

**Title:** Neural correlates of improved recognition of happy faces after erythropoietin treatment in bipolar disorder

**Running title:** EPO: neural response to faces in bipolar disorder

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## **ABSTRACT**

**Objective:** Bipolar disorder is associated with impairments in social cognition including the recognition of happy faces. This is accompanied by imbalanced cortico-limbic response to emotional faces. We found that EPO improved the recognition of happy faces in patients with bipolar disorder. This randomized, controlled, longitudinal fMRI study explores the neuronal underpinnings of this effect.

**Method:** Forty-four patients with bipolar disorder in full or partial remission were randomized to eight weekly **erythropoietin (EPO; 40,000 IU)** or saline (NaCl 0.9%) infusions in a double-blind, parallel-group design. Participants underwent whole-brain fMRI at 3T, mood ratings and blood tests at baseline and week 14. During fMRI, participants viewed happy and fearful faces and performed a gender discrimination task.

**Results:** Thirty-four patients had complete pre- and post-treatment fMRI data (EPO: N=18, saline: N=16). Erythropoietin vs. saline increased right superior frontal response to happy vs. fearful faces. This correlated with improved happiness recognition in the EPO group. Erythropoietin also enhanced gender discrimination accuracy for happy faces. These effects were not influenced by medication, mood, red blood cells or blood pressure.

**Conclusions** Together with previous findings, the present observation suggests that increased dorsal prefrontal attention control is a common mechanism of EPO-associated improvements across several cognitive domains.

**Key words:** Bipolar disorder, neurocognition, randomized controlled trial, neuroimaging

**Significant Outcomes:**

- Erythropoietin increased superior frontal response to happy vs. fearful faces, which correlated with improved happiness recognition in the EPO group.
- The findings highlight key neural correlates of the EPO-associated improvement in happiness recognition.
- Modulation of higher-order prefrontal functioning may be a target for treatments that aim to improve social cognition in bipolar disorder.

**Limitations:**

- The sample size was modest, which could have led to type-2 errors.
- Participants received mood stabilizing and/or antipsychotic medication, which may have non-specific effects on neural activity.
- The therapeutic use of EPO to improve cognition is limited by hematological side-effects and potential associated complications in non-anemic populations.

## INTRODUCTION

Bipolar disorder (BD) is a chronic and debilitating psychiatric disorder occurring in 1-2% of the population and is among the top ten leading causes of disability worldwide according to the World Health Organization (1). A core feature is impairments within social cognition, including the ability to understand and respond appropriately to others' thoughts and feelings (2,3). Social cognitive deficits in BD are most prominent during mood episodes but persist to a moderate degree in periods of remission (3). Specifically, remitted patients show trait-related difficulties with Theory of Mind (2,3) and with recognizing facial displays of emotion (4), particularly of happiness (5).

Functional magnetic resonance imaging (fMRI) studies indicate that patients' difficulties with facial expression recognition are accompanied by aberrant (most commonly *hyper-*) activity in limbic and ventral prefrontal regions and hypo-activity in dorsal prefrontal cortex (PFC) during emotional face processing (2, 6). In depressive states, patients display *hyper-*activity in the amygdala to negative (fearful, angry and sad) faces (7,8,9) and in the ventral PFC to both fearful and happy faces (7). In contrast, patients in manic states show *hypo-*activity in the amygdala and orbitofrontal cortex (OFC) and *hyper-*activity in the dorsolateral PFC (dlPFC) to negative faces (9). Trait-related abnormalities in fronto-limbic response to emotional faces have also been observed. Some studies found that remitted patients display limbic *hyper-*activity to happy and fearful faces within the amygdala (6, 9, 10, 11) and hippocampus (12), whereas others observed no such abnormal limbic responsivity (13, 14). A somewhat more consistent finding is trait-related aberrant PFC activity to emotional faces, with several reports of *hyper-*activity in the medial frontal (6,9) and inferior frontal (15) gyri and *hypo-*activity in dorsal cognitive control regions, including the dorsolateral PFC (dlPFC) (13,16). Taken together, heightened limbic and ventral PFC reactivity to emotional faces coupled with reduced dorsal PFC top-down control constitute putative neural correlates of trait-related facial processing difficulties in BD.

