD; $n = 1$), post-traumatic stress disorder ($n = 1$), panic disorder ($n = 1$), and specific phobia together with GAD ($n = 2$).

Participants were excluded if they reported: (1) treatment of social anxiety in the past 6 months; (2) current serious or dominant psychiatric disorder other than SAD (eg, psychosis, major depressive or bipolar disorder), (3) chronic use of prescribed medication, (4) abuse of alcohol/narcotics, (5) pregnancy, (6) menopause, (7) left handedness, (8) previous PET-examination, and (9) any somatic or neurologic disorder that could be expected to influence the outcome of the study. Study details were registered at www.clinicaltrials.gov (identifier NCT00343707).

Clinical Trials

Data were extracted from three randomized double-blind clinical PET trials evaluating changes in rCBF following treatment with SSRIs and placebo ($N = 144$). All trials were performed at the PET center in Uppsala with the

collaboration of Quintiles AB Uppsala (Sweden), and GlaxoSmithKline Verona (Italy), during 2002–2005. The current study presents data on the pooled SSRI and placebo groups (three SSRI and three placebo groups) with novel comparative analyses and conjunction analyses of responders and nonresponders. Parts of the data set were included in previous publications, the SSRI and placebo groups from the first trial were included in Furmark *et al* (2005), whereas the placebo groups from the second and the third trials were included in Furmark *et al* (2008). There were three treatment arms in the first two trials and six arms in the third (Figure 1). One subject allocated to SSRIs discontinued medication after the first week due to initial nausea. Thus, analyses were based on 35 patients randomized to SSRIs consisting of 42 days of citalopram 40 mg, or 56 days of paroxetine 20 mg or 7.5 mg. There was a matching placebo arm for each SSRI arm. In the present analyses, the placebo arms are grouped into one cohort with a total of 37 patients—see Figure 1.

There were no significant differences across trial 1–3 with regard to demographic characteristics and clinical variables according to χ^2 and t -test (all P 's > 10). Likewise, when statistically comparing SSRI and placebo arms, as well as

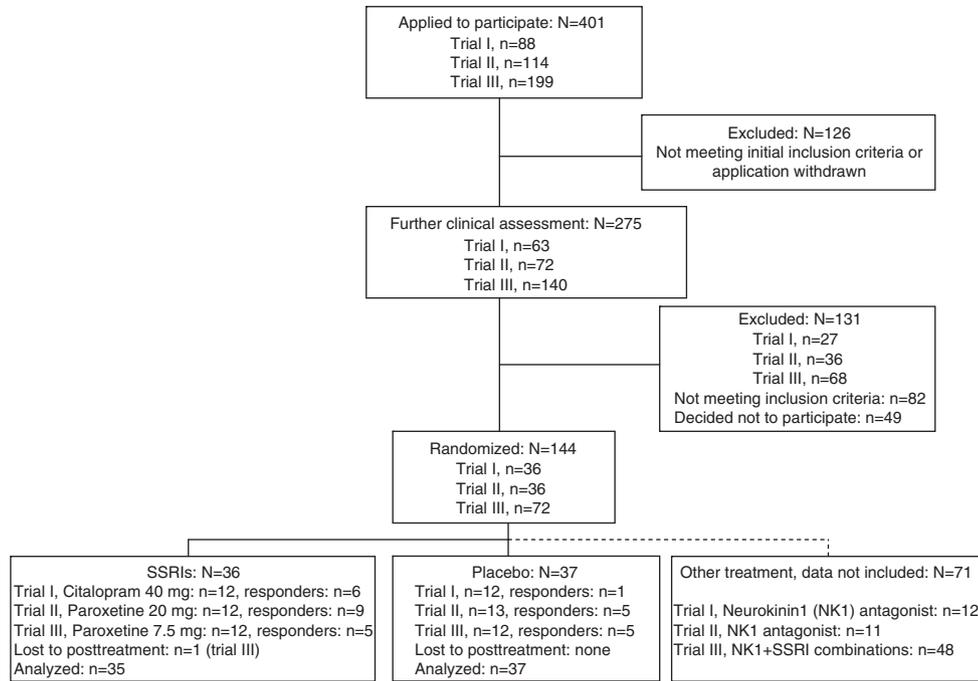


Figure 1 Flow diagram of subject eligibility from screening to statistical analysis. SSRIs, selective serotonin reuptake inhibitors.

