Subchronic SSRI administration reduces insula response during affective anticipation in healthy volunteers

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Abstract

The anterior cingulate cortex (ACC) and insula are important neural substrates for the integration of cognitive, emotional, and physiological information, as well as the coordination of responses to anticipated stimuli. Increased neural activation within these structures has been observed in individuals with anxiety and depressive disorders. Selective serotonin reuptake inhibitors (SSRIs) are among the most effective and frequently prescribed anxiolytic agents, yet it is not known whether ACC or insula underlie the effects of these drugs. We examined whether subchronic administration of a SSRI to healthy volunteers attenuates activation in ACC or insula during anticipation, an important emotional process underlying anxiety. Support for this hypothesis would help to understand where and by what process SSRIs may exert beneficial effects as anxiolytics and would provide further mechanistic evidence for functional magnetic resonance imaging (fMRI) as a biomarker for the development of anxiolytics. Fifteen volunteers participated in a double-blind, placebo-controlled, randomized cross-over study. Participants completed a pleasant and aversive picture-cued anticipation task during fMRI after taking either escitalopram (10 mg) or placebo for 21 d. We found that escitalopram significantly decreased activation in bilateral posterior and middle insula during the anticipation condition irrespective of stimulus valence and in medial prefrontal and ACC during anticipation of aversive vs. pleasant images. Reduced insular and ACC activation in healthy controls during anticipation may be integral to the therapeutic efficacy of SSRIs and may provide a mechanistic approach for the use of pharmaco-fMRI in the identification of novel pharmaco-therapeutic agents in patient populations.

Key words: Anticipation, escitalopram, fMRI, insula, SSRI.

Introduction

Increased emotionality associated with the anticipation of future events is a key feature of anxiety disorders (Eysenck, 1997; Grillon, 2008). Related evidence indicates that heightened anticipatory anxiety is associated with deleterious psychophysiological stress responses (Masaoka & Homma, 2001; Masaoka et al. 2007). Serotonin may well be an important neurotransmitter in this, and other affective processes (Deakin, 1998), as well as playing a role in modulating both psychological (Brown & Hariri, 2006; Graeff et al. 1996; Hariri & Brown, 2006; Hariri et al. 2006) and physiological (Feldman et al. 2003; Hood et al. 2006; Richter et al. 2003) aspects of anticipatory anxiety. Serotonin receptors are widely expressed within the amygdala (Kent et al. 1998), the ventral anterior cingulate cortex (ACC) and the insula (Lanzenberger et al. 2007) – neural substrates that play a critical role in regulating psychological well-being and physiological homeostasis (Craig, 2003; Critchley et al. 2003).

Functional neuroimaging studies have shown that the medial prefrontal gyrus (MPFG), ACC (Chua et al. 1999; Sawamoto et al. 2000; Simpson et al. 2001) and insula (Chua et al. 1999; Ploghaus et al. 1999) are activated during anticipation of an electric shock or a noxious thermal stimulus, and during anticipation...
of feedback in a decision-making task (Critchley et al. 2001). Previously (Simmons et al. 2004), we examined anticipation of aversive images (i.e. spiders and snakes) in healthy volunteers and found anticipation-related activation within the right insula. Furthermore, using that task, we observed greater insula activity in subjects with high trait anxiety (Simmons et al. 2006), as well as in patients with PTSD (Simmons et al. 2008).

In a similar study, Nitschke and colleagues displayed aversive and non-aversive pictures to healthy volunteers and found anticipation-related activation in ventral and dorsal ACC, bilateral insula, and bilateral amygdala (Nitschke et al. 2006). In addition, Bermpoth and colleagues have found that the dorsal MPFG/ACC are particularly sensitive to expectancy while other regions such as the insula and amygdala are more sensitive to emotional intensity of the stimulus (Bermpoth et al. 2006a,b). The dorsal ACC has strong connections with the insula and these areas are often described as being part of a primary ‘default mode’ network (Fox et al. 2005).

