Research Paper

Sad mood induction has an opposite effect on amygdala response to emotional stimuli in euthymic patients with bipolar disorder and healthy controls

Jiri Horacek, MD, PhD; Pavol Mikolas, MD; Jaroslav Tintera, CSc; Tomas Novak, MD, PhD; Tomas Palenicek, MD, PhD; Martin Brunovsky, MD, PhD; Cyril Höschl MD, DrSc; Martin Alda, MD

Background: Aberrant amygdala reactivity to affective stimuli represents a candidate factor predisposing patients with bipolar disorder (BD) to relapse, but it is unclear to what extent amygdala reactivity is state-dependent. We evaluated the modulatory influence of mood on amygdala reactivity and functional connectivity in patients with remitted BD and healthy controls. **Methods:** Amygdala response to sad versus neutral faces was investigated using fMRI during periods of normal and sad mood induced by autobiographical scripts. We assessed the functional connectivity of the amygdala to characterize the influence of mood state on the network responsible for the amygdala response. **Results:** We included 20 patients with remitted BD and 20 controls in our study. The sad and normal mood exerted opposite effects on the amygdala response to emotional faces in patients compared with controls ($F_{1,38}$ = 5.85, p = 0.020). Sad mood amplified the amygdala response to sad facial stimuli in controls but attenuated the amygdala response in patients. The groups differed in functional connectivity between the amygdala and the inferior prefrontal gyrus ($p \leq 0.05$, family-wise error–corrected) of ventrolateral prefrontal cortex (vlPFC) corresponding to Brodmann area 47. The sad mood challenge increased connectivity during the period of processing sad faces in patients but decreased connectivity in controls. **Limitations:** Limitations to our study included long-term medication use in the patient group and the fact that we mapped only depressive (not manic) reactivity. **Conclusion:** Our results support the role of the amygdala–vlPFC as the system of dysfunctional contextual affective processing in patients with BD. Opposite amygdala reactivity unmasked by the mood challenge paradigm could represent a trait marker of altered mood regulation in patients with BD.

Introduction

The neuronal mechanism of mood instability underlying the transitions from remission to mania or depression represents one of the most enigmatic and unexplained questions in bipolar disorder (BD). The residual mood instability,[1] recurrent course of BD and the fact that mood episodes are often triggered by an exaggerated response to emotional stimuli[2,3] indicate that the neuronal substrate for emotional dysregulation and vulnerability for mood episodes persists during remission, but the specific mechanisms responsible for episodic mood changes are yet to be elucidated.

Neuroimaging studies strongly support the role of the amygdala and ventrolateral prefrontal cortex (vlPFC) in emotional dysregulation in patients with BD.[4] The amygdala, a key structure for functional response to emotional stimuli, represents a candidate region for pathological emotional processing in patients with BD.[5] The vlPFC is involved in the top-down regulation of emotional responses in the amygdala.[4,6] Both structures are anatomically interconnected and form a functionally coupled system.[7] Dysfunction of this system may lead to dysregulated amygdala responses in patients with BD. However, neuroimaging studies of amygdala reactivity to emotional stimuli have produced inconsistent findings with decreased, increased or unchanged amygdala reactivity to emotional stimuli.[8] The reasons for these inconclusive results include the heterogeneity of clinical samples, differences in affective cues used for fMRI, neuroimaging analytical methods and, above all, the mood state during which patients were studied.[4] A recently proposed view is that amygdala reactivity follows a state-dependent course in patients with BD and is unchanged in euthymic states, increased in mania and decreased in depressive episodes.[9] To examine the effect of current mood

Correspondence to: J. Horacek, Prague Psychiatric Centre, Ustavni 91, 181 03 Prague 8, Czech Republic EU; horacek@pcp.lf3.cuni.cz

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state we decided to compare different mood conditions in the same individual during 1 scanning session — a design that can substantially reduce the variability of fMRI responses and elucidate the amygdala reactivity in patients with BD.

In our fMRI study we combined the standard affective processing paradigm with the method of transient mood induction to test a specific hypothesis of mood influence on amygdala reactivity. The method, in which transient intense mood is induced by a short autobiographical script, has been validated for neuroimaging studies in patients with major depressive disorder (MDD) and BD in full remission and has been shown to unmask apparent depression trait markers.[10,11]

Our study aimed to answer 2 questions related to the modulation of emotional processing in the amygdala. First, to test the influence of specific mood conditions on emotion processing in the amygdala, participants were exposed to a series of emotional faces within periods of sad and normal mood. Second, to identify the networks responsible for amygdala reactivity to affective stimuli, we analyzed the effects of induced mood on functional connectivity between the amygdala and the whole brain.

We hypothesized that induced sadness has a different impact on amygdala response to affective stimuli and on amygdala functional connectivity in patients with BD and healthy controls.

Methods

Participants and behavioural measures

We recruited participants with BD type I, diagnosed independently by 2 experienced psychiatrists (J.H., T.N.) according to DSM-IV criteria using the Structured Clinical Interview — Patient Version (SCID-I/P), from among patients prospectively followed at the Prague Psychiatric Centre, Prague, Czech Republic. At the time of the study, patients had to have been in clinical remission for a period of at least 3 months, verified by ongoing follow-up at the centre and by mood ratings on the day of scanning. Patients with a history of substance abuse, neurologic disease or head injury, an additional psychiatric diagnosis or a medical disorder requiring active treatment were excluded.

