Published in final edited form as:
Psychopharmacology (Berl). 2008 March ; 196(4): 661–672. doi:10.1007/s00213-007-1004-8.

Escitalopram effects on insula and amygdala BOLD activation during emotional processing

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Abstract

Rationale—The amygdala and insular cortex are integral to the processing of emotionally salient stimuli. We have shown in healthy volunteers that an anxiolytic agent, lorazepam, dose-dependently attenuates activation of limbic structures.

Objective—The current study investigated whether administration of a selective serotonin reuptake inhibitor (SSRI), escitalopram, alters the activation of limbic structures. We hypothesized that subchronic (21 days) SSRI treatment attenuates the activation of the amygdala and insula during processing of emotional faces.

Methods—Thirteen healthy volunteers participated in a double-blind, placebo-controlled, cross-over, randomized study. After 21 days of treatment with either escitalopram or placebo, participants underwent functional magnetic resonance imaging (fMRI) during which all subjects completed an emotion face assessment task, which has been shown to elicit amygdala and insula activation.

Results—Subjects activated the bilateral insula and amygdala following treatment with both escitalopram and placebo. In subjects who were adherent to the protocol (as evidenced by sufficiently high urine concentrations of escitalopram), a reduction in amygdala activation was seen in the escitalopram condition compared to placebo.

Conclusion—The current investigation provides further evidence for the mechanism of action of SSRIs through the attenuation of activation in brain regions responsible for emotion processing and provides support for the use of BOLD-fMRI with pharmacological probes to help identify the specific therapeutic effect of these agents in patients with anxiety and mood disorders.

Keywords

SSRI; escitalopram; insula; amygdala; fMRI; emotion processing

1. Introduction

The combination of fMRI and pharmacological treatment (pharmaco-fMRI) is a novel discipline with the potential to provide a better understanding of the interface between neural systems and drug therapy (Paulus and Stein 2007). In previous studies, we were able to show that the acute administration of an anxiolytic (lorazepam) attenuates limbic and paralimbic structures (amygdala and insula) during emotional face processing (Paulus, Feinstein et al.

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2005) and risk taking (Arce, Miller et al. 2006). Thus, pharmaco-fMRI may be useful to provide a brain signature of anxiolytics that could help in the development of novel drugs (Mckie, Del-Ben et al. 2005). Serotonin is involved in emotion-related processes (Harmer, Rogers et al. 2003;Rogers, Tunbridge et al. 2003) and selective serotonin reuptake inhibitors (SSRIs) are among the most commonly prescribed antidepressants (Masand and Gupta 1999;Nutt, Forshall et al. 1999) initially developed to regulate brain serotonin levels in order to treat affective disorders (Mendlewicz 1999). Moreover, these drugs are also an important aspect of the chronic treatment of individuals with anxiety disorders. Thus, understanding how prolonged (i.e., beyond acute) administration of an SSRI affects limbic and paralimbic structures in the brain provides an important next step in determining the usefulness of pharmaco-fMRI for the development of new drugs.

One of the actions of SSRIs is to block the reuptake of secreted serotonin, preventing it from being transported back into the presynaptic neuron. It is also believed that the antidepressant (and, possibly, the anxiolytic) actions of SSRIs involve effects that extend beyond serotonin reuptake (Carrasco and Sandner 2005;Vaswani, Linda et al. 2003;Waugh and Goa 2003). Nevertheless, it is still unknown how they work to normalize abnormal cognitive and emotional processes. The effects of SSRIs in affective disorders are likely to be the result of the interaction between serotonin (5-HT) pathways with the cortical and subcortical circuitry thought to be involved in the processing of emotional stimuli (Fu, Williams et al. 2004). Their acute/early stage versus chronic treatment effects are sometimes opposite in that an early exacerbation of anxiety symptoms is often followed by an anxiolytic effect if treatment is not interrupted (Harmer, Mackay et al. 2006;Kent, Coplan et al. 1998). Acute oral (Browning et al., 2007) and intravenous (Harmer, Bhagwagar et al. 2003) administration of the SSRI citalopram increases the processing of anxiety-related stimuli in healthy volunteers whereas repeated administration impairs the recognition of fearful stimuli (Harmer, Shelley et al. 2004) and attenuates amygdala activation (Harmer, Mackay et al. 2006).

Serotonin receptors are widely expressed within the amygdala (Kent, Coplan et al. 1998), considered part of the circuitry involved in the detection of emotionally relevant stimuli, which, in turn, is a process targeted by SSRIs (Harmer, Mackay et al. 2006). The amygdala, as revealed by functional neuroimaging studies, is involved in fear conditioning (Buchel, Morris et al. 1998), reward-related processing (Breiter and Rosen 1999), encoding of emotionally salient stimuli (Canli, Zhao et al. 2000), risk taking (Ernst, Bolla et al. 2002), processing positively valenced stimuli (Garavan, Pendergrass et al. 2001), and appetitive or aversive olfactory learning (Gottfried, O'Doherty et al. 2002), as well as in the pathophysiology of anxiety disorders (Charney 2003;Rauch, Shin et al. 2003). The insula, along with the anterior cingulate gyrus and medial prefrontal cortex, plays a crucial role in the detection of emotionally salient stimuli (Morris, Friston et al. 1998;Phillips, Young et al. 1998), generation of an affective response, and regulation of that affective state (Phillips, Drevets et al. 2003). This cortical region has afferent and efferent connections to the medial and orbitofrontal cortex (Ongur and Price 2000), anterior cingulate gyrus, and several amygdalar nuclei as well as the nucleus accumbens (Reynolds and Zahm 2005). Functionally, it has been typically associated with feelings of disgust (Phillips, Young et al. 1998) although more recent studies have suggested its importance in the anticipation of emotional stimuli (Simmons, Matthews et al. 2004), aversive conditioning (Paulus, Rogalsky et al. 2003) as well as physiological processing (Critchley, Wiens et al. 2004). Thus, this area appears to be a confluent point of emotion, cognition and physiology. Given the importance of the insula and amygdala in emotional processing, the functional status of these structures has been proposed to serve as a biomarker to index the effect of anxiolytic and/or antidepressant treatments (Paulus, Feinstein et al. 2005).