In an effort to better understand the mechanism (and brain localization) of SSRI treatments, functional magnetic resonance imaging (fMRI) techniques have recently been applied to measure their effect on neural processing (Fernandez et al. 2001; Harmer et al. 2006; Van der Linden et al. 2000a). We have previously shown that acute administration of a benzodiazepine anxiolytic (lorazepam) attenuates activity of the amygdala, ACC, and insula during risk-taking decision-making (Arce et al. 2006) and emotional face processing (Paulus et al. 2005). Acute doses of SSRI treatment can increase anxiety (Bagdy et al. 2006; Greenwood et al. 2008; Grillon et al. 2007) whereas prolonged administration can attenuate amygdala activation (Harmer et al. 2006) and decrease anxious distress. Although several studies have used pharmaco-fMRI to assess the neural correlates of certain antidepressant agents (Chen et al. 2007; DelBen et al. 2005; Fu et al. 2004, 2007; Harmer et al. 2006; Rose et al. 2006; Sheline et al. 2001; Vollm et al. 2006), to our knowledge, this is the first study to implement a subchronic, placebo-controlled, cross-over (i.e. within-subjects) design using a cohort of healthy volunteers to assess the effects of SSRIs on emotion anticipation. Considering the pivotal role of the insula and ACC in subjective feeling states and interoceptive awareness (Craig, 2002; Critchley, 2004) and their implication in the pathophysiology of anxiety disorders (Critchley, 2004; Etkin & Wager, 2007; Paulus & Stein, 2006), we hypothesized that subchronic administration (3 wk) of the SSRI escitalopram would be associated with attenuated activation in the ACC and insula during an emotional anticipation task. Confirmation of this hypothesis would provide further evidence of the utility of pharmaco-fMRI as a tool to identify the neural substrates important for anxiety and depression (Paulus & Stein, 2007). Once neural substrates are established, changes in their activity can be used as biomarkers for the measurement of efficacy of novel anxiolytics or antidepressants.

**Method**

**Subjects**

Sixteen healthy, non-smoking females provided written informed consent and were paid for their participation in this study, which was approved by the University of California San Diego School of Medicine institutional review board. One subject was excluded because her urine escitalopram level was undetectable during the period of time when she was supposed to have been taking escitalopram, suggesting non-adherence to the protocol. The remaining 15 subjects were aged 19–27 yr (mean ± S.D., 22.3 ± 2.3 yr) with 11–17 yr education (mean ± S.D., 15.5 ± 1.8 yr). Participants did not have medical or psychiatric disorders as determined by medical history and diagnoses according to the Structured Clinical Interview for DSM-IV-TR (First et al. 2002). Subjects had no history of drug or alcohol abuse and no history of previously taking benzodiazepines, SSRIs, monoamine oxidase inhibitors (MAOIs), or neuroleptics. All participants had a negative urine drug screen at baseline. EKG and routine laboratory blood tests, which included a CBC, electrolytes, and liver function tests, were within normal limits. Subjects were instructed to maintain their regular bedtimes and wake times for 1 wk before and throughout the study period.

**Study design**

This study used a randomized, cross-over, double blind design (see Fig. 1). Once it was determined that a subject was eligible for the study, and informed consent was obtained, randomization was performed. Subjects were then assigned to a wash-out period of 3 wk followed by a randomized, cross-over, double blind drug treatment protocol. Subjects were assigned to escitalopram (20 mg) or placebo for 2–4 wk followed by a wash-out period of 3 wk before crossover to placebo or escitalopram, respectively. Subjects were examined twice, initial examination before any drug treatment and last examination after 3 wk of escitalopram treatment. Each examination was separated by a wash-out period of 3 wk. During the drug treatment period, subjects took their assigned medication at 7:00 am daily for 1 wk, followed by 2 wk of placebo. The wash-out period was designed to determine if escitalopram levels were within normal limits. Subjects were instructed to maintain their regular bedtimes and wake times for 1 wk before and throughout the study period.

**Fig. 1. Study design.**

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**Chronic study protocol**

**Randomization**

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**Placebo**

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**Cross-over and wash-out**

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**Escitalopram**

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**Placebo**

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**fMRI test 1**

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**fMRI test 2**

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**3 wk**

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**2-4 wk**

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**3 wk**

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written consent was obtained, the subject was randomized to receive either escitalopram (5 mg/d for the first 3 d, then 10 mg/d for another 18 d) or placebo, administered in identical, capsular form. Subjects were instructed to take the medication each morning throughout each 21-d arm of the study. In between arms, there was a 14- to 28-d tapered wash-out period, during which the medication was reduced from 10 mg/d to 5 mg/d for 3 d, and then discontinued. The study physician (M.P.P.) also met the subjects weekly in order to address any concerns and to ensure that compliance with the medication was maintained.

