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Impact of 5-HTTLPR on SSRI Serotonin Transporter Blockade during Emotion Regulation: A Preliminary fMRI Study

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Abstract

Background: The short ('S') allele of the serotonin transporter (5-HTT)-linked polymorphic region (5-HTTLPR) is associated with increased negative emotion processing bias, and this polymorphism moderates acute effects of selective serotonin reuptake inhibitor (SSRI) treatment. Here In this preliminary study, we explore the moderating effect of 5-HTTLPR on the impact of the SSRI, escitalopram during emotion regulation of negative emotional stimuli.

Method: Thirty-six healthy Caucasian, female participants underwent two fMRI scanning sessions following single dose escitalopram and placebo administration separated by a seven-day washout period according to a double-blind, randomized, placebo-controlled crossover design. Functional connectivity analysis was employed with a left (L) amygdala seed and a right interior frontal gyrus (R IFG) target.

Results: Changes in functional connectivity with emotion regulation and treatment were linearly related to 5-HTTLPR 'L' allele load such that negative R IFG-L amygdala connectivity was increased with an increasing number of 'L' alleles. Therefore, escitalopram may facilitate the effects of reappraisal by enhancing negative functional connectivity, a finding that is greatest in participants homozygous for the 'L' allele and least in those homozygous for the 'S' allele.

Limitations: Sub-samples of the homozygote 'S/S' and 'L/L' 5-HTTLPR groupings were small. However, the within-subjects nature of the experiment and observing changes at the individual subject level increases our confidence in the findings of the present study.

Conclusions: The present study elucidates a potential neural mechanism by which antidepressant treatment produces differential treatment outcomes dependent on the 5-

HTTLPR polymorphism, providing new and important leads for models of antidepressant action.

Keywords: antidepressant; 5-HTTLPR; emotion; serotonin; pharmacogenetics; fMRI

1. Introduction

Affective disorders including major depressive disorder and generalized anxiety disorder are common disabling conditions, associated with a high degree of burden (World Health Organization, 2008). Key underlying cognitive deficits of these disorders are negative emotion processing bias—whereby reactivity to negative emotional stimuli is greater than that to positive stimuli—and dysfunctional emotion dysregulation are key (Beck, 2008; Beck et al., 1979; Gotlib and Joormann, 2010). Together, these deficits reduce inhibition of negatively-valenced emotional stimuli. Antidepressant medication has been shown to ameliorate these deficiencies by decreasing negative emotion processing bias (Harmer, 2012) and facilitating adaptive emotion regulation strategies, namely reappraisal (McRae et al., 2014), which is an emotion regulation strategy that involves reframing of a situation or stimulus to decrease resultant emotional reactivity (Gross, 1998; Gross and Thompson, 2007). However, the variability of therapeutic response with antidepressant treatment is high, with 50% to 70% of patients not responding to first-line treatment (Kemp et al., 2015; 2008; Trivedi, 2006). Previous research on genetic variation at the serotonin transporter-linked promoter region (5-HTTLPR) explains variation in amygdala activity changes associated with decreased negative emotion processing bias with a single dose of the commonly prescribed SSRI escitalopram (Outhred et al., 2014a) and citalopram (Ma et al., 2015). We have also observed that a single dose of escitalopram facilitates the activity of a key neural pathway involved in reappraisal (Outhred et al., 2015). In the present paper, we investigate whether facilitation of this reappraisal neural pathway with escitalopram is modulated by 5-HTTLPR variation. In doing so, the present paper will contribute to understanding the association between 5-HTTLPR and SSRI response and remission

rates (Huezo-Diaz et al., 2009; Kato and Serretti, 2010; Licinio and Wong, 2011; Serretti et al., 2007; Smeraldi et al., 1998).

The serotonin transporter protein (5-HTT) is the key target of commonly prescribed selective serotonin reuptake inhibitors (SSRIs; Klein et al., 2006). 5-HTT is encoded by the gene *SLC6A4*, the expression of which is modulated by a polymorphism in the promoter region, termed 5-HTTLPR (Del-Ben et al., 2005; Heils et al., 1996; Lesch et al., 1996). The short ('S') allele of the 5-HTTLPR is associated with lower transcriptional efficiency of 5-HTT as compared to the long ('L') allele, leading to dysfunctional regulation of 5-HT (Canli and Lesch, 2007; Heils et al., 1996; Lesch et al., 1996; Lesch and Gutknecht, 2005; Smeraldi et al., 1998). Previous research has shown that, in comparison to 'L' allele carriers, 'S/S' homozygotes display lower remission and response rates when treated with SSRIs (Kato and Serretti, 2010; Licinio and Wong, 2011; Serretti et al., 2007).

Although specific cellular mechanisms remain to be illustrated, it is known the 'S' allele is associated with chronic dysregulation of 5-HT with depletion occurring with little reuptake after release as well as 5-HT_{1A} autoreceptor over-sensitivity resulting in little release overtime (Hariri et al., 2005; Risch et al., 2009). Hence, SSRI treatment at the acute stage leads to further dysregulation, reducing 5-HT availability in the synapse (Ruhé et al., 2009). This may be due to reduced maintenance of 5-HT pools in presynaptic neurons for subsequent release as 'S' allele has half the 5-HT reuptake (Lesch et al., 1996), which may then increase 5-HT_{1A} autoreceptor sensitivity (Smeraldi et al., 1998) leading to decreased 5-HT availability in the synapse (Canli and Lesch, 2007; Lesch and Gutknecht, 2005). One potential mechanism is that the acute action of SSRIs in 'S' carriers may further decrease 5-HT availability in the synapse under 5-

HTT blockade through increased negative feedback from increased 5-HT_{1A} autoreceptor sensitivity when 5-HT remains in the synapse after release (Celada et al., 2013). An alternative mechanism is that the low 5-HT pools in 'S' carriers become more exhausted with decreased reuptake with SSRI blockade, leading to further decreases in 5-HT availability in the synapse (Ruhé et al., 2009). Regardless, 5-HTT blockade with SSRIs in 'S' carriers is associated with worsening treatment outcome due to increased 5-HT dysregulation (Ruhé et al., 2009).