For the current investigation we studied the properties of a widely prescribed SSRI, escitalopram. Although several studies have used pharmaco-fMRI to assess the neural correlates of certain antidepressant agents (e.g., Chen, Ridler et al. 2007;Del-Ben, Deakin et al. 2005;Fu, Williams et al. 2007;Harmer, Mackay et al. 2006;Rose, Simonotto et al. 2006;Sheline, Barch et al. 2001;Vollm, Richardson et al. 2006), to our knowledge, this is the first study to implement a sub-chronic, placebo-control cross-over design using a group of healthy volunteers. The goal of this study was to examine whether treatment with escitalopram affects emotional processing in healthy volunteers as measured by activation in the insula and amygdala. Considering the pivotal role of the insula in subjective feeling states and interoceptive awareness (Craig 2002;Critchley, Wiens et al. 2004) as well as its presumed role in anxiety disorders (Critchley, Wiens et al. 2004;Paulus and Stein 2006) and the efficacy of SSRIs to treat such, we hypothesized that escitalopram would attenuate not only amygdala but also insular cortex activation.

This investigation was aimed to determine whether sub-chronic (21 day) administration of an SSRI, escitalopram, would attenuate the blood oxygenation level-dependent functional magnetic resonance imaging (BOLD-fMRI) activation of limbic and paralimbic structures during emotion processing. A modified emotion face assessment task was used to determine whether this attenuation would occur with emotional faces (angry, fearful and happy) when compared to a sensorimotor control task. Support for this hypothesis would provide a neurophysiological link between the clinical efficacy of escitalopram as an anti-anxiety agent and the biological basis of its modulation of limbic and paralimbic structures as key targets for anxiety circuitry.

2. Methods and Materials

2.1. Subjects

The University of California, San Diego School of Medicine institutional review board approved the study procedures. All participants provided written informed consent and were paid for their participation. Sixteen healthy, nonsmoking females completed the study (see Figure 1). Two participants were excluded from the analysis due to excessive movement during the fMRI session (more than 3 instances, at least 4mm every time) and one subject was excluded because her urine escitalopram was undetectable during the period of time when she was to have been taking escitalopram, indicating nonadherence to the protocol. The remaining 13 subjects were females of ages 19 to 27 years (mean ± SD, 22.4 ± 2.5 years) with 13 to 17 years of education (mean ± SD, 15.3 ± 1.3 years). Participants did not have medical or psychiatric disorders as determined by medical history and diagnoses according to the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Revised Fourth Edition. Subjects had no history of drug or alcohol abuse and did not report prior use of benzodiazepines, SSRIs, monoamine oxidase inhibitors (MAOIs), or neuroleptics. All participants passed a urine drug screen at baseline, EKG and routine bloodwork including CBC, electrolytes, and liver function tests. Subjects were instructed to maintain their regular bedtimes and wake times for 1 week before and throughout the study period. During this visit, and prior to the scanning session, subjects completed several self-report questionnaires, including the State-Trait Anxiety Inventory (STAI-S) (Spielberger et al. 1983), Beck Depression Inventory (BDI) (Beck et al. 1961), Social Interaction Anxiety Scale SIAS (Mattick and Clarke 1998), and the Brief Symptom Inventory BSI (Derogatis and Melisaratos 1983) to evaluate their psychological state at that time and provided a urine sample for escitalopram measurement

2.2. Study design

This study was performed in a randomized, cross-over and double blind manner (see Figure 2). Once it was determined that a subject was eligible for the study, and informed consent was

obtained, the subject was randomized to receive either escitalopram (5 mg/d for the first 3 days, then 10 mg/d for another 18 days) or placebo, administered in identical, capsular form. Subjects were instructed to take the medication each morning throughout each 21 day arm of the study. In between arms, there was a 14–28 day wash-out period, during which the medication was be reduced from 10 mg/d to 5 mg/d for 3 days, and then discontinued. The study physician (MPP) also met with the subject weekly in order to address any concerns and to ensure that compliance with the medication was maintained. Following completion of each medication arm, subjects were scheduled for an fMRI visit. During this visit, and prior to the scanning session, subjects completed several self-report questionnaires to evaluate their psychological state at that time and provided a urine sample for escitalopram measurement to assess treatment compliance. The results of the urine assay for escitalopram were not made available to the study personnel until after all subjects had completed participation. The urine samples were processed by a contract laboratory, which used gas chromatography for quantitative detection of the parent drug (test sensitivity within the range of 5 and 2000 Ng/ml).