Mood stabilizing treatments fail to reverse patients' cognitive impairments across memory, attention, executive function and social cognitive domains (17). This highlights a need for novel treatment strategies that target cognition. Growing evidence indicates that cognitive impairments in patients with mood disorders arise from impaired neuroplasticity including cell death, suppression of neurogenesis and decreased cellular resilience (18, 19). Novel candidate treatments with rapid and enduring effects on neuroplasticity are therefore promising candidates to treat cognitive impairments in these disorders. Erythropoietin (EPO) is a multifunctional growth factor that was originally described to be produced in fetal liver and kidney and to play a substantial role in the regulation of red blood cells. However, EPO and its receptor (EPO-R) are also expressed in the brain by neurons and astrocytes and play important roles in neurodevelopment, neuroprotection and cognitive function (20, 21, 22). When administered systemically (intravenously or subcutaneously) in high doses ( $\geq 500$  IU/kg body weight), EPO crosses the blood-brain barrier and increases neuroprotection, neuroplasticity and cognition in animal models of acute brain damage and neurodegenerative conditions (20) and in translational studies of patients with neuropsychiatric disorders (23, 24). We therefore conducted two randomised, placebo-controlled studies of eight weekly EPO infusions on cognitive impairment in partially remitted patients with BD (25) and treatment-resistant depression (26) and associated structural and functional brain changes (27, 28, 29). The studies revealed benefits of EPO across several cognitive domains, which were accompanied by structural increase in the left hippocampus (27) and neural activity changes in attention control regions including the dorsomedial PFC during memory encoding and working memory (28, 29). Regarding social cognition, EPO produced a specific improvement in the recognition of happy facial expressions in partially remitted BD patients (25). This effect is similar to the early enhanced recognition of facial expressions including happiness three days after a single dose of EPO vs. saline to healthy volunteers (30) and occurred in the absence of changes in the recognition of fear or other emotions (25). Interestingly, EPO effects on social cognition seem to be robust across mammals; Mice with transgenic expression

of a constitutively active EPOR isoform in pyramidal neurons of prefrontal cortex and hippocampus exhibit enhanced social memory and attentional capacities (31).

### *Aims of the study*

The aim of this fMRI report was to investigate the neural underpinnings of the improved recognition of happiness in EPO-treated patients from the above study. Based on the aberrant PFC activity to emotional faces in remitted BD patients and predominant effects of EPO on dorsal PFC activity across other cognitive tests, we hypothesized that EPO would modulate PFC response to happy vs. fearful faces (primary outcome), and that this would correlate with the increased recognition of happiness after EPO vs. saline treatment.

## **MATERIAL AND METHODS**

### **Study design**

This fMRI report is part of a randomized, placebo-controlled, double-blind study of the effects of eight weekly EPO vs. saline infusions on cognitive impairments in patients with BD in full or partial remission. Patients and outcome assessors were blinded to patients' treatment allocation throughout the trial (for full study details, see (32, 25). The researcher conducting the fMRI analysis was also blinded to patients' treatment allocation (to this end, patients were simply allocated to groups '1' and '2' for the group comparisons).

### **Participants**

Patients were recruited through the Copenhagen Affective Disorder Clinic and advertisement on relevant websites and were screened with Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (33). Included patients had an ICD-10 diagnosis of BD (**type I or type II**) in full or partial remission (HDRS-17 and YMRS scores  $\leq 14$ ). Participants were on stable medication from minimum two weeks prior to trial start and were unchanged for the duration of the study. For a detailed description of the screening, exclusion criteria, safety monitoring, and approvals, please see the trial protocol (32) and main outcome article (25). The study was conducted in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from all participants after complete description of the study.

### **Randomization and masking**

Pharma Consulting Group (Uppsala, Sweden) performed the block randomization with stratification for age ( $<$  or  $\geq 35$  years) and gender. Randomization list was kept in locked cabinet and where only available for an investigator. Patients and study personnel involved in administration of EPO/saline, regular contact with the patients, outcome assessment and data analyses were kept blinded. The physicians performing weekly monitoring blood test results and side-effects were not involved in the outcome assessments.

