Neural underpinnings of emotion regulation subgroups in remitted patients with recently diagnosed bipolar disorder

Hanne Lie Kjærstad\textsuperscript{a,}*\textsuperscript{*}, Viktoria Damgaard\textsuperscript{a,}\textsuperscript{b}, Gitte M. Knudsen\textsuperscript{c,}\textsuperscript{d}, Maj Vinberg\textsuperscript{a,}\textsuperscript{d,}\textsuperscript{e}, Lars Vedel Kessing\textsuperscript{a,}\textsuperscript{d}, Julian Macoveanu\textsuperscript{a}, Kamilla W. Miskowiak\textsuperscript{a,}\textsuperscript{b}

\textsuperscript{a}Copenhagen Affective Disorder research Centre (CADIC), Psychiatric Centre Copenhagen, Copenhagen University Hospital, Rigshospitalet, Denmark
\textsuperscript{b}Department of Psychology, University of Copenhagen, Denmark
\textsuperscript{c}Neurobiology Research Unit, Copenhagen University Hospital, Rigshospitalet, Denmark
\textsuperscript{d}Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark
\textsuperscript{e}Mental Health Center, Northern Zealand, Copenhagen University Hospital - Mental Health Services CPH, Copenhagen, Denmark

Received 24 November 2021; received in revised form 13 April 2022; accepted 19 April 2022

KEYWORDS
Bipolar disorder; fMRI; Cluster analysis; Subgroups; Emotion regulation

Abstract
Neuroimaging studies of bipolar disorder (BD) generally involve comparison with healthy controls (HC), which may mask neurobiological variability within the disorder. This study aims to assess the neural underpinnings of potential subgroups of BD patients based on functional activity in the emotion regulation network and its relation to illness characteristics and relapse risk. Eighty-seven remitted patients with recently diagnosed BD and 66 HC underwent functional magnetic resonance imaging (fMRI) while performing an emotion regulation task. Patients were re-assessed with clinical interviews after 16 (±5) months. Data-driven hierarchical cluster analysis was employed to investigate “neuronal subgroups” of patients based on their neuronal activity in a pre-defined emotion regulation network. Relations between neuronal subgroups and illness characteristics and relapse rates were examined. Patients were allocated into two subgroups. Subgroup 1 (n=62, 75%) was characterized by exaggerated bilateral amygdala reactivity but normal prefrontal and temporo-parietal activation. Subgroup 2 (n= 22, 25%) showed widespread hypo-activity within all emotion regulation regions. Both subgroups were less successful at down-regulating their emotions than HC ($F(2,146)=5.33, p=.006, \eta_p^2=.07$). Patients

* Corresponding author.
E-mail address: hanne.lie.kjaerstad@regionh.dk (H.L. Kjærstad).

https://doi.org/10.1016/j.euroneuro.2022.04.010
0924-977X/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)
1. Introduction

Bipolar disorder (BD) is a neuropsychiatric disorder characterized by recurrent mood episodes of depression and (hypo)mania (Association American Association, 2013). The distinctive symptoms of BD, including prominent mood swings and affect lability, suggest underlying difficulties with emotion regulation (Phillips et al., 2003; Townsend and Althusler, 2012). Investigating patients’ emotion regulation abilities and their neural underpinnings may thus provide insight into the pathophysiology of BD and inform new treatment approaches.

Successful emotion regulation relies on the interaction between emotion-generating limbic structures (mainly the amygdala) and the prefrontal cortex (PFC) implicated in top-down cognitive control (Buhle et al., 2014). In healthy individuals, voluntary regulation of negative affect is associated with increased PFC activity and decreased amygdala activity, which points to efficient PFC down-regulation of amygdala response to emotional stimuli (Buhle et al., 2014; Frank et al., 2014; Kohn et al., 2014). Neuroimaging studies of patients with BD demonstrated aberrant fronto-limbic activity during emotion regulation in both acute mood episodes and in remission (Kurtz et al., 2021; McTeague et al., 2020; Pico-Pérez et al., 2017; Townsend and Althusler, 2012; Zilverstand et al., 2017), although findings are inconsistent. Specifically, some studies revealed heightened amygdala response coupled with PFC hyper-activity during down-regulation of emotional response to affective stimuli (Corbalán et al., 2015; Morris et al., 2012; Rive et al., 2015), whereas others detected task-related prefrontal hypo-activity (Kjaerstad et al., 2021; Sankar et al., 2021; Townsend et al., 2013; Zhang et al., 2020). This discrepancy is likely due to differences in BD samples across studies (i.e., BD type I vs. II and early vs. late illness stages) rather than differences in applied emotion regulation paradigms (which most often involved IAPS pictures). Together, these findings suggest that aberrant recruitment of regulatory PFC resources (evidence by hyper- or hypo-activity) contributes to patients’ difficulties with emotion regulation.

