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Acute Neurofunctional Effects of Escitalopram in Pediatric Anxiety: A Double-Blind, Placebo-Controlled Trial

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Experts: Dr. Mills served as the statistical expert for this research.

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Abstract

Objective: Amygdala-ventrolateral prefrontal cortex (VLPFC) circuitry is disrupted in pediatric anxiety disorders, yet how selective serotonin reuptake inhibitors (SSRIs), impact this circuitry is unknown. We examined the impact of SSRI on functional connectivity (FC) within this circuit, and whether early FC changes predict treatment response in adolescents with generalized anxiety disorder (GAD).

Method: Resting-state functional MR images were acquired before and after 2-weeks of treatment in 41 adolescents with GAD (age: 12–17) who received double-blind escitalopram or placebo over 8 weeks. Change in amygdala-based whole-brain FC and anxiety severity were analyzed.

Results: Controlling for age, sex and pretreatment anxiety, escitalopram increased amygdala-VLPFC connectivity compared to placebo ($F=17.79$, $p=0.002$ FWE-corrected). This early FC change predicted 76.7% of the variability in improvement trajectory in patients who received escitalopram ($p<0.001$) but not placebo ($p=0.169$); the predictive power of early amygdala-VLPFC FC change significantly differed between placebo and escitalopram ($p=0.013$). Further, this FC change predicted improvement better than baseline FC or demographics. Exploratory analyses of amygdala subfields' FC revealed connectivity of left basolateral amygdala (BLA)-VLPFC ($F=19.64$, $p<0.001$ FWE-corrected) and superficial amygdala-posterior cingulate cortex ($F=22.92$, $p=0.001$ FWE-corrected) were also increased by escitalopram, but only BLA-VLPFC FC predicted improvement in anxiety over 8 weeks of treatment.

Conclusions: In adolescents with GAD, escitalopram increases amygdala-prefrontal connectivity within the first 2 weeks of treatment, and the magnitude of this change predicts subsequent clinical improvement. Early normalization of amygdala-VLPFC circuitry might represent a useful tool for identifying future treatment responders as well as a promising biomarker for drug development.

Trial Registration: ClinicalTrials.gov Identifier: NCT02818751.

Lay summary:

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly used medications to treat teens with anxiety. In this study, the SSRI, escitalopram increased the strength of the connection between two brain regions—the amygdala and the ventrolateral prefrontal cortex—that are overactive in teens with anxiety disorders. Compared to placebo, escitalopram increased the strength of these connections by the second week of treatment and predicted which patients would improve most with treatment.

Keywords

selective serotonin reuptake inhibitors (SSRIs); antidepressant; clinical trial; anxiety disorders; MRI

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) represent an effective treatment for many children and adolescents with anxiety disorders.[1,2] Improvement varies considerably from patient to patient and 6–8 weeks of treatment is often needed to evaluate response to an SSRI.[1,3,4] Early prediction of limited treatment response to this first line treatment could indicate the need for clinicians to consider alternative or adjunctive treatments and thus improve outcomes. Further, because the effects of SSRIs on functional neurocircuitry in youth with anxiety disorders are poorly understood,[5,6] this knowledge would also be important for understanding circuit-level mechanisms of treatment efficacy.[7]

Several magnetic resonance imaging (MRI) studies have examined the treatment effects of SSRIs on brain structure and activity. In open-label trials, SSRIs attenuated insula and amygdala activity, and enhanced posterior cingulate cortex (PCC) activity during emotion perception tasks in adults with anxiety and/or depression. Changes in functional activation in these regions correspond to improvement in anxiety.[8–11] Compared with placebo, SSRIs reduce insula and amygdala reactivity during emotion processing tasks in healthy adults.[12,13] Only two open-label studies have examined the effects of SSRIs on functional brain activity in youth with anxiety disorders. In the first, fluoxetine increased ventrolateral prefrontal cortex (VLPFC) activity in response to angry faces in adolescents with GAD.[6] In the second, effective treatment increased rostral anterior cingulate cortex (ACC) activation, and greater activation was associated with more improvement in anxiety and avoidance symptoms.[14] In addition, SSRI-exposed infants compared with both healthy controls and non SSRI-exposed infants, had gray matter volume expansion in the amygdala and insula, and increased white matter connectivity between amygdala and insula.[15] Taken together, these studies indicate that SSRIs change functional reactivity, structure and connectivity in the amygdala and other regions that subserve emotion processing and these effects might mechanistically relate to SSRI-driven improvement in anxiety. Lower animal studies reveal that SSRIs induce plasticity in fear circuitry and reorganize inhibitory circuits—at the level of the amygdala—through expression of the plasticity-related molecules (e.g., polysialylated form of the neural cell adhesion molecule, [PAS-NCAM]). At the molecular level, these changes enhance interneuronal connectivity as a result of changes in dendritic spines and axonal architecture in the amygdala.[16] Based on these recent findings and the established role of the amygdala in pediatric anxiety,[17] we focused on the amygdala as the seed for connectivity analyses.