Healthy controls were recruited via local advertisement; they had a similar sociodemographic background as the patients with BD, to whom they were matched for age, education and sex. The exclusion criteria for healthy controls were a personal history of any psychiatric disorder or substance abuse and a mood disorder (e.g., BD, MDD) in first- or second-degree relatives.

Affective symptoms on the day of the experiment were assessed using the Young Mania Rating Scale (YMRS),[12] the Montgomery-Åsberg Depression Rating Scale (MADRS)[13] and the Beck Depression Inventory (BDI).[14]

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki. We obtained written informed consent from all participants, and the local ethics committee approved the study.

Procedure of mood induction

For emotional stress modelling we used the technique of autobiographical script,[11] which has been successfully applied in a neuroimaging study of BD.[10] To induce transient intense sadness and a normal (control) mood emotional condition, the participants were asked to prepare 2 short autobiographical scripts: 1 describing a specific sad life event and 1 describing an emotionally neutral event, defined as a specific situation when the participant felt "calm and sense of well-being" (but not happiness or pleasure). Both scripts were tested before scanning to ensure reliability and reproducibility in inducing a sad or normal mood in all participants. To ensure that the control emotion condition was not affected by preceding sadness induction, the neutral script was always presented first. The scripts were displayed through an LCD projector while the participant lay in the scanner until he/she indicated (by pressing a button) that the peak emotion, defined as 6 or 7 on the self-rating Likert scale (0 = emotion is not expressed; 7 = emotion is very much expressed), was reached. Subsequently, the script projection was turned off, and participants were instructed to stay with the emotion but not to actively ruminate on the script content or to cognitively modulate the target feelings.

Affective stimulation and fMRI data acquisition

Imaging was performed using a 3 T Siemens Trio MRI scanner. All participants had their heads firmly immobilized in a 12-channel head coil using foam inserts positioned on either side of the head.

For affective stimulation of the amygdala (within the periods of induced sadness and normal mood), we applied the standard fMRI paradigm of emotional faces processing with contrasting sad versus neutral expression. This contrast exerted very specific amygdala activation compared with other emotional facial expressions (e.g., anger, happiness, fear) or alternative contrasting to fixation.[15] The set of black and white images of male and female actors from the Erwin database[16] making neutral or sad facial expressions were displayed in a block design. Participants were instructed to empathize with the emotional faces displayed by the LCD projector. The sad and neutral faces were presented in 8 alternating 30-second conditions, 4 with sad and 4 with neutral emotions. Each face was presented for 6 seconds, with a 2-second interscan interval (ISI; 3 scans per face).

The fMRI of emotional face expression acquisitions (T_2*-weighted gradient echo-planar imaging sequence) of 30 axial slices (3 mm thickness, no gap) parallel to the anterior and posterior commissure covering the whole brain were imaged as follows: temporal resolution 2 seconds, echo time (TE) 30 ms, repetition time (TR) 2000 ms, matrix 64 × 64, bandwidth 2790 Hz/pixel, flip angle 70°. The field of view (FOV) was 192 mm and the effective inplane functional spatial resolution was 3×3 mm^2. We collected a total of 120 whole brain scans for both measurements. The total acquisition time of each fMRI was 4 minutes, 8 seconds.

To avoid a practice effect, we used 2 different sets of facial images from the same database. We compared the adequacy

of both sets before the experiment in an independent group of 61 volunteers (15 male, 46 female, mean age = 31.4 ± 16.1 yr) who rated each face by the 100 mm analogue scale, with sad and happy as extremes and neutral marked in the middle. We did not detect any difference between the 2 sets of emotional stimuli (means 41.5 ± 0.9 and 39.8 ± 1.0, $t_{58} = 1.27$, $p = 0.21$). Immediately after the second block of fMRI the induced sadness was rated by the same Likert scale to document the ability to keep the induced sadness during fMRI acquisition (Fig. 1).

For the localization of the activated voxels in the amygdala and fMRI data preprocessing the participants were scanned using a T_1-weighted 3D-MPRAGE sequence: TR 2.3 seconds, TE 4.6 ms, bandwidth 130 Hz/pixel, FOV 256 mm, matrix 256 × 256, slice thickness 1 mm, 160 contiguous sagital slices, voxel size 3 × 1 mm, parallel acquisition technique reduction factor 2, total acquisition time 4 minutes, 44 seconds.

Functional MRI data analyses

Emotional face amygdala activation was analyzed first using Statistical Parametric Mapping software version 5 (SPM5; www.fil.ion.ucl.ac.uk/spm/software/spm5) implemented in MATLAB version 7.3 (MathWorks). Statistical analysis of data across all participants proceeded by entering each participant's preprocessed functional data (realigned to correct for movement, normalized and smoothed using a 6 × 6 × 6 mm Gaussian kernel) into a generalized linear model. Low-frequency noise was removed using a highpass filter (128 seconds). The first-level design matrix contained factors modelling regressors for the neutral and sad faces conditions convolved with a canonical hemodynamic response function. The contrast images (linear combinations of β images) were then subjected to second-level analyses to determine task-specific regional responses in the amygdala at a group level.