### **Procedures**

Participants were randomized to receive weekly intravenous infusions of EPO (40,000 IU) or saline (NaCl 0.9%) over eight weeks. Blood tests and blood pressure measurements were taken at a weekly basis during the active treatment phase and six weeks after treatment completion (week 14). Neurocognitive function was assessed at baseline (week 1), post-treatment (week 9) and at the six

weeks follow-up (week 14), and fMRI was performed in weeks 1 and 14. Facial expression recognition was assessed with the facial expression recognition test from the Emotional Test Battery (ETB; Oxford P1Vital) as part of the neurocognitive test battery at weeks 1, 9 and 14 (for details, see (25)). In addition, neural response to emotional faces was assessed during fMRI in weeks 1 and 14.

### **Facial expression recognition task**

Pictures of faces from Ekman and Friesen displaying one of the six emotions: happiness, surprise, sadness, fear, anger and disgust were presented for 500 ms on a laptop computer in randomized order. The expressions were morphed at 10% steps in shape and texture differences between a neutral face (0%) and a full emotion face (100%). A total of 250 stimuli were presented consisting of four examples of every emotion at each intensity level plus a neutral face for every motion. The participants had to press the corresponding key on the keyboard matching the particular emotional expression as quickly and accurately as possible. Accuracy, misclassifications and RTs for correct responses were recorded.

### **Emotional face processing task**

Neural activity to happy and fearful faces was assessed with an incidental face processing task from the Emotional Test Battery (ETB; P1Vital Oxford). Face stimuli were projected from a computer using E-Prime software version 1.2 onto an opaque screen at the foot end of the scanner bed, which could be seen by the participants' through an angled mirror. The pictures was presented in a block paradigm, each lasting 25 s and consisted of 10 pictures of happy or fearful shown on the screen for 200 ms immediately followed by a fixation cross shown for 2300 ms. Total task time were 5 min 28 s composed of four blocks of each emotion condition and eight inter-blocks with a central fixation



cross. The participants were instructed to perform a gender discrimination task by pressing the keys on a response pad with their right middle and index finger for male and female, respectively. **This enabled investigation of neural responses associated with incidental (rather than explicit) processing of facial emotion.** Accuracy and response times (RT) for correct responses were recorded.

### **Other fMRI paradigms**

The effects of EPO on picture encoding and spatial working memory were investigated in the same scan session and are reported elsewhere for clarity (28, 29). We reported previously that EPO had no effect on neural response in the occipital cortex during a visual stimulation control task, which suggests that the effects of EPO on task-related neural activity were not confounded by any global effects of EPO on brain oxygenation or hemodynamic responses (28, 29).

### **MRI acquisition protocol**

The MRI data acquisition was performed using a Siemens Trio MR scanner (Siemens Trio, Erlangen, Germany) with an eight-channel head array coil. BOLD-sensitive fMRI was acquired using a T2\*-weighted gradient echo-planar imaging (EPI) sequence with following parameters: repetition time (TR) = 2.5 s, echo time (TE) = 26 ms, flip angle = 20° to minimize physiological noise (34), 42 slices with a slice thickness of 3 mm, and a field of view (FOV) of 192×192 mm using a 64×64 grid. A total of 129 brain volumes were acquired in a single fMRI session with a duration of 5 min and 23 s. High-resolution 3D structural T1-weighted images were also obtained with following parameters: TI=800, TE=3.93, TR=1540 ms, flip angle 9°, 256×256 FOV, 192 slices. The MRI protocol used for the baseline and follow-up investigations was identical.

## Functional MRI data analysis

### *Volume of interest*

To test our main hypothesis, we initially restricted the search volume to a volume of interest (VOI) which included the PFC, the anterior cingulate cortex (ACC), insula, amygdala, hippocampus, and the fusiform gyri. This VOI enabled investigation of the primary hypothesis that EPO would modulated PFC response to happy vs. fearful faces and assessment of potential additional effects of EPO within this neuro-circuitry involved in emotional face processing. The VOI was defined bilaterally on a standard MNI template included in the FSL package using FSLView 4.0.1. The PFC was defined to include the superior, medial and inferior frontal gyri, the subcallosal cortex, the orbitofrontal and medial frontal cortex and the frontal poles. The ACC, fusiform gyri and the hippocampi were delineated according to the Harvard-Oxford cortical structural Atlas maps implemented in FSLView (35) and thresholded at 5%. All defined regions were added together to form a single VOI mask.