Our previous work demonstrated that single-dose SSRI treatment modulates neural activity by supressing negative emotion processing biases (Kemp et al., 2004a; Outhred et al., 2014b; 2013), and that variability in this effect is explained by 5-HTTLPR allelic variation in a dose-response manner (Outhred et al., 2014a). In this study, we found that 'S/S' homozygote neural responses to affective pictorial stimuli were associated with increased negative emotion bias and 'L/L' homozygote neural responses were associated with decreased negative emotion bias with SSRI treatment (Outhred et al., 2014a). This finding has been independently corroborated with experiments employing the SSRI citalopram and affective facial stimuli (Ma et al., 2015). In the context of prior research (Kato and Serretti, 2010; Licinio and Wong, 2011; Serretti et al., 2007), these findings may have important implications for understanding the variation in response rates explained, at least in part, by the impact of 5-HTTLPR on acute treatment effects on emotion processing and emotion regulation. Hariri and Holmes (2006) implicate 5-HTTLPR in the integrity of the functional pathway between the prefrontal cortex and the amygdala, such that 'S' allele carriers are characterised by diminished inhibitory emotion regulation feedback. Negative functional connectivity between right inferior frontal gyrus (R IFG) and left (L)

amygdala during reappraisal reflects regulation of neural responses to negative stimuli (Aron et al., 2004; Banks et al., 2007; Goldin et al., 2008; Ochsner and Gross, 2005). Heinz and colleagues (2005) found positive amygdala and prefrontal coupling during negative images in 'S' allele carriers than 'L/L' homozygotes, with no association observed for positive images. These authors suggested that the 'S' allele is associated with increased risk of psychopathology due to dysfunctional amygdala-prefrontal connectivity within emotion regulation pathways during the processing of negative stimuli, increasing the saliency of the stimuli (Heinz et al., 2005). More recently, we observed that a single dose of escitalopram is associated with negative L amygdala-R IFG coupling during reappraisal of negative stimuli, suggesting facilitation of emotion regulation within this pathway (Outhred et al., 2015). However, it remains unclear whether allelic variation in 5-HTTLPR moderates this effect.

Building on our previous findings (Outhred et al., 2014a) and the 5-HTT literature showing that 'L' carriers have better treatment outcomes than 'S' carriers both at the cellular and the behavioral levels, we predicted a linear relationship (dose-response) between 5-HTTLPR and L amygdala-R IFG functional connectivity during the reappraisal of negative images after single dose escitalopram relative to placebo. Given that SSRI treatment is associated with increased dysregulation in 'S/S' allele homozygotes (Outhred et al., 2014a), we hypothesise that 'S/S' allele carriers will display more positive L amygdala-R IFG coupling than 'L/L' homozygotes during reappraisal of negative stimuli under escitalopram.

2. Methods

2.1 Participants

Thirty-six right-handed healthy Caucasian female participants (mean age = 25.08; SD = 6.49; range 18-47) were recruited and completed a trial, as previously reported (Outhred et al., 2015; 2014b; 2014a). The present study interrogates the genetic and fMRI findings on an emotion regulation task we employed (an event-related design with reappraisal of negative images). Our previous paper (Outhred et al., 2015) reports on the basic emotion regulation effects and our other papers (Outhred et al., 2014b; 2014a) reported on fMRI findings from a basic emotion processing task (a blocked design viewing negative, positive, and neutral images). Hence, the genetic effects on the emotion regulation task results have not been previously reported. All participants provided informed consent in accordance with the Australian National Health and Medical Research Council (NHMRC) guidelines. The Sydney University Human Research Ethics Committee (13901) and the Northern Sydney Central Coast Area Health Service Human Research Ethics Committee (1105-178M) granted ethical approval for this study. This trial was also registered with the Australian New Zealand Clinical Trials Registry (ANZCTR, available here: http://www.anzctr.org.au; ACTRN12611000719932). Participants were determined to be free from medication (other than hormonal contraceptives), physical and psychiatric illness, major depressive disorder (PHQ-9 assessment; Kroenke et al., 2001) or generalized anxiety disorder symptoms (GAD-7 assessment; Spitzer et al., 2006). Additionally, participants were free from illicit drug use and heavy alcohol use (abstaining for at least 24 hours), smoking, brain injury, neurological disorders, loss of consciousness for longer than five minutes, and contraindications for fMRI scanning. Finally, participants abstained from

caffeine on the morning of the experiment and no participant tested positive on pregnancy tests conducted at each session. See Table 1 for a breakdown of participant demographics by 5-HTTLPR grouping with statistical tests showing no between group differences.

2.2 Genotyping

DNA was extracted from saliva samples and 5-HTTLPR and rs25531 genotypes were determined, given the differential impact of the La and Lg genotypes (Dannlowski et al., 2007; Hu et al., 2005; Kato et al., 2013). The Lg variant of rs25531 and the 'S' 5-HTTLPR allele are similar in function; thus, the Lg variant is considered low expressing (Hu et al., 2005). 5-HTTLPR and rs25531 were determined according to protocols described previously (Bryant et al., 2010; Quinn et al., 2012). Genotypes were scored independently by two researchers. See the Supplement for 5-HTTLPR and rs25531 grouping number breakdowns. To summarize, the functional 5-HTTLPR genotypes (taking into account rs225531) were categorized as 'S/S' (n = 8; 22%), 'S/L' (n = 21; 58%), and 'L/L' (n = 7; 19%), and were found to be in Hardy-Weinberg equilibrium, χ^2 (1) = 1.011, p = 0.315.

2.3 Experimental and Emotion Regulation Task design

All participants were tested under placebo (saccharin) and escitalopram (20 mg; *per os*) conditions using a randomized, double-blind, placebo controlled cross-over design, with a washout period of one week (or five half-lives t1/2 = 26.7 hours; Alphapharm, 2012; Sogaard et al., 2005). A crossover design and checks for correct experimental manipulation (instead of a mixed-models approach) were used, as previously recommended and discussed (Mills et al., 2009; Senn et al., 2004). An equal number of participants had either treatment in their first testing session. fMRI during an

emotion regulation task was conducted four hours post-treatment to coincide with expected peak pharmacokinetic effects of escitalopram (mean tmax = 4.0 hours, tmax = 3.0 ± 1.5 hours; Alphapharm, 2012; Sogaard et al., 2005). An event-related emotion regulation task was constructed with instruction prompts ('Think Objectively'; 'Watch'), high-arousal negative valance pictures, low-arousal neutral valance pictures, a negative valence rating scale prompt, an arousal rating scale prompt, and fixation crosses (see Supplement). This task was based on that used by Goldin and colleagues (Goldin et al., 2008). Stimuli were selected from the International Affective Picture System (Lang et al., 2008), based on the normed valence and arousal ratings that are provided in the IAPS manual. The task consisted of trials with a 2-second instruction (either 'Think Objectively' or 'Watch') followed by a 4-second high arousal negative IAPS image, a 2-second negative valence rating, a 2-second arousal rating, a 2-second 'Watch' instruction, a 4-second low arousal neutral IAPS image, and a jittered fixation cross (average duration of 4 seconds; see Supplement for further details on the task administered to participants). The valence and arousal rating scales were on a five-point scale from "0. 'not at all negative/arousing'" to "4.'overwhelmingly negative/arousing'". During the 'Think Objectively' trials, participants were asked to assume the perspective of a medical professional watching an instructional video, focusing on technical aspects of the film, so as to decrease emotional reactivity as per previously published study (Goldin et al., 2008). During the 'Watch' trials, participants were asked to view the negative image and to 'feel' the emotions associated with each of the pictures. Attention to experiencing the emotional stimuli applies to both the 'Watch' and 'Think Objectively' conditions. In the 'Think Objectively' condition, however, participants are asked to reappraise the stimuli in order to decrease emotional