At the end of each 21-d medication arm (prior to taper), subjects were scheduled for a fMRI visit. Prior to medication administration and prior to both scanning sessions, 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 & Clarke, 1998), and the Brief Symptom Inventory (BSI; Derogatis & Melisaratos, 1983) to evaluate their psychological state at that time, and also provided a urine sample for escitalopram measurement.

**Task**

The task combined a continuous performance task (CPT), similar to a task described previously (Huettel et al. 2002), with the interspersed presentation of aversive affective stimuli. During the CPT, subjects were asked to press a ‘LEFT’ mouse button whenever they saw a blue circle and a ‘RIGHT’ mouse button whenever they saw a blue square on the screen. Stimuli were presented at a visual angle of 4 degrees at a rate of 0.5 Hz. Simultaneously, a 250-ms long 500-Hz tone was presented every 2 s. Subjects were instructed prior to the task that a switch from a blue to a green circle or square accompanied by a 250-Hz tone would indicate that a positive image was going to appear on the screen. In contrast, a switch from blue to red stimuli together with a 1000-Hz tone signalled an impending negative image. The picture stimuli were comprised of 17 positive (i.e. pleasant) and 17 negative (i.e. unpleasant, or aversive) images taken from the International Affective Picture System (IAPS; Lang et al. 1998), which consisted of superficial physical injuries, assaults, traffic accidents or other common traumatic events. The anticipation periods during the task (red and green shapes) lasted 6 s and the image presentation lasted 2 s. The baseline CPT task was interspersed for variable duration averaging about 8 s in between these task components. The total duration of the task was 580 s. No response from subjects was required when a picture stimulus was presented on the screen.

Response accuracy and response latency were obtained for the CPT, anticipation of a positive image (API), and anticipation of a negative image (ANI). To examine the behavioural effect of anticipation, we examined the difference between behavioural measures during the API and ANI.

**Image acquisition**

During the task, one fMRI run sensitive to blood oxygenation level-dependent (BOLD) contrast was collected for each subject using a Signa Excite (GE Healthcare, USA) 3.0 T scanner (T2*-weighted echo-planar imaging, TR=2000 ms, TE=32 ms, FOV = 230 × 230 mm³, 64 × 64 matrix, thirty 2.6-mm axial slices with a 1.4-mm gap, 290 scans). fMRI acquisitions were time-locked to the onset of each trial. During the same experimental session, a high-resolution T1-weighted image [spoiled gradient recalled (SPGR), TI = 450, TR = 8 ms, TE = 4 ms, flip angle = 12°, FOV = 250 × 250, ~1 mm³ voxels] was obtained for anatomical reference.

Data were preprocessed and analysed with the Analysis of Functional NeuroImages (AFNI) software package (Cox, 1996). Preprocessed time-series data for each individual were analysed using a multiple regression model. Regressors of interest included four orthogonal regressors that were constructed to quantify the neural substrates contributing to the different components of the task: (1) the API, capturing the anticipation of a positive image, (2) the ANI, capturing the anticipation of a negative image, (3) the positive image (PI) phase, which assesses the processing of positive stimuli, and (4) the negative image (NI) phase, which assesses the processing of negative stimuli. In addition, six nuisance regressors were entered into the linear regression model: three movement-related regressors used to account for residual motion (in the roll, pitch, and yaw direction), a white-matter mask to control for physiological noise (Strigo et al. 2006), and regressors for baseline and linear trends used to eliminate slow signal drifts. The CPT task (blue shapes) provided the baseline condition and was accounted for by the baseline regressor. Percent signal change was calculated by dividing the regressor of interest by the baseline regressor. Subsequently, simple contrasts were constructed on an individual subject level for all anticipation (ANI + API) and differential anticipation (DA) of negative vs. positive...
(ANI—API). A Gaussian filter with full-width-half-maximum 6 mm was applied to the voxel-wise percent signal change data to account for individual variations in the anatomical landmarks. Data of each subject were normalized to Talairach coordinates.