responders vs nonresponders, no significant differences were noted on demographic variables, or pretreatment clinical variables described in Table 1, including distributions for subtypes and comorbid/noncomorbid cases (all P 's > 0.10). To check for pretreatment differences in rCBF, we extracted mean values from all *a priori* regions of interest (ROI) and found no significant main effects of treatment regimen (ie, SSRIs/placebo), response (ie, responders/nonresponders), or regimen \times response interactions ($F_{1,68} = 0.005\text{--}2.05$; $P = 0.99\text{--}0.12$).

GlaxoSmithKline Verona supplied daily doses of study drugs and matching placebo for the three consecutive PET trials with a fixed dosing schedule performed under double-blind conditions. The first dose was given on the same day as the first PET examination and the final dose was administered 2–4 h before the final PET assessment after 42 or 56 days (post-treatment). Subjects did not receive any other form of treatment during the study period. Patients visited the clinic biweekly for assessments of compliance, side effects, and to receive new supplies of medication. After study completion, patients were offered further psychiatric consultation and pharmacotherapy.

Clinical Outcome Measurements

Response rate was determined by the Clinical Global Impression improvement item (CGI-I; Zaider *et al*, 2003) administered by an experienced psychiatrist. Patients having a score of 1 or 2 (very much or much improved) on the CGI-I at posttest were classified as responders, whereas those having scores of ≥ 3 were considered to be nonresponders. Additional changes in the social anxiety symptomatology were assessed by the Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987). Symptom experience during public speaking was assessed using the Spielberger

State Anxiety Inventory (STAI-S; Spielberger *et al*, 1970) and subjective fear ratings was evaluated by a 0–100 (min–max) visual analog scale administered after each public speaking challenge. Measures were collected by independent and blinded assessors.

PET Assessments

The procedure for rCBF assessments, using $[H_2^{15}O]\text{-PET}$, has been described elsewhere (Furmark *et al*, 2008). Before and after treatment, all patients were scanned during an anxiogenic public speaking task, ie, patients gave a $2\frac{1}{2}$ -min speech about a travel experience, while lying in the camera, surrounded by a silently observing audience of 6–8 persons. We used a 32-ring ECAT EXACT HR + scanner (Siemens/CTI, Knoxville, USA), which enables acquisition of 63 contiguous planes of data with a distance of 2.46 mm, resulting in a total axial field of view of 155 mm.

Patients fasted for 3 h, and refrained from tobacco, alcohol, and caffeine 12 h before PET-investigations. Patients were positioned in the scanner with the head gently fixated and a venous catheter for tracer injections was inserted. Patients were instructed to prepare a $2\frac{1}{2}$ -min speech about a vacation or travel experience about 20 min before the initial emission scan. A 10-min transmission scan was performed using three retractable ^{68}Ge rotating line sources. Following intravenous administration of the ^{15}O -water tracer, ~ 10 MBq/kg body weight, the emission scan (three 30 s frames, 3-D mode) started automatically when the bolus reached the brain (50 000 counts/s).

Immediately after tracer injection, patients were asked to start their speech and continue until they received instructions to stop. The speech was performed in presence of a standing silently observing audience of 6–8 persons. Patients were instructed to observe the audience while

giving the speech that was being videotaped, to increase observational anxiety. Directly after the speech, state anxiety and fear ratings were obtained, ie, subjects rated how they felt during scans. The PET procedure was the same at post-treatment, but the speech topic was different.

Emission scans were reconstructed with a filter back projection using an 8 mm Hanning filter, resulting in a spatial resolution of about 5 mm in the field of view. Data were corrected for photon attenuation, decay, scattered radiation, and random coincidences. After reconstruction, a summation image of the three frames was made in order to obtain a better statistical reference for realignment and subsequent analyses.

Statistical Analysis

Behavioral data. Demographic and clinical data were analyzed using IBM SPSS 19 (IBM, Somers, NY, USA) with the significance level set at $P < .05$. Group differences in clinical response were evaluated using χ^2 -test and analyses of covariance (ANCOVA) with the pretreatment value as covariate.