Next, to test our a priori hypothesis, we analyzed amygdala activity using 2 alternative approaches. First, we calculated the parameter estimates (mean β values) representing percentage blood oxygen level–dependent (BOLD) signal differences between the sad and neutral faces blocks for all conditions for the whole amygdala as a region of interest (ROI) defined according to the automated anatomical labelling (AAL) atlas.[17] The overlap of ROI with the individual anatomic position of the amygdala was verified on normalized T_1-weighted images in all participants. Second, the full factorial SPM5 model was used to identify the local maxima of significant interaction between conditions (scripts) and groups as factors within the ROI of the bilateral amygdala. For this purpose, we accepted a statistical threshold of $p < 0.05$ for voxels within the volume of amygdala. This approach has been successfully applied in previous analyses of modulatory influences on amygdala reactivity.[18]

We analyzed the β values for both the whole amygdala and the local maxima using analysis of variance (ANOVA) to determine mood challenge–specific regional responses at the group level. We used a repeated-measures model with group, laterality of amygdala (left v. right) and condition (normal v. sad mood challenge) as factors to compare amygdala activation between patients and controls (parameter estimates for sad v. neutral facial expression pictures).

Functional connectivity was analyzed for both neutral and sad face blocks following each mood induction using a seed-driven approach with CONN connectivity software (www.nitrc.org/projects/conn/). Data were preprocessed with the same parameters as for SPM5. Physiologic and other spurious sources of noise (signal from a region in the cerebrospinal fluid, white matter and the whole brain signal) were estimated using the implemented component-based method and removed together with movement-related covariates. The residual BOLD time series were band-pass filtered over a low-frequency window of interest (0.004–0.08 Hz).

Correlation maps produced by computing Pearson correlation coefficients between the residual BOLD time course from the bilateral whole amygdala conjunction seed and all other grey matter voxels were converted to normally distributed Z scores using Fisher transformation. The Z maps were submitted to a random-effects full factorial model to address the interaction between the groups, mood challenge (normal v. induced sadness) and valence of emotional faces (neutral v. sad) using a family-wise error (FWE)–corrected threshold of cluster level $p < 0.05$. For β values extracted from the regions of significant interaction, we performed Tukey post hoc tests to further investigate within-subjects and between-group differences in amygdala connectivity.

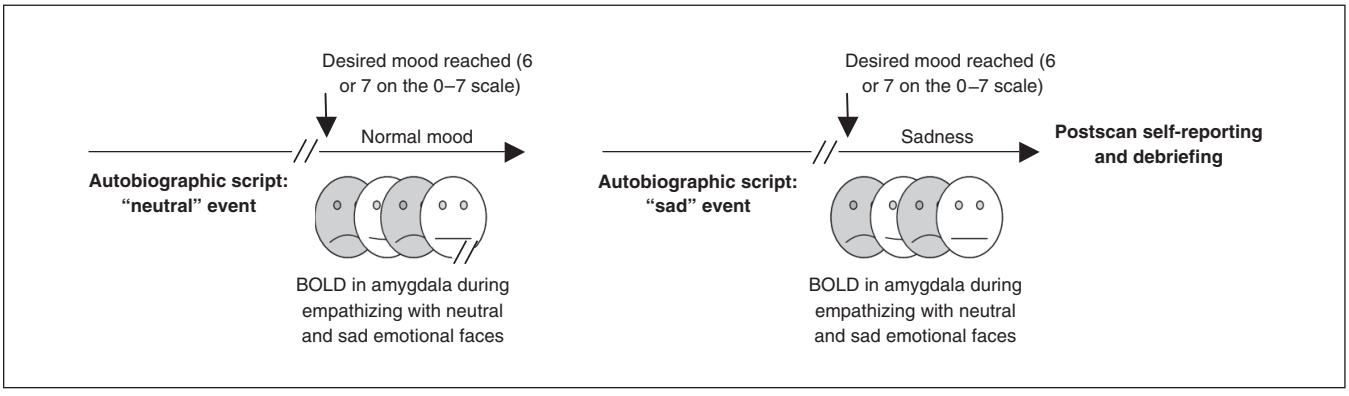


Fig. 1: Protocol for mood induction and fMRI affective stimulation. BOLD = blood oxygen level–dependent.

Behavioural measure statistics

We compared age; education in years; and Likert scale, MADRS, BDI and YMRS scores between patients and controls using independent sample t tests. Sex distribution was compared using the χ^2 test.

Results

Participants and behavioural measures

We included 20 patients with BD and 20 controls matched for age, sex and education in our study. Patients and controls did not differ in demographic characteristics (Table 1); all participants were right-handed. All patients with BD were on long-term medication (Table 1).