reactivity. Therefore, the contrast between the two conditions allows for neural changes associated with thinking objectively about the stimuli to be determined, by partialling out the attention to the experience component. Analysis of the behavioral valence and arousal ratings (see Supplement) demonstrated that the 'Think Objectively' condition successfully reduced emotional reactivity to the stimuli relative to the 'Watch' condition, providing an important validation of the task on which neural changes were determined.

A variety of hormonal, behavioral, and neurophysiological manipulation checks were performed. Analyses revealed that our results reported below were not confounded by side effects or menstrual phase (see Supplement).

2.4 fMRI data acquisition

Using a 3.0 T Siemens Trio scanner, imaging was performed at Advanced Research and Clinical Highfield Imaging (ARCHI, the University of Sydney; a dedicated research facility). Twenty-nine consecutive axial slices (4 mm thickness with 1 mm gap) parallel to the anterior–posterior commissure covering the whole brain were imaged using a T2*-weighted gradient echo EPI sequence (echo time [TE] = 32 ms; repetition time [TR] = 2000 ms; matrix = 64 × 64; flip angle = 70°). The field of view was 240 mm and the effective in plane functional spatial resolution was 3.75 mm. For each functional run, 305 volumes were collected, after which the first five were discarded to allow for magnetic saturation effects. Participant movement was minimised by securing the head within the scanner coil using foam padding.

2.5 fMRI data analyses

The imaging data from the two treatment sessions were inspected, pre-processed and analyzed using standard image processing routines implemented within the

statistical parametric mapping software package, SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/; Wellcome Trust Centre for Neuroimaging). Images for each subject were realigned (and unwarped) and spatially normalized into a standard stereotactic space (Montreal Neurologic Institute template) and smoothed with a Gaussian kernel (FWHM 8 mm) in order to minimize anatomical differences, and slice timing correction was performed. Realignment parameters were inspected and subjects had movement less than the size of a voxel of 3.75 mm, thus no data was considered to be problematic. The BOLD response at each voxel was modelled with a canonical hemodynamic response function and its temporal derivative, with the events reappraise negative and watch negative events modelled across each session and all other trials events modelled as baseline, along with realignment regressors of no interest. The default high-pass filter of 128 seconds was applied and did not cut off experimental variance. Generalized Psychophysiological Interaction analysis (gPPI; McLaren et al., 2012) was conducted to determine experimental condition dependent functional connectivity. Following gPPI routines, the deconvolved time series from a functionally derived L amygdala seed region (drawn as a sphere 6 mm in radius at previously identified coordinates [-20, -4, -20]; Outhred et al., 2015) was extracted to create the physiological variable for each participant. Though it would have been possible to examine the right amygdala in another analysis, this would be based on the assumption that the activation is the same on the right side. To avoid relying on this assumption and creating further multiple comparisons issues, we restricted our analysis to an ROI guided by our previous findings with the left amygdala (Outhred et al., 2015; 2014b). Though we did not intend to test a laterality hypothesis, prior studies show a consistent pattern of left amygdala lateralization in emotional processing (Baas et al.,

2004). Selection of the L amygdala, over the R IFG, as a seed region is consistent with previous research (Banks et al., 2007; Foland et al., 2008; Kanske et al., 2011; Payer et al., 2012; Townsend et al., 2013). Furthermore, the L amygdala-R IFG functional connectivity estimate has shown robust modulation with reappraisal (Aron et al., 2004; Buhle et al., 2014; Goldin et al., 2008; Ochsner and Gross, 2005; Ochsner et al., 2012) and treatment administration (Kemp et al., 2004a; Outhred et al., 2015; 2014b; 2014a; 2013). The condition onset times for the 'reappraise negative' and 'watch negative' events under each treatment session were each convolved with the canonical hemodynamic response function, creating two task regressors for each treatment session. The physiological variable and the 'reappraise negative' (and 'watch negative' task) regressors were then multiplied to obtain the 'reappraise negative' (and 'watch negative') PPIs. These two PPIs were contrasted each participant, with the resultant contrast representing reappraisal-associated modulation of functional connectivity. Thus each participant had contrasts for reappraisal-associated modulation of functional connectivity for each treatment session. In order to extract estimates of the direction and magnitude of functional connectivity, beta coupling estimates for participants' reappraisal PPI contrasts for each treatment session were extracted from the R IFG region using the MarsBaR toolbox (Brett et al., 2002) for the following analyses. For reference, results of the previously reported regions of interest analyses (an a priori R IFG analysis, and a wider frontal region analysis for illustration of connectivity with other regulatory regions) and the functional connectivity (gPPI) analysis are provided in the Supplement, and are reported and discussed in detail in our previous work (Outhred et al., 2015). Hence, we extended these analyses in the present study to determine the between-subjects effects of 5-HTTLPR allele loading on the effect of escitalopram on

reappraisal using the previously determined coupling estimates. For illustrative purposes only, averaged beta coupling estimates for each treatment session are presented in Figure 1C, alongside those split and averaged for each 5-HTTLPR group. In this context, negative functional connectivity indicates that decreases in L amygdala activity are correlated with (not caused by) increases in R IFG activity under reappraisal (a relationship that is associated with effective emotion regulation during reappraisal), where as positive functional connectivity indicates increases in L amygdala activity are correlated with increases in R IFG (a relationship that is associated with ineffective emotion regulation during reappraisal).