PET data. Imaging data were analyzed using Statistical Parametric Mapping Software (SPM2 - Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 7.3.0. (MathWorks, Natick, MA). PET images were realigned to correct for different positions between scans (pre vs post-treatment) and normalized to the Montreal Neurological Institute's (MNI) stereotactic template. Images were then smoothed using an 8 mm Gaussian kernel and scaled to give all scans the same global signal.

Effects on rCBF were evaluated at the voxel level (1 voxel = $2 \times 2 \times 2$ mm) with results described as xyz coordinates in MNI space. ROI analyses were performed with the following *a priori* regions: amygdala, hippocampus, hypothalamus, brainstem, insula, ACC, OFC, and vmPFC, defined by the Wake Forest University School of Medicine PickAtlas (Maldjian et al, 2003). In addition, exploratory whole-brain analyses were conducted. Statistically significant changes were examined at $P < .05$ corrected family wise (FWE) for multiple comparisons. Additional uncorrected P -values are reported for the amygdala region ($P_{uncorr} < .05$). Anatomical localization was guided by the Talairach atlas (Talairach and Tournoux, 1988), the Talairach Daemon (Lancaster et al, 2000), and the brain atlas of Mai (Mai et al, 2004).

SPM-analyses were performed in six steps:

- (1) Clinical effects on brain activity were initially investigated by contrasting rCBF changes, measured during public speaking before and after treatment, in responders and nonresponders within each treatment regimen (SSRI and placebo).
- (2) To further evaluate the specific treatment effects of SSRI and placebo on the brain activity, between-group comparisons were conducted in the form of subgroup \times time interaction analyses, eg, (SSRI responders_{pre}-SSRI responders_{post})-(SSRI nonresponders_{pre}-SSRI nonresponders_{post}).
- (3) To identify common rCBF changes in SSRI and placebo responders, the conjunction from the within-group contrasts was conducted, ie, (SSRI responders pre-

post) and (placebo responders pre-post). Conjunction analysis presumes a common effect in all contrasts tested. To exclude the impact of nonspecific treatment effects, (eg, repeated testing), we used the corresponding rCBF changes in SSRI and placebo nonresponders as an exclusion mask. Thus, we only considered the voxels that were common for treatment responders, regardless of modality, while excluding voxels that changed with repeated testing but were unrelated to clinical improvement.

- (4) To identify unspecific SSRI-neurophysiological effects (unrelated to symptom improvement), we computed the conjunction of both SSRI within-group contrasts, ie, (SSRI responders pre-post) and (SSRI nonresponders pre-post). To exclude the potential impact of repeated testing and symptom improvement, we used rCBF changes from pre- to post-treatment occurring within placebo responders and nonresponders as an exclusion mask.
- (5) To track nonspecific rCBF changes occurring with repeated testing over time (pre-post), we estimated the conjunction of all subgroups, ie, (SSRI responders pre-post), (placebo responders pre-post), (SSRI nonresponders pre-post), and (placebo nonresponders pre-post).
- (6) In addition, we extracted the maximum correspondent voxel values resulting from the conjunction analyses and correlated the rCBF change with corresponding measures of state anxiety (STAI-S, fear) and clinical severity (LSAS).

RESULTS

Behavioral Measures

After unblinding, it was revealed that there were 20 SSRI responders (57%) and 11 placebo responders (30%), ie, a higher response rate in the merged SSRI group as compared with placebo ($\chi^2(1) = 5.51$, $P = 0.02$). On clinical outcome measures, ANCOVAs confirmed significantly greater improvement in SSRI responders in comparison with SSRI nonresponders (LSAS: $F_{1,32} = 30.24$, $P < .0001$; STAI-S: $F_{1,32} = 6.44$, $P = 0.016$; fear: $F_{1,32} = 17.08$, $P = 0.0002$), whereas responders in the SSRI and placebo arms did not differ significantly ($F_{1,28} = 0.34-2.18$, $P = 0.56-0.15$). Placebo responders were more improved than placebo nonresponders on the LSAS ($F_{1,34} = 34.71$, $P < .0001$) and STAI-S ($F_{1,34} = 5.48$, $P = 0.025$) but not on fear ratings ($F_{1,34} = 2.15$, $P = 0.15$). Finally, the two nonresponder subgroups did not differ on LSAS or fear ratings ($F_{1,38} = 0.23-0.66$, $P = 0.63-0.42$), but a difference (SSRI > placebo) was noted on the STAI-S ($F_{1,38} = 4.59$, $P = 0.039$)—see Table 1.