Emerging evidence indicates that BD patients show heterogeneity within emotion regulation abilities (Kjaerstad et al., 2019; Varo et al., 2021). At the behavioral level, our group identified subgroups in patients with mood disorders based on their emotional cognition profile. Here, we found that patients who presented with a generally ‘emotionally blunted’ profile were less successful in down-regulating emotions in pleasant social scenarios compared to ‘emotionally preserved’ patients and healthy controls (HC) (Varo et al., 2021). Further, we demonstrated that cognitively impaired BD patients were less successful in down-regulating their emotions compared to cognitively intact patients and HC (Kjaerstad et al., 2019). Taken together, this suggests that BD is associated with differential impairments in emotional cognition. However, there is a paucity of studies investigating variability in neuronal activity within emotion regulation circuitries in BD. One functional magnetic resonance imaging (fMRI) study of 86 remitted patients with BD type I and 80 HC by Njau and colleagues revealed two distinct subgroups based on differential emotion regulation circuitry activations (Njau et al., 2020). Specifically, one subgroup showed increased amygdala and VLPFC activations during reappraisal of unpleasant stimuli, whereas the second subgroup displayed blunted activity in the amygdala and several prefrontal regions (Njau et al., 2020). Accordingly, subgroup 1 was associated with more hospitalizations for depression compared to subgroup 2 (Njau et al., 2020). However, this is the only published study of variability in neuronal activation patterns during emotion regulation in BD-I and the findings therefore warrant replication in a broader group of patients with BD type I and II in addition to investigation of behavioral measures of emotion regulation in relation to patients’ neural response. The identification of differential neural subgroups carries the potential to reveal potential biological variation within BD, which may account for some of the heterogeneity observed in bipolar symptomatology.

The aim of the present fMRI study was therefore to investigate the variability in the neural underpinnings of emotion regulation in sample of recently diagnosed fully or partially remitted patients with BD type I or II using a data-driven approach as well as its association with illness characteristics and relapse risk. We hypothesized that we would identify two distinct neuronal subgroups in line with the results from Njau et al. (2020) and that these would differ with respect to illness characteristics and subsequent relapse rates. Finally, we wanted to investigate the relations between emotion regulation and associated neural activity on patients’ overall functioning, quality of life, and childhood trauma, highlighting the clinical relevance of targeting emotion regulation abilities.
2. **Experimental procedures**

2.1. **Study design and participants**

The present study is a cross-sectional investigation of baseline data from the ongoing longitudinal Bipolar Illness Onset (BIO) study work package 3, which aims to investigate neurocognitive and neuroimaging biomarkers of BD (Kessing et al., 2017). Patients with BD were recruited exclusively from the Copenhagen Affective Disorder Clinic, where they were diagnosed with BD within two years prior to study enrolment. The clinical diagnoses were validated using the semi-structured interview the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (Wing et al., 1990), which was completed by MDs or MSc in psychology. For the present BIO-3 sub-study, patients were included in full or partial remission only, defined as a total score ≤ 14 both the Hamilton Depression Rating Scale (HDRS-17) (Hamilton, 1960) and the Young Mania Rating Scale (YMRS) (Young et al., 1978) and were diagnosed with BD according to the SCAN interview using International Classification of Diseases (ICD-10) criteria (World Health Organization, 1992). Patients’ daily use of psychotropic medication was recorded. Healthy control persons, with no personal or familial (up to first-degree relatives) history of mental disorders or substance abuse, were recruited from the University Hospital, Rigshospitalet, Blood Bank. Participants in the BIO-3 sub-study with a history of neurological disorder (including dementia), severe brain injury, severe somatic illness, daily use of benzodiazepines ≥22.5 mg, and/or substance abuse disorder were excluded. The study protocol was approved by the Committee on Health Research Ethics of the Capital region of Denmark (protocol number: H-7-2014-007) and the Danish Data Protection Agency, Capital Region of Copenhagen (protocol number: RHP-2015-023). Informed consent was provided by all participants prior to inclusion in the study.

2.2. **Measures**

2.2.1. **Emotion regulation paradigm**

During fMRI, participants performed a well-established paradigm of emotion regulation (Banks et al., 2007; Phan et al., 2005), involving the voluntary down-regulation of emotion elicited by 24 neutral and 48 unpleasant pictures from the International Affective Picture System (IAPS) (Lang et al., 1997). Participants were instructed to either simply view the images (“passive view” conditions) or try to lower their emotional response to unpleasant images (“decrease” condition). The paradigm thus comprised three conditions: passive view of neutral images, passive view of unpleasant images, and down-regulation of unpleasant images. Sets of four images were presented in each condition and different sets of unpleasant images were used in the passive view and decrease conditions, which were matched for valence (p=0.54) and arousal (p=0.56) consistent with the IAPS normative ratings (Lang et al., 1997). Each of the three conditions was randomly shown six times, interleaved by a 16 s fixation cross on a blank black screen. Each condition comprised an instruction to “view” or “decrease” (4 s), followed by the presentation of four images of the corresponding condition (4 s each), and finally a rating of unpleasantness (4 s) on a range from 1 (not at all unpleasant) to 5 (very unpleasant) (4 s), in which participants indicated by pressing a button on a response box. In order to allow participants to choose the emotion regulation strategy that they habitually employ in similar real-life situations, they were not trained or instructed as to which possible emotion regulation strategies to use during the “decrease” conditions. The total time of the paradigm was 12 min.

2.2.2. **Measures of functioning, quality of life, and childhood trauma**

Participants’ functional impairment was rated using the 24-item semi-structured interview Functional Assessment Short Test (FAST) (Rosa et al., 2007), quality of life was assessed with the European Quality of Life 5 Domain (EQ-5D) (EuroQol, 1990) questionnaire and childhood trauma with the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 1998).