For the present study, the PPI beta coupling estimates from each participant at the escitalopram session was subtracted from those of the placebo session. The resultant values represented the within-subjects relative change in L amygdala-R IFG functional connectivity with escitalopram treatment during reappraisal relative to placebo, with positive beta coupling change values representing increased connectivity with treatment relative to placebo. As 5-HTTLPR allele loading prediction is consistent with a dosage model of 5-HTT expression (Caspi et al., 2003; Freidlin et al., 2002), it is a common data analysis strategy (Hariri et al., 2005; Outhred et al., 2014a; Risch et al., 2009). Consequently, linear regressions were performed in IBM SPSS 21 for OSX with 5-HTTLPR ‘L’ allele loading as a predictor variable and the beta coupling change values as a dependent variable. For the purposes of this study, 5-HTTLPR allele loading prediction was performed in order to illustrate and determine the extent of the effect of 5-HTTLPR allele loading has on modulation of functional connectivity between the L amygdala-R IFG functional connectivity during reappraisal with escitalopram. For verification purposes, this linear regression analysis was supported on a background of a

significant analysis of variance (ANOVA) testing for 5-HTTLPR group differences on the PPI beta coupling change values, $F(2, 33) = 12.792$, $p < 0.001$, partial $\eta^2 = 0.437$. Significant behavioral analysis on modulation functional connectivity during reappraisal with escitalopram was previously reported, but was not significant for 5-HTTLPR group differences. In order to illustrate that the regression models are consistent with the aforementioned 5-HTT expression dose-response model, Cook's Distances (Cook and Weisberg, 1982) were calculated for each data point. The Cook's Distance values were checked for values greater than one, which would indicate data points that have a specific influence on (or drove the significance of) the determined regression slope. In order to determine whether any 5-HTTLPR group had a specific influence on—or had a significantly greater loading, thereby driving significance of—the determined regression slopes, one-way ANOVAs were performed on the Cook's Distance values from each regression slope and checked for significant differences between 5-HTTLPR groups.

3. Results

3.1 fMRI results: 5-HTTLPR on functional connectivity (gPPI) with reappraisal for escitalopram and placebo sessions

Functional connectivity (gPPI) results are displayed in Figure 1. For illustrative purposes only, the average gPPI beta coupling estimates between the L amygdala (Figure 1A) and R IFG (Figure 1B) for the escitalopram and placebo sessions for the whole sample ($N = 36$; as previously reported) are shown along side those across each 5-HTTLPR group ('S/S', 'S/L', 'L/L'; Figure 1C for the present report). For the whole sample, negative functional connectivity (decreased L amygdala activity paired with increased R IFG activity) was heightened with escitalopram, relative to placebo. Descriptively, differential responses were observed when the sample was split by 5-

HTTLPR groups ('S/S', 'S/L', 'L/L'; see Figure 1C). In the 'S/S' group, functional connectivity was positive under placebo and this positive connectivity was heightened with escitalopram administration. The 'S/L' group displayed positive functional connectivity under placebo and showed negative functional connectivity with escitalopram. Finally, the 'L/L' group showed negative functional connectivity under placebo, which was heightened with escitalopram.

3.2 fMRI results: 5-HTTLPR on functional connectivity (gPPI) during reappraisal with escitalopram

The linear regression on the gPPI beta coupling change values with 5-HTTLPR grouping as the predictor was significant, $r^2 = 0.324$, adjusted $r^2 = 0.304$, $F(1, 34) = 16.277$, $p < 0.001$. Descriptively, this observation suggests that under escitalopram, an increasing number of 'L' alleles is associated with increasing negative L amygdala-R IFG functional connectivity during reappraisal. In turn, this finding suggests that escitalopram facilitates the effects of 'L' alleles by enhancing negative functional connectivity during reappraisal. See Figure 1D for a visualisation of this result. Consistent with a dose-response model, no data point was significantly influential (all Cook's Distances < 1) and no 5-HTTLPR group was specifically influential on the determined regression slope ($F[2, 33] = 1.615$, $p = 0.214$).

4. Discussion

The present study examined the impact of 5-HTTLPR on neural responses during reappraisal of highly arousing negative pictures after a single dose of escitalopram. Consistent with predictions, 5-HTTLPR allelic variation accounted for significant variance (adjusted $r^2 = 0.304$) in the functional connectivity between the L amygdala and the R IFG during reappraisal under escitalopram. Specifically, with

increasing number of ‘L’ alleles, the negative functional connectivity between these regions was increased. With escitalopram treatment, the functional connectivity in the ‘S/S’ group was more positive, the ‘S/L’ group was more negative, and the ‘L/L’ group was more negative further still (as illustrated in Figure 1D). Though preliminary, these findings suggest that 5-HTTLPR may influence the acute effects of an SSRI through facilitation of the functional connections between the L amygdala and R IFG during reappraisal. Such responses may underlie changes in emotional bias and have consequences for therapeutic effects (see Figure 2).

Building on our earlier pharmaco-fMRI findings (Outhred et al., 2015; 2014b; 2013) and previous pharmacogenetic-fMRI study (Ma et al., 2015; Outhred et al., 2014a), the present pharmacogenetic-fMRI study found that ‘L’ alleles were associated with a negative L amygdala-R IFG functional connectivity during reappraisal with escitalopram, which may reflect regulation of amygdala responses during processing of negative stimuli (Aron et al., 2004; Banks et al., 2007; Goldin et al., 2008; Ochsner and Gross, 2005). These observations suggest that, in those with more ‘L’ alleles, a single dose of a commonly prescribed SSRI facilitates a positive information bias through emotion regulation circuitry, consistent with cognitive neuropsychological models of antidepressant action (Harmer et al., 2009; Outhred et al., 2014b; 2014a; 2013; Pringle et al., 2011; Roiser et al., 2012). Additionally, in probing the serotonergic system with an acute dose of an SSRI, we provide *in vivo* support for Hariri and Holmes’s (2006) model of 5-HTTLPR and emotion regulation circuitry in that 5-HTTLPR accounted for variation in circuitry response to 5-HT augmentation. Acute improvement in cognitive neuropsychological processes with antidepressant treatment may form the basis for downstream changes and symptom amelioration with clinical therapeutic administration

seen not only until after weeks of treatment (Harmer et al., 2009; Pringle et al., 2011; Roiser et al., 2012). Though acute SSRI administration has measurable neural effects, the manner in which these translate to clinical benefits is yet to be fully elucidated; however, it is speculated that facilitation and use of more adaptive emotional regulation strategies within the environment, along with reducing negative emotion processing bias, leads to improved mood symptoms overtime, inline with cognitive models of affective disorder (Beck, 2008; Beck et al., 1979; Gotlib and Joormann, 2010). The present findings suggest emotion regulation-related neural processing may improve with more 'L' alleles; thus providing a potential, at least partial, explanation for the manner in which more 'L' alleles predicts improved response to, and remission with antidepressant treatment (Kato and Serretti, 2010; Licinio and Wong, 2011; Ruhé et al., 2009; Serretti et al., 2007). Hence, future research should examine whether differential acute effects of antidepressants on emotion regulation associated with 5-HTTLPR variation are predictive of longitudinal therapeutic changes with chronic treatment in patients with affective disorders. Given that 5-HTT is a target of antidepressants at which initial changes occur, 5-HTTLPR is a likely candidate for explaining variation in treatment outcomes (Licinio and Wong, 2011). However, 5-HTTLPR alone is unlikely to predict the downstream changes that occur with chronic, therapeutic administration. Additional candidate genes accounting for downstream changes include those involved in the expression of neurotropic factors such as brain derived neurotrophic factor (BDNF) Val66Met (Chen et al., 2011).

