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ARTICLE

Amygdala-frontal couplings characterizing SSRI and placebo response in social anxiety disorder

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Abstract

In patients with social anxiety disorder (SAD) it has been reported that selective serotonin reuptake inhibitors (SSRIs) and placebo induce anxiolytic effects by attenuating neural activity in overlapping amygdala subregions, i.e. left basolateral and right ventrolateral amygdala. However, it is not known whether these treatments inhibit amygdala subregions via similar or distinct brain pathways. As anxiolytic treatments may alter amygdala-frontal couplings we investigated differences and similarities in amygdala-frontal functional co-activation patterns between responders and nonresponders to SSRIs and placebo in patients with SAD. Positron emission tomography (PET) with oxygen-15-labeled water was used to measure anxiety-related regional cerebral blood flow in 72 patients with SAD before and after 6–8 wk of treatment under double-blind conditions. Functional couplings were evaluated with a seed region approach using voxel values from the left basolateral and right ventrolateral amygdala. Responders and nonresponders to SSRIs and placebo showed different treatment-induced co-activations between the left amygdala and the dorsolateral prefrontal cortex (dlPFC) as well as the rostral anterior cingulate cortex (ACC). Conjunction analysis suggested shared anxiolysis-dependent inverse co-activations in SSRI and placebo responders between the left amygdala-dlPFC and left amygdala-rostral ACC, and a shared positive co-activation between left amygdala-dorsal ACC. We demonstrate that amygdala-frontal co-activation patterns differentiate effective from ineffective anxiolytic treatments and that SSRI and placebo responders share overlapping neuromodulatory paths that may underlie improved emotion regulation and reduced expression of anxiety.

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Introduction

Social anxiety disorder (SAD) is among the most common psychiatric anxiety conditions in western societies (Furmark, 2002). First-line treatment for SAD usually includes pharmacotherapy with selective serotonin reuptake inhibitors (SSRIs) and/or cognitive-behavioral therapy (CBT) (Stein and Stein, 2008). Clinical trials have also shown that placebo yields considerable anxiolytic effects (Oosterbaan et al., 2001; Furmark et al., 2008; Faria et al., 2012). With no active pharmacodynamic component as in SSRI-treatment, and without involving explicit cognitive strategies as in CBT, placebo appears to rely on expectancy-induced psychological mechanisms (Benedetti et al., 2005; Faria et al., 2008). However, the neural processes underlying placebo-induced, in comparison to pharmacologically induced, anxiety alleviation are not well understood and the neural mechanisms separating responders from nonresponders in anxiety treatment need further characterization. Here we describe and contrast brain co-activation patterns induced by successful and unsuccessful treatment with SSRIs and placebo.

The amygdala, a putative neural target for treatment interventions, seems to be crucially involved in the pathophysiology of anxiety disorders, including SAD (Etkin and Wager, 2007). Imaging studies have shown that SAD is associated with hyper-responsivity of the amygdala during aversive stimulation (Freitas-Ferrari et al., 2010; Miskovic and Schmidt, 2012), which is down-regulated both by successful pharmacological and psychosocial treatments (Furmark et al., 2002, 2005, 2008). However, although animal studies have shown that the

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amygdala is neither structurally nor functionally homogenous (LeDoux, 2007), human neuroimaging research has typically treated the amygdala as a single unit, overlooking the independent functions and connectivity patterns of its subregions (Swanson and Petrovich, 1998; Roy et al., 2009).

In a recent positron emission tomography (PET) study we demonstrated that effective treatments of SAD inhibit reactivity in the same amygdala subregions (Faria et al., 2012). The left basomedial/basolateral (BM/BLA) and right ventrolateral (VLA) amygdala were commonly deactivated in responders to prolonged treatment with either SSRIs or placebo but not in nonresponders. Anxiety reduction, regardless of treatment, was associated with decreased reactivity exclusively in these amygdala subregions, which parallels findings in animals especially regarding the BM/BLA (Green and Vale, 1992; Bueno et al., 2005). Although effective treatments with SSRIs and placebo seem to inhibit reactivity in the same amygdala subregions, it remains unknown whether they influence these subregions via similar or different neural pathways.