Clinical and demographic characteristics predict treatment response in pediatric anxiety disorders.[1] In the largest trial of an SSRI in youth with anxiety disorders, the Child and Adolescent Anxiety Multimodal Study (CAMS), patients who were younger, were non-minority, had less severe anxiety and fewer co-morbidities were more likely to remit.[18] Additionally in this sample, anxiety severity and caregiver strain were significantly related with treatment response to sertraline.[19] To date, studies have failed to predict response to SSRIs with pharmacokinetic or pharmacogenetic parameters in youth with mixed anxiety and depressive disorders treated with sertraline or escitalopram.[20,21] Neuroimaging studies in youth with GAD have reported pretreatment amygdala activation being correlated with improvement following fluoxetine treatment.[5] Increased activation of the dorsolateral

prefrontal cortex, VLPFC and precentral/postcentral gyri before treatment have also been associated with greater treatment-related improvement in anxiety in children and adolescents with anxiety disorders.[22] In adults with social anxiety disorder, pre-treatment dorsal ACC reactivity separated treatment responders from non-responders (83% accuracy) who received an SSRI.[23,24] Studying the connectome of these regions is a promising next step, as the amygdala and prefrontal regions are widely considered to influence the intensity and modulation of emotions respectively.[25]

To date, several studies have examined SSRI-related changes in brain function in open-label trials, as well as baseline neurofunctional predictors of SSRI response in anxiety disorders. However, acute SSRI effects on functional connectivity (FC) in youth with anxiety and the potential utility of early treatment-related FC changes (vs clinical characteristics) to predict subsequent clinical outcomes have never been examined in a double-blind, placebo-controlled trial. With these considerations in mind, the primary aim of this study is to examine early treatment effects of escitalopram on amygdala-based FC, and then to explore the relationship of these neurofunctional treatment effects and improvement in adolescents with GAD. Since the amygdala comprises several subfields that might have specific connectivities and are differentially involved in anxiety,[26] we further investigated the effect of escitalopram on amygdala subfields' connectivity. As an exploratory analysis, we also examined whether the SSRI-related FC change at week 2 predicted improvement of anxiety better than demographic/clinical characteristics and baseline (*i.e.*, pretreatment) FC. Based on previous findings, we hypothesized that treatment would increase amygdala-prefrontal FC and that early, treatment-related increases in amygdala-prefrontal FC would predict subsequent improvement in anxiety over the course of the trial.

METHOD

Participants

This randomized clinical trial aimed to identify neurofunctional predictors of treatment-response in adolescents with GAD (ClinicalTrials.gov Identifier: NCT02818751). The protocol was approved by the Institutional Review Board of the University of Cincinnati and conducted in accordance with Good Clinical Practice guidelines. This study was conducted at a single academic site in the United States from February 2015 to November 2018. Outpatients aged 12–17 years who met *DSM-IV-TR* criteria for GAD, assessed using the Anxiety Disorders Interview Schedule (ADIS), had a Pediatric Anxiety Rating Scale (PARS) score ≥15 at screening and baseline visits; and had a Clinical Global Impression of Severity (CGI-Severity) score ≥4 were eligible. Patients with secondary diagnoses of separation or social anxiety disorder or panic disorder and/or agoraphobia were enrolled, provided that GAD was the primary diagnosis; however, patients with current MDD or any history of bipolar disorder, psychotic disorder, obsessive compulsive disorder or post-traumatic stress disorder were excluded. Concomitant psychotherapy was allowed during the study provided the psychotherapy was stable prior to study entry and remained stable, no new psychotherapy was allowed during the trial. Other exclusion criteria included: a contraindication to MRI, pregnancy, a history of alcohol and drug abuse or any major medical or neurological disorder. Additionally, the original proposal, as reflected in

clinicialtrials.gov, proposed a sample size of 64 patients with GAD, although only 51 were randomized. Further, in the original conception of the study, 20 healthy controls were to have been enrolled (not part of this analysis).

Treatment and Assessments

Patients were randomized to an 8-week double-blind placebo-controlled clinical trial with escitalopram, the most serotonergically selective SSRI, or placebo (1:1) which was delivered in identically-appearing purple capsules. Treatment was assigned by investigational pharmacists using a random number generator and randomization was stratified by sex. Patients, caregivers, and investigational staff were blind to treatment assignment. As previously described,[27] escitalopram was initiated at 5 mg daily for 2 days and then 10 mg daily for 7 days and then 15 mg daily. At week 4 and 6 visits, escitalopram could be titrated to 20 mg daily.

