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## Brief Report

# A preliminary functional magnetic resonance imaging study of prefrontal-amygdalar activation changes in adolescents with bipolar depression treated with lamotrigine

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***Objectives:*** Hypotheses regarding mood dysregulation in bipolar disorder (BD) have centered on limbic overactivity with relative prefrontal underactivity during mood episodes. Therefore, we hypothesized that adolescents with bipolar depression successfully treated with lamotrigine would show decreases in amygdalar activation, and increases in prefrontal activation.

***Methods:*** Eight adolescents with BD underwent functional magnetic resonance imaging (fMRI) at baseline and after eight weeks of lamotrigine treatment. Blocks of negatively and neutrally valenced emotional pictures were presented during scanning, and subjects were asked to rate how each picture made them feel. Activation in bilateral amygdalae and dorsolateral prefrontal cortices (DLPFC) for negative minus neutral pictures was correlated with Children's Depression Rating Scale (CDRS) scores.

***Results:*** Mean (SD) CDRS scores decreased significantly, from 53.0 (10.6) at baseline to 26.3 (5.3) at Week 8. This clinical improvement was correlated with decreased right amygdalar activation ($r = 0.91$, $p = 0.002$). At Week 8, but not baseline, CDRS score was positively correlated with bilateral amygdalar activation ($r = 0.85$, $p = 0.007$). DLPFC activation was not correlated with change in CDRS score.

***Conclusions:*** These preliminary results indicate that adolescents with BD treated with lamotrigine demonstrated less amygdalar activation when viewing negative stimuli as depressive symptoms improved. Larger controlled studies are needed to confirm these findings.

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Hypotheses regarding mood dysregulation in bipolar disorder (BD) have centered on limbic overactivity with relative prefrontal underactivity (1–3), indicating decreased prefrontal control over limbic structures that mediate mood symptoms. Studies of adolescents with BD have reported abnormal prefrontal-subcortical activation patterns (2, 4, 5), supporting these hypotheses. The amygdala has been particularly implicated in the pathophysi-

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ology of pediatric BD, as studies have consistently reported decreased amygdalar volume compared to controls (6–9). However, it is unclear how the function of the amygdala is affected by a relatively decreased volume. While adults with BD demonstrate abnormal amygdalar activation (3, 10, 11), only one pediatric study has shown the same (12). Furthermore, no studies have indicated whether amygdalar activation varies according to mood state, such as depression.

Adults with bipolar depression have consistently exhibited decreased prefrontal blood flow and metabolism (13, 14). However, functional magnetic resonance imaging (fMRI) studies using a variety of tasks have found adults with bipolar depression to have overactivation in prefrontal regions when performing affective or cognitive tasks (1, 15). Thus, while the effects of mood state on brain activation in pediatric BD have not been studied, it might appear that prefrontal regions may show over- or underactivation, whereas limbic structures may demonstrate increased activation (2). In an earlier study, we demonstrated that lamotrigine treatment was efficacious for bipolar depression in adolescents (16). In order to better understand the effects of bipolar depression and symptom resolution on activity within the amygdala and prefrontal cortex, we performed fMRI on a subgroup of these subjects before and after treatment with lamotrigine. We hypothesized that adolescents with bipolar depression successfully treated with lamotrigine would show decreases in amygdalar activation, and increases in prefrontal activation in response to an emotional probe.

## Methods

Clinical results of an eight-week open-label trial of lamotrigine monotherapy or adjunct therapy in adolescents with bipolar depression were detailed in a previous report (16). For the current study, 11 consecutive subjects in the clinical trial were scanned with fMRI at baseline and after eight weeks of lamotrigine. Informed written and verbal consent was obtained from the parent or legal guardian of the subjects, and written and verbal assent was obtained from the subjects. The protocol was approved by the Stanford University Panel on Human Subjects in Medical Research and carried out in accordance with the Helsinki Declaration of 1975.