Based on the present study's findings within the context of the aforementioned literature, a framework was developed for understanding the potential impact of 5-HTTLPR on acute SSRI administration on differential change in emotional biases

though emotion regulation circuitry, having consequences for downstream changes and symptom amelioration over weeks of treatment (see Figure 2). Building on previous models (Harmer et al., 2009; Outhred et al., 2014b; 2014a; 2013; Pringle et al., 2011; Roiser et al., 2012), a potential mechanism is proposed as follows. 5-HTT expression plays a role in the manner in which increases in 5-HT with SSRI 5-HTT blockade are regulated, though the more subordinate cellular mechanism is undetermined (Ruhé et al., 2009). Decreased 5-HT levels in 'S' carriers with acute SSRI treatment in contrast to increased 5-HT levels in 'L' carriers are hypothesized to be related to differential 5-HT_{1A} autoreceptor sensitivity or 5-HT pooling, or both (Ruhé et al., 2009). Regardless, individuals with the low expressing 'S' allele have increased dysregulation of 5-HT with treatment leading to L amygdala-R IFG functional connectivity modulation consistent with an increased negative emotion bias. In contrast, those with the high expressing 'L' allele have increased regulation of 5-HT with treatment, leading to connectivity modulation consistent with an increased positive emotion bias. This mechanism may account for variation in downstream changes, symptom amelioration, and thus therapeutic treatment responses by 5-HTTLPR variation. In sum, the facilitation of functional connectivity between the L amygdala and R IFG—as an emotion regulation circuit—may be a key target of SSRIs, and differential modulation of this emotion regulation circuit with SSRIs may explain differential therapeutic outcomes between 5-HTTLPR groups.

4.1 Limitations

With the preliminary nature of the present study, a limitation was the small sub-samples of the homozygote 'S/S' and 'L/L' 5-HTTLPR groupings. However, the within-subjects nature of the experiment—with both treatment and task conditions—and

observing the within-subjects changes at the individual subject level increases our confidence in the findings of the present study. A second possible limitation was that a female-only sample was employed, findings, therefore, may not generalize to the male population. While this can also be considered a strength, as gender differences in neurophysiological responses to affective stimuli (Kemp et al., 2004b; Kret and De Gelder, 2012) and antidepressant administration (Khan et al., 2005; Young et al., 2009) are widely reported, future studies need to investigate whether similar effects are observed in males. Nevertheless, the employment of a homogenous, well-characterized sample within-subjects design and extensive manipulation checks (see Supplement), along with, increase our confidence in the observed findings. Examining responses within healthy and homogenous samples enables a high degree of control, and these findings will inform future studies in clinical populations, with both acute and long-term treatment. While the gPPI analysis provided important insights into functional connectivity modulation, causal inferences cannot be made and modulations maybe mediated by other regions or pathways. While 5-HTTLPR accounts for expression in the 5-HTT SSRI target, other variation on other genes are likely to account for responses, particularly with longer-term treatment and downstream changes.

4.2 Conclusion

We believe this is the first study to demonstrate a pharmacogenetic effect within the brain's emotion regulation circuitry following a single dose of the commonly prescribed SSRI escitalopram. Variation in acute neural changes with SSRI treatment facilitates reappraisal of negative stimuli, an effect that may, in part, be explained by 5-HTTLPR. Specifically, the *'S' allele* was related to modulation of L amygdala-R IFG activity consistent with emotion dysregulation, while the '*L' allele* related to L

amygdala-R IFG activity consistent with improved emotion regulation. Though preliminary, these findings are likely to extend the understanding of the pharmacogenetics of acute SSRI treatment in that a foundation on which future research in clinical samples could be based was provided. Extrapolation of this work into the clinical arena and further study of the pharmacogenetics of antidepressant treatment at acute and chronic stages in patient samples will provide important leads towards personalized medicine in affective disorders.

Supplementary materials

Supplementary data to this article can be found online at <url>

References

Alphapharm, 2012. Loxalate: Product Information. Alphapharm, Sydney, Australia.

Aron, A.R., Robbins, T.W., Poldrack, R.A., 2004. Inhibition and the right inferior frontal cortex. Trends Cogn Sci 8, 170–177. doi:10.1016/j.tics.2004.02.010

Baas, D., Aleman, A., Kahn, R.S., 2004. Lateralization of amygdala activation: a systematic review of functional neuroimaging studies. Brain Research Reviews 45, 96–103. doi:10.1016/j.brainresrev.2004.02.004

Banks, S.J., Eddy, K.T., Angstadt, M., Nathan, P.J., Phan, K.L., 2007. Amygdala frontal connectivity during emotion regulation. Soc Cogn Affect Neurosci 2, 303–312. doi:10.1093/scan/nsm029

Beck, A.T., 2008. The evolution of the cognitive model of depression and its neurobiological correlates. Am J Psychiatry 165, 969–977. doi:10.1176/appi.ajp.2008.08050721

Beck, A.T., Rush, A.J., Shaw, B.F., Emery, G., 1979. Cognitive therapy of depression. Guilford Press, New York.