Regional Cerebral Blood Flow

Within-group treatment effects. Results from within-group analyses of responders and nonresponders to SSRI or placebo treatments are displayed in Supplementary Table 1. Notably, there was evidence of reduced amygdala response (pre > post) in all responder/nonresponder subgroups, suggesting that lowered amygdala responsivity is not exclusively related to clinical improvement. However, only

SSRI and placebo responders exhibited reduced neural activity in the left basomedial/basolateral (BM/BLA) and right ventrolateral (VLA) amygdala subregions, suggesting that these areas are related to anxiety relief.

Between-group treatment effects. In the between-group analysis (Table 2) there was evidence, mostly at the uncorrected p -level, that amygdala rCBF was more attenuated (pre > post) in responders relative to nonresponders both in SSRI- and placebo-treated subjects. These effects were noted in the left BM/BLA and right VLA. A cluster with its peak in the left lateral amygdala showed a tendency for greater deactivation (pre > post) in SSRI nonresponders than in SSRI responders, suggesting that lowered responsiveness in this amygdala subregion is unrelated to clinical improvement. Differential rCBF changes between responders and nonresponders to SSRIs were additionally noted in frontal regions (ACC, OFC, and Insula), whereas brainstem differences were observed between the two placebo groups (Table 2). There were no rCBF differences between responders to SSRIs and placebo.

Table 2 Brain Regions that Changed between Responders and Nonresponders as a Function of SSRI and Placebo Treatments

Brain region	MNI coordinate ^a			z Value	P-value
	x	y	z		
<i>SSRI resp < SSRI nonresp (n = 20/15)</i>					
R amygdala	26	-4	-26	2.08	0.019 ^b
L amygdala	-16	-6	-14	1.65	0.049 ^b
R orbitofrontal cortex (11)	24	42	-14	4.04	0.005
L anterior cingulate cortex (32)	-14	40	16	3.33	0.047
<i>SSRI nonresp < SSRI resp (n = 15/20)</i>					
L amygdala	-26	-8	-14	2.52	0.006 ^b
L insula	-46	-14	2	3.61	0.028
<i>Placebo resp < placebo nonresp (n = 11/26)</i>					
L amygdala	-16	-6	-14	3.15	0.012
R amygdala	28	-2	-26	2.42	0.008 ^b
<i>Placebo nonresp < placebo resp (n = 26/11)</i>					
R brainstem, pons	12	-16	-30	3.43	0.049
<i>SSRI resp < placebo resp (n = 20/11)</i>					
NS					
<i>Placebo resp < SSRI resp (n = 11/20)</i>					
NS					

Abbreviations: L, left side; R, right side; SSRI, selective serotonin reuptake inhibitor; nonresp, nonresponders; NS, not significant; resp, responders. Approximate Brodmann's areas within parentheses.

^aBased on the Montreal Neurologic Institute coordinate system.

^bP-value uncorrected for multiple comparisons. All analyses are regions of interest (ROI)-based.

Anxiolytic effects common to SSRIs and placebo. Conjunction analysis revealed a common reduction of amygdala activity in SSRI and placebo responders, with statistical peaks in the right VLA ($x = 28, y = -2, z = -26, Z = 2.95, P_{FWE} < .05$) and left BM/BLA ($x = -16, y = -6, z = -14, Z = 2.49, P_{uncorr} < .005$; see Figures 2 and 3 and Supplementary Results). No overlapping rCBF changes were observed outside the amygdala region.

The rCBF change from pre- to post-treatment in the left BM/BLA and right VLA subregions of the amygdala (peak voxels) correlated with corresponding changes in LSAS (left: $r = 0.40, P < .001$; right: $r = 0.34, P < .005$), state anxiety (left: $r = 0.40, P < .001$; right: $r = 0.22, P = 0.062$), and fear ratings (left: $r = 0.33, P < .005$; right: $r = 0.21, P = 0.065$), suggesting that neural alterations in these regions underlie anxiety reduction regardless of treatment modality.

Non-anxiolytic effects.