The mood-provocation protocol produced the desired behavioural effect; all participants reached scores of 6 or 7 on a Likert scale in both normal and sad conditions and remained in a state of substantial sadness throughout the fMRI following the sad mood challenge; this was verified by postscan self-reporting and debriefing. Sadness rated on the Likert scale at the end of fMRI remained substantial without significant difference between the groups ($t_{38} = 0.47$, $p = 0.64$, Table 1). All participants confirmed that they successfully avoided active ruminations or attempts to actively modulate their negative mood state after the script was turned off and that they were able to focus on empathizing with the emotional faces.

The influence of mood challenge on amygdala response to affective stimuli

The sad and normal mood inductions by emotional faces (sad v. neutral) exerted opposite effects on amygdala activation in patients with BD compared with controls. Transient sadness increased the activation by sad compared with neutral faces in the whole amygdala in controls and decreased the activity in patients with BD (Fig. 2). This result is reflected in the significant group × condition interaction ($p = 0.020$; Table 2).

Neither the individual factors (group: $p = 0.87$; condition: $p = 0.76$; laterality of amygdala: $p = 0.06$) nor the interactions of laterality of the amygdala (group × laterality: $p = 0.62$; condition × laterality: $p = 0.70$; group × condition × laterality: $p = 0.60$) were significant (Table 2). The post hoc tests did not yield any significant results.

In the second step, we identified the local maxima of significant interaction between the condition (scripts) and the group within the ROIs of the amygdala. The full factorial model with diagnosis and induced mood as factors revealed significant interactions for 1 cluster in the right (42 voxels, $t_{1,75} = 2.82$, $p = 0.003$) and the left (13 voxels, $t_{1,75} = 2.23$, $p = 0.016$) amygdala. Similar to the whole amygdala, we detected a significant group × condition interaction ($p < 0.001$) and no effects of individual factors (group: $p = 0.90$; condition: $p = 0.58$; laterality: $p = 0.95$). We did not find any significant interactions with laterality (group × laterality: $p = 0.74$; condition × laterality: $p = 0.94$; group × condition × laterality: $p = 0.84$, Table 2).

The within-group post hoc comparisons confirmed that in patients with BD the sad autobiographical script decreased amygdala activation by sad faces in the right ($t_{19} = 3.09$, $p = 0.006$) and the left ($t_{19} = 2.80$, $p = 0.011$) amygdala. The direction for the opposite effect of the sad script was detected in the control group, but it did not reach statistical significance ($t_{19} = 1.68$, $p = 0.11$ in the right and $t_{19} = 1.68$, $p = 0.11$ in the left amygdala).

Between-group comparisons showed that amygdala activation was greater in patients than controls after the neutral script ($t_{38} = 2{,}07$, $p = 0.001$ for the right and $t_{38} = 2.55$, $p = 0.015$ for left amygdala), but sad mood induction led to lower right amygdala activation in patients ($t_{38} = 2.07$, $p = 0.05$), with the same direction on the left side ($t_{38} = 1.71$, $p = 0.15$).

Amygdala functional connectivity during induced sadness and normal mood

The full factorial analysis with group, emotional challenge, laterality and facial expression at the whole brain level showed a significant interaction in the left (Montreal

Table 1: Demographic characteristics and behavioural results.

Characteristic	Group, mean ± SD*		p value
	Control, $n = 20$	BD, $n = 20$	
Sex, men:women	7:13	7:13	> 0.99
Age, yr	39.1 ± 13.2	41.9 ± 12.9	0.64
Education, yr	17.1 ± 2.5	17.2 ± 3.8	> 0.99
Age at onset of BD, yr	—	23.2 ± 6.8	—
Lithium/valproate/carbamazepine/lamotrigine, no.	—	11/4/2/2	—
Atypical antipsychotics/antidepressants, no.	—	5/5	—
Sadness rating postscanning	4.9 ± 1.2	4.7 ± 1.4	0.64
MADRS score, mean ± SD (IQR)	2.0 ± 2.9 (0–2)	2.5 ± 3.5 (0–3.5)	0.59
BDI score, mean ± SD (IQR)	1.9 ± 2.4 (0–4)	3.3 ± 4.5 (0–5.5)	0.23
YMRS score, mean ± SD (IQR)	1.6 ± 2.8 (0–2)	1.4 ± 2.2 (0–2)	0.80

BD = bipolar disorder; BDI = Beck Depression Inventory; IQR = interquartile range; MADRS = Montgomery–Åsberg Depression Rating Scale; SD = standard deviation; YMRS = Young Mania Rating Scale.
*Unless otherwise indicated.

Neurological Institute [MNI] space: x, y, z = –38, 22, –18; 81 voxels) and right (MNI space: x, y, z = 46, 28, –08; 71 voxels) inferior frontal gyrus (IFG), corresponding to the Brodmann area (BA) 47 (cluster $p < 0.05$, FWE-corrected; Fig. 3 and Table 2).

For the regions of significant interaction, we performed Tukey post hoc tests to identify the influence of the facial expression valence and mood challenge on connectivity with group differences tested for corresponding conditions. In healthy controls, the sad mood induction bilaterally decreased the functional connectivity between the amygdala and clusters in the IFG ($p = 0.049$ for the left and $p = 0.001$ for the right IFG) during exposure to sad faces and increased connectivity between the right amygdala and the ipsilateral IFG cluster ($p = 0.002$) during exposure to neutral faces (Fig. 3).