The PARS was used to measure the anxiety symptom severity and was administered at each study visit (week 0, 1, 2, 4, 6, and 8) to provide a trajectory of symptom change. The PARS was selected as it has been utilized as the primary outcome measure in the majority of psychopharmacologic treatment studies in youth with anxiety disorders.[17] The PARS includes a 50-item symptom checklist which encompasses social anxiety/performance anxiety (9 items), separation anxiety (10 items), generalized anxiety (8 items), specific phobia (4 items), physical/somatic symptoms (13 items) as well as “other” items (6 items). However, the PARS score is determined from 7 severity and impairment items that are rated on a 6-point scale, with higher scores representing more severe symptoms and impairment. The PARS has established, acceptable, convergent validity with 3 anxiety rating scales.[28] For patients who discontinued treatment prior to week 8 (endpoint), a last observation carried forward (LOCF) approach was utilized for missing PARS scores (3 participants in each group discontinued, p=1.0).

Image Acquisition

Resting-state functional MR images (rsfMRI) and high-resolution anatomical images were acquired on a 3-T scanner (Achieva; Philips, USA) with a 32-channel phased-array head coil at baseline and 2 weeks after beginning treatment. Scanner noise was attenuated with earplugs and headphones; head motion was restricted with foam padding. Functional images were obtained using a single shot, fast Fourier echo, echo planar (FFE-EPI) sequence with the following parameters: repetition time (TR) = 2000 ms; echo time (TE) = 30 ms; number of axial slices = 40; resolution = 2.8 mm × 2.8 mm; slice thickness = 3 mm; flip angle = 75°; matrix = 80 × 80; field of view = 224 mm × 224 mm. High-resolution anatomical images were obtained using a three dimensional T1-weighted Turbo field echo (T1-TFE) sequence with the following parameters: TR = 6.8 ms; TE = 2.9 ms; number of sagittal slices = 160; resolution = 1 mm × 1 mm; slice thickness = 1 mm; flip angle = 9°; matrix = 256 × 256; field of view = 256 mm × 256 mm. Images were reviewed by a pediatric neuroradiologist to exclude cases with gross structural abnormalities and image artifacts.

Functional Connectivity of Amygdala and Amygdala-subfields

Functional images were preprocessed with the SPM12 package (www.fl.ion.ucl.ac.uk/spm) and DPABI toolbox.[29] For each patient, the first 10 volumes were discarded to ensure signal stabilization. The remaining images were corrected for slice time. Head motion was corrected by regression of 24 head motion parameters, mean framewise displacement (FD) of every subject was <0.5 mm and did not different between groups (pretreatment, t=0.70, p=0.49; week 2, t=0.48, p=0.63). Functional images were spatially normalized to standard Montreal Neurological Institute space using unified segmentation on individual T1 images, and each voxel was resampled to 3×3×3 mm^3. The normalized images were then smoothed with a 6-mm full-width at half-maximum Gaussian kernel. Linear trends and nuisance signals (six motion parameters, white matter signal, and cerebrospinal fluid signal) were removed with linear regression and a temporal band pass filter (0.01–0.08 Hz) was utilized to exclude high and low frequency signals. Four amygdala subfields (amygdalostriatal transition [AStr], basolateral [BLA], centralmedial [CMA] and superficial amygdala [SFA]) were identified using a cytoarchitectonically-defined probabilistic map of the amygdala.[30] Seed-based resting-state FC analyses were conducted for the left and right amygdala and their subfields separately (10 seeds in total including bilateral amygdala, AStr, BLA, CMA, and SFA) using the Resting-State fMRI Data Analysis Toolkit (http://resting-fmri.sourceforge.net). Specifically, the time series of voxels within each region of interest were extracted and averaged, then voxel-wise correlation analyses were performed with the rest of the brain to obtain FC maps. Correlation coefficients were transformed to *z*-value images using the Fisher *r*-to-*z* transformation for statistical analyses.

Statistical Analysis

The treatment-by-time interaction was examined using a full factorial analysis in SPM12, with FC maps as dependent variables, treatment (*i.e.*, placebo or escitalopram) and time (baseline or week 2) as independent variables, and age, sex and baseline PARS score as covariates. Family-wise error (FWE) was applied to correct for multiple comparisons, with thresholds of $p<0.001$ at the voxel level and $p<0.05$ at the cluster level. Bonferroni correction was utilized to control type 1 error given that 10 seeds we used in current study (corrected $p<0.005=0.05/10$). FC strength was extracted from clusters with significant treatment-by-time interactions, and a *post-hoc* analysis was performed to examine the change of FC from baseline to week 2 within the escitalopram and placebo groups respectively.

