SUPPLEMENTARY INFORMATION

Title: "Regional fMRI Hypoactivation and Altered Functional Connectivity During Emotion Processing in Nonmedicated Depressed Patients With Bipolar II Disorder."

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Methods section

Participants

All participants completed the Structured Clinical Interview for DSM-IV Axis I Disorders, Research Version (SCID) (1). Subjects who met DSM-IV criteria for Bipolar II Disorder in a current Major Depressive Episode, and scored ≥ 22 on the 30-item Inventory of Depressive Symptomatology, Clinician Rated (2) were eligible to participate. Course of illness information (i.e., bipolar illness duration, prior history of hypomanic and depressive episodes) was obtained by self-report and confirmed by reference to psychiatric care records when available. Because persons with Borderline Personality Disorder are at risk of being misdiagnosed with bipolar disorder (3), subjects meeting borderline personality criteria using the Personality Diagnostic Questionnaire (4) and confirmed via a clinical interview were excluded. Bipolar subjects with a past history of alcohol or drug use disorder could participate if they were sober for >3 months, as confirmed by self-report and urine toxicology tests. Exclusion criteria for all subjects included left-handedness, head injury with loss of consciousness > 5 min, unstable medical illness (e.g., hyperthyroidism), ferrous metal implants, neurologic illness, pregnancy and current use of medications with psychotropic effects.

Experimental Stimuli and Paradigm

The functional MRI paradigm consisted of two experimental conditions: 1) "match emotions," where subjects selected one of two emotional faces that best matched the emotion of the target face and 2) "identify emotions," where subjects selected one of two presented words (e.g. *angry*, *afraid*) to describe an emotional target face. All target faces displayed a negative expression (anger or fear) and were selected from a standardized stimulus set (5). A control task, "match forms," involved subjects selecting one of two geometric shapes at the bottom of the screen matching a target shape above. To select their response, subjects held a button box in their right hand and pressed a left or right button corresponding to the spatial location of the stimuli displayed on the computer screen. The conditions were presented in nine "blocks": two blocks of each experimental condition and five blocks of the control condition. There were 6 trials per condition block, and each trial was shown for 5 seconds. Each block lasted 32.5 seconds for a total scan length of 4:58 min, including a 5 second rest at the beginning of the scan.

Functional Image Acquisition

Functional imaging data was acquired on a 3T Magnetom Allegra scanner (Siemens, Erlangen, Germany) using an echo planar T2*-weighted gradient echo sequence (TR: 2500 ms, TE: 35 ms, flip angle: 90°, matrix: 64 x 64, number of slices: 28, slice thickness: 3mm, in-plane resolution: 3.12 mm, 1mm gap). To allow for scanner equilibration, 2 TRs at the beginning of the scan were discarded. High-resolution structural images aligned to the anterior and posterior commissure were acquired with the following parameters: (TR: 5000 ms, TE: 33 ms, flip angle: 90°, matrix: 128 x 128, number of slices: 28, slice thickness: 3mm, in-plane resolution: 1.56, 1mm gap).

Behavioral Data Analysis

Accuracy and response times for all three conditions were calculated separately for each group. Since the response times were not normally distributed due to outliers, their group differences were assessed using the Mann-Whitney U test, a non-parametric analogue of the two-sample t-test. The accuracy measures could not reasonably be treated as continuous variables because only a few distinct values were observed. This appeared to be due to a ceiling effect with the majority of subjects making no errors. The accuracy scores were therefore dichotomized as high or low performance and group differences were assessed using Fisher's exact test.