2.3. **fMRI data acquisition**

Neuroimaging data were collected at the Copenhagen University Hospital, Rigshospitalet using a 3-Tesla Siemens Prisma scanner and a 64-channel head-neck coil. During the performance of the emotion regulation paradigm, blood oxygen level dependent (BOLD) fMRI was acquired using a T2*-weighted gradient echo spiral echo-planar (EPI) sequence with an echo time (TE) of 30 ms, repetition time (TR) of 2s, and flip angle of 90°. A total of 366 volumes were acquired, each consisting of 32 slices with a slice thickness of 3mm with 25% gaps in-between, and a field of view (FOV) of 230 × 230 mm using a 64 × 64 grid. The BOLD images were registered to T1-weighted structural images (TR=1900 ms; TE=2.58 ms; flip angle=9°; distance factor=50%; FOV=230 × 230 mm; slice thickness=0.9 mm). A standard B0 field map sequence was also acquired with the same FOV and resolution as the fMRI sequence (TR=400 ms; TE=7.38 ms; flip angle=60°) and used for geometric distortions correction of the BOLD images. Good image quality was ascertained by visual inspection of individual images.

2.4. **fMRI data analysis**

Data pre-processing and first-level analysis were conducted using fMRI Expert Analysis Tool (FEAT) version 6.0 (Woolrich et al., 2001) from the FMRIB Software (FSL; http://www.fmrib.ox.ac.uk/fsl). Pre-processing included brain extraction, B0 field distortion correction based on field map image, motion correction, linear and nonlinear registration to structural space, spatial normalization to the Montreal Neurological Institute (MNI) standard space, and spatial smoothing (Gaussian kernel full width half maximum = 5 mm). All participants’ registrations were visually inspected to ascertain a good fit. The time series in each session were high pass-filtered (to min 0.008 Hz). The first-level analysis was conducted using a general linear model (GLM) with the three events of interest: ‘passive view neutral’, ‘passive view negative’, and ‘decrease negative’. The events
were modelled as blocks convolved with a canonical hemodynamic response function with added temporal derivative. The main contrast of interests was emotion regulation (‘decrease negative’ > ‘passive view negative’). We secondly calculated an emotion reactivity contrast (‘passive view negative’ > ‘passive view neutral’) for comparison purposes (results for emotion reactivity are presented in the supplement). The GLM model further included standard motion parameters to account for head movement and participants with average relative movement >.2 mm were excluded from the analyses.

2.4.1. Regions of interest
Selection of regions of interest (ROIs) was based on peak activations previously found in healthy individuals during emotion regulation (regulate > control conditions) using IAPS images in a recent meta-analysis (Morawetz et al., 2017) (see Table S1 for MNI coordinates). Eleven 10 mm spheres were constructed in FSLeyes, covering the bilateral inferior frontal gyrus/VLPFC (Brodmann area [BA] 47), bilateral middle frontal gyrus/DLPFC (BA 6/8), bilateral superior frontal gyrus/DMPFC/LDPC (BA 6/9), bilateral angular gyrus (BA 40/39), left middle temporal gyrus (BA 21) and posterior cingulate cortex (BA23). Mean percent BOLD signal change during the emotion regulation (‘decrease negative’ > ‘passive view negative’) and emotion reactivity (‘passive view negative’ > ‘passive view neutral’) contrasts were extracted using featquery tool in FSL for each participant from each of the eleven ROIs as well as from anatomically defined left and right amygdalae based on the probabilistic Harvard-Oxford subcortical structural atlas thresholded at 30% (De-sikan PMID: 16530430).

### 2.5. Statistical analyses

Mean percent BOLD signal change during down-regulation was extracted for all participants from the ROIs. We employed a hierarchical cluster analysis (HCA) with squared Euclidian Distance and Ward’s linkage as agglomeration procedure to examine variability among patients in the extracted BOLD response during emotion regulation. The resulting dendrogram was visually inspected to establish the number of clusters to be retained. Discriminant function analyses (DFA) with leave-one-out classifications were also conducted in order to test the validity of the clustering solutions (see supplementary material).

The identified neuronal subgroups of patients and the HC were compared on mean percent BOLD signal change in all ROIs separately using analysis of variance (ANOVA) with Sidak correction for multiple comparisons. Demographic, clinical, and functional variables were compared between these subgroups using non-parametric tests of Kruskal-Wallis H, Mann-Whitney U tests and Pearson’s chi-square test. Subsequent total number and duration of episodes were compared between these subgroups using analysis of covariance.
controlling for the duration between baseline MRI-scan and episode data collection.

Extracted BOLD signal change in the ROIs during down-regulation in the (1) bilateral amygdala; (2) PFC (mean average of bilateral VLPFC, DLPFC, MDPFC); and (3) temporoparietal regions (mean average of bilateral angular gyri and left middle temporal gyrus) were averaged. Exploratory Pearson’s correlation analyses were conducted to investigate the associations between this averaged BOLD signal change and participants’ ratings of negative emotions elicited by the unpleasant images, subsyndromal symptoms (HDRS, YMRS), functioning, quality of life, and childhood trauma across the entire sample of BD and HC, as well as number and duration of previous episodes of depression, (hypomania), and mixed episodes in the patient sample.

2.5.1. Prediction of clinical course

Finally, follow-up episode data was available for a majority of patients (n=60; 69%). Follow-up episode data was missing for 27 participants as nine patients withdrew from the study, seven patients temporarily paused study participation, six were unable to be reached, one was symptomatic, one was pregnant, and follow-up was delayed for unknown reasons for three patients. To investigate whether neural activity during emotion regulation is associated with episode relapse, we employed (i) binomial logistic regression with relapse (yes/no) as outcome variable; and (ii) multiple regression analyses with total number of episodes and total duration of episodes at follow-up, respectively, as outcome variables. Extracted mean percent BOLD signal change in the bilateral amygdala, PFC, and temporoparietal regions, respectively, were entered at predictor variables, controlling for age, sex, subsyndromal symptoms, and duration between fMRI scan and episode data collection. Statistical analyses were conducted using SPSS version 25.