Inclusion criteria were: male or female adolescents, aged 12–17 years, with a DSM-IV diagnosis of BD type I or II (BDI and BDII, repectively), or not otherwise specified (NOS) by the WASH-U-KSADS (Washington University in St Louis Kiddie Schedule for Affective Disorders and Schizophrenia) (17). For the purposes of this study, BD NOS was defined as having a period of significant elevated, irritable, or expansive mood for at least two days, with at least two other symptoms of mania during that time (three if the predominant mood was irritable). Subjects were required to be experiencing a depressive episode by DSM-IV criteria and needed to have a score of 36 or greater on the Children's Depression Rating Scale-Revised (CDRS) (18, 19) before beginning lamotrigine treatment.

Mood and general improvement was assessed weekly. The primary measure for response was a score of 1 or 2 on the Clinical Global Impression Scale-Improvement (CGI-I) at Week 8. The secondary measure for positive response was defined as at least a 50% decrease in the CDRS score from baseline to Week 8. We refer to the original study for details regarding more specific inclusion/exclusion criteria, laboratories obtained, concomitant medications allowed, and titration schedule of medication (16).

### International Affective Picture System Task

Negative (e.g., a mutilated dog), positive (e.g., puppies) and neutral (e.g., a plate) pictures were selected from the International Affective Picture System (IAPS), and were deemed acceptable for a pediatric population. The three types of stimuli were organized in blocks, each with six stimuli, with each stimulus presented for 4,500 msec with a 500 msec inter-stimulus interval. Subjects were asked to indicate how each picture 'made them feel' by pressing 1 of 3 buttons corresponding to 'negatively', 'neutrally' and 'positively'. For this study, we only used responses to positive and neutral blocks. Stimuli were projected onto a screen using a custom-built magnet compatible projection system (Sanyo, San Diego, CA, USA). A custom-built button box was used to measure subjects' behavioral responses.

### fMRI data acquisition

Images were acquired on a 3T GE Signa scanner (GE Healthcare, Buckinghamshire, UK) using a standard GE whole head coil. The following spiral pulse sequence parameters were used: repetition time (TR) = 2,000 msec, echo time (TE) = 30 msec, flip angle = 80° and 1 interleave. To reduce field inhomogeneities, an automated high-order shimming method based on spiral acquisitions was used before acquiring functional MRI scans (20).

To aid in localization of the functional data, high-resolution T1-weighted spoiled grass gradient recalled (SPGR) 3D MRI sequences with the following parameters were used: TR = 35 msec, TE = 6 msec, flip angle = 45°, field of view = 24 cm, 124 slices in coronal plane, 256 × 192 matrix.

### Image preprocessing

Functional MRI data were preprocessed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm). Images were corrected for movement using least square minimization without higher-order corrections for spin history, and normalized (21) to Montreal Neurological Institute (MNI) coordinates. Images were then resampled every 2 mm using sinc interpolation and smoothed with a 4-mm Gaussian kernel to decrease spatial noise. MNI coordinates were transformed to stereotaxic Talairach coordinates using a nonlinear transformation.

### fMRI region-of-interest (ROI) analysis

Spherical ROIs (5 mm radius) were created for the amygdala and dorsolateral prefrontal cortex (DLPFC). Right [22, –2, –20] and left [–22, –2, –20] amygdala ROIs were visually placed by one research assistant on a group-averaged SPGR scan and examined by two trained neuroscientists to verify accuracy of placement. The most superior white matter tract extending from the temporal lobe marked the inferior border, cerebrospinal fluid marked the medial border, endorhinal sulcus marked the superior border, and a thick, central white matter tract of the temporal lobe was used as the lateral border of amygdala. Placement of right [48, 16, 22] and left [–48, 12, 28] DLPFC ROIs was based on prior loci of activation of the same task and condition from a previous study (2). These foci of activation were within Brodmann Areas 9/45 (DLPFC) and were the areas in which subjects with BD demonstrated greater activation compared to healthy controls when performing the IAPS task, negative minus neutral pictures.