The effect of sad mood induction was different in patients with BD. The sad mood challenge increased connectivity during the period of sad face processing compared with normal mood induction (significant on the left, $p = 0.034$) and neutral faces displayed during sadness induction (significant on the right, $p = 0.040$).

The opposite effects of sad mood challenge in patients and controls correspond with between-group comparisons. During sad face processing, patients showed lower functional connectivity than controls under the normal mood (significant on the left, $p = 0.044$), but greater functional connectivity than controls under the sad mood challenge bilaterally, although more prominently on the right side ($p = 0.013$ on the right and $p = 0.05$ on the left side; Fig. 3). Interestingly, for the control task of neutral faces, both groups differed significantly only after sad mood induction (lower connectivity in patients than controls on the right side, $p = 0.006$, Fig. 3).

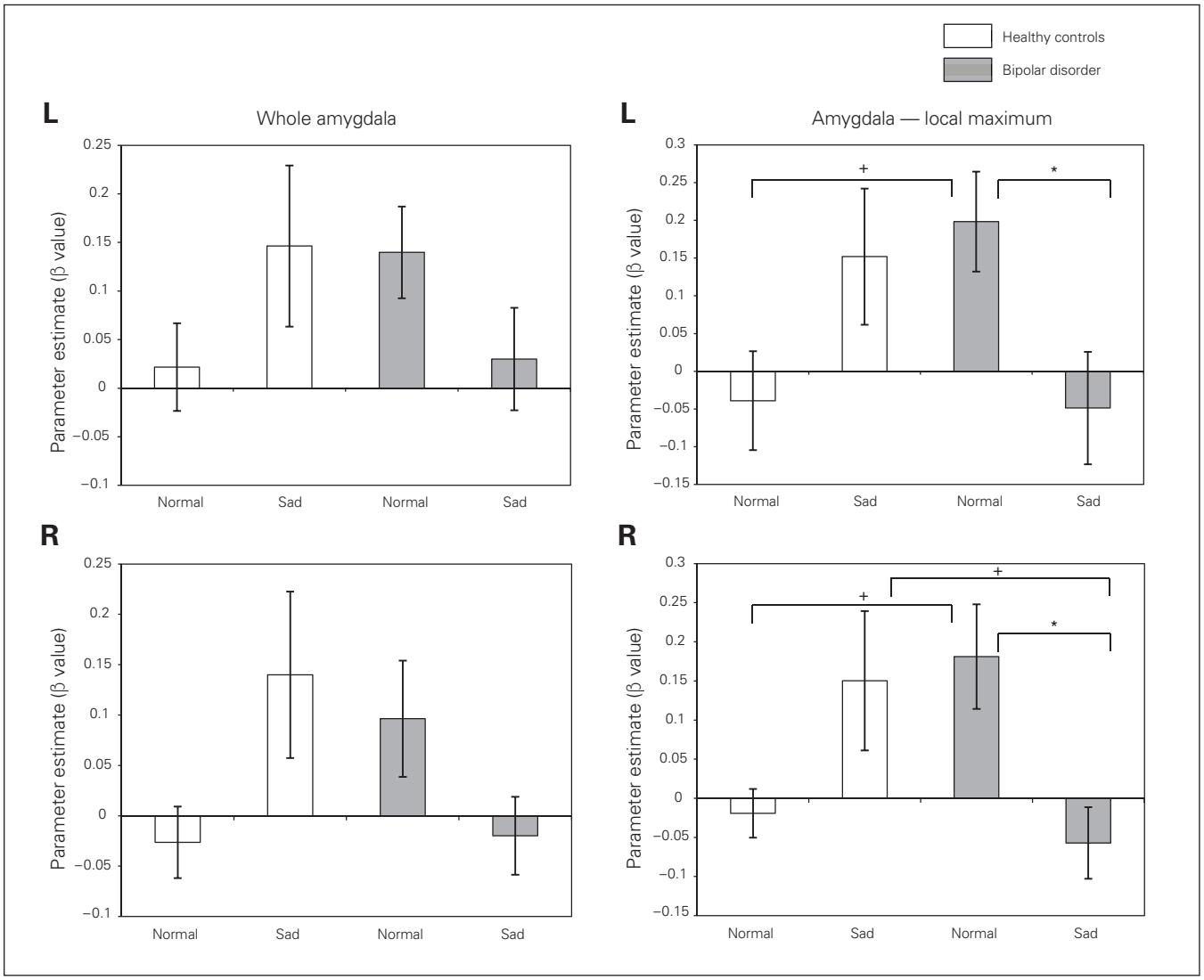


Fig. 2: Effect of mood challenge on amygdala activation by emotional faces. Parameter estimates (β values) for regions of interest (ROIs; whole amygdala and local maxima of the effect) representing percentage blood oxygen level–dependent (BOLD) signal differences between sad and neutral faces in the control (white bars) and bipolar disorder (BD; grey bars) samples. *$p < 0.05$ for within-group post hoc tests, †$p < 0.05$ for between-groups. Error bars represent standard errors of the mean.