3. Results

3.1. Participants

One patient was excluded due to excessive head motion, resulting in a total sample of 153 participants entering the fMRI analysis: 87 newly diagnosed outpatients with BD and 66 HC. For comparison between the entire patient group and HC, see supplementary materials.

3.2. Neuronal subgroups of patients with bipolar disorder

Results obtained from the HCA and series of clustering indices indicated that remitted patients with BD were optimally clustered into two distinct subgroups based on their neural activation in the emotion regulation network during voluntary down-regulation of unpleasant emotions: a subgroup 1 that included 75% (n=65) of patients and a subgroup 2 including 25% (n=22) of patients (see supplementary material for dendrogram, graphical agglomeration of the subgroups, and results from the less optimal three-cluster solution for comparison purposes). Results from the DFA revealed one discriminant function; Wilks’ λ =.37, χ² (13) =77.87, p < .001. Activity in the right inferior frontal gyrus/VLPFC (BA47) contributed most to clustering (r =.68).

The classification results revealed high sensitivity with all 98.9% of patients being correctly classified.

3.3. Comparisons of neural activation within the emotion regulation network between the identified subgroups and healthy controls

Neuronal activation in the emotion regulation network significantly differed between the two neuronal subgroups of patients and HC within all emotion regulation regions: the bilateral amygdala (left amygdala: F(2, 150)=20.06, p < .001, \( \eta^2 = .28 \); right amygdala: F(2, 150)=15.78, p < .001, \( \eta^2 = .17 \)), the bilateral inferior frontal gyrus/VLPFC (left: F(2, 150)=24.64, p < .001, \( \eta^2 = .25 \); right: F(2, 150)=31.40, p < .001, \( \eta^2 = .30 \)), the bilateral middle frontal gyrus/DLPFC (left: F(2, 150)=21.79, p < .001, \( \eta^2 = .23 \); right: F(2, 150)=22.91, p < .001, \( \eta^2 = .23 \)), the bilateral superior frontal gyrus/DMPFC/DLPFC (left DMPFC: F(2, 150)=20.02, p < .001, \( \eta^2 = .21 \); right DLPFC: F(2, 150)=16.26, p < .001, \( \eta^2 = .18 \)), the left middle frontal gyrus/DMPFC (F(2, 150)=13.08, p < .001, \( \eta^2 = .15 \)), the posterior cingulate gyrus (F(2, 150)=19.77, p < .001, \( \eta^2 = .21 \)), the bilateral angular gyrus (left: F(2, 150)=27.17, p < .001, \( \eta^2 = .27 \); right: F(2, 150)=8.00, p < .001, \( \eta^2 = .10 \) and left middle temporal gyrus (F(2, 150)=38.42, p < .001, \( \eta^2 = .34 \)).

The 65 patients assigned to subgroup 1 were comparable to HC in neural activity in the PFC and temporoparietal regions implicated in emotion regulation (p> .08). Yet, they exhibited hyper-activity in the bilateral amygdala (p= .03) during down-regulation of unpleasant emotions compared to HC and to the subgroup 2 (p < .001). The 22 patients in subgroup 2 were characterized by hypo-activity within all ROIs within the emotion regulation network, including the bilateral VLPFC, DLPFC, MDPFC, the posterior cingulate gyrus, bilateral angular gyrus, left middle temporal gyrus, and bilateral amygdala relative to controls and subgroup 1 (p<.001) (Table 1; Fig. 1). For information regarding activity during ‘decrease’ vs. ‘view’ conditions, see supplementary materials (Fig. S4).

3.4. Comparisons between the neuronal subgroups of patients and healthy controls for negative emotions following attempts to dampen emotions

There was a statistically significant difference in the success of emotion down-regulation between the three groups (F(2, 146)=5.33, p=.006, \( \eta^2 = .07 \)). This was driven by the patients being less successful at downregulating their negative emotional response to unpleasant images compared to HC (subgroup 1 vs. HC: p=.02; subgroup 2 vs. HC: p=.03). There were no significant differences between the two neuronal subgroups of patients (p=.91) (Fig. 2).
3.5. Comparison of neuronal subgroups of patients and healthy controls for demographic and clinical variables

The two neuronal subgroups were comparable to HC in age and sex (ps ≥ .38) (Table 2). Patients in subgroup 1 had undergone fewer years of education than HC (p = .02), whereas patients in subgroup 2 did not differ from HC or subgroup 1 (p = .08). Although patients were in full/partial remission during time of testing, they still presented with...
subsyndromal depressive and manic symptoms relative to HC (ps≤.001), with no significant difference between the two neuronal subgroups (ps≥.86).

Regardless of subgroup, patients presented with more impairments in global and interpersonal functioning (ps≤.001), lower quality of life (ps≤.001), and more childhood trauma (ps≤.003) than HC. There was no significant difference between the two patient subgroups in overall functioning, quality of life, or childhood trauma (ps≥.65).

With regards to clinical characteristics, the two neuronal subgroups of patients were comparable in their use of antidepressant, antipsychotic, anticonvulsant, and lithium medication (ps≥.28). Patients assigned to subgroup 2 had experienced more and longer mixed episodes compared to patients in subgroup 1 (number of mixed episodes: p=..01; duration of mixed episodes: p=.008). In contrast, there were no statistically significant differences between the two subgroups in number or duration of depressive, hypomanic, or manic episodes (ps≥.12), nor other clinical characteristics including illness duration, type of BD diagnosis (BD-I vs. BD-II), number of prior suicide attempts, or hospitalizations (ps≥.38).