Using a mixed effects model (with log trend specification), we examined the relationship between change in FC at week 2 and the trajectory of anxiety improvement (*i.e.*, PARS score) using week 0, 1, 2, 4, 6 and 8 ratings in both escitalopram and placebo group. Each model was estimated with covariates (*e.g.*, age, sex) and refined as previously described, using the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC), to obtain the most parsimonious response prediction model.[3] Predicted variation in response models was measured by R^2; adjusted R^2 (R^2_{adj}), a model selection criterion, was not used as it is superseded by AIC and BIC and does not directly measure the proportion of variation explained by the model. As an exploratory analysis of potential superiority of the change in FC as a predictor of outcome, models that included baseline FC and demographic

characteristics were also examined and compared. Non-imaging statistical analyses were performed using *R* (version 3.1.2) and *SPSS* (version 22), *p*-values <0.05 were considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

Fifty-one participants were randomized to treatment and detailed demographic and clinical characteristics have been reported previously.[31] Ten participants (5 from escitalopram and 5 from placebo group) were excluded from this fMRI analysis, 8 of them did not complete baseline and week 2 scans, 1 had orthodontia artifacts, and 1 was scanned with a different MR sequence. Thus, forty-one participants were included in the analysis (escitalopram: *n*=21; placebo: *n*=20, Table 1 and Figure 1). There were no significant differences between groups in age, sex, IQ, comorbidity and baseline anxiety severity (*i.e.*, PARS score). Seventy six percent of escitalopram-treated patients and 81% of patients who received placebo were antidepressant naïve (p=0.73). Moreover, no pretreatment amygdala/amygdala-subfield based FC difference between the two groups was found. Escitalopram-treated patients had significantly greater improvement (PARS score), at endpoint and over time, compared to those who received placebo.

Escitalopram Increases Amygdala Functional Connectivity

Adolescents with GAD showed a significant treatment-by-time interaction in the FC between left amygdala and left VLPFC (cluster size: 144 voxels; peak coordinate: x=−45, y=20, z=24; *F*=17.79, *p*=0.002 FEW- and Bonferroni-corrected). *Post-hoc* analysis revealed that escitalopram significantly increased functional amygdala-VLPFC connectivity compared to placebo. Specifically, the amygdala-VLPFC connectivity increased after 2 weeks of escitalopram treatment (F=3.62, p=0.064) (Figure 2).

In the exploratory analyses, a significant treatment-by-time interaction was observed in the FC between left BLA and left VLPFC (cluster size: 198 voxels; peak coordinate: x=−45, y=6, z=21; *F*=19.64, *p*<0.001 FEW- and Bonferroni-corrected). BLA-VLPFC connectivity increased significantly after 2 weeks of escitalopram treatment (*F*=4.84, *p*=0.034). Specifically, the BLA-VLPFC connectivity increased significantly after 2 weeks' escitalopram treatment (F=4.84, p=0.034) and decreased significantly in youth who received placebo (F=12.33, p=0.001) (Figure 2). A significant treatment-by-time interaction was also found in the FC between left SFA and PCC (cluster size: 161 voxels; peak coordinates: x=6, y=−48, z=24; *F*=22.92, *p*=0.001 FEW- and Bonferroni-corrected). SFA-PCC connectivity increased significantly after 2 weeks of escitalopram treatment (*F*=45.97, *p*<0.001) and marginally decreased in youth receiving placebo (*F*=4.12, *p*=0.049) (Figure 2). No connectivity was changed by escitalopram with right amygdala and its subfields as seeds.

Increased Amygdala Functional Connectivity Predicts Improvement in Anxiety

The change in left amygdala-VLPFC connectivity from baseline to week 2 predicted the trajectory of improvement in PARS scores from baseline to week 8 in escitalopram-treated patients (β=3.706, *p*<0.001) but not those who received placebo (β=1.179, *p*=0.169). For

escitalopram treated patients, this response model explained >76% of the variation in improvement (i.e, R^2=0.767) compared to just 41% in patients who received placebo. Likewise, the change in left BLA-VLPFC (β=−4.340, p<0.001) also predicted the trajectory of PARS score in adolescents taking escitalopram, while the change of SFA-PCC FC did not (β=−1.356, p=0.087). The predictive power of early amygdala-VLPFC FC change significantly differed between patients who received placebo and those who received escitalopram (β=2.685, p=0.013). This difference in predictive power of the two interventions (*i.e.*, placebo, escitalopram) also significantly differed for the BLA-VLPFC (β=−3.672, p=0.005) and the SFA-PCC (β=−2.709, p=0.023) (Table 2).

Escitalopram-related Change in Functional Connectivity Predicts Treatment Response Better than Clinical Features and Pre-treatment Functional Connectivity

Consistent with previous studies, sex predicted the trajectory of anxiety symptoms in escitalopram-treated patients (Table 2). Including baseline FCs with sex and age did not improve model fit (F=0.974, p=0.408). However, including *change* in FC in the demographic model significantly improved model fit (F=4.08, p=0.020) and provided the *best* prediction of improvement in anxiety symptoms based on model fit statistics (BIC and AIC).