Brett, M., Anton, J.-L., Valabregue, R., Poline, J.-B., 2002. Region of interest analysis using an SPM toolbox, in:. Presented at the 8th International Conference on Functional Mapping of the Human Brain, Sendai, Japan, pp. Supp. 1 xxxvi–lxvi. doi:10.1016/S1053-8119(02)90056-X

Bryant, R.A., Felmingham, K.L., Falconer, E.M., Pe Benito, L., Dobson-Stone, C., Pierce, K.D., Schofield, P.R., 2010. Preliminary evidence of the short allele of the serotonin transporter gene predicting poor response to cognitive behavior therapy in posttraumatic stress disorder. Biological Psychiatry 67, 1217–1219. doi:10.1016/j.biopsych.2010.03.016

Buhle, J.T., Silvers, J.A., Wager, T.D., Lopez, R., Onyemekwu, C., Kober, H., Weber, J., Ochsner, K.N., 2014. Cognitive Reappraisal of Emotion: A Meta-Analysis of Human Neuroimaging Studies. Cerebral Cortex 24, 2981–2990. doi:10.1093/cercor/bht154

Canli, T., Lesch, K.P., 2007. Long story short: the serotonin transporter in emotion regulation and social cognition. Nat Neurosci 10, 1103–1109. doi:10.1038/nn1964

Caspi, A., Sugden, K., Moffitt, T., Taylor, A., Craig, I., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene. Science 301, 386–389. doi:10.1126/science.1083968

Celada, P., Bortolozzi, A., Artigas, F., 2013. Serotonin 5-HT1A Receptors as Targets for Agents to Treat Psychiatric Disorders: Rationale and Current Status of Research. CNS Drugs 27, 703–716. doi:10.1007/s40263-013-0071-0

Chen, E.S., Ernst, C., Turecki, G., 2011. The epigenetic effects of antidepressant treatment on human prefrontal cortex BDNF expression. Int J Neuropsychopharmacol 14, 427–429. doi:10.1017/S1461145710001422

Cook, R.D., Weisberg, S., 1982. Residuals and Influence in Regression. Chapman & Hall, New York.

Dannlowski, U., Ohrmann, P., Bauer, J., Kugel, H., Baune, B.T., Hohoff, C., Kersting, A., Arolt, V., Heindel, W., Deckert, J., Suslow, T., 2007. Serotonergic genes modulate amygdala activity in major depression. Genes Brain Behav. 6, 672–676. doi:10.1111/j.1601-183X.2006.00297.x

Del-Ben, C.M., Deakin, J.F.W., McKie, S., Delvai, N.A., Williams, S.R., Elliott, R.,

Dolan, M., Anderson, I.M., 2005. The effect of citalopram pretreatment on neuronal responses to neuropsychological tasks in normal volunteers: an FMRI study. Neuropsychopharmacology 30, 1724–1734. doi:10.1038/sj.npp.1300728

Foland, L.C., Altshuler, L.L., Bookheimer, S.Y., Eisenberger, N., Townsend, J., Thompson, P.M., 2008. Evidence for deficient modulation of amygdala response by prefrontal cortex in bipolar mania. Psychiatry Research: Neuroimaging 162, 27–37. doi:10.1016/j.pscychresns.2007.04.007

Freidlin, B., Zheng, G., Li, Z., Gastwirth, J.L., 2002. Trend tests for case-control studies of genetic markers: power, sample size and robustness. Hum. Hered. 53, 146–152.

Goldin, P.R., McRae, K., Ramel, W., Gross, J.J., 2008. The Neural Bases of Emotion Regulation: Reappraisal and Suppression of Negative Emotion. Biological Psychiatry 63, 577–586. doi:10.1016/j.biopsych.2007.05.031

Gotlib, I.H., Joormann, J., 2010. Cognition and depression: current status and future directions. Annu. Rev. Clin. Psychol. 6, 285–312. doi:10.1146/annurev.clinpsy.121208.131305

Gross, J.J., 1998. Antecedent-and response-focused emotion regulation: Divergent consequences for experience, expression, and physiology. J Pers Soc Psychol 74, 224–237. doi:10.1037/0022-3514.74.1.224

Gross, J.J., Thompson, R.A., 2007. Emotion regulation: Conceptual foundations, in: Gross, J.J. (Ed.), Handbook of Emotion Regulation, Handbook of Emotion Regulation. The Guilford Press, New York, pp. 3–24.

Hariri, A.R., Drabant, E.M., Munoz, K.E., Kolachana, B.S., Mattay, V.S., Egan, M.F., Weinberger, D.R., 2005. A susceptibility gene for affective disorders and the response of the human amygdala. Arch Gen Psychiatry 62, 146–152. doi:10.1001/archpsyc.62.2.146

Hariri, A.R., Holmes, A., 2006. Genetics of emotional regulation: the role of the serotonin transporter in neural function. Trends Cogn Sci 10, 182–191. doi:10.1016/j.tics.2006.02.011

Harmer, C.J., 2012. Emotional Processing and Antidepressant Action. Current Topics in Behavioral Neurosciences 14, 209–222. doi:10.1007/7854_2012_210

Harmer, C.J., Goodwin, G.M., Cowen, P.J., 2009. Why do antidepressants take so long to work? A cognitive neuropsychological model of antidepressant drug action. Br J Psychiatry 195, 102–108. doi:10.1192/bjp.bp.108.051193

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., Lesch, K.P., 1996. Allelic variation of human serotonin transporter gene expression. J. Neurochem. 66, 2621–2624.

Heinz, A., Braus, D.F., Smolka, M.N., Wrase, J., Puls, I., Hermann, D., Klein, S., Grüsser, S.M., Flor, H., Schumann, G., Mann, K., Büchel, C., 2005. Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. Nat Neurosci 8, 20–21. doi:10.1038/nn1366

Hu, X., Oroszi, G., Chun, J., Smith, T.L., Goldman, D., Schuckit, M.A., 2005. An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. Alcohol. Clin. Exp. Res. 29, 8–16.

Huezo-Diaz, P., Uher, R., Smith, R., Rietschel, M., Henigsberg, N., Marušič, A., Mors, O., Maier, W., Hauser, J., Souery, D., Placentino, A., Zobel, A., Larsen, E.R., Czerski, P.M., Gupta, B., Hoda, F., Perroud, N., Farmer, A., Craig, I., Aitchison, K.J., McGuffin, P., 2009. Moderation of antidepressant response by the serotonin transporter gene. Br J Psychiatry 195, 30–38.

Kanske, P., Heissler, J., Schönfelder, S., Bongers, A., Wessa, M., 2011. How to regulate emotion? Neural networks for reappraisal and distraction. Cerebral Cortex 21, 1379–1388. doi:10.1093/cercor/bhq216

Kato, M., Nonen, S., Serretti, A., Tetsuo, S., Takekita, Y., Azuma, J., Kinoshita, T., 2013. 5-HTTLPR rs25531A > G Differentially Influence Paroxetine and Fluvoxamine Antidepressant Efficacy. J Clin Psychopharmacol 33, 131–132. doi:10.1097/01.jcp.0000426182.66701.76

Kato, M., Serretti, A., 2010. Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder. Mol Psychiatry 15, 473–500. doi:10.1038/mp.2008.116

Kemp, A.H., Brunoni, A.R., Machado-Vieira, R., 2015. Predictors of treatment response in major depressive disorder, in: Carvalho, A.F., McIntyre, R.S. (Eds.), Treatment-Resistant Mood Disorders. Oxford University Press, Oxford, pp. 53–60. doi:10.1093/med/9780198707998.003.0005

Kemp, A.H., Gordon, E., Rush, A.J., Williams, L.M., 2008. Improving the prediction of treatment response in depression: integration of clinical, cognitive, psychophysiological, neuroimaging, and genetic measures. CNS Spectrums 13, 1066–86; quiz 1087–8.