### *fMRI analysis*

Functional MRI data processing was performed with the FMRI Expert Analysis Tool (FEAT; version 6.00) part of FMRIB's Software Library (FSL; [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). Pre-processing involved image realignment, non-brain removal, spatial normalization to an MNI (Montreal Neurologic Institute) template and spatially smoothing (Gaussian kernel, 5 mm full-width-half-maximum). The time series in each session were high pass-filtered (to max 0.008 Hz). For the subject level, we modeled the paradigm using a general linear model with local autocorrelation correction (36) and defined two conditions, *fearful faces* and *happy faces*, which were convolved with a canonical hemodynamic response function (37).

**To capture the valence-specific effect of EPO on happy faces observed in the original behavioral outcome paper (25), we decided to examine how EPO influenced the neural activity in response to happy vs. fearful faces.** For each participant, we computed a differential contrast representing happy>fear at both baseline (before EPO/ saline treatment) and follow-up (6 weeks after treatment completion). Using these differential contrast images, we computed *longitudinal contrast* images representing the change from baseline and follow-up measurements in BOLD response to happy >fearful faces.

At the group level, we first calculated task activations at baseline across all participants by entering the happy faces, fearful faces and the differential images (happy vs. fear) in a one-sample t-test. Second, we modeled group-by-time interaction effects by entering the *longitudinal contrasts* into a two-sample t-test to assess group differences in the *change* over time in neural response to happy vs. fearful faces. The analysis thereby involved adjustment for any baseline neural activity differences between groups. Third, we modeled non-specific longitudinal effects on emotional processing by entering the longitudinal contrasts of all participants in a one-sample t-test. The statistical models were estimated using nonparametric permutation-based inference (n=5000) using the ‘randomize’ algorithm implemented in FSL (38, 39). The search volume was initially restricted to the defined VOI mask. We also explored effects at whole brain level by estimating the models again using identical parameters, but without a brain mask. In addition, for exploratory purposes, in order to have increased sensitivity in detecting group-by-time interaction effects, we estimated the longitudinal model using the distinct regions included in the VOI mask as separate search volumes (ACC, amygdala and hippocampus, and fusiform gyrus) without correcting for multiple comparisons. Finally, we examined in additional exploratory purposes any potential group differences in neural response within the VOI for fear>happy given evidence for effects of single dose EPO on fear-relevant neural activity (30, 40). Significant clusters were identified using the Threshold-Free Cluster

Enhancement method at corrected  $P < 0.05$ . The mean percent BOLD signal change in clusters showing significant differences between groups was extracted for visualization purposes.

### **Statistical analyses of behavioural, demographic and mood data**

Accuracy and speed during the recognition of happy and fearful facial expressions (outside the scanner) and gender discrimination (during fMRI) were examined using ANOVA with time and group as independent variables (all tests were two-tailed). Significant interactions were followed up by simple main effect analyses. We corrected for non-sphericity using the Greenhouse-Geisser correction. For significant interactions, we also conducted post-hoc ANCOVA with adjustment for subsyndromal mood symptoms to examine whether the observed effects were mood-independent. Change in mood symptoms were analysed using ANOVA with time and group as independent variables. Demographic variables and medication status in EPO vs. saline groups were examined with simple main effect analyses (independent samples t-tests for parametric data and Pearson's Chi Square and Fisher's Exact tests for non-parametric data). Haemoglobin and blood pressure and lithium dose were examined with simple main effect analyses.

To investigate the functional relevance of EPO-associated effects on BOLD activity, we conducted exploratory Pearson's correlations between the *change* from baseline to follow-up in BOLD response within the predefined VOI and changes in behavioural measures (accuracy during facial expression recognition accuracy and gender discrimination, respectively). Statistical analyses were performed in the Statistical Package for Social Sciences (SPSS; version 22; IBM Corporation, Armonk, New York, United States).

## RESULTS

### Participant flow and characteristics

Table 1 and the CONSORT chart (Supplementary Figure S1) display the participant flow and characteristics. The first participant was randomized in September 2009 and the last assessment was completed in October 2012. Of the 114 screened participants, **70 were excluded before entry to the trial because they did not meet the safety inclusion criteria (n=37; 53%), refused to participate (n=16; 23%) or for other reasons (n=17; 24%) as described in the original trial outcome paper (25). Hence**, 44 participants were randomised to EPO (N=23) or saline (N=21).