Prefrontal cortical regions play an important role in regulating or inhibiting excessive amygdala responsivity (Ochsner and Gross, 2005; Hartley and Phelps, 2010; Kim et al., 2011). Hence, anxiety disorders may arise from abnormal cortical-subcortical interactions, resulting in exaggerated fear responses (Cammarota et al., 2007). Consistent with this notion, abnormalities in functional connectivity between the frontal cortex (FC) and the amygdala have been reported in patients with SAD (Guyer et al., 2008; Hahn et al., 2011; Miskovic and Schmidt, 2012; Prater et al., 2012). For example, connectivity between the amygdala and the rostral anterior cingulate (rACC) and the dorsolateral prefrontal (dlPFC) cortices is reduced (Prater et al., 2012). Moreover, SSRIs have been shown to successfully alter cortico-limbic aberrant couplings both in SAD and depressed patients (Chen et al., 2008; Giménez et al., 2013; Phan et al., 2013).

Treatment of anxiety may involve direct inhibition of the amygdala via the ventromedial PFC (vmPFC), medial orbitofrontal (mOFC) and rACC cortices, and indirect inhibition through the dorsolateral PFC (dlPFC), as demonstrated in studies of emotion regulation (Milad and Rauch, 2007; Hartley and Phelps, 2010; Etkin et al., 2011). Anxiety relief could thus be associated with increased negative coupling between these fronto-cortical regions and the amygdala. Other prefrontal sectors, however, like the lateral OFC (lOFC) and ventrolateral PFC (vlPFC) seem to be activated during states of negative affect (Milad and Rauch, 2007). A recent review suggested the dorsal ACC (dACC) and dorsomedial PFC (dmPFC) to be critically involved in appraisal and expression of negative emotion (Etkin et al., 2011). It is therefore possible that these frontal regions are attenuated concomitantly with the amygdala, i.e. a positive correlation, following anxiolytic treatment as fear expression and exaggerated evaluation of threat stimuli decreases.

The present study aimed at evaluating differential and shared amygdala-frontal co-activation patterns in responders and nonresponders to SSRIs and placebo. We examined functional alterations in brain couplings in patients with SAD, focusing specifically on the amygdala subregions that were earlier tied to reduced state anxiety after treatment (Faria et al., 2012). Based on previous findings we hypothesized that, for both treatment modalities, anxiolytic amygdala subregions would show increased negative coupling (responders>nonresponders) with putative regulatory prefrontal regions (e.g. vmPFC, dlPFC) and increased positive coupling with putative expressive frontal regions (e.g. dACC, dmPFC). We used conjunction analysis to examine overlapping amygdala-FC co-activations in SSRI and placebo responders and between-group contrasts to test if these treatments, when effective, involve different neuromodulatory pathways.

Methods

Participants

For a full description of the method we refer to Faria et al. (2012). Here we compared amygdala-FC co-activation patterns, during an anxiogenic task, in 72 patients with SAD before and after treatment with SSRIs or placebo. All patients fulfilled the DSM-IV (APA, 1994) criteria for SAD, established through the Structured Clinical Interview for DSM Disorders (First et al., 1998). Exclusion criteria were: treatment for social anxiety in the preceding 6 months, current serious or dominant psychiatric disorders other than SAD, chronic use of prescribed medication, alcohol or narcotics abuse, pregnancy, menopause, left handedness, previous PET examination and any somatic or neurologic disorder that could influence the outcome of the study. All participants gave written informed consent. The study was approved by the Swedish Medical Products Agency as well as the local ethics and radiation safety committees.

Clinical trials

Data were extracted from three double-blind randomized controlled clinical trials (RCTs) evaluating changes in regional cerebral blood flow (rCBF) following administration of SSRIs and placebo, performed in collaboration with Uppsala PET center, Quintiles AB Uppsala, and GlaxoSmithKline during 2002–2005 (Furmark et al., 2005, 2008; Faria et al., 2012). At pretreatment, there were no significant demographic, behavioral or neural differences across these trials (all $ps>0.10$) supporting that data could be merged to increase the statistical power – see Supplementary Table S1 for demographic and clinical variables. There were 35 patients randomized to SSRIs, and 37 patients randomized to placebo – see Supplementary Figure S1.