In addition, a region-of-interest (ROI)-based analysis was performed on several a-priori areas of interest: the bilateral insula, bilateral amygdala, ventral ACC, and dorsal ACC. These corrected voxel probabilities are based on Monte Carlo simulations via AFNI’s program AlphaSim, using the filtered data and the a-priori defined ROIs. Stereotactic coordinates of the ROIs were based on standardized Talairach atlas locations (Talairach & Tournoux, 1998). This resulted in minimum clusters sizes of 128 μl for the amygdala ROIs and 256 μl for all remaining ROIs. While the cluster significance is p < 0.05 for the ROIs, the corrected voxel-wise probabilities are as follows: amygdala (p < 0.012), insular cortex (p < 0.00007), ventral medial prefrontal cortex (p < 0.00014), and dorsal medial prefrontal cortex (p < 0.00014). The average percent signal difference was extracted from regions of activation that were found to survive this threshold/cluster method and the t values were calculated with and without education as a covariate.

To allow for inspection of non-a-priori regions we performed two additional whole-brain t tests: (1) for the task effect in the placebo condition alone we did a one-tailed p < 0.05 analysis of ANI> API and (2) for the drug effect using a two-tailed p < 0.05 analysis for significant differences between ANI and API between conditions. Monte Carlo simulations suggested a minimal cluster size of 1448 μl for both of these analyses. The results of these analyses are reported in the Supplementary section (available online). Correlational analyses were also conducted for the placebo minus escitalopram effects for particular contrasts of interest, including imaging, behavioural, and self-report data. All analyses for the behavioural data were performed with SPSS 12.0 (SPSS Inc., USA).

Results

Behavioural analysis

Subchronic administration of escitalopram had no significant effect on task performance during the different task conditions (CPT, ANI, API), as measured by response latency or accuracy [F(1,14) = 2.30, p = n.s.; F(1,14) = 0.007, p = n.s., respectively]. Escitalopram did not alter self-report measures of various types of anxiety symptoms or depression [i.e. BDI, BSI, SIAS, STAI-S; see Supplementary Table 1 (online)] in this group of healthy volunteers.

Brain activation analysis

Task effect

ROI analysis of the task-related activation (combined placebo and escitalopram arms) revealed an effect of differential anticipation (DA: ANI—API) in the bilateral anterior insula (La) [right La: Brodmann Area (BA) 13; x = 38, y = 14, z = 4; F(1,14) = 4.65, p = 0.001; left La: BA 13; x = 32, y = 19, z = 6; F(1,14) = 4.01, p = 0.001; see Fig. 2a]. The bilateral anterior insula regions did not differ significantly across conditions. There was no significant effect of task in other ROIs. There did not appear to be significant effects of order in the task effect for the right insula [(t(15) = 0.654, p = 0.523] and left insula [t(15) = 1.001, p = 0.333]. In addition, the whole-brain effects of task in the placebo condition reflected the importance of the insula and also showed frontal and temporal activations [see Supplementary Table 2 (online)].

SSRI effect

There was a main effect of subchronic administration of escitalopram, which was due to a relative deactivation during differential anticipation (ANI—API) in the pregenual ventral ACC [BA 32; 2624 μl, x = 5, y = 32, z = 12; F(1,14) = 3.26, p = 0.005; see Fig. 2b]. Moreover, individuals after escitalopram administration relative to placebo showed relative deactivation for all anticipation (ANI+ API) trials in the right posterior insula [BA 13; 1344 μl, x = 42, y = 19, z = 3; F(1,14) = 4.50, p = 0.001], left inferior posterior insula [BA 13; 1088 μl, x = 42, y = 17, z = 2; F(1,14) = 2.86, p = 0.05], left superior posterior insula [BA 13; 832 μl, x = −41, y = −13, z = 25; F(1,14) = 3.03, p = 0.01], and left middle insula [BA 13; 1216 μl, x = −40, y = 6, z = −9; F(1,14) = 3.01, p = 0.01] (see Fig. 3). There were no significant effects of escitalopram in other ROIs. There does not appear to be significant effects of order in these in the left insula [t(15) = −0.09, p = 0.929] and the ventral ACC [t(15) = 0.11, p = 0.914]. In addition, the whole-brain effects of drug pointed to frontal and temporal deactivations [see Supplementary Table 3 (online)].