One participant (saline) discontinued treatment in the second week because of increased thrombocyte levels and terminated treatment in week 5, and fMRI data was lost either at baseline or follow-up for 9 participants (EPO: N=5; saline: N=4). Complete pre- and post-treatment fMRI data was thus available and analysed for 34 participants (EPO: N=18, saline: N=16).

There were no baseline differences between EPO and saline groups in demographic or clinical variables ( $p \geq 0.21$ ; see table 1 for details). Consistent with our previous findings in an overlapping cohort, there were no effects of EPO vs. saline on subsyndromal mood symptoms ( $p \geq 0.09$ ), blood pressure ( $p \geq 0.10$ ) or haemoglobin ( $p \geq 0.81$ ).

### Behavioural results

#### Facial expression recognition

There was an improvement across the entire cohort in facial expression recognition accuracy from baseline to weeks 9 ( $F(1,30)=7.36$ ,  $p=0.01$ ) and 14 ( $F(2,60)=7.36$ ,  $p=0.001$ ) and in recognition speed (weeks 1, 9:  $F(1,30)=9.18$ ,  $p=0.005$ ; weeks 1, 9, 14:  $F(2,60)=5.92$ ,  $p=0.004$ ).

EPO increased accuracy during recognition of happy faces from baseline to week 9 in comparison with saline ( $F(1,30)=4.98$ ,  $p=0.03$ ). The partial eta-squared ( $\eta^2=0.14$ ) was of a large size. **The effect remained significant after post-hoc adjustment for subsyndromal depression and mania symptoms (HDRS-17 and YMRS scores) in week 9 ( $F(1,29)=4.64$ ,  $p=0.04$  and  $F(1,29)=4.21$ ,  $p=0.049$ , respectively).** This effect was reduced to a non-significant trend at the 6 weeks follow-up assessment ( $F(2,60)=2.98$ ,  $p=0.06$ ), in line with the previously reported findings in the complete patient sample (25). In contrast, there was no effect of EPO vs. saline on the recognition of fearful faces from baseline to weeks 9 or 14 ( $p\geq 0.72$ ) or on general accuracy or speed of facial emotion recognition at any of these times ( $p\geq 0.24$ ), as reported in the entire cohort.

### **Gender discrimination accuracy**

Speed and accuracy of gender discrimination during fMRI showed no baseline differences between EPO and saline groups ( $p\geq 0.21$ ). **Gender discrimination accuracy was near ceiling levels (mean $\pm$ SD:  $95\pm 6\%$ ) and showed no improvement with repeated testing across the entire cohort ( $p\geq 0.75$ ).** Nevertheless, EPO increased the accuracy of gender discrimination for happy faces compared to saline ( $F(1,30)=4.76$ ,  $p=0.04$ ; post-hoc t-tests non-significant) (see Figure 1.A). **The effect remained significant after post-hoc adjustment for subsyndromal mania symptoms ( $F(1,29)=4.66$ ,  $p=0.04$ ) but was reduced to a non-significant trend after adjustment for subsyndromal depression symptoms in week 14 ( $F(1,29)=3.90$ ,  $p=0.058$ ).** This effect occurred in the absence of significant changes between groups in the gender discrimination accuracy for fearful faces ( $p\geq 0.08$ ) (Figure 1.B) or across all emotional faces ( $p\geq 0.08$ ), or in the gender discrimination speed for happy or fearful faces ( $p\geq 0.07$ ).

### **fMRI results**

### *Volume of interest analysis*

At baseline, happy and fearful faces activated bilateral fusiform gyrus, superior frontal gyrus and insula within the VOI across all participants (Table 2). There were no significant differences in the BOLD response to happy vs. fearful faces across all participants at baseline.

Comparison of neuronal activity change within the VOI from baseline to follow-up revealed a significant effect of EPO vs. saline in the right superior frontal gyrus (Figure 2.A, Table 2), which showed increased response to happy vs. fearful faces in the EPO vs. saline treated patients (Figure 2.B). There were no treatment independent differences between the baseline and follow-up task activations (i.e., no non-specific effects of repeated testing).

### *Exploratory findings*

As expected the happy and fearful faces activated additional regions outside the VOI mask at baseline (for coordinates for peak cluster activations, see Table 2), but no regions showed greater response to happy>fearful faces or vice versa (Table 2). There were no additional regions showing group-by-time interaction effects at the whole-brain level. For the contrast fear>happy, there were no regions showing non-specific changes in neural activity across both groups.