Pharmacodynamic effects of SSRIs: The conjunction of SSRI responders and nonresponders revealed common rCBF decrement (pre > post) peaking in the left lateral amygdala ($x = -26, y = 0, z = -18; Z = 3.38; P_{FWE} < .005$)—see Figures 2 and 3. The resultant amygdala peak value did not correlate significantly ($P > 0.10$) with any behavioral anxiety measure, supportive of a symptom-unrelated pharmacodynamic effect.

Effects related to repeated testing: The conjunction of all subgroups, including nonresponders, suggested a common deactivation (pre > post) with the statistical peak in the left lateral amygdala, ($x = -28, y = 0, z = -22; Z = 2.14; P_{uncorr} < .01$)—see Figures 2 and 3. As expected, no significant correlations were observed between this amygdala region and behavioral anxiety measures ($P > 0.10$).

DISCUSSION

The present study found evidence of common amygdala targets for effective SSRI and placebo treatments of SAD. Between-group and conjunction analyses showed that amygdala clusters with peak values in the left BM/BLA and right VLA sections were deactivated in SSRI responders and placebo responders alike, but not in nonresponders. Attenuated neural activity in these amygdala regions differentiated responders from nonresponders regardless of treatment modality, and correlated with behavioral measures of reduced anxiety.

Alleviation of social anxiety has been associated with lowered amygdala responsiveness after successful CBT (Furmark et al, 2002), pharmacological (Furmark et al, 2002, 2005), and placebo treatments (Furmark et al, 2008). Interestingly, in the present study, we found no rCBF differences between responders to SSRI and placebo treatments. Together, these results are consistent with the notion that pharmacological and psychosocial treatments exert their beneficial effects, at least partly, by targeting the same brain regions (Faria et al, 2008). Cognitive factors such as expectations and beliefs are thought to have key roles in treatment outcomes (Colloca and Miller, 2011). Generally, pharmacological therapies and placebo share the cognitive factor associated with expectancies of symptom