GlaxoSmithKline Verona (Italy) supplied daily doses of study drugs and matching placebo for the three consecutive clinical PET-trials with a fixed dosing schedule performed under double-blind conditions. The first dose was supplied on the same day as the baseline PET-examination, and the final dose was administered 2–4 h before the final PET-assessment after 42 or 56 d of treatment. Subjects did not receive any other form of treatment during the study period.

Clinical improvement

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 $\geqslant 3$ were considered to be nonresponders. In total, there were 20 out of 35 (57%) SSRI-responders, and 11 out of 37 (30%) placebo responders (Faria et al., 2012). In addition, symptom experience during the state-like anxiety inducing public speaking task was assessed using the Spielberger State Anxiety Inventory (STAI-S) (Spielberger et al., 1970). Other anxiety assessments, none of which indicating differences between SSRI and placebo responders, were also obtained but not included herein.

PET assessments

The procedure for rCBF-assessments in patients with SAD, using PET and ^{15}O water, has been described in detail elsewhere (Furmark et al., 2005, 2008). Before and after treatment, all patients were scanned during an anxiogenic public speaking task. We used a 32-ring ECAT EXACT HR+ scanner (Siemens/CTI, 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 were positioned in the scanner with the head gently fixated and a venous catheter for tracer injections was inserted. Twenty minutes before the initial emission scan, patients were instructed to prepare a 2½-min speech about a vacation or travel experience. The PET procedure was the same at pre- and post-treatment, but the speech topic was different. A 10-min transmission scan was performed using three retractable ^{68}Ge rotating line sources. Following intravenous administration of the ^{15}O-water tracer, approximately 10 MBq/kg body weight, the emission scan (three 30 s frames, 3D 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 video-taped speech was performed in the presence of a standing silently observing audience of 6–8 persons. Directly after the speech, state anxiety ratings (STAI-S) were obtained.

Emission scans were reconstructed with a filtered 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.

Preprocessing and statistical analyses

Imaging data were analyzed using Statistical Parametric Mapping Software (SPM8-Wellcome Department of Cognitive Neurology, UK) implemented in Matlab 7.3.0 (MathWorks, USA). 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. Difference images were computed by subtracting the post-treatment from the pre-treatment scan, reflecting treatment response, using the SPM function Imcalc. These difference images were used in all subsequent analyses.

To investigate the functional coupling between anxiolysis-related amygdala subregions (Faria et al., 2012) in the left BM/BLA ($x=-16$, $y=-6$, $z=-14$) and right VLA ($x=28$, $y=-2$; $z=-26$) we extracted the corresponding maximum voxel change values (post – pre) and used them as seeds or covariates in multiple regression analyses. The amygdala parameters from SSRI as well as placebo responders and nonresponders were entered as separate columns in the design matrix, enabling comparisons of responders and nonresponders within each treatment modality and conjunction analysis of responders. Altered amygdala co-activations were examined specifically in relation to frontal cortical regions previously associated with expectancies and emotion regulation (Ochsner and Gross, 2005; Milad and Rauch, 2007; Hartley and Phelps, 2010; Etkin et al., 2011; Prater et al., 2012). The frontal search volume (17 022 voxels) encompassed the vmPFC, vlPFC, OFC, ACC, dmPFC and dlPFC (corresponding Brodmann areas 9, 10, 11, 12, 24, 25, 32, 45, 46, 47) as defined by the Wake Forest University School of Medicine PickAtlas (Maldjian et al., 2003).

To evaluate treatment-related co-activation patterns, regression analyses were performed with the left and right amygdala seeds in distinct steps.

Differential couplings between subgroups of responders and nonresponders

To examine amygdala-FC couplings related to clinical improvement in each treatment modality, the differential co-activation patterns between responders and nonresponders were contrasted within the SSRI and placebo groups separately.

Table 1. Frontal brain regions showing differential anxiolysis-dependent co-activation with the left amygdala seed within and between each treatment modality

Contrasts and brain regions	MNI coordinates			Maximum Z value	*p* value
	x	y	z		
SSRI: responders>nonresponders					
Negative co-activation					
rACC	−20	32	22	3.05	0.001
Positive co-activation					
dmPFC	−18	54	34	3.00	0.001
Placebo: responders>nonresponders					
Negative co-activation					
dlPFC	−48	30	24	3.42	0.000
	−40	52	14	3.32	0.000
Responders: placebo>SSRI					
Negative co-activation					
vmPFC	6	58	−10	3.12	0.001
dlPFC	44	20	14	3.00	0.001
Positive co-activation					
dmPFC	6	60	28	3.07	0.001

SSRI, selective serotonin reuptake inhibitor; rACC, rostral anterior cingulate cortex; dmPFC, dorsomedial prefrontal cortex; dlPFC, dorsolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex.