The separate longitudinal analyses for the three regions comprising the a priori VOI (the ACC, the amygdala-hippocampus complex, and fusiform gyrus) showed group-by-time interaction effects (for cluster maxima, see Table 2 and Figure 3). Since we did not control for multiple comparisons i.e. the number of regions, we report the results as trends.

### **Task-related activity scaling with improved recognition of happy faces**

Within the EPO group, the increased right SFG response to happy vs. fearful faces from weeks 1 to 14 correlated with increased recognition of happy faces in the facial expression recognition test ( $r=0.63$ ,  $p=0.007$ ) (Figure 2.C). This correlation was not observed in the saline group ( $p\geq 0.75$ ) (Figure 2.C) and was reduced to a trend when including the entire cohort ( $r=0.32$ ,  $p=0.07$ ). **In contrast**, the increased right SFG response to happy vs. fearful faces did not correlate with the improved gender discrimination accuracy for happy faces in the EPO group ( $p\geq 0.68$ ). However, across the entire cohort, there was a strong trend correlation between increased right SFG response to happy vs. fearful faces and better gender discrimination accuracy for happy faces ( $r=0.34$ ,  $p=0.054$ ).

Within the three exploratory ROIs, we observed no correlation between changes in neural activity to happy vs. fearful faces and in happiness recognition or gender discrimination accuracy for happy faces within the EPO group ( $p\geq 0.07$ ). However, across all participants, there was a correlation between increase in hippocampal response to happy faces and in the recognition of happy faces from weeks 1 to 14 ( $r=0.39$ ,  $p=0.03$ ). There was also a correlation between increased response in the ACC cluster to happy vs. fearful faces over time and better gender discrimination accuracy for happy faces in the entire cohort ( $r=0.39$ ,  $p=0.03$ ).

### **No influence of change in haematocrit, mood or medication**

Post-hoc exploratory ANCOVA of extracted mean percent BOLD signal change from the SFG cluster indicated that the observed significant effect of EPO vs. saline on neural response to happy vs. fearful faces was unchanged after adjustment for concomitant medication, or for changes in subsyndromal mood symptoms, blood pressure or haematocrit ( $p\leq 0.001$ ). These analyses also revealed no separate effects of medication or change in depressive symptoms on SFG response to fearful vs. happy faces ( $p\geq 0.13$ ). There was a non-significant trend towards an effect of change in subsyndromal mania symptoms on activity change in this region ( $F(1,31)=3.75$ ,  $p=0.06$ ). Correlation analyses showed no general association between change in subsyndromal mania symptoms and in



SFG response across the entire cohort ( $p=0.38$ ). However, within the EPO group alone, increase in subsyndromal mania symptoms from weeks 1 to 14 correlated with increased SFG response to happy vs. fearful faces ( $r=-0.60$ ,  $p=0.008$ ).

## DISCUSSION

This randomized, controlled, longitudinal fMRI study examined the neuronal underpinnings of the EPO-associated improved recognition of happy facial expressions in partially or fully remitted BD patients. Eight weekly EPO infusions increased right SFG response to happy vs. fearful faces from baseline to follow-up in comparison with saline, and this effect correlated strongly with improved recognition of happy faces in the EPO group. Erythropoietin also enhanced gender discrimination accuracy for happy faces in the implicit facial emotion processing task during fMRI. Additional exploratory analyses indicated trend-level increases in neural response to happy vs. fearful faces in the ACC, hippocampus-amygdala complex and fusiform gyrus in EPO vs. saline treated patients. The effects of EPO were not influenced by concomitant medication or changes in subsyndromal mood symptoms, haematocrit or blood pressure from baseline to follow-up. Within the EPO group, the enhanced SFG response to happy vs. fearful faces correlated with increase in subsyndromal mania symptoms.