2.3. Task

During fMRI, subjects were tested using a slightly modified version of the emotion face assessment task, along with a general sensorimotor task (for details on the task see Paulus, Feinstein et al. 2005). Each 5-second trial consisted of a target face (on the top of the computer screen) and two probe faces (on the bottom of the computer screen). Subjects were instructed to match the emotion (angry, fearful, happy) of the target and probe faces. The trials were block designed, such that each block consisted of 6 consecutive trials in which the target face was either angry, fearful, or happy. During the sensorimotor task, subjects were presented with an analogous configuration of ovals and circles, and were instructed to match the shapes of the target and the probe. Each block of the faces and sensorimotor task was presented 3 times in a pseudorandom order, and a fixation cross was interspersed between each block. Response accuracy and time data were obtained for each trial.

2.4. Image Acquisition

The BOLD-fMRI data were collected during the task using a Signa EXCITE 3.0 Tesla-GE scanner (T2*-weighted echo planar imaging, TR=2000 ms, TE=32 ms, FOV = 230×230 mm^3, 64 × 64 matrix, thirty 2.6mm axial slices with a 1.4mm gap, 256 scans). For anatomical reference, a high resolution T1-weighted image (SPGR, TI = 450, TR = 8 ms, TE = 4 ms, FOV = 250×250 mm^3, flip angle = 12°, 172 sagittally acquired slices, ~1 mm^3 voxels) was obtained during the same session. For preprocessing, voxel time series were interpolated to correct for non-simultaneous slice acquisition within each volume. These interpolated values were then corrected for 3-dimensional motion.

2.5. fMRI Analysis Pathway/Image Processing

All structural and functional image processing was done with the Analysis of Functional Neuroimages software package (AFNI). The echoplanar images were realigned to a base using a Fourier transform—using the AFNI program 3dvolreg—and then time-corrected for slice acquisition order. Preprocessed time series data for each individual were analyzed using a multiple regression model. For this model, the four orthogonal regressors of interest were (1) happy, (2) angry, (3) fearful, and (4) circle/oval sensorimotor condition. These regressors were convolved with a modified gamma variate function to account for the delay and the dispersion of the hemodynamic response of the BOLD-fMRI signal. Additionally, five nuisance regressors were used to account for residual motion (roll, pitch, and yaw) and to eliminate slow signal drifts (baseline and linear trend). These nine regressors were applied to the AFNI program 3dDeconvolve in order to calculate the estimated voxelwise response amplitude. To

account for individual variation of anatomical landmarks, a Gaussian filter with 6mm full width at half maximum was applied to the voxelwise percent signal change data.

Data for each subject were normalized to Talairach coordinates. A priori regions of interest—including the amygdala, cingulate cortex, and insula—were used as masks. Based on these three areas, a voxel-wise a priori probability of 0.05 was determined via simulations, which resulted in a corrected cluster-wise activation probability of 0.05 using a minimum volume of 128 μL and two connected voxels (for an amygdala cluster), or 256 μL and four connected voxels (for a cingulate or insular cortex cluster). Using the thresholds and cluster sizes defined above, the corrected voxel-wise probabilities are as follows: amygdala $p<0.012$, cingulate cortex $p< 0.00610129$, and insular cortex $p<0.00006859$. The areas of interest were superimposed on each individual's voxel-wise percent signal change brain image. Stereotatic coordinates of the ROIs were based on standardized atlas locations (Talairach and Tournoux, 1998) and appropriate clustering sizes were determined using the AFNI function AlphaSim. Only the activations within the areas of interest that survived the volume and voxel connection criteria were extracted and used for further analysis. A task effect was calculated by performing a one sample t-test on the placebo condition for the contrast of interest (i.e., all face types versus all shape types). Subjects with detectable escitalopram urine levels were included in a linear regression function using the AFNI program RegAna to assess for the relationship between escitalopram concentrations and BOLD activation.

2.6. Statistical Analysis

All analyses were carried out with SPSS 12.0 (Norusis 1990). A mixed model ANOVA with subjects as a random variable and treatment condition (placebo or escitalopram) as a within subjects variable was used to analyze the behavioral measures and neural activation patterns. Behavioral measures are reported as an interaction between treatment type and task condition. Self-report measures are reported as direct comparisons between the placebo and escitalopram conditions. Correlational analyses were conducted for the escitalopram condition by examining the relationship between activation in the bilateral insula, amygdala and anterior cingulate cortex during the viewing of emotional faces versus the sensorimotor condition and escitalopram urine concentration as well as behavioral and self-report measures.

3. Results

3.1. Behavioral results

Behavioral results showed that subjects performed the task with nearly perfect accuracy (mean +/−SD, 97.2%+/−0.7%), which was not affected by escitalopram administration [$F(1,12)=4.64$, $p>0.05$, partial eta2=0.003]. Similarly, latency was not affected by treatment condition [$F(1,12)=0.04$, $p>0.05$, partial eta2=0.179]. There was a main effect of face type, indicating longer reaction times when matching fearful or angry faces as opposed to happy faces and shapes [$F(3,36)=48.7$, $p<0.001$, partial eta2=0.802].

Escitalopram did not affect levels of anxiety or depressive symptoms in this healthy control sample as measured by the STAI-S [$t(12)=0.53$, $p>0.05$], BDI [$t(12)=0.48$, $p>0.05$], SIAS [$t(12)=0.3$, $p>0.05$], BSI Somatization [$t(12)=0$, $p>0.05$], BSI Depression [$t(12)=1$, $p>0.05$], BSI Anxiety [$t(12)=0.3$, $p>0.05$], or BSI total [$t(12)=0.44$, $p>0.05$].

3.2. Neuroimaging results

Task effect—As observed in a previous investigation using the same task (Paulus, Feinstein et al. 2005), subjects demonstrated bilateral amygdala, anterior and posterior insula, visual cortex and subgenual cingulate activation irrespective of emotion type or drug condition when

compared to the sensorimotor control task. Task-related deactivation was detected in the ventral anterior cingulate (see Table 1).