To investigate the unique amygdala-FC couplings segregating successful treatment modalities, regressions were contrasted between responders of SSRIs and placebo. To exclude the potential impact of nonspecific treatment effects, the corresponding rCBF co-activation in SSRI and placebo nonresponders was used as an exclusive mask.

To exclude amygdala co-activations that were not dependent on a change in state anxiety, we orthogonalized the amygdala change scores with respect to STAI-S change scores, and recomputed the amygdala couplings. We then removed the co-activations that were present *both* when using the original amygdala change scores *and* the orthogonalized values, leaving only co-activations that were dependent on a change in state anxiety. The orthogonalized amygdala values were computed as suggested by Andrade et al. (1999), using a custom-made Matlab (MathWorks, USA) script. Explained in regression terms, the orthogonalization procedure used here amounts to the same as performing a linear regression with amygdala change scores as the dependent variable and STAI-S change scores as the independent variable. The residuals from this regression analysis are the amygdala change scores orthogonalized with respect to STAI-S.

Shared couplings between responders to SSRIs and placebo

To examine overlapping co-activation patterns, reflecting a common mechanism of action, a conjunction analysis of SSRI- and placebo responders was conducted. We ensured that the co-activations were related to anxiety reductions by masking out co-activations that were present in non-responders and removing co-activations that were also present when we used amygdala change scores that were orthogonalized with respect to changes in STAI-S (for more details, see section on Differential couplings between subgroups of responders and nonresponders).

Due to our restricted focus on amygdala-FC co-activations, results were evaluated at the voxel level (1 voxel=2×2×2 mm) with the statistical threshold at $p<0.001$ uncorrected for multiple comparisons. Because conjunction analysis assume a common significant effect in all contrasts tested, it was evaluated at a more lenient statistical threshold ($Z>2$ at $p<0.05$ uncorrected). Results are described as xyz coordinates in MNI space. 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).

Results

Differential couplings between subgroups of responders and nonresponders

In SSRI responders, in comparison to SSRI nonresponders, the left amygdala (BM/BLA) showed a stronger anxiolysis-dependent negative co-activation to the left rostral ACC and a stronger positive co-activation to the left dmPFC – see Table 1. In placebo responders, the