Activation in the ROIs was quantified as the percent of voxels within the ROI that surpassed ($Z > 1.67$; $p < 0.05$). We used this threshold, and did not use overall mean activation, to ensure that we would detect variation in activation, instead of any present activation leading to values of 100% activation in all subjects. ROI activation for negative *minus* neutral pictures was computed for baseline and Week 8. Percent voxel activation for each ROI was correlated with CDRS score using Spearman's non-parametric correlation. Due to our a priori hypotheses, a one-tailed alpha was set at 0.10 but, due to the two ROIs compared (amygdala and DLPFC), we used Bonferroni correction to set the alpha at 0.05. In addition to correlations between brain activation and clinical measures, the relationship between activation in the DLPFC and activation in the amygdala and contributions of right versus left structure were tested as exploratory analyses.

## Results

Our standard exclusion criteria excluded subjects who had movement > 3 mm during more than 10% of the task, or who did not respond to over 60% of the trials. Thus, of the 11 scanned subjects, 8 were included in the analysis.

### Clinical response

Of the 8 scanned subjects, there were 3 subjects with BDI, 3 with BDII disorder and 2 with BD NOS. Mean (SD) age was 15.9 (1.4) years, and there were 4 girls and 4 boys. Six children had a comorbid diagnosis of attention-deficient hyperactivity disorder, 4 had oppositional defiant disorder, 2 had generalized anxiety disorder, 1 had conduct disorder, and 2 had a history of psychotic symptoms. There were 3 (37.5%) subjects taking adjunctive medications: 1 taking divalproex sodium, 1 taking methylphenidate, and 1 taking aripiprazole. Subjects were all Caucasian.

All subjects completed the study. Mean (SD) baseline CDRS was 53.0 (10.6); Week 8 CDRS was 26.3 (5.3). Mean (SD) final dose of lamotrigine was 131.3 (29.1) mg/day. All 8 subjects (100%) were considered responders by primary criteria, and 5 (62.5%) responded by secondary criteria. Remission, considered a 28 or less on the CDRS and Clinical Global Impression Scale-Severity of 1 or 2, was achieved by 5 out of 8 (62.5%) subjects.

### Behavioral response to IAPS stimuli

Subjects demonstrated satisfactory distinction between negative and neutral pictures, in the expected directions (baseline $t = 3.5$, df = 7, $p = 0.001$; Week 8: $t = 4.5$, df = 7, $p = 0.003$) (Table 1). There were no significant differences between mean ratings of valenced picture sets at baseline and after eight weeks of lamotrigine treatment (negative pictures: $t = 27$, df = 7, $p = 0.79$; neutral pictures: $t = 0.36$, df = 7, $p = 0.73$).

Table 1. Subjects' behavioral ratings of International Affective Picture System (IAPS) pictures

| Subject no. | Age (years) | Hand | Gender | Average rating[a] (baseline/follow up) | | |
|---|---|---|---|---|---|---|
| | | | | Positive | Neutral | Negative |
| 1 | 15.9 | R | M | 2.20/2.18 | 2.00/2.00 | 1.78/1.40 |
| 2 | 13.8 | R | M | 2.11/1.94 | 1.95/1.95 | 1.63/1.50 |
| 3 | 17.9 | R | F | 2.85/2.94 | 2.57/2.45 | 1.07/2.00 |
| 4 | 16.4 | L | M | 2.50/2.40 | 2.05/2.00 | 1.90/1.50 |
| 5 | 16.8 | R | M | 3.00/2.89 | 2.00/2.42 | 1.00/1.00 |
| 6 | 15.6 | R | F | 2.45/2.31 | 1.89/1.83 | 1.60/1.70 |
| 7 | 17.2 | R | F | 2.54/2.62 | 2.20/2.07 | 1.67/1.60 |
| 8 | 14.6 | R | F | 2.44/2.94 | 2.33/2.07 | 1.00/1.27 |
| Mean | 16.2 | | | 2.52/2.47 | 2.09/2.10 | 1.52/1.53 |

[a]Ratings based on 1 = negative; 2 = neutral; 3 = positive.