Drug condition effect—There were no significant differences in BOLD activity between the placebo and escitalopram conditions during emotional face processing for each of the emotions considered separately (data not shown) nor when collapsing across emotions. The average of all three emotions versus shapes is reflected on Figure 3. Within group two-tailed paired-sample t-tests revealed no significant differences for any region of interest. Therefore, we extracted the % signal change for the anatomically defined regions of interest which lead to no significant differences between conditions: bilateral amygdala [left: $t(12)=1.06$, $p>0.1$; right $t(12)=0.45$] and insula [left: $t(12)=1.99$, $p>0.05$; right $t(12)=1.17$], as well as vACC [$t(12)=0.68$], dACC [$t(12)=1.1$, $p>0.1$], and bilateral fusiform gyrus [left: $t(12)=0.38$, $p>0.1$; right: $t(12)=0.54$, $p>0.1$] (see Figure 3).

Correlations between behavioral measures and functional neuroimaging results—There were no significant correlations between response latency or accuracy, or any self-report measures (i.e., BDI, BSI, SIAS, STAIS) and the degree of activation in the insula or amygdala during the emotion face assessment task (data not shown).

Correlations between escitalopram urine concentration and functional neuroimaging results—Three subjects who had detectable urine escitalopram levels nonetheless had concentrations lower than 500 ng/ml, which was far below the level of all other subjects. Regression analysis was performed to assess the relationship between escitalopram concentration in urine and BOLD activation during emotional processing. All subjects were included in a linear regression function using the AFNI program RegAna. Spearman-rank non-parametric correlations were run to correlate the extracted % signal change and urine concentration levels. Results (see Figure 4) suggested that higher escitalopram concentrations predicted lower levels of BOLD activation in the bilateral amygdala (coordinates (x,y,z): 27,−6,−14 [$rho=-0.76$, $p<0.01$] and −18,−7,10 [$rho=-0.75$, $p<0.05$]), bilateral insula (43,−14,11 [$rho=-0.8$, $p=0.01$]; 40,−26,14 [$rho=-0.75$, $p=0.01$]; −34,−33,16 [$rho=-0.88$, $p<0.001$]) as well as subgenual anterior cingulate cortex (0,15,−4 [$rho=-0.9$, $p=0.01$]). There was no significant effect of escitalopram on activation in the bilateral visual or motor cortex (BA 2 and 4).

Urine concentrations >500 ng/ml—Following the relationship between urine concentration and limbic and paralimbic attenuation, we completed a series of post-hoc analyses in the 10 subjects who had urine levels of escitalopram that were well above the lower detection limit. Thus, in this analysis we only included individuals with urine concentrations above 500 ng/ml (n=10). An ANOVA of face-type × treatment condition did not reveal a significant interaction. To further investigate a possible effect of treatment, we performed between-condition t-tests for each of the emotion contrasts. No clusters survived thresholding for the happy – oval contrast. Angry - oval revealed 4 clusters of greater activation, two in the right [39, 8, 19, vol= 1984μL, $t(9)=4.2$, $p<0.01$ and 41, −3, 12, vol=896μL; $t(9)=3.5$, $p<0.01$)] and two in the left insula [−51, 34, 18, vol=704μL $t(9)=4.5$; $p<0.01$ and −41, 12, 17, vol=512μL $t(9)=3.95$, $p<0.01$] during the escitalopram condition. However, extracting the previous four clusters and masking these functionally defined regions onto the other two contrasts (i.e., fearful-oval and happy-oval) showed no significant activation for the fearful or happy emotions (data not shown). The fearful – oval comparison reflected a cluster in the left amygdala (−25, −4, −15; vol=320μL) suggesting attenuation of this area following escitalopram treatment [$t(9)=3.2$, $p=0.01$]. When this latter cluster was used to extract activation in the happy and angry contrast, a marginal effect of escitalopram was detected for anger [$t(9)=2.1$, $p=0.07$)] but not for happy [$t(9)=1.3$, $p>0.1$], suggesting an effect of emotional

valence (**see** Figure 5). Interestingly, the strongest task activation was observed for the fearful – oval contrast (data not shown).

4. Discussion

The aim of this study was to investigate whether subchronic (21 days of) escitalopram treatment would attenuate emotion processing related activation in limbic and paralimbic structures. Escitalopram did not affect behavior on this task nor did it change levels of anxiety in these healthy volunteers. The escitalopram treatment phase (compared to the placebo treatment phase) was not associated with an altered BOLD response to the task when including all subjects in the analysis. However, when urinary concentrations of escitalopram (which had been collected primarily as an indicator of SSRI adherence) were considered, strong concentration-dependent relationships between amygdala and insular activity were observed. An additional set of analyses including only the 10 of 13 subjects with concentrations of escitalopram in urine that were well above the assay detection threshold (which we interpreted as indicative of good compliance with the escitalopram administration) revealed a significant attenuation for fearful faces as well as a marginal attenuation for angry but not for happy faces. Taken together, these observations suggest that there was an attenuation of BOLD activity in the bilateral insula and amygdala (but not in the fusiform gyrus) directly related to bioavailability of escitalopram, and a direct attenuation for negative faces in those who most likely complied with chronic administration of escitalopram. Because we had not measured plasma concentrations of escitalopram (and metabolites) at various phases of the study, there is some uncertainty as to whether blood (and, by inference, brain) concentrations of escitalopram do, indeed, correlate with the observed BOLD fMRI responses. Thus, future work will be required, using pharmacodynamic modeling, to confirm these suspected effects.