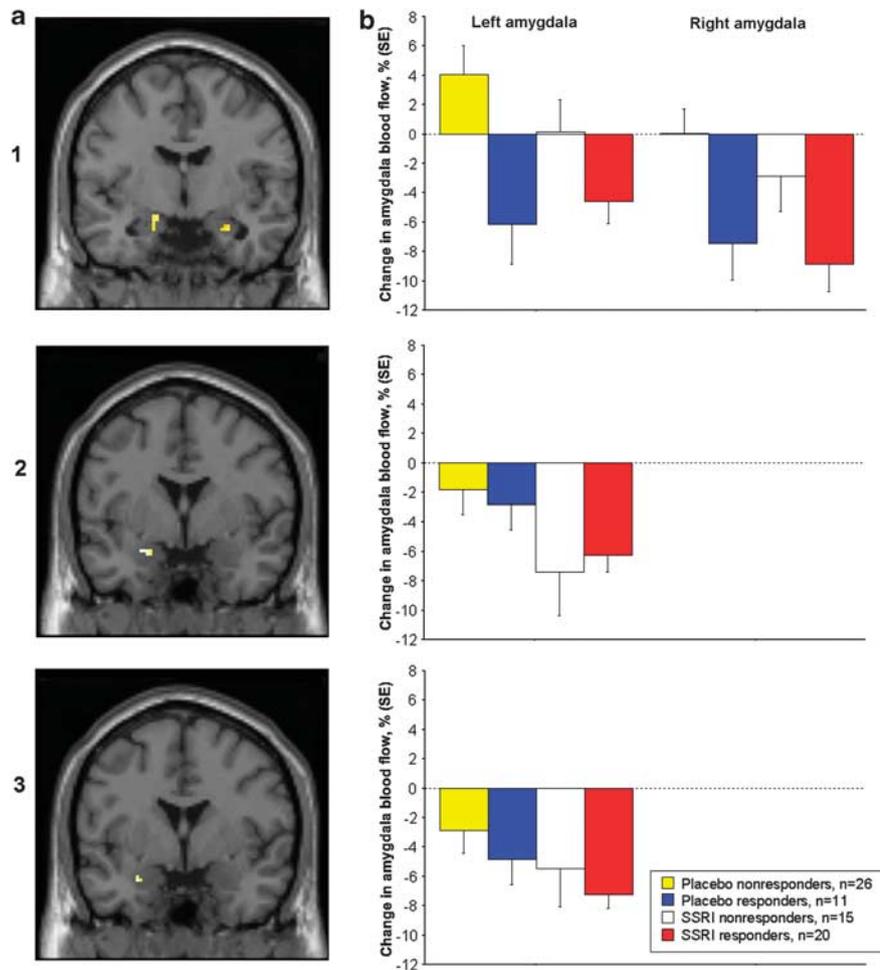


Figure 2 (a) Coronal images displaying decreased cerebral blood flow with treatment (pre–post) in three amygdala clusters, resultant from conjunction analyses. (1) The conjunction of selective serotonin reuptake inhibitor (SSRI) and placebo responders (with nonresponders to SSRIs and placebo as exclusive mask) showed overlapping amygdala deactivations with statistical peaks in the right ventrolateral and left basomedial/basolateral regions, indicating a common anxiolytic effect. (2) The conjunction of SSRI responders and SSRI nonresponders (with placebo responders and nonresponders as exclusive mask) revealed a common deactivation of the left lateral section of the amygdala, indicating a nonanxiolytic pharmacodynamic effect. (3) The conjunction of all groups, ie, SSRI and placebo responders/nonresponders, revealed a common deactivation of the left lateral amygdala, indicating an effect related to repeated testing over time. (b) Corresponding plots of percent change (\pm SE) in amygdala blood flow in responders and nonresponders in the three amygdala clusters related to (1) common anxiolytic effect of SSRIs and placebo; (2) non-anxiolytic pharmacodynamic effect of SSRIs, and (3) effect related to repeated testing.

improvement. Hence, the common deactivation of the left BM/BLA and the right VLA sectors of the amygdala, observed only in responders, might result from patients' belief in the effectiveness of the treatment, giving fuel to the speculation that SSRIs and placebo operate via similar belief-driven psychological mechanisms. Compelling evidence for expectancy-induced anxiety relief was found in anxious post-operative patients who after an open administration of diazepam reported significantly lower levels of anxiety in comparison to the ineffective hidden administration of benzodiazepine (Colloca *et al*, 2004). Concordantly, controversial meta-analytic studies in depression reported that placebos account for $\sim 75\%$ of the improvement found in active pharmacological treatments (Kirsch and Sapirstein, 1998) and that the remaining portion could be explained by the use of inactive rather than active placebos (Kirsch *et al*, 2008; Moncrieff *et al*, 2004). However, our study cannot properly evaluate the impact of such psychological factors on neural systems because

active placebos were not used and expectancies were not measured. Evidence from the animal literature, however, shows an inhibitory effect of SSRIs on amygdala reactivity and fear-related behaviors (Inoue *et al*, 2004; Izumi *et al*, 2006). Therefore, it is likely that the common response-related amygdala subregions constitute anxiolytic targets under the influence of both pharmacodynamic and psychological effects.

Even though certain parts of the amygdala were commonly deactivated in responders, and correlated with anxiety measures, the pattern within the broader amygdala region was complex. Decreased amygdala perfusion was noted in all subgroups including nonresponders. SSRI responders and SSRI nonresponders shared a common area of deactivation in the left lateral amygdala that was unrelated to anxiety relief, ie, a symptom-unrelated pharmacodynamic effect. Moreover, in the left, more ventral part of the lateral amygdala a common anxiety-unrelated deactivation was noted in all subgroups including