### fMRI activation

Greater activation in the left DLPFC (but not bilateral DLPFC) was significantly correlated with higher CDRS scores at baseline ($r$ = 0.766, p = 0.027). Correlations for CDRS score and activation of the right DLPFC and the right and left amygdala did not reach significance. No relationship was found between activation in the DLPFC and activation in the amygdala at baseline.

At Week 8, CDRS score was positively correlated with bilateral amygdalar activation ($r$ = 0.707, p = 0.05), an effect that was driven primarily by the correlation between CDRS score and activation in the left amygdala ($r$ = 0.766, p = 0.027) (Fig. 1). DLPFC activation was not correlated with CDRS score. However, left DLPFC activation was positively correlated with activation in the left amygdala ($r$ = 0.731, p = 0.04).

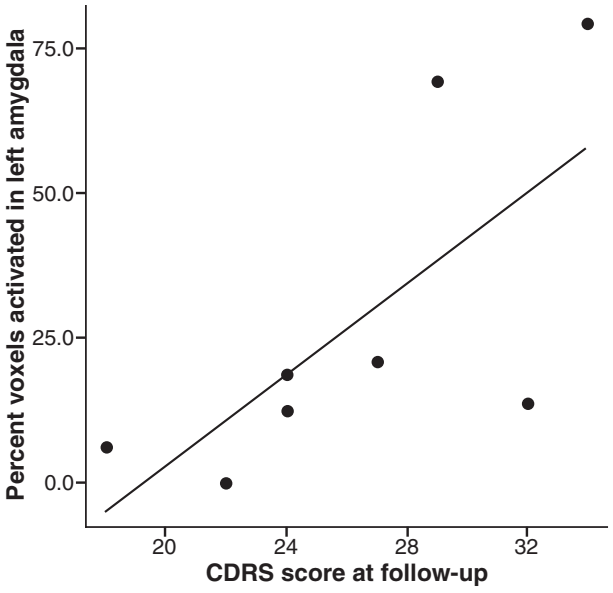


*Fig. 1.* Amygdalar activation versus Children's Depression Rating Scale (CDRS) score at Week 8.

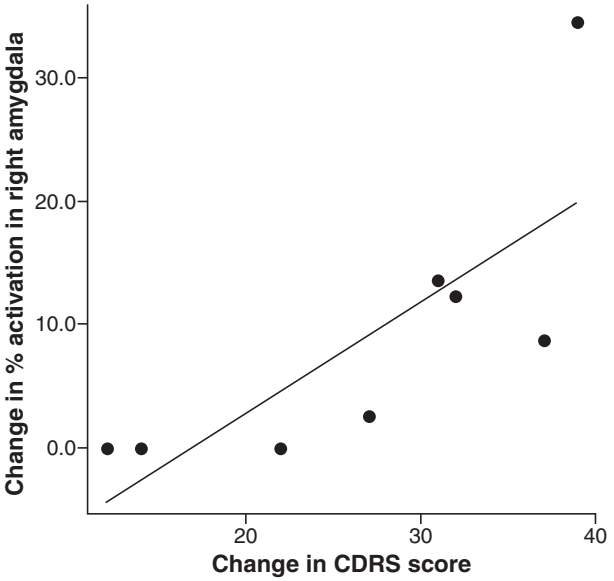


*Fig. 2.* Change in right amygdalar activation versus change in Children's Depression Rating Scale (CDRS) score over eight weeks.

For the comparison of baseline to Week 8 activation, greater decreases in CDRS score were positively correlated with greater decreases in bilateral amygdalar activation ($r$ = 0.738, p = 0.037) (Fig. 2). This effect was driven primarily by the right amygdala ($r$ = 0.878, p = 0.004). Change in DLPFC activation was not correlated with change in CDRS score. There were no significant correlations between change in activation between the DLPFC and the amygdala.