Exploratory analyses of relationships between clinical variables and BOLD response

To investigate the relationship between ventrolateral prefrontal cortex or amygdala reactivity and bipolar II illness characteristics, we employed an anatomically-defined ROI analysis of our *a priori* regions defining the left and right inferior frontal gyrus (corresponding to Brodmann's areas 44, 45, and 47) using the Brodmann atlas from MRIcron (http://www.cabiatl.com/mricro/mricro/lesion.html#brod) (6). The Harvard-Oxford probabilistic atlas was utilized to create left and right amygdala masks (thresholded at 25% probability). The time course from these regions during "match emotions" versus "match forms" trials was extracted separately for each subject and used for the calculation of mean percent signal change using FEATQuery. Correlational analyses were conducted in SPSS to examine if activity in these regions correlated with illness characteristics ($P < 0.05$) in our patients. Exploratory clinical variables included: current depression severity (using the Hamilton Rating Scale for Depression); age of onset of bipolar illness; duration of bipolar illness, and duration of current

depressive episode. Prior to conducting these correlational analyses, consistent with recent fMRI work (7), an outlier correction was applied in which outlying values greater than 2.5 SDs were winsorized by moving the data point to 2.5 SDs from the group mean without that value included in the estimate of the mean.

Results section

Within-Group: whole-brain GLM results

Figure 1 displays within-group regional patterns of activation ($Z>2.0$, $p < 0.05$ corrected) for the "match emotions" versus "match forms" comparison. Consistent with previous studies using this paradigm, control subjects (**Figure 1A**) demonstrated robust activation in bilateral amygdalae (MNI xyz coordinates: -20, -2, -18, Z statistic=5.00; xyz: 28, 0, -22, Z=5.40) and bilateral inferior frontal gyri corresponding to Brodmann's area 47 (xyz: -48, 42, -14, Z=4.98; xyz: 44, 24, -4, Z=5.24), and Brodmann's area 45/46 (xyz: -48, 34, 8, Z=5.86; xyz: 54, 40, 8, Z=5.35). Bipolar II depressed subjects (**Figure 1B**) similarly demonstrated significant activation in bilateral amygdalae (xyz: -22, -6, -20, Z=4.64; xyz: 20, -6, -16, Z=4.72) and bilateral inferior frontal gyri corresponding to Brodmann's area 47 (xyz: -40, 32, -4, Z=3.34; xyz: 46, 28, -8, Z=3.63), and Brodmann's area 45 (xyz: -48, 22, 22, Z=6.05; xyz: 52, 28, 18, Z=5.21).

Additional activated brain regions in both groups included the right putamen as well as left and right regions of the thalamus, hippocampus, middle temporal gyrus, superior temporal gyrus, cingulate cortex, insula, fusiform gyrus, and occipital gyri.

Functional connectivity results

Within-Group Negative Connectivity

In the healthy comparison group, activity in the right amygdala was negatively correlated with ventral and dorsal ACC and other frontal regions including bilateral superior frontal gyrus

(BA 10), middle frontal gyrus (BA 8 and BA 10) and bilateral dorsolateral prefrontal frontal cortex (DLPFC) (BA 46) (**Figure 2B**; **Table 3**). That is, the higher the activation in these prefrontal/frontal cortical regions, the lower the activation in the amygdala or alternatively, the higher the activation in the amygdala, the lower the activation in these frontal regions. Activity in the right amygdala was also negatively correlated with posterior regions including the inferior and superior parietal lobules, as well as occipital regions including the precuneus and cuneus. For the bipolar depressed group, activity in the right amygdala was negatively correlated with a similar network as the control group but did not include the right superior frontal gyrus (BA 10) and right middle frontal gyrus (BA 10) (**Figure 2C**; **Table 3**).

Within-Group Positive Connectivity

In the control group, activity in the right amygdala activity was positively correlated with activity in portions of the temporal lobe including the hippocampus, superior temporal gyrus, left amygdala as well as putamen (**Figure 2B**; **Table 3**)**.** Right amygdala activity was also positively correlated with activity in the left insula and left ventrolateral prefrontal cortex (BA 47) (**Figure 2B**; **Table 3**). For the bipolar depressed group, amygdala activity was positively correlated with a similar network as the control but the bipolar subjects also displayed positive connectivity between the right amygdala and activity in both the right precentral gyrus and right postcentral gyrus (**Figure 2C**; **Table 3**).

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