Follow-up episode data was available for 60 patients (subgroup 1 n=45; subgroup 2 n=15), of which 41 (68%) experienced a mood episode (depression, (hypo)mania, or mixed episode) within the mean 16 (±5) month follow-up period. 67% of patients in subgroup 1 and 73% of patients in subgroup 2 experienced at least one mood episode in the
follow-up period. The two neuronal subgroups did not differ in total number or duration of subsequent mood episodes during the follow-up time (ps ≥ .67; Table 3).

3.6. Associations between BOLD fMRI, emotion ratings, subsyndromal symptoms, functioning, quality of life, childhood trauma, and history of mixed episodes

Across the entire sample, self-reported lower success of emotion down-regulation was associated with more functional impairments (r = −.25, p = .002) and lower quality of life (r = −.20, p = .02). Childhood trauma was significantly correlated with more hyper-activity in PFC (r = −.18, p = .03) and temporo-parietal regions (r = −.19, p = .03) (but not amygdalae, p = .61) during emotion regulation. A history of more mixed mood episodes (r = −.28, p = .008) and longer episodes of depression (r = −.32, p = .005) were associated with more amygdalae hyper-activity (but not PFC or temporo-parietal activity: ps ≥ .19). However, success of emotion regulation, subsyndromal symptoms, functioning, quality of life, and (hyper)manic episodes did not correlate with activity in the amygdalae, PFC, or temporo-parietal regions (ps ≥ .07).

3.7. Prediction of clinical course

Across all patients, binomial logistic regression analysis was performed to investigate the effect of amygdala, PFC, and temporo-parietal neural activity implicated in emotion regulation on the likelihood of episode relapse. The regression model, including mean percent BOLD signal in the bilateral amygdalae as predictor variable, was statistically significant ($\chi^2(6) = 20.71$, p = .002). The model explained 41.0% (Nagelkerke $R^2$) of the variance and correctly classified 78% of cases. Increased amygdala activity during emotion regulation was significantly associated with an increased likelihood of subsequent relapse across all BD patients (β = 3.36, 95% CI [1.49; 5.50], p = .03). The regression models with activity in the PFC and temporo-parietal regions as predictor variables revealed significant models (PFC: $\chi^2(6) = 15.49$, p = .02; temporo-parietal: $\chi^2(6) = 15.45$, p = .02). However, activity in the PFC and temporo-parietal regions, respectively, did not significantly predict the likelihood of relapse (p = .52 and p = .54).

Multiple regression analyses investigating whether activity in the PFC, temporo-parietal cortex or amygdalae predict total number and duration of mood episodes at follow-up all revealed significant models. However, activity in these ROIs did not significantly predict the number or duration of episodes (all ps ≥ .23) during the 16-months follow-up time.

4. Discussion

In this large fMRI study of 87 patients and 66 HC, we revealed two distinct neuronal subgroups among remitted patients with recently diagnosed BD based on their fMRI BOLD response in predefined nodes of an emotion regulation brain network. Specifically, the first and largest subgroup (n = 65) was characterized by heightened bilateral amygdala reactivity to aversive pictures during attempts to dampen negative emotions, whereas their PFC and temporo-parietal activation was comparable to HC. In contrast, subgroup 2 (n = 22) showed wide-spread hypo-activity in all ROIs in the emotion regulation network. Both neuronal subgroups reported being less successful at down-regulating their negative emotions than HC, as reflected by more negative emotional reactions to the pictures. Importantly, impaired emotion regulation was associated with poorer functioning and quality of life across all participants. Patients in subgroup 2 had a history of more and longer mixed episodes, which was associated with more blunted amygdala activity. In contrast, amygdala hyper-activity during emotion regulation was associated with increased likelihood of subsequent mood episodes across all patients during the 16-month follow-up period.

Our demonstration of two distinct neuronal subgroups in recently diagnosed remitted BD patients is consistent with a recent report on neural activity patterns during reappraisal of negative images in remitted patients with BD type I (Njau et al., 2020). Here, the authors identified two subgroups: one characterized by blunted prefrontal and amygdala activity, whereas the second showed widespread hyper-activity in an emotion regulation network (Njau et al., 2020). In particular, the previous and current findings point to the existence of two neuronal subgroups that differ consistently in the direction of amygdala responsibility during emotion regulation. However, while a large subgroup of patients exhibited ‘normal’ activations within the prefrontal and temporo-parietal regions of the network in our study, the previously studied cohort exhibited either hypo- or hyper-activity in these regions (Njau et al., 2020). The discrepancies may be explained by differences in patient characteristics (patients with BD type I in full remission in the previous study versus a mixed group of patients with either BD type I or II and more subsyndromal symptoms...
in the current study). Nonetheless, the two extant studies consistently point to an existence of two neurally distinct subgroups of patients with BD. This new approach is likely to explain the inconsistent findings regarding the direction of aberrant neuronal activity during emotion regulation in studies comparing BD with HC (Green et al., 2020).