In line with a recent fMRI study by Harmer and colleagues (2006), our results suggested that subchronic SSRI administration attenuates amygdala activation (when compared against placebo) to negatively but not positively valenced emotional faces.. Similarities with the current study include same population type (i.e., healthy volunteers) and sub-chronic SSRI treatment. Nevertheless, some important differences should be noted. Regarding the behavioral paradigm, the current participants were presented with unmasked (versus masked in the Harmet et al. 2006 study) presentations of facial expressions which also differed in the emotion depicted (anger, fear and happiness as opposed to threat and happiness). Additionally, the fMRI task used by the Harmer et al group did not include a sensorimotor condition and subjects were asked to judge the gender of the face versus matching its affect. Duration of treatment is three times longer in the current study (21 versus 7 day administration). Finally, and perhaps most importantly, the current study is a double-blind placebo-control crossover versus a double-blind between groups design in which treatment compliance was not reported.

Our results are consistent with a presumed role for amygdala activation in anxiety and depression. The amygdala is involved during fear conditioning (Charney 2003) and it has been suggested that its malfunctioning is a stable feature of depression (Mayberg, Liotti et al. 1999) and anxiety disorders (Rauch, Shin et al. 2003) such as social anxiety (Stein, Goldin et al. 2002) or posttraumatic stress disorder (Rauch, Whalen et al. 2000). Thus, the attenuation of amygdala activity following escitalopram treatment provides evidence for the direct action of SSRIs in emotion processing structures regardless of symptom attenuation (Fu, Williams et al. 2004;Harmer, Mackay et al. 2006) and bridges a direct translation of previous findings of serotonin-induced cell firing inhibition in animal amygdala (Harmer, Mackay et al. 2006;Stutzmann and Ledoux 1999).

An unexpected opposite effect (greater activation with escitalopram) was revealed in the insula. However, several aspects may account for this result. First, this effect was restricted to the

emotion of anger (as opposed to the amygdala attenuation that was valence dependent). Second, the task effect for angry was not as robust as for the fear contrast, suggesting that it may be the result of lack of power due to the reduced number of subjects (n=10). Furthermore, results from the regression analysis suggested that escitalopram attenuates activation in the insular cortex on a urine concentration-dependent basis. A recent review paper published by our group postulates that individuals prone to anxiety disorders present an altered interoceptive predictive signal (Paulus and Stein 2006). Interoception involves the self-perception of bodily signals that are important for internal body integrity and the connections that are crucial for allocating attention, contextual evaluation, and action planning. The insula is the structure tasked with evaluating the impact that certain stimuli may have in the body state. Insula activation correlates with anxiety indices during a risk-taking task (Paulus, Rogalsky et al. 2003), is heightened in subjects with specific phobia when viewing fearful faces (Wright, Martis et al. 2003), and in subjects with high trait anxiety (Simmons, Strigo et al. 2006;Stein, Simmons et al. 2006;Stein, Simmons et al. 2007). Because activation in the insular cortex has been associated with the processing of affective modulation, cognitive processing during learning, and interoception of aversive stimuli, the insula emerges as a structure that links emotion, cognition and behavior. In summary, the link between altered insular function and several anxiety disorders (Hoehn-Saric, Schlund et al. 2004;Lorberbaum, Kose et al. 2004;Malizia, Cunningham et al. 1998;Mataix-Cols, Rauch et al. 1999;Rauch, Savage et al. 1997;Wright, Martis et al. 2003) suggests a common denominator that could be used as a biomarker for their treatment.

To our knowledge, this is the first study to show that an antidepressant drug can reduce not only amygdala but possibly insular cortex activation. Similarly to other studies which have detected ventral anterior cingulate cortex attenuation following SSRI treatment in normal volunteers (Harmer, Mackay et al. 2006) and those who successfully recovered from depression (Mayberg, Brannan et al. 2000), our findings support a role for this area in the modulation of affective material (Whalen, Bush et al. 1998). Our results also provide another piece of evidence for the potential utility of pharmaco-fMRI (Arce, Miller et al. 2006;Harmer, Mackay et al. 2006;Paulus, Feinstein et al. 2005) to delineate the neurocircuitry involved the treatment of anxiety and affective disorders.

SSRIs were developed to regulate serotonin levels within the central serotonin 5-HT2 receptor in order to treat affective disorders (Mendlewicz 1999). Rodent studies have suggested that escitalopram may be effective in reducing aggressive behavior (Sanchez, Bergqvist et al. 2003), panic-like anxiety (Hogg, Michan et al. 2006) as well as reverse conditioned fear-related behaviors (Sanchez, Gruca et al. 2003). The current findings of reduced limbic and paralimbic activity following escitalopram treatment contribute to our understanding of how SSRIs can be effective therapeutic agents in the treatment of a broad array of disorders such as anxiety and depression. That is, the selective attenuation of emotion processing structures, as opposed to overall brain activation changes (i.e., lack of attenuation in motor or fusiform cortex), provides evidence for the specificity of SSRIs in targeting relevant processes in the pathophysiology of anxiety and mood disorders. Studies with depressive as well as anxious individuals should be conducted to explore whether the current findings can be translated from healthy volunteers to clinical populations.