There were no significant correlations between the change in response latency or accuracy and self-report measures (i.e. BDI, BSI, SIAS, STAI-S), or the change in the degree of activation in the insula or cingulate during the task across drug conditions (data not shown).
Discussion

This experiment yielded three main findings. First, subchronic administration of ‘therapeutic doses’ of the SSRI escitalopram resulted in significant relative deactivation of the ventral ACC during anticipation of negative compared to positive visual stimuli. Second, escitalopram reduced middle to posterior bilateral insula activation during anticipation regardless of the valence of the stimulus. Third, we confirmed previous observations (Simmons et al. 2004, 2006) that bilateral anterior insula is important for anticipation of negative (aversive) compared to positive (pleasant) visual stimuli. Taken together, these results suggest that escitalopram modulates anticipation by attenuating the insula and ventral ACC, which provide compelling mechanistic evidence for process by which SSRIs may act as anxiolytics. Therefore, therapeutic effects of SSRIs as anxiolytics may be due to cues that signal expected emotional states contributing less significantly to emotion processing. The use of healthy controls allows an understanding of the neurobiology of these drugs in a well-controlled and homogeneous sample and highlights that these brain effects are detectable even without symptom remediation. These observations add to the growing literature that pharmac-fMRI may be useful in revealing effects of well-established anxiolytics and antidepressants in the brain, and could thus be a useful tool in the development of novel therapeutics (Paulus & Stein, 2007).

The present study replicated the relative increases in the bilateral insula during anticipation seen in our previous work (Simmons et al. 2004, 2006, 2008). The insula, a part of the extended limbic system, can be subdivided into anterior agranular (Ia), central/middle dysgranular (Id) and posterior granular (Ig) subregions based on function and cytoarchitectural structure (Augustine, 1996; Dupont et al. 2003). The anterior insula has efferent connections with ventral frontal brain regions such as the ACC and orbital frontal cortex (OFC), as well as with periamygdaloid areas. Its middle region has strong connections with
the amygdala body, OFC and secondary somatosensory areas (Augustine, 1996; Bermudez-Rattoni et al. 2004; Devinsky et al. 1995). The posterior insula has afferent projections from the frontal cortex, the temporopolar cortex, and secondary somatosensory area (Dupont et al. 2003). Recent literature has suggested that the rostral parts of the insula (Ia and anterior Id) motivate action while the caudal insula (Ig and posterior Id) is involved in monitoring the physiological condition of the body (Craig, 2002, 2005). In comparison, the ACC – particularly the ventral subdivision – plays a similar role in emotional and physiological processing (BA 24a). Its ventral region has projections to the anterior insula (Augustine, 1996; Dupont et al. 2003; Ongur et al. 1998, 2003; Shi & Cassell, 1998) and the amygdala (An et al. 1998; Likhtik et al. 2005; Ongur et al. 1998; Phan et al. 2003; Quirk et al. 2003) exerting top-down regulation on these structures (Likhtik et al. 2005; Quirk et al. 2003). The ventral ACC is involved in fear conditioning (Phan et al. 2003; Quirk et al. 2003; Sotres-Bayon et al. 2006), in the pathophysiology of anxiety disorders (Britton et al. 2005; Phan et al. 2006), self-relevant cognition (Amodio & Frith, 2006; Beauregard et al. 2001; Gusnard et al. 2001; Heatherton et al. 2006; Johnson et al. 2005; Keenan et al. 2000; Mars et al. 2005; Northoff & Bermpohl, 2004; Wicker et al. 2003), and error processing (Bremner et al. 2004; Bush et al. 2000; Mars et al. 2005; Whalen et al. 1998). Given the importance of the various subdivisions of the ACC in the integration of physiological and psychological processes, changes in their activity are potentially useful neural biomarkers for the efficacy of pharmacotherapies (Paulus et al. 2005).