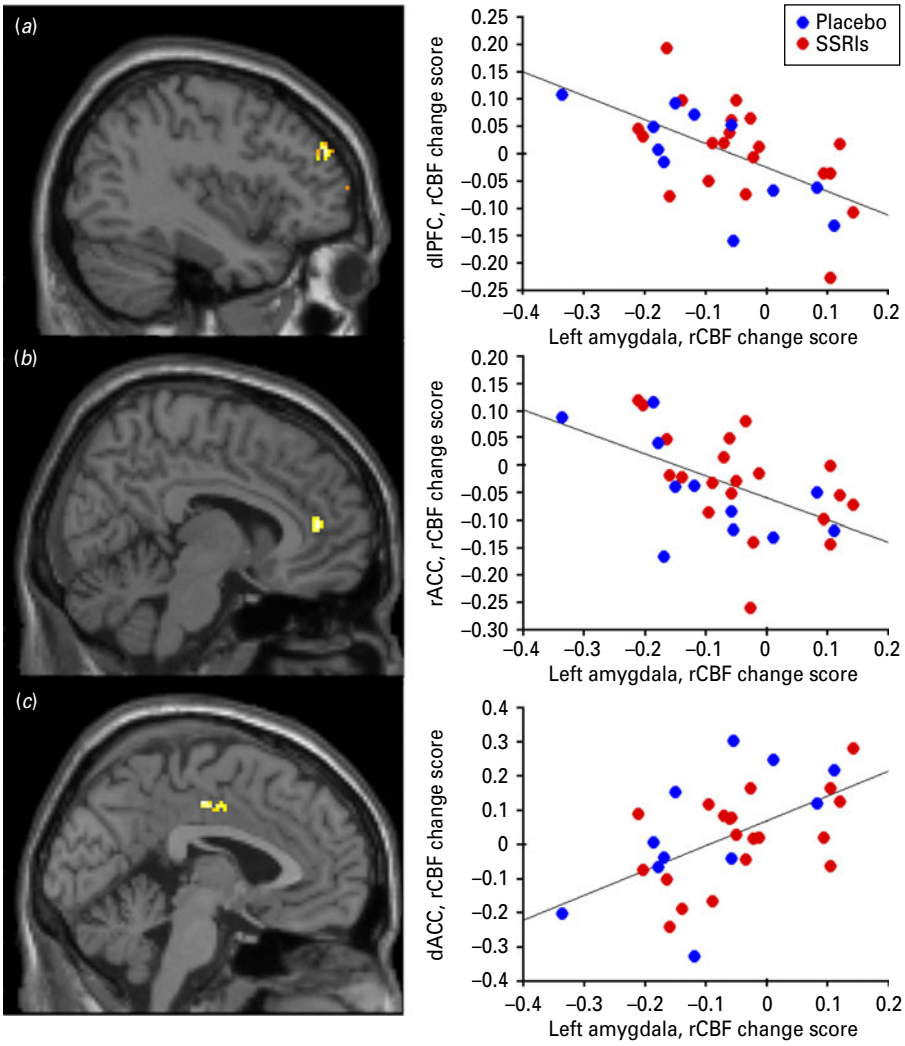

Fig. 1. Sagittal images of shared anxiolytic co-activation patterns in SSRI- and placebo responders revealed by conjunction analysis. In both treatment modalities the left amygdala seed region showed increased treatment-induced negative co-activation with (*a*) the dlPFC and (*b*) the rACC, as well as increased positive co-activation with (*c*) the posterior portion of the dACC. Plots show correlations between change-scores (post-pre) rCBF in the corresponding amygdala-frontal areas. All correlations were significant collectively ($|r| \geqslant 0.52$, $p \leqslant 0.002$) and within each treatment modality ($|r| \geqslant 0.52$, $p \leqslant 0.04$). SSRI selective serotonin reuptake inhibitors, dlPFC dorsolateral prefrontal cortex, rACC rostral anterior cingulate cortex, dACC dorsal anterior cingulate cortex, rCBF regional cerebral blood flow.

left amygdala showed a stronger negative co-activation to the left dlPFC, compared to placebo nonresponders – Table 1. There was no significant anxiolysis-related co-activation between the right amygdala (VLA) seed and the FC that differentiated responders from non-responders, neither in the SSRI nor in the placebo group.

In comparison to SSRI responders, placebo responders exhibited stronger negative co-activation between the left amygdala and the right vmPFC and dlPFC, as well as stronger positive co-activation between the left amygdala and the right dmPFC (Table 1). Results were unaffected when the corresponding connectivity voxels for SSRI and placebo nonresponders were masked out.

Shared couplings between responders to SSRIs and placebo

No significant co-activations were observed at the $p<0.001$ *a priori* threshold. At a more lenient statistical level ($Z>2$), conjunction analysis suggested shared anxiolysis-dependent negative couplings between the left amygdala seed and the left dlPFC [$(xyz)=(-38\ 48\ 30)$; $Z=2.45$; $p=0.007$] and right rACC [$(xyz)=(8\ 44\ 10)$; $Z=2.05$; $p=0.02$], respectively – see Fig. 1. Shared positive co-activation between left amygdala and the posterior portion of the dACC [$(xyz)=(-2\ -18\ 44)$; $Z=2.16$; $p=0.01$] was also noticed in responders (Fig. 1). The left amygdala activation changes from pre to post correlated significantly with all these frontal regions (Fig. 1)

and the findings persisted when masking out the corresponding co-activations for the nonresponder subgroups (SSRI and placebo), suggesting that the conjunction clusters were specific for responders. For within group co-activation results see Supplementary Table S2.