This study has a number of limitations. Foremost among these is the lack of escitalopram blood levels. Thus, while we found a robust relationship between escitalopram urine levels and activation in emotion-processing brain areas, we cannot be certain that higher urine levels can be considered to reflect higher blood (and brain) levels. The relationship between urine and plasma levels can be supported by a pharmacokinetic report that suggested consistency between both $t_{1/2}$ of urine and plasma (Sogaard, Mengel et al. 2005). We have also used urine levels to make inferences about compliance with escitalopram administration. This resulted in us excluding subjects with non-detectable or very low urine escitalopram levels, under the

assumption that such subjects were non-compliant with the protocol. The limited sensitivity of the urine analysis test (5 to 2000 Ng/ml) may have caused a possible range restriction effect given that 5 out of 13 individuals presented scores within the upper limit of the detection range. However, if anything, this would have reduced the power to detect correlations. Nevertheless, we have performed non-parametric tests to minimize the influence of this effect. Future pharmaco-imaging studies may incorporate urine and blood assays with greater assiduity to monitor compliance and determine the relationship between plasma levels and brain effects. Another consideration for future studies is that highly arousing and graphic images may be able to more effectively probe the therapeutic effects of pharmacological agents by assuring greater pretreatment levels of limbic and paralimbic hyperactivity which can be reduced after treatment.

In summary, the current investigation provides additional neuroimaging evidence of a change in brain regions critical for the mediation of anxiety and depression induced by a well-established pharmacological agent, an SSRI. This study offers further support for the use of BOLD-fMRI in combination with pharmacological probes to assess neurophysiological models proposed for anxiety and depression, and highlights the a role for fMRI in the evaluation of novel pharmacological treatments.

Acknowledgments

We would like to acknowledge the assistance of Ryan Pepin.

Financial disclosure: This study was supported by grants from NIMH (MH65413 and MH075792). Dr. Stein is a consultant for Forest Laboratories, the makers of escitalopram. He is also a consultant to numerous other pharmaceutical companies that market antidepressants and other psychotropic medications.

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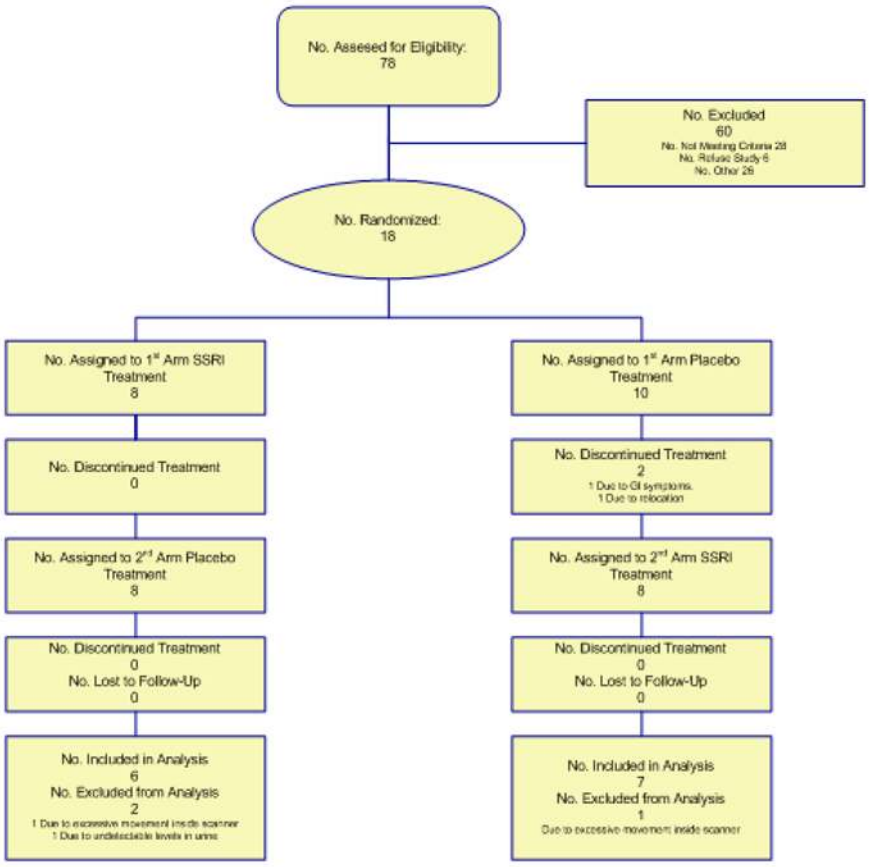

Figure 1.
Flowchart of study participants.