DISCUSSION

This is the first prospective, double-blind, placebo-controlled examination of *early* SSRI-related changes in FC in anxiety disorders. Within two weeks, escitalopram increased amygdala FC relative to placebo (amygdala-VLPFC, BLA-VLPFC and SFA-PCC). Further, in escitalopram-treated (but not placebo-treated) patients, the magnitude of increases in amygdala-VLPFC and BLA-VLPFC FC predicted the trajectory of anxiety symptom reduction over 8 weeks, and did so better than baseline amygdala FC and demographic characteristics. Taken together, these findings demonstrate acute, neurofunctional effects of escitalopram on amygdala FC and the promising role of these FC changes in predicting treatment response in adolescents with anxiety disorders.

Hyperactivity in the amygdala and its attenuated FC with VLPFC have been observed in task-based fMRI studies of anxious youth.[32,33] Specifically, the VLPFC known to be involved in emotion regulation has decreased connectivity with amygdala compared to healthy youth in cross-sectional studies of adolescents with GAD.[34,35] Our findings indicate that escitalopram increases this impaired connectivity preceding full treatment response, suggesting that normalizing this FC produces early restorative effects on the neuropathophysiology of deficient emotion regulation in GAD. Further, prior fMRI studies using emotional processing tasks in adolescents with GAD revealed increased amygdala and VLPFC activation when viewing angry faces, and the increased VLPFC activation was associated with attenuated amygdala activation and less anxiety.[36,37] By increasing amygdala-VLPFC FC, escitalopram may enhance VLPFC's regulation of amygdala activity and thus reduce anxiety in youth with GAD. This interpretation is consistent with previous findings showing that both fluoxetine and cognitive behavior therapy increase VLPFC activation in response to angry faces in adolescents with GAD.[6] Further, in adults with high state anxiety, real-time FC-informed neurofeedback training during threat exposure increases

amygdala-VLPFC connectivity and decreases anxiety.[38] Thus, our findings are consistent with the view that reduced functional integration of amygdala-VLPFC circuitry may weaken regulation of amygdala-driven emotional responses to threatening stimuli and thus to manifest anxiety. Our findings indicate that increasing amygdala-VLPFC connectivity may improve this top-down emotion regulation and relieve anxiety symptoms, and represent a target for interventions aiming to reduce anxiety.

In our study, significant effects of escitalopram on amygdala-VLPFC FC were identified in the BLA amygdala subfield. Importantly, the BLA receives information about the external environment from the sensory thalamus and neocortices, and reciprocally projects to cortical regions implicated in the pathophysiology of anxiety disorders.[39] This study extends previous findings by providing evidence that amygdala FC can be successfully modified by treatment to reduce anxiety and that greater BLA-VLPFC FC predicts better treatment response. Additionally, the importance of the BLA subfield is consistent with lower animal models showing the importance of this region for anxiety and the role of 5-HT in regulating BLA neuronal response to aversive sensory cues.[40–42]

In addition to escitalopram-related changes in amygdala-VLPFC FC, treatment-related changes in SFA-PCC were identified. The effects of SSRI treatment are not limited to the amygdala-prefrontal circuitry but involve its connections to the default mode network (DMN). Decreased DMN activity and decreased FC between DMN hubs and amygdala have been observed in anxiety disorders and in individuals with high state anxiety.[26,43–47] Recently, an examination of individual-specific FC of the amygdala subdivisions revealed connectivity between SFA and PCC.[48] The SFA processes socially-relevant information and modulates approach-avoidance behavior.[49,50] The PCC is involved in evaluating the affective valence of external stimuli.[51] In healthy adults, escitalopram has been shown to decrease PCC activity and anxiety during a self-evaluation task.[52] Thus, our findings suggest that SSRIs may impact amygdala-PPC FC to modulate the evaluation of emotional stimuli by decreasing the affective valence of threatening stimuli and reducing avoidance.

Since about 50% of adolescents with anxiety disorders fail to respond to SSRIs,[1] there is an urgent need to identify biomarkers that predict treatment response prior to or early in the course of a treatment so that long trials of treatments that are unlikely to be effective can be avoided. Previous studies reveal that demographic factors and baseline activation in the amygdala, PFC, and ACC during emotional processing are associated with improvement in anxiety symptoms.[5,22,53] Our findings extend this work by revealing that SSRI-related normalization of FC of the amygdala could better predict treatment response than pretreatment neuroimaging biomarkers and demographic factors, and could do so early after treatment initiation. Further, the early FC changes observed herein relate to recent data suggesting that—early in the course of treatment—SSRIs attenuate the negative bias in processing emotionally salient information in patients with anxiety. It has been hypothesized that this change facilitates the clinical improvement that occurs later.[54] Finally, it is also possible that these early FC changes could relate to tolerability, including “activation,” a common side effect of SSRIs in youth with anxiety and affective disorders.[55,56] Importantly, activation which consists of transient increases in anxiety, restlessness and insomnia emerges

early in the course of treatment is related to plasma blood levels of SSRIs and may relate to effects of SSRIs on amygdala-prefrontal circuits.[55,57]