Kemp, A.H., Gray, M.A., Silberstein, R.B., Armstrong, S.M., Nathan, P.J., 2004a. Augmentation of serotonin enhances pleasant and suppresses unpleasant cortical electrophysiological responses to visual emotional stimuli in humans. NeuroImage 22, 1084–1096. doi:10.1016/j.neuroimage.2004.03.022

Kemp, A.H., Silberstein, R.B., Armstrong, S.M., Nathan, P.J., 2004b. Gender differences in the cortical electrophysiological processing of visual emotional stimuli. NeuroImage 21, 632–646. doi:10.1016/j.neuroimage.2003.09.055

Khan, A., Brodhead, A.E., Schwartz, K.A., Kolts, R.L., Brown, W.A., 2005. Sex differences in antidepressant response in recent antidepressant clinical trials. J Clin Psychopharmacol 25, 318–324.

Klein, N., Sacher, J., Geiss-Granadia, T., Attarbaschi, T., Mossaheb, N., Lanzenberger, R., Pötzi, C., Holik, A., Spindelegger, C., Asenbaum, S., Dudczak, R., Tauscher, J., Kasper, S., 2006. In vivo imaging of serotonin transporter occupancy by means of SPECT and [123I]ADAM in healthy subjects administered different doses of escitalopram or citalopram. Psychopharmacology 188, 263–272. doi:10.1007/s00213-006-0486-0

Kret, M.E., De Gelder, B., 2012. A review on sex differences in processing emotional signals. Neuropsychologia 50, 1211–1221. doi:10.1016/j.neuropsychologia.2011.12.022

Kroenke, K., Spitzer, R.L., Williams, J.B.W., 2001. The PHQ-9. Validity of a Brief Depression Severity Measure. J Gen Intern Med 16, 606–613. doi:10.1046/j.1525-1497.2001.016009606.x

Lang, P.J., Bradley, M.M., Cuthbert, B.N., 2008. International affective picture system (IAPS): Affective ratings of pictures and instruction manual. Technical Report A-8. University of Florida, Gainesville, FL.

Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Müller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274, 1527–1531.

Lesch, K.P., Gutknecht, L., 2005. Pharmacogenetics of the serotonin transporter. Prog

Neuropsychopharmacol Biol Psychiatry 29, 1062–1073. doi:10.1016/j.pnpbp.2005.03.012

Licinio, J., Wong, M.-L., 2011. Pharmacogenomics of antidepressant treatment effects. Dialogues Clin Neurosci 13, 63–71.

Ma, Y., Li, B., Wang, C., Zhang, W., Rao, Y., Han, S., 2015. Allelic variation in 5-HTTLPR and the effects of citalopram on the emotional neural network. Br J Psychiatry. doi:10.1192/bjp.bp.114.150128

McLaren, D.G., Ries, M.L., Xu, G., Johnson, S.C., 2012. A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. NeuroImage 61, 1277–1286. doi:10.1016/j.neuroimage.2012.03.068

McRae, K., Rekshan, W., Williams, L.M., Cooper, N., Gross, J.J., 2014. Effects of antidepressant medication on emotion regulation in depressed patients: An iSPOT-D report. J Affect Disord 159, 127–132. doi:10.1016/j.jad.2013.12.037

Mills, E.J., Chan, A.-W., Wu, P., Vail, A., Guyatt, G.H., Altman, D.G., 2009. Design, analysis, and presentation of crossover trials. Trials 10, 27. doi:10.1186/1745-6215-10-27

Ochsner, K.N., Gross, J.J., 2005. The cognitive control of emotion. Trends Cogn Sci 9, 242–249. doi:10.1016/j.tics.2005.03.010

Ochsner, K.N., Silvers, J.A., Buhle, J.T., 2012. Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. Ann. N. Y. Acad. Sci. 1251, E1–24. doi:10.1111/j.1749-6632.2012.06751.x

Outhred, T., Das, P., Dobson-Stone, C., Felmingham, K.L., Bryant, R.A., Nathan, P.J., Malhi, G.S., Kemp, A.H., 2014a. The impact of 5-HTTLPR on acute serotonin transporter blockade by escitalopram on emotion processing: Preliminary findings from a randomised, crossover fMRI study. Aust N Z J Psychiatry 1–38. doi:10.1177/0004867414533837

Outhred, T., Das, P., Felmingham, K.L., Bryant, R.A., Nathan, P.J., Malhi, G.S., Kemp, A.H., 2015. Facilitation of emotion regulation with a single dose of escitalopram: A randomized fMRI study. Psychiatry Research: Neuroimaging 233, 451–457. doi:10.1016/j.pscychresns.2015.07.018

Outhred, T., Das, P., Felmingham, K.L., Bryant, R.A., Nathan, P.J., Malhi, G.S., Kemp, A.H., 2014b. Impact of acute administration of escitalopram on the processing of emotional and neutral images: a randomized crossover fMRI study of healthy women. J Psychiatry Neurosci 39, 267–275. doi:10.1503/jpn.130118

Outhred, T., Hawkshead, B.E., Wager, T.D., Das, P., Malhi, G.S., Kemp, A.H., 2013. Acute neural effects of selective serotonin reuptake inhibitors versus noradrenaline reuptake inhibitors on emotion processing: Implications for differential treatment efficacy. Neurosci Biobehav Rev 37, 1786–1800. doi:10.1016/j.neubiorev.2013.07.010

Payer, D.E., Baicy, K., Lieberman, M.D., London, E.D., 2012. Overlapping neural substrates between intentional and incidental down-regulation of negative emotions. Emotion 12, 229–235. doi:10.1037/a0027421

Pringle, A., Browning, M., Cowen, P.J., Harmer, C.J., 2011. A cognitive neuropsychological model of antidepressant drug action. Prog Neuropsychopharmacol Biol Psychiatry 35, 1586–1592. doi:doi: 10.1016/j.pnpbp.2010.07.022