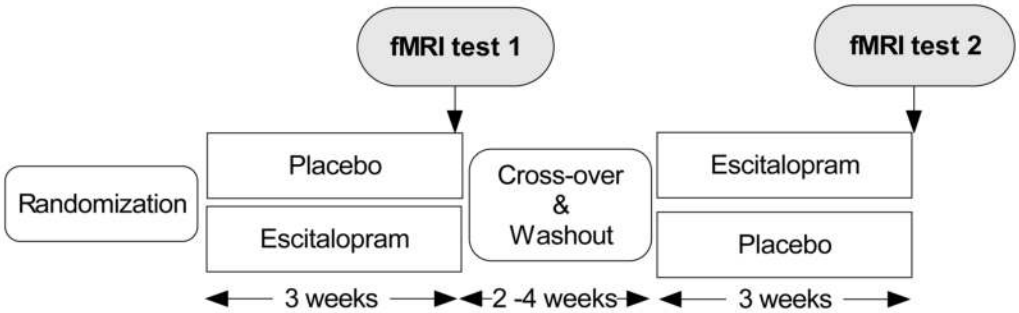

Figure 2.
Study design

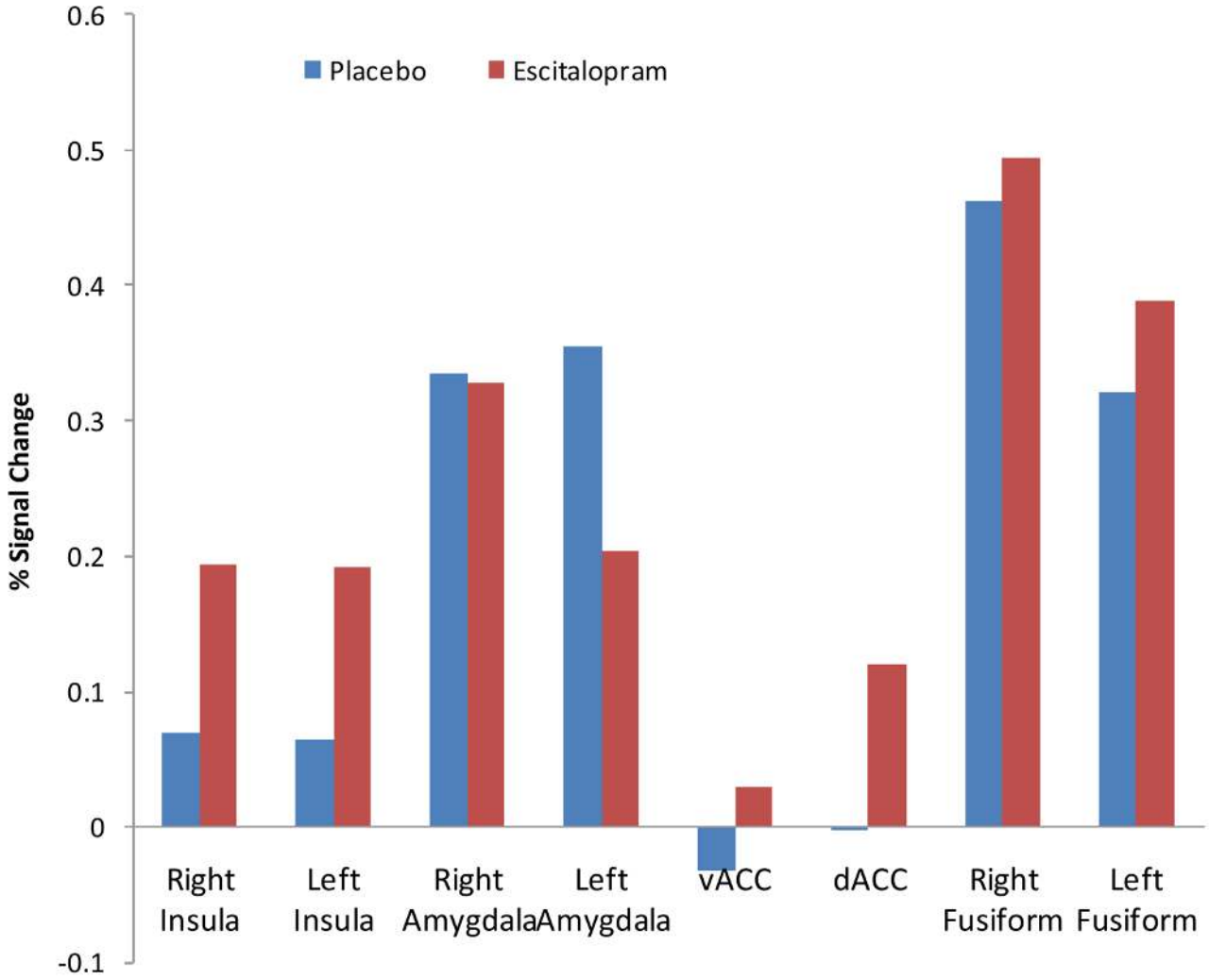

Figure 3.
Extraction of percent signal change in anatomically defined regions of interest during all faces types – all shape types, for each treatment condition.