The normal prefrontal and temporo-parietal activations in subgroup 1 suggests that patients were able to recruit top-down cognitive control recourses implicated in emotional down-regulation (Buhle et al., 2014). Indeed, these patients showed increased prefrontal activity during ‘decrease negative’ compared to ‘passive view negative’ conditions (Fig. S4). Yet, the exaggerated bilateral amygdala reactivity and less successful down-regulating of negative emotional response in this subgroup indicates that they were nevertheless unable to use these prefrontal resources to efficiently lower the activity in the emotion-generating amygdalae. This indicates that patients in this subgroup fail to compensate for the exaggerated amygdala responsivity; indeed, their prefrontal activity during emotion regulation was comparable to but not greater than that in controls. Interestingly, exaggerated amygdala activity during emotion down-regulation also predicted subsequent mood episodes during a 16-month follow-up period across the entire patient sample, even after controlling for subsyndromal symptoms. However, subgroup 1 (with heightened amygdala responsivity) did not develop more mood episodes compared to subgroup 2. This lack of difference in clinical trajectories between the two subgroups was unexpected given the across-patient association between amygdala hyper-activity and increased likelihood of relapse. It is possible that we did not see this difference due to the small sample sizes of the two subgroups (n=45 and n=15, respectively). Nevertheless, it is possible that heightened amygdala responsivity (and inability to decrease this when required to do so) may be a latent vulnerability marker that increases patients’ sensitivity when they encounter stressors in everyday life (i.e., situations that require emotion regulation), which may trigger full-blown mood episodes. In keeping with this, heightened amygdala reactivity in healthy individuals has previously been shown to predict more depressive symptoms over time (Mattson et al., 2016) and increased psychological vulnerability (i.e., depressive and anxiety sympotms) to life stressors (Swartz et al., 2015). If this association is replicated also at the level of the individual patient (e.g., prediction of individual-level prognosis by use of machine learning using neuroimaging (Schnack, 2019)), exaggerated amygdala reactivity during emotion regulation could potentially serve as a neurocircuitry marker of relapse risk and thereby guide decision making regarding targeted prophylactic treatment for these patients. However, current machine learning prediction models have poor precision at an individual level and thus limited clinical use. Additional large-scale prospective longitudinal studies are warranted to identify neural activity biomarkers reliably related to illness course and treatment response (Schnack, 2019).

In contrast, the wide-spread neural hypo-activity observed in subgroup 2 suggests a reduced ability to efficiently recruit prefrontal and temporo-parietal resources implicated in successful emotion regulation. This is in line with previous studies showing PFC hypo-activity during emotion regulation in BD (Kjaerstad et al., 2021; Sankar et al., 2021; Townsend et al., 2013; Zhang et al., 2020). Indeed, these patients exhibited less activity in PFC and temporo-parietal regions when instructed to down-regulate vs. passively viewing negative images. Although these patients presented with blunted amygdala response, they still reported difficulties with down-regulating their negative emotions elicited by the aversive pictures. This subgroup of patients also experienced more and longer prior mixed episodes, which was associated with more amygdala hypo-activity, specifically. Prior studies from our group has shown that the prevalence of mixed episodes increases from the first episode to the tenth episode in bipolar disorder (Kessing, 2008). Specifically, mixed episodes may progressively attenuate the modulation of the amygdala by the prefrontal cortex, resulting in a blunted amygdala reactivity to emotional stimuli. Indeed, evidence suggest that mixed episodes are associated with failure to exhibit normative increase in amygdala volume during adolescence (Bitter et al., 2011) and blunted amygdala response during emotional processing (Strakowski et al., 2011). However, neuroimaging studies investigating the progressive effects of mixed episodes are essentially lacking and a majority of these studies do not differentiate between mania and mixed episodes.

The observed variability in neuronal activity was not simply an effect of psychotropic medication, because the subgroups did not differ with respect to use of lithium, anti-convulsants, or anti-psychotics. Instead, it is possible that patients applied different emotion regulation strategies, which could have affected their neural responses. Specifically, subgroup 1 may have used more cognitive reappraisal strategies (i.e., reinterpreting the meaning of the negative stimuli, for example thinking of the people in aversive pictures as actors with makeup on). Indeed, cognitive reappraisal is typically associated with increased prefrontal top-down control of amygdala reactivity and is an effective strategy to down-regulate emotion (Buhle et al., 2014; Cutili, 2014; Diekhof et al., 2011). Nevertheless, patients in this subgroup still failed to down-regulate their amygdala response and negative emotional response. This is consistent with the idea that while BD patients apply great regulatory efforts, their attempts at emotion regulation are often unsuccessful (Gruber et al., 2012). In contrast, subgroup 2 may have used more crude and less adaptive - emotion regulation strategies, such as avoidance (Alldao et al., 2010). Specifically, they may avoid the negative stimuli by looking away or focusing on something else, resulting in a blunted amygdala response and, consequently, less necessity to recruit the emotion regulation network. While avoidance may be adaptive short term, it has been found less efficient in reducing emotional arousal in the long run (Alldao et al., 2010). Notably, these patients in subgroup 2 displayed less neural activity during ‘decrease negative’ compared to ‘passive view negative’ conditions, further suggesting they did not recruit their emotion regulation network when asked to down-regulate their emotions.