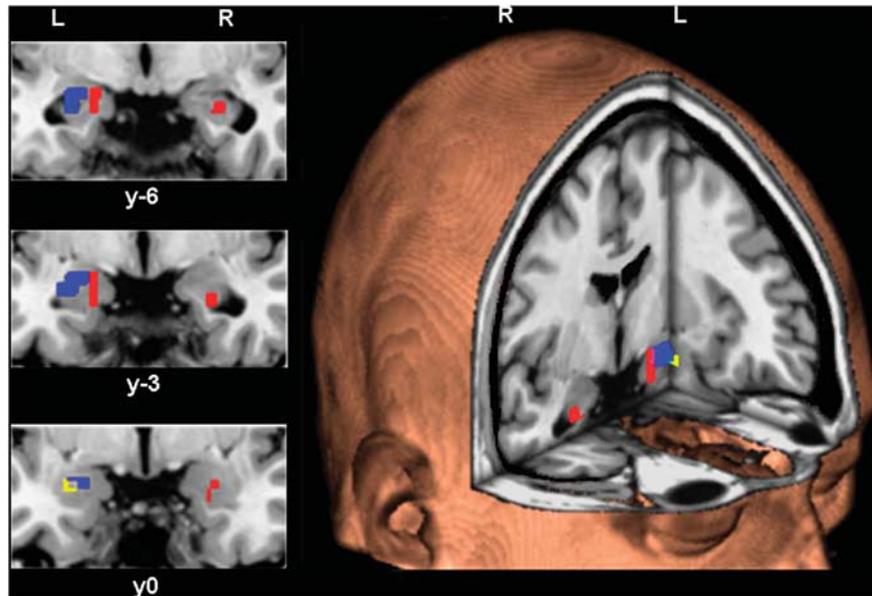


Figure 3 The spatial extent of left (L) and right (R) amygdala clusters changing significantly with treatment. Red displays the common anxiolytic effect in selective serotonin reuptake inhibitor (SSRI) and placebo responders. Blue displays the nonanxiolytic pharmacodynamic effect of SSRIs noted in responders as well as nonresponders. Yellow displays the effect related to repeated testing noted in all four groups.

nonresponders, reflecting repeated testing which is a confounding factor in clinical trials. Importantly, our results indicate that only certain parts of the amygdala mediate anxiety relief, whereas other amygdala deactivations reflect nonspecific effects of intervention.

Although usually treated as a single unit in human imaging studies, the amygdala is composed by multiple and functionally heterogeneous, but heavily interconnected, subnuclei (Aggleton, 1985; Pitkänen *et al*, 1997). Animal studies have reported differential distributions of the serotonin transporter protein (Rourke and Fudge, 2006) as well as serotonin-1A and other receptors (Pazos *et al*, 1987) across these subnuclei, which may underlie variation in treatment effects on amygdala firing. Damaging neurons of the central, basal, and lateral nuclei yields different mediation of fear-related behaviors in animals (Lázaro-Muñoz *et al*, 2010). Pharmacological studies have shown that the BLA and central amygdala, in particular, have distinct roles in the anxiolytic effect of benzodiazepines (Green and Vale, 1992). The highest concentration of benzodiazepine receptors appears to be in the BLA (Niehoff and Kuhar, 1983), which is involved in the modulation of inhibitory avoidance, characteristic for anxiety disorders (Bueno *et al*, 2005). Microinjection studies further support a central role of BLA in the anxiolytic effects of SSRIs (Inoue *et al*, 2004; Izumi *et al*, 2006). Moreover, stimulating BLA terminals in the central nucleus produced anxiolytic effects in a recent optogenetic study (Tye *et al*, 2011). Also in humans, BLA reactivity to unconscious stimuli has been shown to predict individual differences in trait anxiety (Etkin *et al*, 2009), making it a plausible target for anxiolytic treatments. Together these findings underscore the different roles of distinct amygdalar subnuclei in treatment response and the importance of BLA in anxiety.

The specific roles for amygdala subnuclei in conditioning (Morris *et al*, 2001) and social learning (Davis *et al*, 2010)

have begun to be documented. For example, a recent fMRI study demonstrated that the right VLA was more reactive to negative faces, and was more resistant to habituation, in comparison with medial and dorsal amygdala loci (Davis *et al*, 2010). Broadly this is in line with our results, ie, the right VLA was tied to negative experience. Neuroimaging attempts to map interactions between distinct amygdala subregions and other brain regions also point to a functional complexity of the amygdala reflected by different connectivity patterns (Bach *et al*, 2011; Ressler, 2010; Roy *et al*, 2010). The question whether our observed anxiety-related amygdala subregions have similar or different connectivity networks in SSRI- and placebo-treated patients will be addressed in a separate report. Moreover, it is possible that the genetic control of neural activity differs across amygdala subregions, which in turn could be related to SSRI treatment outcome as previously suggested for placebo (Furmark *et al*, 2008). This will also be addressed in a separate report.