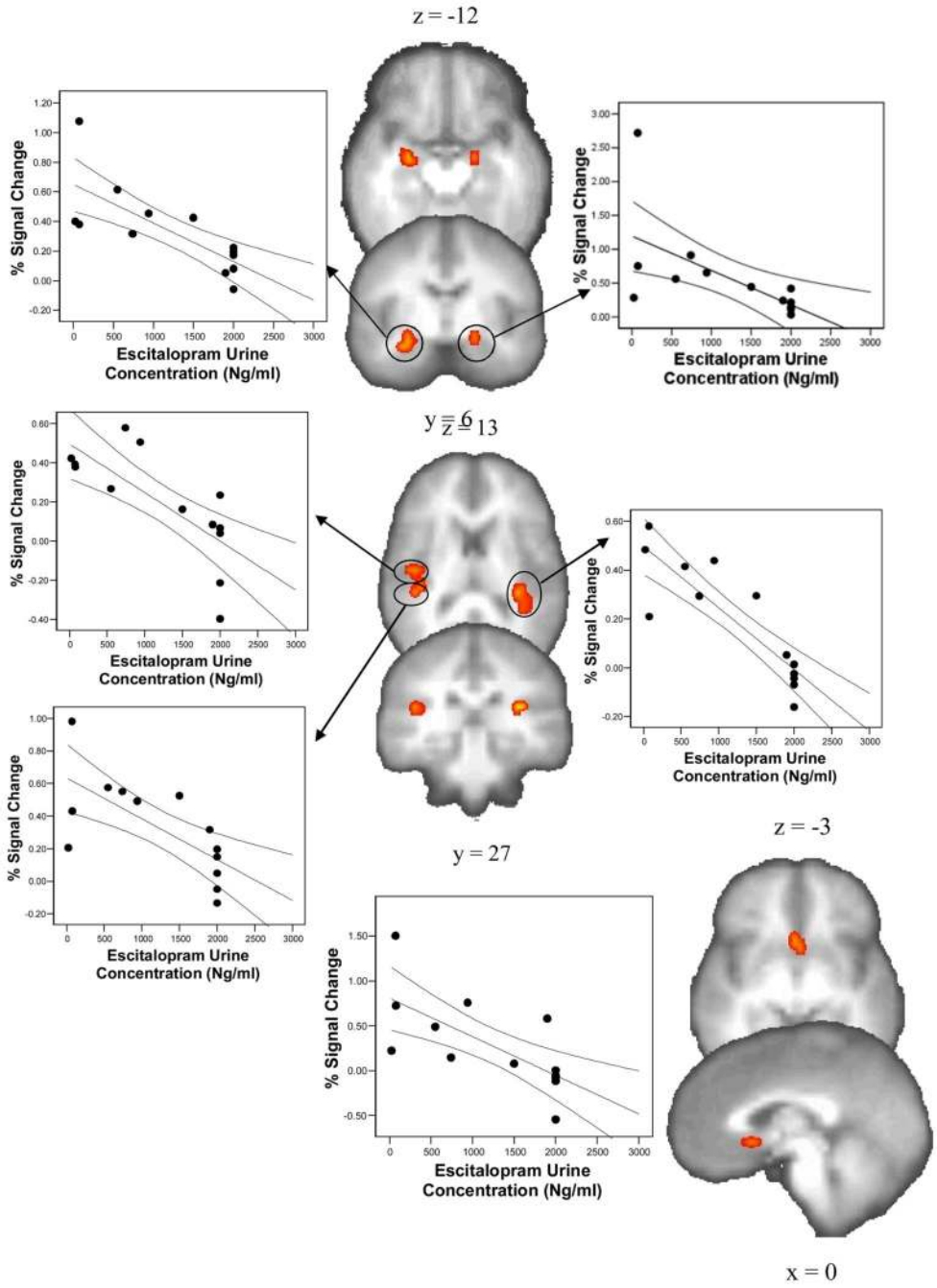

Figure 4.
Brain areas that significantly correlated with regions of interest related to urine concentration levels during escitalopram administration in 13 subjects.

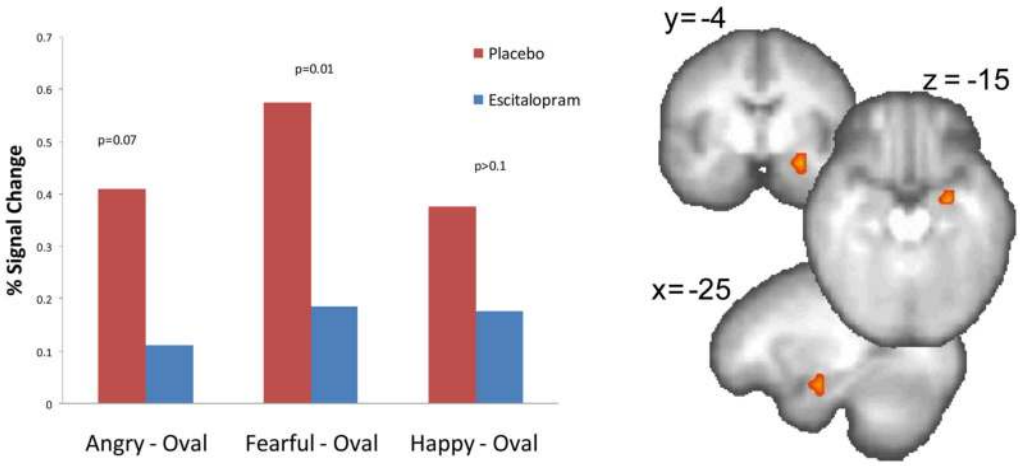

Figure 5. Amygdala attenuation due to sub-chronic escitalopram administration in 10 subjects
Note: Brain regions depicted in the figure resulted from between-conditions (Placebo vs Escitalopram) Fearful-Oval Contrast in 10 subjects. Areas were masked onto Happy-Oval and Angry-Oval contrasts to obtain the above % signal change.

Psychopharmacology (Berl). Author manuscript; available in PMC 2010 March 16.

Table 1

Task activation (or deactivation, as indicated by a negative t value) during all face emotion types versus sensorimotor condition based on a region of interest analysis.

Cortical Areas (within cluster)	BA	L/R	Coordinates (center of cluster)*			Cluster Volume (μL)	t value**
			x	y	z		
Fusiform Gyrus	18, 19, 37	R	37	−57	−13	6656	6.1
Fusiform Gyrus	18, 19, 37	L	−31	−64	−12	4480	5.7
Post Insula	13	R	38	−19	11	2624	4.3
Insula	13	R	37	13	2	2496	4.5
Post Insula	13	L	−38	−19	13	1536	5.4
Insula	13	L	−38	10	−1	1024	4.1
Amygdala		L	−23	−6	−14	960	8.3
Amygdala		R	24	−5	−15	896	5.5
VACC	32	L	−7	43	4	448	−3
Subgenual ACC	25	R, L	1	19	−4	320	3.7

*All coordinates are Talairach coordinates (x, y, z). Cortical areas are based on Talairach Daemon software (Lancaster et al., 2000).

**degrees of freedom (25), $p < 0.05$