While this is the first investigation of acute effect of escitalopram on FC in adolescents, several limitations warrant additional discussion. First, the sample size is relatively small and limits our ability to characterize the effects of sex on the neurocircuitry of treatment response. Specifically, the sample size is smaller than our planned sample size and 6 patients discontinued treatment prior to endpoint, although for trajectory analyses, a last observation carried forward approaches was used.[57] Second, the stability of FC changes cannot be determined based on our study design; the acute changes in FC may represent transient, persistent or progressively increasing treatment-related changes. Third, the dependent variable in the prediction models—PARS score—which reflects general anxiety burden could relate to a clinician's decision to titrate escitalopram from 15 to 20 mg at week 4 or 6. However, the clinician's decision to titrate escitalopram at week 4 or 6 is unlikely to correlate with the change in FC during the first two weeks of treatment. In other words, if Y represents the change in PARS score from baseline to endpoint, X represents the change in FC during the first two weeks of treatment and Z represents a clinician's decision to titrate at week 4 or 6, then if X and Z do not correlate, the statistical relationship between Y and X is unaffected by the presence or exclusion of Z in the model. Thus, omitted variable bias related to a decision to titrate dose at week 4 or 6 is unlikely. Fourth, the stereotactic positions of amygdala subfields vary across patients and template-based approaches to measure amygdala sub-regional connectivity may lack precision and affect generalizability, although we would point out that amygdala sub-regional FC patterns match known subfield anatomic connectivity patterns. Finally, the resolution of functional images, compared to anatomical images, may limit precision of the segmentation of amygdala subfields and then result in imprecise connectivity assessment; however, this approach has been used in prior pediatric studies.[58] The significant result in our study comes from the left BLA, the largest subfield of amygdala, which has 85 voxels with voxel size of $3\times3\times3$ mm^3. Moreover, our BLA-VLPFC connectivity map overlapped with our amygdala-VLPFC connectivity map.

In summary, escitalopram increases amygdala-VLPFC connectivity during the first 2 weeks of treatment, which is driven by changes in FC with the basolateral amygdala. The magnitude of these changes predict subsequent reduction of anxiety in adolescents with GAD. These findings reveal a potential neural systems mechanism of escitalopram efficacy in GAD, providing a target engagement model for novel medications and a potential early means to identify SSRI responders. Finally, this study provides a preliminary rationale for future studies of acute neurofunctional changes to guide treatment selection in youth with anxiety disorders, and perhaps, those with other internalizing disorders.

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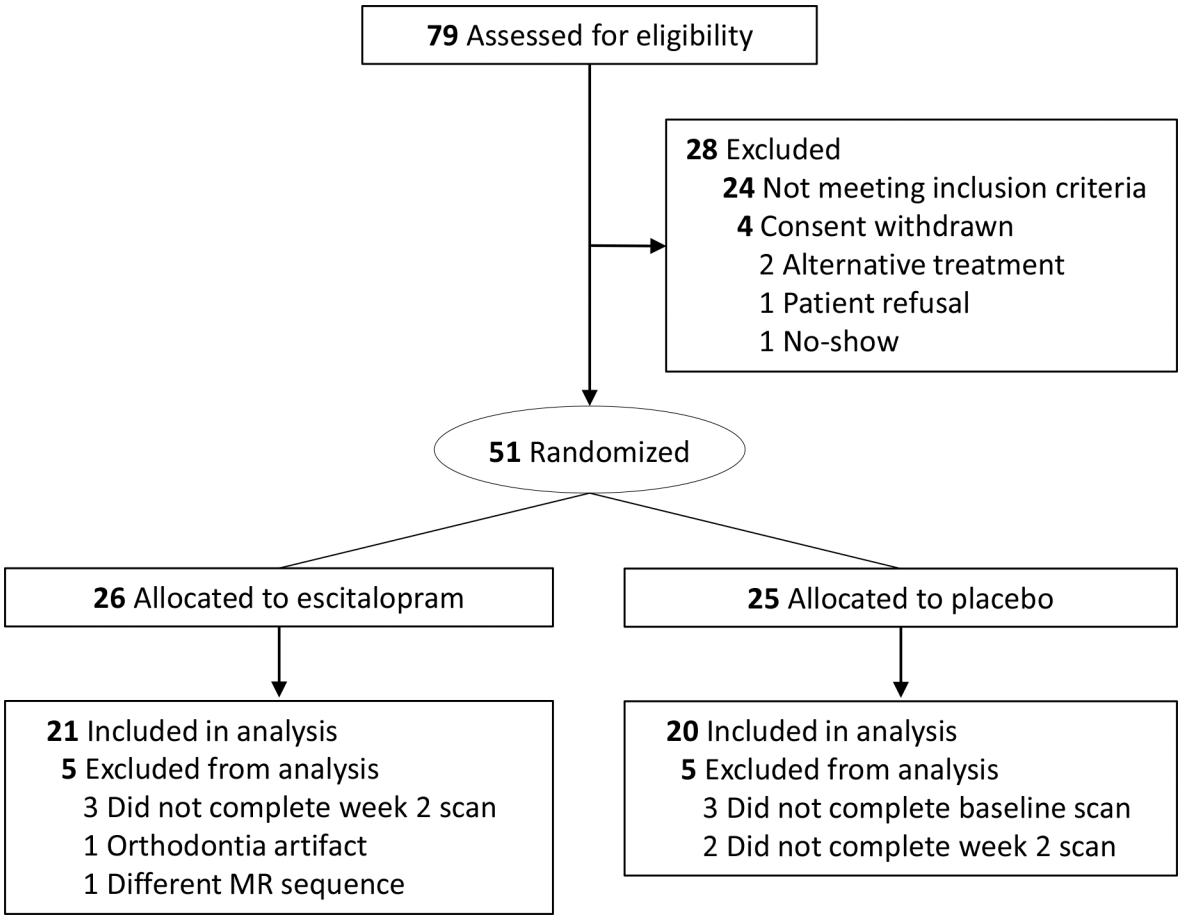