4.1. Strengths and limitations

Strengths of the present study include the large sample of well characterized newly diagnosed BD patients and HC in addition to the data-driven approach that did not assume
neural homogeneity in patients. Thus, the data-driven clustering of twodistinct neuronal subgroups among remitted patients with recently diagnosed BD was based on their fMRI BOLD response in nodes of a predefined emotion regulation brain network highlighting that the findings in the study is not a result of selective reporting. Another strength was that we compared in-scanner behavioral emotion regulation data in relation to neuronal activity responses unlike previous study by Njau et al. (2020). A limitation was that follow-up episode data was only available for 60 patients, preventing separate prediction analyses on clinical course for the two subgroups. This further limited the within-group comparison of subsequent mood episodes differentiating between types of episodes (i.e., depression, hypomania, mania, or mixed episodes). Accordingly, types of patients’ most recent episode were not recorded, which may have differed between the subgroups and influenced the results. Secondly, although patients were newly diagnosed they still retrospectively had their first affective episode approximately seven years prior to being diagnosed with BD (mean untreated illness = 7.3, SD = 7.9), reflecting the difficulty in recruiting patients early in the illness course as the average delay between illness onset and diagnosis in BD is 5-10 years (Fritz et al., 2017). Nonetheless, we cannot exclude that previous illness history besides the number and duration of mixed episodes (i.e., other episodes and medications etc.) could have affected their neural response. Finally, we did not assess participants’ daily use of habitual emotion regulation strategies, which may have influenced their chosen strategy to down-regulate the negative stimuli and associated neural activity response.

5. Conclusions

In this large fMRI study, we revealed evidence of two subgroups distinguished by neuronal activity during emotion regulation among remitted patients with recently diagnosed BD. The subgroups were primarily defined by differences in neural activity in the amygdala, prefrontal, and temporoparietal regions. Across all BD patients, blunted amygdala activity was associated with more and longer previous mixed episodes, whereas amygdala hyper-activity was associated with increased likelihood of new episodes during the 16-month follow-up period. Our findings warrant future investigations into neurobiological heterogeneity in BD to reveal information about the underlying pathophysiology and guide personalized treatment approaches.

Contributors

KWM, LVK and MV were principle investigators of the BIO study. KWM was responsible for the original study design and draft of protocol. HLK was responsible for participant recruitment, conducting diagnostic interviews, rating of mood symptoms, neuropsychological testing, MR-scanning, and data collection, under the supervision of KWM. JM and GMK were responsible for MRI acquisition. VD and HLK wrote the initial manuscript draft. HLK and JM conducted the fMRI analyses. All authors contributed to interpretation of the data. All authors have approved the final manuscript.

Declaration of Competing Interest

MV has received consultancy fees from Lundbeck, Sunovion and Janssen-Cilag in the past three years. LVK has within the preceding three years been a consultant for Lundbeck and Teva. GMK has received honoraria as speaker and consultant for Sage Therapeutics/Biogen and from Sanos. JM has received honoraria as speaker for Lundbeck for the last three years. KWM has received consultancy fees from Lundbeck and and Janssen-Cilag in the past three years. The remaining authors declare no conflicts of interest.

Acknowledgment

The Research Fund of the Mental Health Services - Capital Region of Denmark has provided HLK’s post-doctorate salary. KWM holds a five-year Lundbeck Foundation Fellowship (grant no. R215-2015-4121).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2022.04.010.

References

Aldao, A., Nolen-Hoeksema, S., Schweizer, S., 2010. Emotion-regulation strategies across psychopathology: A meta-analytic review. Clin. Psychol. Rev. 30, 217-237. doi:10.1016/j.cpr.2009.11.004.

Association, American Psychiatric, 2013. Diagnostic and statistical manual of mental disorders (DSM-5®). Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Pub.

Banks, S.J., Eddy, K.T., Angstadt, M., Nathan, P.J., Luan Phan, K., 2007. Amygdala-frontal connectivity during emotion regulation. Social Cognitive and Affective Neuroscience 2, 303-312. doi:10.1093 SCAN/nsm029.

Bernstein, D.P., Fink, L., Handelsman, L., Foote, J., 1998. Childhood trauma questionnaire. Assessment of Family Violence: A Handbook for Researchers and Practitioners.

Bitter, S.M., Mills, N.P., Adler, C.M., Strakowski, S.M., Delbello, M.P., 2011. Progression of amygdala volumetric abnormalities in adolescents after their first manic episode. J. Am. Acad. Child Adolesc. Psychiatry 50, 1017-1026. doi:10.1016/j.jaac.2011.07.001.

Buhrle, J.T., Silvers, J.A., Wage, T.D., Lopez, R., Onyemekwu, C., Kober, H., Webe, J., Ochsner, K.N., 2014. Cognitive reappraisal of emotion: a meta-analysis of human neuroimaging studies. Cereb. Cortex 24, 2981-2990. doi:10.1093/cercor/bht154.

Corbalán, F., Beaulieu, S., Armony, J.L., 2015. Emotion regulation in bipolar disorder type I: an fMRI study. Psychol. Med. 45, 2521-2531. doi:10.1017/s0033291715000434.

Cutuli, D., 2014. Cognitive reappraisal and expressive suppression strategies role in the emotion regulation: An overview on their modulatory effects and neural correlates. Front. Syst. Neurosci. 8, 1–6. doi:10.3389/fnsys.2014.00175.

Diekhof, E.K., Geier, K., Falkai, P., Gruber, O., 2011. Fear is only as deep as the mind allows. A coordinate-based meta-analysis of neuroimaging studies on the regulation of negative affect. Neuroimage 58, 275-285. doi:10.1016/j.neuroimage.2011.05.073.
Moris, R.W., Sparks, A., Mitchell, P.B., Weickert, C.S., Green, M.J., 2012. Lack of cortico-limbic coupling in bipolar disorder and schizophrenia during emotion regulation. Transl. Psychiatry 2, e90.e99. doi:10.1038/tp.2012.16.

Njau, S., Townsend, J., Wade, B., Hellemann, G., Bookheimer, S., Narr, K., Brooks, J.O., 2020. Neural subtypes of euthymic bipolar I disorder characterized by emotion regulation circuitry. Biol. Psychiatry Cognit. Neurosci. Neuroimaging 5, 591-600. doi:10.1016/j.bpsc.2020.02.011.