The demonstration of EPO-associated increase in right SFG response to happy vs. fearful faces—and its correlation with improved recognition of happiness—is interesting since remitted BD patients have been found to display right-lateralised dorsal PFC hypo-activity to happy faces (13) and impaired recognition of happy expressions (5). Modulation of right SFG response may thus represent a key neuronal target for treatment-related improvement of happiness recognition in BD. Given the involvement of dorsal PFC in aspects of executive function related to selective attention and attention switching, the effect of EPO on SFG response to happy vs. fearful faces could represent

greater allocation of attention resources to positive social information consistent with the EPO-associated improved happiness recognition. Enhanced mood-congruent processing of positive expressions could contribute to this effect given the correlation between increased SFG response to happy vs. fearful faces and more subsyndromal mania symptoms in the EPO group. Although this effect could be interpreted as a risk marker of manic switch, we observed no evidence for **mania inducing** effects of EPO in our patients (25). **Notably, this correlation should be interpreted with caution given the constrained distribution in the subsyndromal mania symptoms with most patients scoring 0-3 and only a few scoring 3-6.** An alternative interpretation is that the increased SFG response represents better attention control during the gender discrimination task, which required participants to disregard faces' emotional signals. This interpretation is consistent with EPO-treated patients' specific improvement in gender discrimination accuracy for happy faces, as well as with an association between greater right dorsal PFC activity and better performance on a facial processing task requiring selective attention in patients with autism spectrum disorders (40). Conversely, dorsal PFC hypo-activity during emotional face processing in partially remitted BD may thus reflect failure to attend to the facial configurations associated with particular emotional states (thus leading to difficulties with emotion identification) rather task-related disinhibition of limbic regions *per se*. Beyond EPO, the present finding suggests that social cognition in BD may be improved by treatments that specifically strengthen prefrontal attentional control mechanisms.

The increased task-related SFG response after eight weekly EPO vs. saline infusions in our partially remitted BD patients — but only trend-level effects in limbic and fusiform face processing regions — contrasts with the pattern of *early* neuronal effects after a single dose of EPO vs. saline to healthy volunteers (30). Specifically, single EPO administration to healthy volunteers produced broad modulation of activity in limbic and fusiform regions to happy vs. fearful faces, consistent with facilitated bottom-up perception of happy expressions (30). Nevertheless, the selective SFG activity increase in this eight week study is remarkably similar to the predominant dorsomedial and

dorsolateral PFC activity increase across picture encoding and spatial working memory tasks after long-term EPO treatment in an overlapping (larger) cohort of patients with BD or treatment-resistant unipolar depression (28, 29). These similar patterns of neural activity change during affective and non-affective cognitive tests after long-term EPO vs. saline treatment suggest that a common neural correlate for the EPO-associated improvements across attention, memory and social cognition is increased efficiency of dorsal PFC top-down regulation and attention control.

The effect of EPO on neural response to emotional faces is likely to be mediated through multiple neurobiological mechanisms. While EPO treatment upregulated red blood cells in the acute treatment phase, this was normalised at the time of the follow-up fMRI scan 6 weeks after treatment completion. In addition, the EPO-associated SFG activity increase was not influenced by concomitant medication or changes in red blood cells, blood pressure or subsyndromal mood symptoms. The findings are therefore likely to reflect direct neurobiological actions of EPO. Specifically, EPO and its receptor are expressed across various cortical regions including the PFC in rodent, monkey and human brain tissue (42, 43) and play a key role in synaptic plasticity and neuronal differentiation (22). In addition, exogenous administered EPO increases neuroplasticity through activation of anti-inflammatory, anti-apoptotic and anti-oxidant signalling cascades (20) and inhibition of glycogen synthase kinase 3 beta (GSK3 $\beta$ ) (44). Given this, the effects of EPO on the recognition of happy expressions and associated SFG activity change may be mediated by restoration of synaptic and neuronal plasticity in the PFC regions affected by atrophy and loss in BD. This is consistent with evidence for direct beneficial effects of EPO on social cognition from studies of transgenic mice that overexpress EPOR in pyramidal neurons in the PFC (31).