Although neural changes did not differ significantly between the responder subgroups, prefrontal differences were noted when comparing SSRI responders and nonresponders. Enhanced activity in OFC and ACC was observed in SSRI nonresponders, relative to responders, whereas the reverse pattern was noted in the insula. The meaning of these findings is unclear as the direction of the changes were generally contrary to what could be predicted from emotional regulation and processing theories (Goldin *et al*, 2008; Ochsner and Gross, 2005; Shin and Liberzon, 2010). However, other lines of research have shown that altered responsivity in these areas correlate with emotional experience or changes in ruminative and self-focused thoughts, ie, vulnerability factors in mood and anxiety disorders (Bar, 2009; Cooney *et al*, 2010). Placebo responders showed increased activity, relative to placebo nonresponders in the pons. This region has been reported

as being involved in the opioid network affected by placebo analgesia (Petrovic *et al*, 2002).

The present study has some important limitations. First, to improve the generalizability of the SSRI results and to enable comparisons between responders and nonresponders with sufficient statistical power, data from patients treated with different SSRIs, different doses and different durations were pooled even though the clinical efficacy may vary (Telles-Correia *et al*, 2007). In particular, paroxetine 7.5 mg could be regarded as a subtherapeutic dose. Further analyses showed, however, that paroxetine 7.5 mg was neurophysiologically different from placebo but not deviant from paroxetine 20 mg in terms of reduced blood flow in the left lateral section of the amygdala that was demonstrated to be a general target for SSRIs (see Supplemental results). Statistically no behavioral or neural differences were found across the three SSRI arms, supporting that data could be merged. Second, because our paradigm did not include a neutral control task, we were not able to properly evaluate pretreatment amygdala reactivity and to exclude that attenuated amygdala activity after treatment reflects lowered trait-like activity. A previous study from our group suggested, however, that amygdala deactivation is related to state anxiety reduction (Furmark *et al*, 2005). Third, as we did not have a natural history or waiting list control group, it may be argued that our placebo results could be due to confounding variables such as spontaneous remission. However, this is unlikely as SAD appears to be a chronic condition that does not resolve without treatment (Yonkers *et al*, 2001). Concordantly, in a previous, similarly designed, PET study we did not observe clinical improvement or altered amygdala activity in waiting list controls (Furmark *et al*, 2002). Our randomized double-blind design protected against response biases and regression to the mean is also unlikely as there were no pretreatment differences between placebo responders and nonresponders on clinical variables or brain activity. Fourth, the PET technique has limited spatial resolution, which does not allow for a precise delineation of the amygdala subnuclei involved in anxiolytic and non-anxiolytic treatment responses. However, in spite of suboptimal anatomical precision and filtering (FWHM = 8 mm), we were still able to separate functionally different clusters within the amygdaloid complex. Also, results were virtually the same when using a more narrow 6-mm filter. Importantly, the smallest interpeak distance between the reported anxiolytic and nonanxiolytic amygdala subregions was above 8 mm, indicating these clusters did not overlap. Moreover, PET has the advantage of being less sensitive to movement, which allows the implementation of an ecologically valid anxiogenic public speaking challenge that would be difficult to employ with more sensitive techniques such as fMRI. Nonetheless, high-resolution fMRI could be used with other anxiogenic paradigms to confirm and define, with better anatomical precision, the amygdala subnuclei that mediate reduced anxiety. The present findings need replication in other cohorts and further studies are also needed to clarify whether the neural effects are lateralized as our data suggest.

We conclude that only certain amygdala sections, the left BM/BLA and right VLA, were commonly deactivated in clinically improved patients treated with SSRIs or placebo, and may constitute brain targets that mediate successful

anxiety reduction regardless of treatment modality. In contrast, deactivations of lateral amygdala sections, at least in the left hemisphere, appear to reflect nonspecific effects of intervention including nonanxiolytic pharmacodynamics and repeated testing. To our knowledge this is the first study showing that effective and ineffective treatments target different areas of the amygdala, shedding new lights on the neuromediators underlying anxiety relief. The amygdala is heterogeneous with respect to anxiety and should ideally be treated as such in human neuroimaging studies.

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DISCLOSURE

Massimo Bani, Paolo Bettica, and Emilio Merlo Pich were full-time employees at GlaxoSmithKline at the time of the design and conduct of this study. None of the other authors declare conflict of interest.

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