Discussion

In this study we found that patients with BD and healthy controls had opposite amygdala responses to the mood challenge. The sad mood provocation amplified amygdala response to sad facial stimuli in controls but attenuated the amygdala response in patients with BD. This finding indicates that BD, even in remission, could be characterized by specific patterns of interaction between emotional setting (mood) and the processing of current emotional stimuli (affect). These results correspond with both the concept of trait dysregulation of the corticolimbic network in patients with BD[20] and with the recently formulated assumption that amygdala reactivity in patients with BD follows a state-dependent course with decreased response to negative affective stimuli in depression.[9] It has been shown repeatedly that depression in individuals with BD type I[21] and in those with BD type II[22] is characterized by amygdala hypoactivation during negative affect face processing. Similarly, induced sad mood in our experiment attenuated the amygdala response in patients with fully remitted BD and mirrored bipolar depression. Therefore, blunted amygdala activation may represent a state marker of a substantial change of amygdala reactivity in both bipolar depression and experimentally induced sadness. This finding could also clarify the inconsistencies among previous fMRI studies focused on amygdala response to facial emotion activation.[8]

To elucidate the network regulation responsible for the opposite reactivity of the amygdala in patients with BD, we analyzed the functional connectivity of the amygdala. We identified a significant group × condition interaction in the vlPFC (IFG), which is considered essential for top–down emotional regulation.[6,9] Interestingly, a meta-analysis of 50 primary fMRI studies has documented that patients with BD, regardless of current mood state, exhibit underactivation in the right IFG during both emotional and cognitive tasks.[8] The role of the IFG in BD is also supported by morphometric studies that replicated reduced right IFG volumes in patients with BD.[23–27,32,38] The vlPFC connections to the amygdala are critical for regulation of amygdala activation, and decoupling of these regions has been demonstrated in relation to emotion dysregulation.[28] The recent model assumes that BD arises from disruption in early brain development leading to decreased functional and/or structural connectivity between the vlPFC and amygdala.[5,28] However, the functional connectivity between both regions depends also on the valence of emotional stimuli.[29,30]

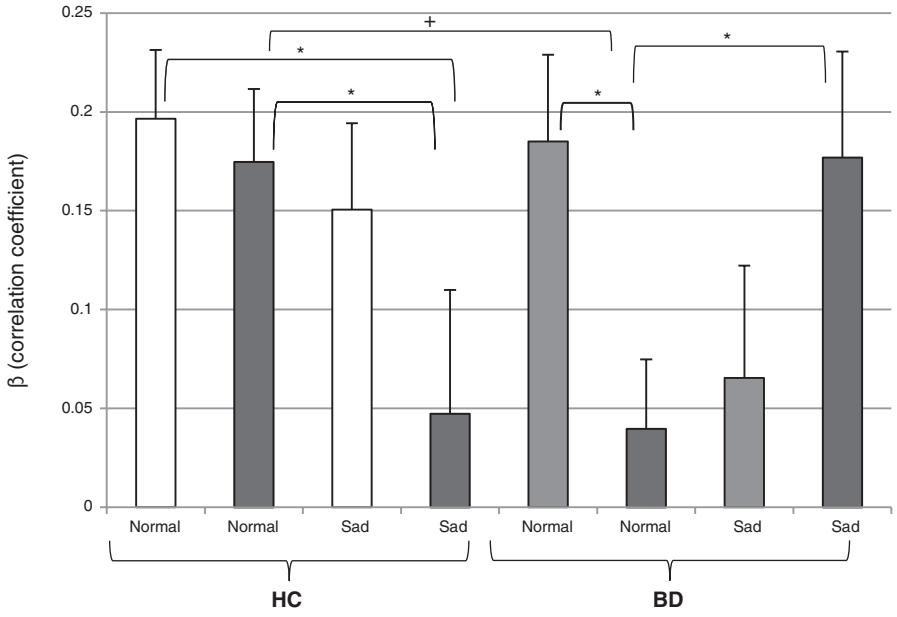
We showed that coupling of the amygdala and IFG in patients with BD was differently influenced by both emotional valence of facial stimuli and mood. In healthy controls, sad mood induction reduced the functional connectivity between the amygdala and IFG (bilaterally) during exposure to sad faces. The opposite was detected in patients with BD, in whom the sad mood challenge increased connectivity during the period of sad face processing with a subsequent blunted amygdala response. We need to stress, however, that our data alone cannot resolve the direction of altered functional connectivity; for instance, the decreased coupling of vlPFC and the amygdala in controls during the sad mood challenge could be responsible for the exaggerated amygdala response to sad stimuli. Taken together, our data are congruent with the assumption that the amygdala–vlPFC connectivity in patients with BD is state-dependent. During periods of sad face processing, patients with BD had lower connectivity after the neutral script but increased connectivity during sad mood induction (Fig. 3). Our observation that sad mood induction in patients with BD increases amygdala–vlPFC connectivity in response to sad stimuli is in line with the finding that depressed but not euthymic patients with BD have greater amygdala–vlPFC connectivity than controls, specifically during exposure to sad faces.[29]

Several studies of BD have documented increased connectivity in a resting state.[20,31] However, the fact that amygdala–vlPFC

Table 2: Repeated-measures ANOVA results of amygdala activation (parameter estimates) at the level of the whole amygdala, the local maxima of the effect, and the effect of mood challenge on amygdala–IFG functional connectivity identified at the whole brain level.