FIGURE 1.
CONSORT Diagram

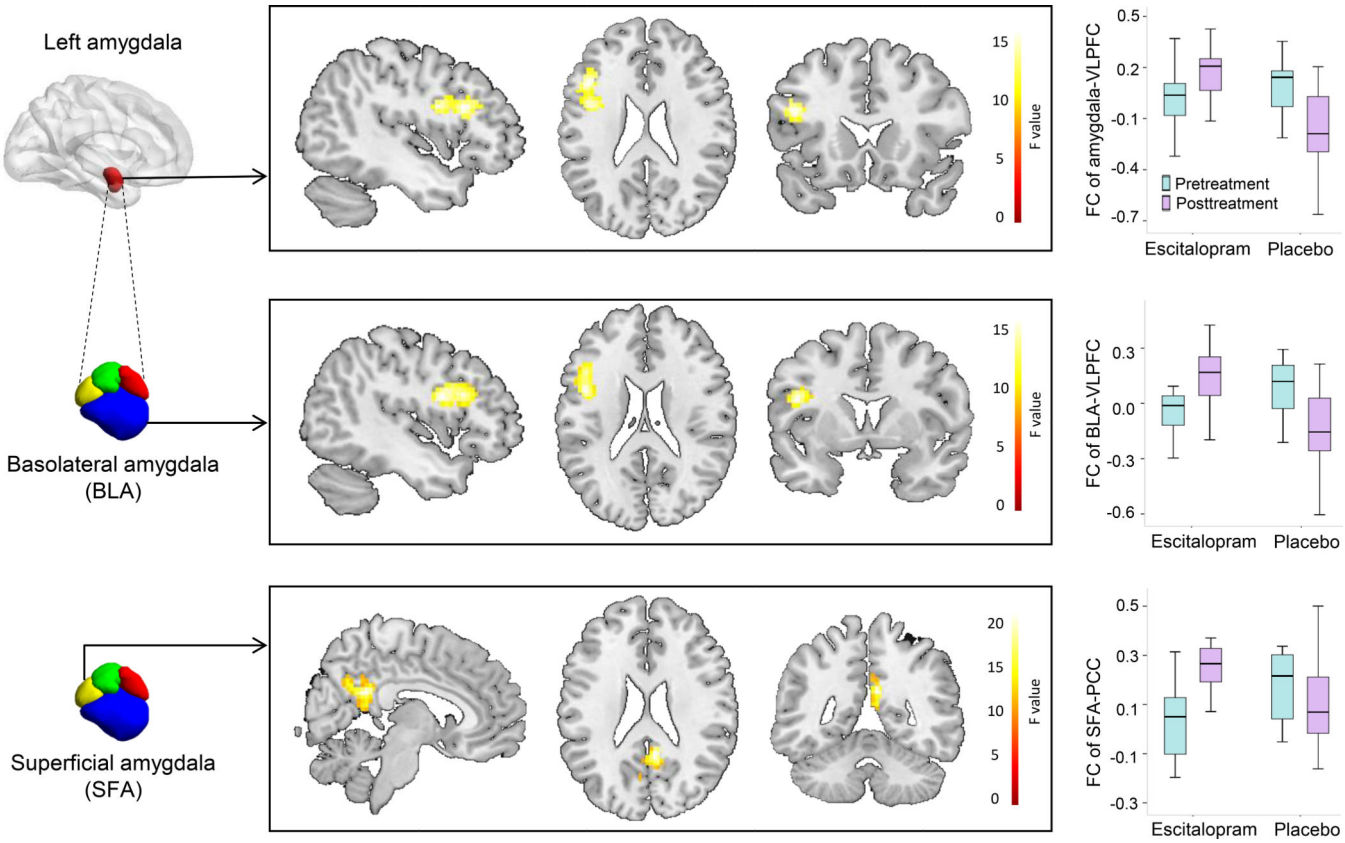

FIGURE 2. Treatment-by-Time Interaction in Amygdala- and Amygdala Subdivision-based Functional Connectivity (FC) in Adolescents with Generalized Anxiety Disorder.