Discussion

The present study examined altered amygdala-frontal functional couplings related to SSRI and placebo response, focusing on amygdala subregions previously tied to symptom improvement (Faria et al., 2012) and functional couplings directly associated with reduced anxiety after treatment. Left amygdala (BM/BLA)-frontal couplings differentiated subgroups of responders and nonresponders in both treatment modalities. There was also suggestive evidence of shared left amygdala-frontal co-activations in SSRI and placebo responders.

Comparisons of responders *vs.* nonresponders within each treatment modality showed an increased negative co-activation between left amygdala-dlPFC in placebo responders, and also between left amygdala-rACC in SSRI responders, relative to the corresponding nonresponder subgroups. Thus, both treatments affected amygdala-FC couplings previously reported to be disrupted in SAD (Hahn et al., 2011; Prater et al., 2012) and specifically in patients who improved significantly with treatment. Both the dlPFC and rACC are known to play important roles in emotion regulation, exerting inhibitory influences on the amygdala (Banks et al., 2007; Phillips et al., 2008; Etkin et al., 2011). Consistent with the present results, the rostral section of the ACC, together with dlPFC were reported to be negatively coupled with the amygdala after SSRI treatment of depression (Chen et al., 2008), supporting the notion that pharmacological treatments enhance cortical regulation of abnormal limbic activity (Mayberg, 2002; Anand et al., 2005). Activity in the rACC, has been shown to predict a favorable pharmacological treatment response in patients with anxiety disorders (Whalen et al., 2008) and depression (Mayberg et al., 1997, 1999; Pizzagalli et al., 2001, 2003; Korb et al., 2011). In addition, rACC has also been implicated in placebo responses across disorders (Faria et al., 2008; Bingel et al., 2011; Korb et al., 2011; Petrovic et al., 2002, 2005, 2010).

Moreover, our conjunction analysis suggested, albeit at a lenient statistical level, that there are shared anxiolytic mechanisms of action for SSRI and placebo responders since both responder groups showed increased negative co-activation between the left amygdala seed and the left dlPFC as well as the right rACC. These co-activation patterns were specific for responders as they fell outside the masks generated from the nonresponder groups. The overlapping negative amygdala-dlPFC/rACC couplings are consistent with an emotion regulatory role for these prefrontal regions. Conceivably, cognitive expectancy of improvement is a common denominator for treatment responders. The dlPFC has been demonstrated to be crucially involved in placebo responses (Benedetti et al., 2006; Krummenacher et al., 2010; Atlas and Wager, 2012) and might be the cognitive locomotive region underlying expectancy-induced improvement that also plays an important role in pharmacotherapy (Colloca et al., 2004). Since the dlPFC is connected to the amygdala mostly via other PFC regions including the ACC (Gashghaei et al., 2007; Ray and Zald, 2012) it could be speculated that the dlPFC inhibits the amygdala via the rACC. Indeed, with dense projections to the BLA and extensive input from the FC (Salzman and Fusi, 2010; Kim et al., 2011), the rACC could be the interface between cognition and emotion through which dlPFC exert expectancy-driven inhibitory influences on the BLA. However, because treatment expectancies were not assessed, and given the correlative nature of the present findings, we could not demonstrate direct evidence for this.

Also, in support of a common mechanism of anxiolytic action, conjunction analysis suggested shared positive coupling between the left amygdala seed and the left posterior dACC in SSRI and placebo responders, i.e. decreased amygdala activity with treatment was accompanied by decreased activation of the posterior dACC. However, since the anatomical connectivity between amygdala and the posterior portion of the dACC is limited (Etkin et al., 2011), the observed positive co-activation might not reflect a direct pathway between the two regions. Neuroimaging studies have nonetheless shown that the dACC/mPFC area is involved in expression of negative emotion (evaluative function) in contrast to the regulatory role (inhibitory function) of the rACC (Etkin et al., 2011). Moreover, amygdala-dmPFC crosstalk during anxious states has recently been demonstrated in healthy volunteers (Robinson et al., 2012). The current findings are broadly consistent with this line of research. Notably, SSRI responders also showed enhanced positive co-activation, relative to SSRI nonresponders, between the left amygdala-dmPFC. Hence, after successful treatment, decreased dmPFC and dACC activity in concert with amygdala attenuation may reflect reduced expression of fear/anxiety.