Quinn, C.R., Dobson-Stone, C., Outhred, T., Harris, A., Kemp, A.H., 2012. The contribution of BDNF and 5-HTT polymorphisms and early life stress to the heterogeneity of major depressive disorder: A preliminary study. Aust N Z J Psychiatry 46, 55–63. doi:10.1177/0004867411430878

Risch, N., Herrell, R., Lehner, T., Liang, K.-Y., Eaves, L., Hoh, J., Griem, A., Kovacs, M., Ott, J., Merikangas, K.R., 2009. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. JAMA 301, 2462–2471. doi:10.1001/jama.2009.878

Roiser, J.P., Elliott, R., Sahakian, B.J., 2012. Cognitive mechanisms of treatment in depression. Neuropsychopharmacology 37, 117–136. doi:10.1038/npp.2011.183

Ruhé, H.G., Ooteman, W., Booij, J., Michel, M.C., Moeton, M., Baas, F., Schene, A.H., 2009. Serotonin transporter gene promoter polymorphisms modify the association between paroxetine serotonin transporter occupancy and clinical response in major depressive disorder. Pharmacogenet. Genomics 19, 67–76. doi:10.1097/FPC.0b013e32831a6a3a

Senn, S., D'Angelo, G., Potvin, D., 2004. Carry-over in cross-over trials in bioequivalence: theoretical concerns and empirical evidence. Pharmaceut. Statist. 3, 133–142. doi:10.1002/pst.111

Serretti, A., Kato, M., De Ronchi, D., Kinoshita, T., 2007. Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. Mol Psychiatry 12, 247–257. doi:10.1038/sj.mp.4001926

Smeraldi, E., Zanardi, R., Benedetti, F., Di Bella, D., Perez, J., Catalano, M., 1998. Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. Mol Psychiatry 3, 508–511.

Sogaard, B., Mengel, H., Rao, N., Larsen, F., 2005. The pharmacokinetics of escitalopram after oral and intravenous administration of single and multiple doses to healthy subjects. J Clin Pharmacol 45, 1400–1406. doi:10.1177/0091270005280860

Spitzer, R.L., Kroenke, K., Williams, J.B.W., Löwe, B., 2006. A brief measure for assessing generalized anxiety disorder: the GAD-7. Arch. Intern. Med. 166, 1092–1097. doi:10.1001/archinte.166.10.1092

Townsend, J.D., Torrisi, S.J., Lieberman, M.D., Sugar, C.A., Bookheimer, S.Y., Altshuler, L.L., 2013. Frontal-Amygdala Connectivity Alterations During Emotion Downregulation in Bipolar I Disorder. Biological Psychiatry 73, 127–135. doi:10.1016/j.biopsych.2012.06.030

Trivedi, M.H., 2006. Evaluation of Outcomes With Citalopram for Depression Using Measurement-Based Care in STAR*D: Implications for Clinical Practice. Am J Psychiatry 163, 28–40. doi:10.1176/appi.ajp.163.1.28

World Health Organization, 2008. The global burden of disease: 2004 update. World Health Organization.

Young, E.A., Kornstein, S.G., Marcus, S.M., Harvey, A.T., Warden, D., Wisniewski, S.R., Balasubramani, G.K., Fava, M., Trivedi, M.H., John Rush, A., 2009. Sex differences in response to citalopram: A STAR* D report. J Psychiatr Res 43, 503–511.

Table 1

Participant demographics and functional connectivity change with treatment by 5-HTTLPR group

	5-HTTLPR			
	'S/S' (n = 8)	'S/L' (n = 21)	'L/L' (n = 7)	Group Differences
Age (years; SD)	22.50 (3.67)	24.95 (6.32)	28.43 (8.66)	$F(2, 33) = 1.623$ $p = 0.213$
Education (years; SD)	16.88 (3.14)	16.95 (2.67)	18.14 (3.02)	$F(2, 33) = 0.514$ $p = 0.603$
BMI (kg/m^2; SD)	21.79 (2.54)	22.88 (3.17)	21.64 (2.30)	$F(2, 33) = 0.708$ $p = 0.500$
PHQ-9 (SD)	1.75 (1.04)	1.48 (1.21)	1.00 (1.00)	$F(2, 33) = 0.830$ $p = 0.445$
GAD-7 (SD)	1.00 (1.15)	1.10 (1.14)	1.14 (0.90)	$F(2, 33) = 0.029$ $p = 0.971$
Functional Connectivity (gPPI Δ)	1.72 (1.15)	-0.69 (1.34)	-0.96 (0.88)	$F(2, 33) = 12.792$ $p < 0.001$

Note. SD = standard deviation; BMI = body mass index; PHQ-9 = Patient Health Questionnaire; GAD-7 = Generalized Anxiety Disorder questionnaire; gPPI Δ = generalized psychophysiological interaction change with treatment: PPI escitalopram – PPI placebo beta coupling estimates (positive values = decreased negative connectivity with escitalopram; negative values = increased negative connectivity with escitalopram).

Figure 1. The impact of 5-HTTLPR on functional connectivity (gPPI) between the left amygdala seed and the right inferior frontal gyrus target during reappraisal of negative stimuli under escitalopram treatment. Panel A. The L amygdala seed region (6 mm sphere at [-20 , -4, -20]). Panel B. The cluster activation of the right inferior frontal gyrus negatively correlated with left amygdala activation during reappraisal under escitalopram treatment. Panel C. Average beta coupling estimates under each treatment session for the whole sample (N = 36, as reported in Outhred et al., 2015), and then split by 5-HTTLPR groups (for the present study). Panel D. The negative relationship between 'L' alleles and functional connectivity signal change during reappraisal of negative images with escitalopram treatment (dashed lines represent the 95% confidence interval of the regression slope).

Figure 1 is attached separately.

Figure 2. An extended framework for understanding the impact of 5-HTTLPR on acute SSRI administration on differential change in emotional biases though emotion regulation circuitry, having consequences for downstream changes and symptom amelioration. 5-HTT expression plays a role in the manner in which increases in 5-HT with SSRI 5-HTT blockade are regulated. Individuals with low expressing 'S' allele have increased dysregulation of 5-HT with treatment leading to modulation of emotion regulation circuitry consistent with an increased negative emotion bias. In contrast, those with the high expressing 'L' allele have increased regulation of 5-HT with treatment, leading to modulation of emotion regulation circuitry consistent with an increased positive emotion bias. The facilitation of functional connectivity between the L amygdala and the R IFG is a key target of SSRIs (rather than modulation of each region themselves; shown in white). Further, the differential modulation of the emotion regulation circuit with SSRIs thus explains differential therapeutic outcomes between 5-HTTLPR groups.

Figure 2 is attached separately