Activation, source of variation	Statistic	*p* value
Whole amygdala		
Between-subjects		
Group	$F_{1,38} = 0.03$	0.87
Within subjects		
Laterality	$F_{1,38} = 3.67$	0.06
Condition	$F_{1,38} = 0.09$	0.76
Laterality × group	$F_{1,38} = 0.25$	0.62
Laterality × condition	$F_{1,38} = 0.15$	0.70
Condition × group	$F_{1,38} = 5.85$	0.020
Laterality × condition × group	$F_{1,38} = 0.28$	0.60
Local maxima of amygdala activation		
Between-subjects		
Group	$F_{1,38} = 0.01$	0.90
Within-subjects		
Laterality	$F_{1,38} = 0.00$	0.95
Condition	$F_{1,38} = 0.32$	0.58
Laterality × group	$F_{1,38} = 0.11$	0.74
Laterality × condition	$F_{1,38} = 0.01$	0.94
Condition × group	$F_{1,38} = 14.40$	< 0.001
Laterality × condition × group	$F_{1,38} = 0.04$	0.85
Functional connectivity between amygdala and IFG (vlPFC)		
Between-subjects		
Group	$F_{1,114} = 0.47$	0.50
Within-subjects		
Laterality	$F_{1,114} = 1.76$	0.19
Condition	$F_{3,114} = 0.78$	0.51
Laterality × group	$F_{1,114} = 0.19$	0.67
Laterality × condition	$F_{3,114} = 2.43$	0.07
Condition × group	$F_{3,114} = 7.41$	< 0.001
Laterality × condition × group	$F_{3,114} = 0.69$	0.56

ANOVA = analysis of variance; IFG = inferior frontal gyrus; vlPFC = ventrolateral prefrontal cortex.



x,y,z	k (vx)	*p* (FWE)
-38, +22, -18	81	0.024
+46, +28, -08	71	0.043

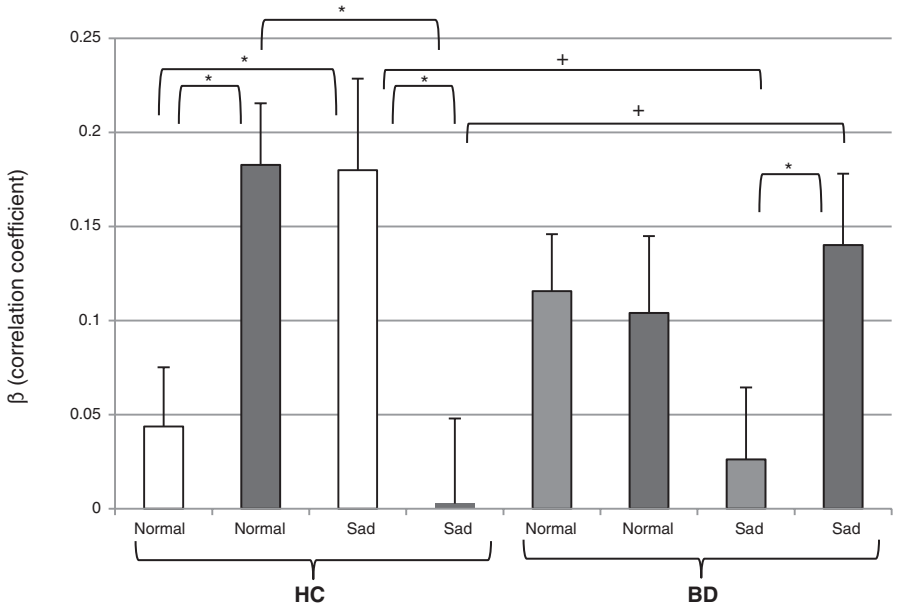


Fig. 3: Effect of mood challenge on functional connectivity with the amygdala as a seed during emotional face processing. Middle panel: The whole brain *F* test results with significant regions of interaction (group × emotional challenge × laterality × emotional faces) in left and right inferior frontal gyrus (IFG; Brodmann area 47). The graphs show the *Z* scores of functional connectivity between left (upper panel) and right (lower panel) amygdala and corresponding IFG clusters during exposure to neutral and sad faces. Coordinates of the regions of interaction are in Montreal Neurological Institute space. BD = bipolar disorder; HC = healthy controls; k (vx) = number of voxels. **p* < 0.05 for within-group post hoc tests, †*p* < 0.05 for between-groups. *p* values are family-wise error (FWE)–corrected at the cluster level. Error bars represent standard errors of the mean.

coupling in our sample depended on mood induction does not support the explanation that the increased connectivity in patients with BD results from the sensitization of these connections.[20] If hyperconnectivity was a result of simple sensitization of anatomic connections, it would be detectable either in all conditions tested or specifically during exposure to sad emotional faces during both mood inductions. Considering the mood-affective stimuli interaction in our sample, it is also unlikely that aberrant white matter tracts from the vlPFC are responsible for amygdala dysregulation in patients with BD. Together our findings point to the vlPFC (specifically IFG) as the primary region of dysfunctional contextual affective processing in patients with BD leading to aberrant amygdala response. The crucial role of the vlPFC in these patients is supported by voxel-based morphometry studies that revealed the greatest effect size of grey matter reduction in the right IFG at the whole brain level.[27] The accelerated volume reduction of the right IFG correlating with disease burden[27,32] indicates that the IFG could be a candidate region for sensitization by mood episodes.