Altered anticipatory processing is a key feature of many of the anxiety disorders, reflected by greater activation in the insula (Chua et al. 1999; Lorberbaum et al. 2004; Nitschke et al. 2006; Simmons et al. 2006), medial frontal gyrus (Bystritsky et al. 2001; Kalisch et al. 2006; Lorberbaum et al. 2004; Nitschke et al. 2006; Simmons et al. 2006), and amygdala (Lorberbaum et al. 2004; Masaoka et al. 2007; Nitschke et al. 2006). Due to the high comorbidity anxiety and depression (Mineka et al. 1998) alleviating anticipatory symptoms may benefit both conditions. SSRIs are among the first-line treatments for anxiety and depression (Burke et al. 2002; Deakin, 1998; Kent et al. 1998; Masand & Gupta, 1999; Nutt et al. 1999; Taylor et al. 2006; van der Linden et al. 2000b; Vaswani et al. 2003; Wade et al. 2002; Waugh & Goa, 2003). Although SSRIs block synaptic neuronal reuptake of secreted serotonin (Klein et al. 2006, 2007), current theories posit that the antidepressant (and, possibly, the anxiolytic) actions of SSRIs involve effects that extend beyond serotonin reuptake (Carrasco & Sandner, 2005; Vaswani et al. 2003; Waugh & Goa, 2003). The effects of SSRIs in
affective disorders may be the result of modulation by serotonin pathways of the cortical and subcortical circuitry involved in the processing of emotional stimuli (Fu et al. 2004). The acute effects of SSRIs are sometimes opposite the chronic effects, in that an early elevation of anxiety symptoms is often followed by an anxiolytic effect if treatment is continued (Harmer et al. 2006; Kent et al. 1998). Acute oral (Browning et al. 2007) and intravenous (Bhagwagar et al. 2006) administration of a SSRI (i.e. citalopram) has been shown to increase the processing of anxiety-related stimuli in healthy volunteers and patients. Although these effects are not consistent as others have found that acute SSRI administration has been associated with decreased activation during affective image processing (Takahashi et al. 2005) and during a go/no-go task (Del-Ben et al. 2005). Prolonged administration has more consistently been associated with attenuation of the recognition of fearful stimuli (Harmer et al. 2004) and amygdala activation (Harmer et al. 2006). Thus the role of SSRIs in brain may be highly dependent on the task used to probe the brain and the length of drug administration. Although effects of SSRIs on emotion-processing networks are under intense investigation, much remains unknown about how these substances work to normalize abnormal cognitive and emotional processes.

The insula has been suggested to play a key role in evaluating the impact that environmental stimuli may have on the interoceptive body state (Craig, 2003; Paulus & Stein, 2006). Activity in this region relates to anxiety during risk-taking decision-making (Paulus & Stein, 2006), and is associated with increased perfusion in patients with panic disorder (Malizia et al. 1998). Taken in combination, these studies suggest that altered insula activity may be a common denominator that could be used as a biomarker for treatment effects. The anterior subdivision of the insula has been highlighted as an important region for the integration of physiological and psychological self (Craig, 2002; Paulus & Stein, 2006), and is of particular importance in down-modulating the posterior insula (Augustine, 1996).

In the present study, activity in the posterior insula was reduced by the administration of escitalopram, suggesting that SSRIs may contribute to central reduction in physiological reactivity during emotional anticipation. Escitalopram did not affect processing within the anterior insula, which is important for the integration of cognitive, affective, and physiological processes (for review see Craig, 2002). In comparison, escitalopram attenuated the more posterior aspects of the insula, which are important for the physiological representation of potentially aversive emotional experiences. Therefore, escitalopram may have a more subtle ‘bottom-up’ effect, i.e. modulate the physiological associations of anticipatory stimuli, rather than a ‘top-down’ modulation, i.e. the cognitive attributes of anticipatory stimuli, which is consistent with models proposed by Mayberg and colleagues (Mayberg, 2002; Ressler & Mayberg, 2007). In fact, the model proposed by Mayberg focuses on the subgenual cingulate, directly inferior to the region found in the present study, as being a critical path for the treatment effects of SSRIs given the high serotonin density of this region (Goldapple et al. 2004; Kennedy et al. 2001; Mayberg, 2002; Meyer et al. 2001; Ressler & Mayberg, 2007).

Slight discrepancy in location of the effects of SSRI in the present study may be, in part, due to the selection of an anticipatory task to probe brain functioning. As a cautionary note, however, it is important to point out that activation differences between the anticipation and baseline condition in posterior insula regions were mostly negative. This may be due to uncorrected physiological effects such as breathing (Strigo et al. 2006) or, alternatively, there may be a dampening of somatic information during anticipation (Obrist et al. 1969). We also found a significant attenuation of the ventral ACC during anticipation of negative vs. positive visual stimuli. This region is often linked with self-focus and emotional evaluation (Amodio & Frith, 2006; Beauregard et al. 2001; Gusnard et al. 2001; Heatherton et al. 2006; Johnson et al. 2005; Keenan et al. 2000; Mars et al. 2005; Northoff & Berns, 2004; Wicker et al. 2003), as well as anticipatory processing (Bystritsky et al. 2001; Chua et al. 1999; Nitschke et al. 2006; Porro et al. 2002). Thus, the attenuating effects of escitalopram in this region may reflect decreases in self-focus during the anticipation of aversive stimuli or conversely allow increased focus on self during anticipation of a non-aversive stimuli. Similar but less pronounced deactivations exist in the posterior regions of the insula during placebo as well. Numerous studies have found an anticipatory activation in the dorsal ACC, particularly in contrast with uncued anticipatory phases (Berns, 2006a,b); considering the strong connections between the insula and dorsal ACC/MPFG (Dupont et al. 2003).
this relationship may play a part in top-down modulation of interoceptive processing.