Note: In the box and whisker plots, the horizontal line inside the box represents the median FC strength, the bottom and top edges reflect interquartile range (25th and 75th percentiles, respectively) and the whiskers extend to the furthest datum within 1.5 times the interquartile range. BLA, basolateral amygdala; FC, functional connectivity; L, left; PCC, posterior cingulate cortex; SFA, superficial amygdala; VLPFC, ventrolateral prefrontal cortex. *, p<0.05; **, p<0.001.

Table 1.

Demographic and Clinical Characteristics of Patients Receiving Escitalopram and Placebo

Baseline Characteristics	Escitalopram (*n*=21)	Placebo (*n*=20)	Summary statistic	p-value
Age, mean (SD), year	14.9±1.7	15.0±1.6	−0.18	0.86
Female, n (%)	16 (76)	14 (70)	0.20	0.66
Full scale IQ, mean (SD)	106±10	104±11	0.48	0.64
Race			1.51	0.87
Asian	0 (17)	1 (6)		
Black and African American	1 (0)	1 (6)		
Caucasian	19 (83)	17 (81)		
Other	1 (0)	1 (6)		
Hispanic or Latino	2 (0)	0 (0)		0.49
PARS score, baseline	17±2	17±3	0.17	0.86
PARS score, week 8/ET	7±6	15±3	−2.37	0.02
CGI-Severity score, median	4	4		0.22
Secondary diagnoses				
Separation anxiety disorder	3 (14)	4 (20)		0.70
Panic disorder	10 (48)	12 (60)		0.54
Agoraphobia	6 (29)	6 (30)		1.00
ADHD	4 (19)	4 (20)		1.00
Specific phobia	7 (33)	2 (10)		0.13
Prior SSRI/SNRI treatment, n (%)	5 (24)	6 (19)	0.20	0.73

CGI-S, Clinical Global Impression Scale-Severity; CDRS-R, Children's Depression Rating Scale-Revised; PARS, Pediatric Anxiety Rating Scale; ADHD, Attention/Deficit-Hyperactivity Disorder; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin-norepinephrine reuptake inhibitor.

Table 2.

Regression Models of Improvement in Pediatric Anxiety Rating Scale (PARS) Score over 8-weeks in Adolescents Receiving Escitalopram and Placebo

Trajectory of anxiety symptoms, escitalopram (n=21)				
	Estimate	**Std. Error**	**t value**	**p-value**
Week (log)	−1.862	1.214	−1.534	0.128
Amygdala-VLPFC FC change	3.706	0.747	4.961	**<0.001**
BLA-VLPFC FC change	−4.340	1.046	−4.150	**<0.001**
SFA-PCC FC change	−1.356	0.785	−1.724	0.087
Age	−0.043	0.086	−0.502	0.617
Sex, female	0.969	0.318	3.046	**0.003**
Trajectory of anxiety symptoms, placebo (n=20)				
	Estimate	**Std. Error**	**t value**	**p-value**
Week (log)	−3.476	1.213	−2.866	**0.005**
Amygdala-VLPFC FC change	1.179	0.850	1.387	0.169
BLA-VLPFC FC change	−0.920	0.864	−1.064	0.290
SFA-PCC FC change	1.191	0.867	1.373	0.173
Age	0.161	0.081	1.994	**0.049**
Sex, female	0.493	0.273	1.806	0.074
Trajectory of anxiety symptoms, all patients (n=41)				
	Estimate	**Std. Error**	**t value**	**p-value**
Week (log)	−2.162	0.880	−2.457	**0.015**
Tx x week	−1.008	0.303	−3.331	**0.001**
amygdala-VLPFC change	0.982	0.855	1.149	0.252
BLA-VLPFC FC change	−0.778	0.886	−0.879	0.381
SFA-PCC FC change	1.231	0.905	1.360	0.175
Tx by amygdala-VLPFC change	2.685	1.075	2.498	**0.013**
Tx by BLA-VLPFC FC change	−3.672	1.283	−2.861	**0.005**
Tx by SFA-PCC FC change	−2.709	1.183	−2.290	**0.023**
age	0.064	0.059	1.081	0.281
sex	0.676	0.209	3.243	**0.001**

VLPFC, ventrolateral prefrontal cortex; FC, functional connectivity; BLA, basolateral amygdala; SFA, superficial amygdala; PCC, posterior cingulate cortex