## Discussion

To our knowledge, the present study is the first to demonstrate neural changes after resolution of

bipolar depression. However, due to the relatively small sample size and lack of a control group, these findings should be considered preliminary. First, we found a positive correlation of level of amygdalar activation to negatively valenced pictures with severity of depressive symptoms. Next, we demonstrated that those subjects with greater improvement in depressive symptoms had greater decreases in amygdalar activation by eight-week follow-up. We were unable to directly test our hypothesis concerning 'successfully treated' subjects because all scanned subjects were responders, and thus we could not compare responders to non-responders.

Studies of adults with unipolar depression have indicated that the degree of depression is correlated with amygdalar activity (22, 23). It is possible that this correlation extends to bipolar depression as well. However, another fMRI study in pediatric BD did not find that amygdalar activity varied by mood state, although only 4 out of 22 subjects were depressed, limiting the power to detect differences between groups (12). Regarding the lack of such a correlation at baseline in the present study, it is possible that more severe depression results in a ceiling effect for amygdalar activation, so that more subtle variations in activation are not detectable. When we removed one outlier, the correlation became significant ($p < 0.04$), so this negative finding might be due to a type II error.

The correlation between resolution of depressive symptoms with degree of amygdalar deactivation is consistent with another study reporting that adults with BD taking mood stabilizers had less amygdalar activation than those not taking medications (10). Furthermore, studies of resolution of unipolar depression also indicate that relative decreases in limbic (including amygdalar) overactivity are seen with symptom resolution (24, 25). Thus, it appears that limbic deactivation associated with symptom resolution may be a consistent finding in both bipolar and unipolar depression.

We did not see a correlation of increased DLPFC activation with clinical improvement, as hypothesized, and DLPFC activation was correlated with degree of amygdalar activation at Week 8 only. In emotion regulation, prefrontal regions have been shown to correlate with degree of amygdalar activation (26, 27). Therefore, it is possible that, at baseline of the present study, when subjects were more depressed, the DLPFC was not contributing to regulation of mood and amygdalar activation as expected. This would also explain why DLPFC activation was *positively* instead of negatively correlated with degree of depression at baseline, contrary to our hypotheses. Week 8 findings might indicate that, after lamotrigine treatment and with clinical improvement, the synchronization between amygdalar and DLPFC activation was restored. Nonetheless, the relationship between DLPFC and amygdalar activation in depressed states is not clear (28), and there may be other reasons for this disconnect.

We cannot be sure whether lamotrigine directly decreased amygdalar activation, leading to resolution of depression. Alternately, lamotrigine may not have had a direct effect on the amygdala, but was involved in the resolution of depressive symptoms via other mechanisms. Lamotrigine may also have affected the hemodynamic response in areas around and including the amygdala. Additionally, as this was an open study of lamotrigine, subjects may have improved independent of lamotrigine.

The limitations of the present study include a small sample size, so some of our negative findings may have been due to insufficient power. However, the correlations found with only eight subjects were robust and consistent with our hypotheses. Furthermore, the power of the study was somewhat increased by performing within-subjects longitudinal comparisons. The effects of comorbid conditions could not be explored, again due to small sample size. We used percent instead of mean activation of our ROI, which tests for the amount of voxels activated instead of overall degree of activation, a subtle but potentially important difference. Decreases in amygdalar activation might have been due to habituation effects (29), but our within-group analyses correlating with depressive symptom change should be independent of any such effects. While concomitant medications are a concern, there were no changes in dose of these medications during the eight weeks of the trial, so it is not likely that these medications contributed to changes in activation. A healthy control group would help to clarify whether amygdala and prefrontal activation was greater than, similar to, or less than controls at baseline and follow-up. Further fMRI studies of placebo-controlled medication trials in bipolar depression are necessary to support and clarify these findings.

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