In placebo responders relative to SSRI responders, the left amygdala showed enhanced negative co-activation with the right vmPFC/dlPFC areas, and enhanced positive co-activation with the right dmPFC. In terms of emotion regulation (Phillips et al., 2008; Etkin et al., 2011) this could suggest a stronger regulatory neural path in the placebo group, which is in line with a recent fMRI study reporting reduced resting state couplings in SAD patients treated with SSRI as compared to placebo (Giménez et al., 2013). Notably, there were no signs of enhanced coupling in SSRI relative to placebo responders in our study. However, the clinical relevance of these results remains uncertain as the different

neuromodulatory paths in the two treatment modalities could not be tied to differential clinical outcome, i.e. levels of anxiety reduction were highly similar in placebo and SSRI responders.

It should be noted that we could only demonstrate anxiolysis-related co-activation results for the left amygdala (BM/BLA) seed. Alterations in amygdala-prefrontal connectivity in SAD have been previously reported to be left lateralized (Hahn et al., 2011; Phan et al., 2013). Interestingly, Chen et al. (2008) also noted that the SSRI-effects on amygdala-PFC coupling in depressed patients were more salient for the left amygdala than for the right, possibly indicating lateralized differences in the susceptibility of amygdala-frontocortical projections to modulation by treatments. Thus, future studies may consider comparing reactivity and connectivity patterns for the left and right amygdala.

There are several limitations that should be considered in the present study. The PET-methodology that we used has limited spatial and temporal resolution. Functional couplings are better assessed with fMRI-time series although with fMRI it would have been difficult to use our ecologically valid public speaking challenge. To enable contrasting responders with nonresponders with sufficient power, data from patients treated with different doses and durations of SSRIs were pooled, and even though 7.5 mg of paroxetine could be considered a subtherapeutic dose, previous neurophysiological and behavioral analysis have shown no differences between these SSRI arms, supporting that data could be merged (see Faria et al., 2012 – supplementary material). Still, our sample-sizes were rather modest when comparing subgroups of responders and nonresponders, increasing the risk for type-II errors. Even though our findings are supported from a theoretical and empirical standpoint, and effects were generally large with *Z*-scores >3, they did not survive FWE correction for multiple comparisons and should be therefore interpreted with caution. This especially concerns the conjunction results (at $p<0.05$ uncorrected). Also, the present analyses were correlative in nature and do not allow for causal inferences about driving *vs.* receiving structures. For cause-effect inferences, effective connectivity analyses are needed. Moreover, the use of single voxel seeds might constitute another limitation since it can result in noisy outcomes (Cole et al., 2010). However, to increase the signal to noise ratio data were smoothed with an 8 mm FWHM Gaussian kernel. Hence, the extracted voxel values contain information about the values of neighboring voxels, increasing the reproducibility of the findings. It should also be noted that absolute rCBF quantification was not preformed. However, due to the present focus on individual differences in amygdala-frontal coupling, the relation between amygdala-frontal cortex becomes more relevant than the absolute values. The present study focused on amygdala-frontal co-activations only, leaving out other regions such as the insula, ventral striatum and brainstem, which may also be involved in anxiolytic treatments. Moreover, because we did not include a healthy control group, we cannot determine whether the observed neural changes reflect normalization or compensatory effects, and without a neutral control task to the public speaking challenge we cannot properly discriminate between state and trait-like neural changes. Despite these limitations, the present study is, to our knowledge, the first one to investigate anxiety-relevant co-activation patterns that segregate and unite SSRI and placebo responders.

In summary, we demonstrate that responders and nonresponders to SSRIs and placebo exhibit distinct amygdala-frontal couplings, pointing towards restored emotion regulation and reduced fear expression regardless of treatment modality. Treatment-induced, and anxiolysis-dependent, negative couplings between amygdala and dlPFC/rACC areas that overlapped in SSRI and placebo responders could reflect a similar cognitive or expectancy-related mechanism leading to improved emotion regulation, while increased positive coupling between amygdala and dACC/dmPFC areas suggest general treatment effects on appraisal and expression of anxiety. Our data suggest a dual role for the relation between the FC and the amygdala both in pharmacological and placebo contexts and that amygdala-frontal couplings are useful neuromarkers, differentiating between successful and unsuccessful anxiolytic treatments. The current findings may be of essential importance for clinical trial design and interpretation of treatment outcomes in anxiety disorders.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145714000352

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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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