Phan, K.L., Fitzgerald, D.A., Nathan, P.J., Moore, G.J., Uhde, T.W., Tancer, M.E., 2005. Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. Biol Psychiatry 57, 210-219. doi:10.1016/j.biopsych.2004.10.030.

Phillips, M.L., Drevets, W.C., Rauch, S.L., Lane, R., 2003. Neurobiology of emotion perception II: implications for major psychiatric disorders. Biol. Psychiatry 54, 515-528. doi:10.1016/S0006-3223(03)00171-9.

Picó-Pérez, M., Radua, J., Steward, T., Menchón, J.M., Soriano-Mas, C., 2017. Emotion regulation in mood and anxiety disorders: a meta-analysis of fMRI cognitive reappraisal studies. Prog. Neuropsychopharmacol. Biol. Psychiatry 79, 96-104. doi:10.1016/j.pnpbp.2017.06.001.

Rive, M.M., Mocking, R.J.T., Koeter, M.W.J., Van Wingen, G., De Wit, S.J., Van Den Heuvel, O.A., Veltman, D.J., Ruhé, H.G., Schene, A.H., 2015. State-dependent differences in emotion regulation between unmedicated bipolar disorder and major depressive disorder. JAMA Psychiatry 72, 687-696. doi:10.1001/jamapsychiatry.2015.0161.

Rosa, A.R., Sánchez-Moreno, J., Martínez-Aran, A., Salamero, M., Torrent, C., Reinares, M., Comes, M., Colom, F., Van Riel, W., Ayuso-Mateos, J., Kapczinski, F., Vieta, E., 2007. Validity and reliability of the functioning assessment short test (FAST) in bipolar disorder. Clin. Pract. Epidemiol. Ment. Health 3, 1-8. doi:10.17455/1745-0179-3-5.

Sankar, A., Purves, K., Colic, L., Cox Lippard, E.T., Millard, H., Fan, S., Spencer, L., Wang, F., Pittman, B., Constable, R.T., Gross, J.J., Blumberg, H.P., 2021. Altered frontal cortex functioning in emotion regulation and hopelessness in bipolar disorder. Bipolar Disord. 23, 152-164. doi:10.1111/bip.12954.

Schnack, H.G., 2019. Improving individual predictions: Machine learning approaches for detecting and attacking heterogeneity in schizophrenia (and other psychiatric diseases). Schizophr. Res. 214, 34-42. doi:10.1016/j.schres.2017.10.023.

Strakowski, S.M., Eilussen, J.C., Lamy, M., Cerullo, M.A., Allendorfer, J.B., Madore, A., Lachman, J.H., Welge, J.A., Delbello, M.P., Fleck, D.E., Adler, C.M., 2011. Functional magnetic resonance imaging brain activation in bipolar mania: evidence for disruption of the ventrolateral prefrontal-amygual-dala emotional pathway. Biol Psychiatry 69, 381-388. doi:10.1016/j.biopsych.2010.09.019.

Swartz, J.R., Knodt, A.R., Radtke, S.R., Hariri, A.R., 2015. A neural biomarker of psychological vulnerability to future life stress. Neuron 85, 505-511. doi:10.1016/j.neuron.2014.12.055.

Townsend, J., Altshuler, L.L., 2012. Emotion processing and regulation in bipolar disorder: a review. Bipolar Disord. 14, 326-339. doi:10.1111/j.1399-5618.2012.01021.x.

Townsend, J.D., Torrisi, S.J., Lieberman, M.D., Sugar, C.A., Bookheimer, S.Y., Altshuler, L.L., 2013. Frontal-Amygdala connectivity alterations during emotion downregulation in bipolar i disorder. Biol. Psychiatry 73, 127-135. doi:10.1016/j.biopsych.2012.06.030.

Varo, C., Kjærstad, H.L., Poulsen, E., Meluken, I., Vieta, E., Kessing, L.V., Vinberg, M., Miskowiak, K.W., 2021. Emotional cognition subgroups in mood disorders: associations with familial risk. Eur. Neuropsychopharmacol. 51, 71-83. doi:10.1016/j.euroneuro.2021.05.003.

Wing, J.K., Babor, T.,Brugha, Burke, T., Cooper, J., Giel, J.E., X. R., Sartorius, N., 1990. SCAN. Schedules for clinical assessment in neuropsychiatry. Arch. Gen. Psychiatry 47, 589-593.
Woolrich, M.W., Ripley, B.D., Brady, M., Smith, S.M., 2001. Temporal autocorrelation in univariate linear modeling of FMRI data. Neuroimage 14, 1370-1386. doi:10.1006/nimg.2001.0931.

World Health Organization, 1992. The ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines. World Health Organization, Geneva.

Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: Reliability, validity and sensitivity. Br. J. Psychiatry 133, 429-435. doi:10.1192/bjp.133.5.429.

Zhang, L., Ai, H., Opmeer, E.M., Marsman, J.B.C., Van Der Meer, L., Ruhé, H.G., Aleman, A., Van Tol, M.J., 2020. Distinct temporal brain dynamics in bipolar disorder and schizophrenia during emotion regulation. Psychol. Med. 50, 413-421. doi:10.1017/S0033291719000217.

Zilverstand, A., Parvaz, M.A., Goldstein, R.Z., 2017. Neuroimaging cognitive reappraisal in clinical populations to define neural targets for enhancing emotion regulation. A systematic review. Neuroimage 151, 105-116. doi:10.1016/j.neuroimage.2016.06.009.