We can speculate that greater amygdala–vlPFC (BA 47) coupling in patients with BD within the periods of sad stimuli presented during a sad mood might be associated with abnormal overappraisal of such stimuli at the level of the vlPFC with subsequent blunted amygdala response in this condition (Fig. 2). An alternative explanation is that patients with BD exposed to a sad mood challenge process sad and neutral stimuli in a similar way and that the vlPFC–amygdala system does not discriminate the emotional valence of stimuli. In other words, patients with BD lack the typical mood-congruent processing bias where sad mood facilitates the response to sad stimuli. In this regard, BD appears different from MDD, in which the mood-congruent processing bias in the amygdala (depression amplifies response to sad faces) has been consistently replicated.[33–36] Future research should clarify if the effect of mood on emotional stimuli activation observed in our sample is specific to sad faces and not to other negative facial emotions (e.g., anger, fear). This specificity for sad faces (v. fearful faces) in mood-congruent processing bias was identified in patients with MDD.[36]

The differences between patients with BD and healthy controls in vlPFC–amygdala coupling could be mediated also by a third region: the anterior cingulate (ACC). The ACC has been implicated in mood disorders, and it has been documented that this region could mediate the hyperconnectivity between the vlPFC and amygdala in patients with BD type I.[20] However, our amygdala connectivity analysis revealed only the IFG as significant. In a previous study, the mediatory role of the ACC in amygdala–vlPFC coupling was found in resting fMRI,[20] whereas in our study we used an affective activation fMRI paradigm. Further investigation is necessary to elucidate the role of the ACC (and other regions, such as the hippocampus) in the regulation of the vlPFC–amygdala complex. We did not find any substantial amygdala lateralization effect in our sample in terms of both activation and vlPFC connectivity, and the patterns of mood challenge × emotional stimuli interaction were similar on both sides. The lack of lateralized effect observed in our study could be related to the applied combination of sad mood challenge, which has exerted effect almost exclusively in the right hemisphere,[10] with the activation by sad faces (v. other negative faces), which is lateralized to the left amygdala.[15]

Limitations

There are several limitations to the present study. First, all patients with BD were stabilized on long-term medication. However, a recent systematic review documented that the impact of medications on imaging data in patients with BD is limited and that this impact, if any, is "normalizing" (i.e., medicated patients with BD are more similar to unmedicated controls, with a lower probability of type-I error).[37] Second, our study maps only 1 emotional pole of BD. We did not challenge the manic reactivity because happy mood induction is more problematic, has not been validated for mood disorders and might be difficult to achieve along with sad mood provocation during a single scanning session. The baseline neutral script was always presented before the sad mood challenge, and we cannot rule out the effect of order and carry-over effect. This is a tradeoff for being able to study the same participants in 2 different mood states during a single scanning session. However, the design was the same for all participants, and the very pronounced subjective emotional differences between both conditions were confirmed by postscan debriefing. We did not control for IQ, but its effect on our findings is unlikely because the groups did not differ in education level. Finally, we used an AAL atlas to define ROI for the amygdala and, despite the visual control, certain errors might occur. However, both local maxima of group × condition interactions within these ROIs were significant, even in the whole brain SPM analysis ($p < 0.05$). On the other hand, the less clearly anatomically bordered vlPFC regions were functionally defined strictly as clusters of significant group × condition interactions of functional connectivity with the amygdala at the whole brain level.

Conclusion

Our results of inverse amygdala reactivity to the mood challenge are in accordance with those of prior studies of bipolar depression. That the sad mood challenge attenuates amygdala response to sad stimuli in euthymic patients with BD suggests that a mood challenge can unmask the vulnerability to abnormal mood regulation in these patients and that they differ from patients with remitted MDD, which is characterized by mood-congruent processing bias. The changes in amygdala reactivity are paralleled by differences between patients with BD and healthy controls in the amygdala–vlPFC connectivity. The amygdala–vlPFC hyperconnectivity in response to negative (sad) stimuli during the mood challenge is congruent with previous findings in studies of bipolar depression and may be responsible for the blunted amygdala response to sad stimuli.

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Affiliations: Prague Psychiatric Centre, Prague, Czech Republic (Horacek, Mikolas, Novak, Palenicek, Brunovsky, Höschl); Third Faculty of Medicine, Charles University, Prague, Czech Republic (Horacek, Novak, Palenicek, Brunovsky, Höschl, Alda); Department of Psychiatry, Dalhousie University, Halifax, Canada (Horacek, Alda); Institute for Clinical and Experimental Medicine, Prague, Czech Republic (Tintera).

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