Our findings are based on results from healthy volunteers who did not report significant subjective changes during subchronic escitalopram administration. Nevertheless, imaging of healthy volunteers is an important step in proof of concept in drug discovery (Wise & Tracey, 2006). In particular, the use of a relatively homogenous, healthy population may allow for the use of a smaller group to detect neural changes as a result of administration of a compound. It should be noted that this group may show brain changes despite the lack of behavioural changes (Wise & Tracey, 2006).

Although escitalopram is an approved, marketed drug, proving that subchronic doses of SSRIs act on specific neural pathways can provide biomarkers for efficacy in similar drugs entering phases I or II (i.e. safety/efficacy studies). In comparison, the advantage of using patient samples may be the ability to determine the relationship between the neural substrate effects of potential therapeutics and subjective or objective changes in mood state.

In terms of the mechanistic action of SSRIs, these findings suggest that direct or indirect serotonergic modulations of insula, among other regions, results in relative deactivation of affective neural substrates during anticipation. Specifically, the mechanism of action could be explained as a reduction of the affective/physiological reactivity to anticipation that may then result in decreased feelings of anxiety and/or depression. Reduction of interoceptive attention during negative anticipation may relate to diminished concern about the internal body state. Given the importance of somatic reactivity in both anxiety and anticipatory processing (Masaoka & Homma, 1997, 2001; Masaoka et al. 2007), this mechanism may be considered as a potentially effective way to modulate affect through particular pharmacological interventions. This model will help to explain why SSRIs are effective at mood modification in conditions of distress, such as anxiety. In the present study, this is mimicked when the individual is momentarily provoked by an affective anticipatory state that can induce psychological and physiological distress.

The present study has several limitations. First, this study was conducted with healthy female volunteers and, thus, generalizability to patient and male samples still need to be established. In particular, we did not observe changes in subjective ratings on scales measuring anxiety or depression. This range restriction in the emotional state of healthy volunteers may explain the lack of significant correlations between change in psychological measures and change in BOLD signal in functional ROIs during SSRI treatment. Because physiological reactivity to anticipatory anxiety is greater in anxious individuals (Masaoka & Homma, 1997; Masaoka et al. 2007), future studies with patients suffering from anxiety disorders may reveal amplified BOLD changes during SSRI treatment. While steps were taken to reduce the impact of these effects in the methods, it is possible that physiological changes may contribute to the pervasive BOLD signal reduction observed in the present study. Furthermore, the neural expression of escitalopram appears to be task dependent both in the mechanism and strength of the effect (Arce et al. 2007), suggesting caution regarding the generalizability of its effects. The small sample may limit the effects of randomization, while the main effects do not show significant differences when contrasted by order a larger sample may help further limit this effect in future studies. In the present study, we did not control for subjects’ menstrual cycle or familial mental health history. Future studies could benefit on determining the influence of these affects.

In summary, our results suggest that treatment with escitalopram results in attenuation of the neural response to affective anticipation in brain regions responsible for the integration of physiological and affective well-being. Specifically, subchronic SSRI treatment may reduce the anticipatory reactivity to emotional – including aversive – stimuli. We anticipate that through understanding the neurobiology of escitalopram in healthy controls we can both suggest a mechanism of action and provide a contrast for future studies that investigate pertinent clinical groups. These findings can have important implications for the development of pharmacological interventions to treat anxiety and mood disorders, suggesting that BOLD signal in the insula and ACC during anticipatory anxiety may be a useful biomarker for measuring psychopharmacological effects of extant and novel anxiolytic and antidepressant agents.

Note
Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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Statement of Interest
None.

References


Bagdy G, Graf M, Anheuer ZE, Modos EA, Kantor S (2001). Anxiety-like effects induced by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with the 5-HT2C receptor antagonist SB-242084 but not the 5-HT1A receptor antagonist WAY-100635. International Journal of Neuropsychopharmacology 4, 399–408.


somatosensory stimulation in the anterior cingulate cortex and parietal operculum/posterior insula: an event-related functional magnetic resonance imaging study. *Journal of Neuroscience* 20, 7438–7445.