The randomized, placebo-controlled design was a key methodological strength since this could accommodate for effects of repeated testing and scanning. A limitation was that patients were treated with psychotropic medication since this could have produced non-specific change in global brain

activity. Nevertheless, EPO and saline groups showed no differences in psychotropic medication status or baseline neural response, which speaks against such confounding effects of psychotropic medication. Post-hoc analyses also revealed no effects of lithium, antipsychotic or antidepressant treatment on neural activity in the SFG. Given the lack of a healthy control group in the present study, it cannot be determined if our patients displayed aberrant activity to happy vs. fearful faces or deficits in happiness recognition at baseline or whether the EPO-related SFG activity change represents normalisation. Given the lack of a healthy control group it is also unclear whether the effect of EPO was specific to bipolar disorder patients. However, the pro-cognitive and/or mood-modulating effects of EPO are clearly non-disease specific. We have in the past observed beneficial effects in disorders as different as schizophrenia, multiple sclerosis, major depression or bipolar disorder (23-26) as well as in healthy individuals (45). Nevertheless, in disorders with defined problems in the EPO-responsive areas (as exemplified here with facial expression recognition), the application of this compound is likely beneficial. It should also be noted that this fMRI study of the effects of EPO on neural circuitry activity during face processing was *exploratory* in nature according to our original published trial protocol (32). While the primary trial efficacy outcomes were analyzed with intention-to-treat analysis in the original trial (25), we explored the effects on neural activity using complete data sets. Nevertheless, the risk of bias is low since almost all (98%) participants completed both fMRI scans and the main mechanism for missing data was technical difficulties. The relatively small sample size (n=34) was also a limitation, although it was comparable to previous cognition trials previous with prospective fMRI investigations that involved 20-30 participants (46, 47). Finally, the results may not be representative for all BD patients given the extensive exclusion criteria that were necessary to ensure safety of EPO treatment.

The present findings link improved recognition of happy facial expressions in EPO vs. saline treated patients to increased SFG activation to happy vs. fearful faces. This dorsal PFC effect of EPO during face processing is remarkably similar to the effects of EPO on neural activity during memory

encoding and working memory performance. Together, these findings highlight enhanced dorsal PFC top-down control and attention control as a common neuronal correlate of EPO-associated improvements across several cognitive domains.

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## **Declaration of interest**

KWM reports having received consultancy fees from Lundbeck. MV within the last three years consultancy fees from Lundbeck. CJH has received consultancy fees from P1vital Ltd, Lundbeck, Johnson and Johnson, Servier and Eli-Lilly. CJH has also received grant income from GlaxoSmithKline, UCB Pharma, Janssen Inc, Lundbeck, Servier and Astra Zeneca. GMK received within the last 3 years honoraria as field editor for Int J Neuropsychopharmacology and as scientific advisor for Lundbeck. LVK reports having been a consultant for Sunovion within the last 3 years. HRS has received honoraria as member of an ad-hoc scientific advisory board for Lundbeck A/S, as

speaker from Biogen Idec, Denmark A/S, Genzyme, Denmark and MerckSerono, Denmark, and as senior and reviewing editor for Neuroimage (Elsevier Publishers, Amsterdam, The Netherlands) and book editor from Springer Publishing, Stuttgart, Germany. He has received travel support from MagVenture, Denmark. HE, JM, and NP report no biomedical financial interests or potential conflicts of interest.

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**Table 1.** Participant characteristics

	<b>EPO</b> <b>(N = 18)</b>	<b>Saline</b> <b>(N = 16)</b>	<b>p-value</b>
Age, Mean (SD)	39 (12)	39 (12)	0.89
Gender, No. Women (%)	13 (72)	11 (69)	0.82
Education, Mean (SD)	15 (2)*	14 (3)	0.30
HDRS baseline, Mean (SD)	9 (4)	7 (5)	0.21
follow-up, Mean (SD)	7 (5)	9 (7)	0.51
BDI baseline, Mean (SD)	18 (10)	16 (9)	0.62
follow-up, Mean (SD)	12 (10)	15 (13)	0.42
YMRS baseline, Mean (SD)	2 (3)	2 (2)	0.60
follow-up, Mean (SD)	2 (2)	2 (2)	0.93
No. prior depressions, Mean (SD)	6 (5)	6 (4)	0.85
No. prior (hypo)manias, Mean (SD)	4 (4)	4 (4)	0.73
Bipolar subtype, No. of type II (%)	<b>10 (56)</b>	<b>8 (50)</b>	<b>0.75</b>
Haemoglobin baseline, Mean (SD)	8.5 (0.7)	8.4 (0.8)	0.82
follow-up, Mean (SD)	8.4 (0.8)	8.3 (1.0)	1.0
Blood pressure, Mean (SD)			
systolic/diastolic, baseline	122(14)/79 (11)	124(16)/79 (10)	0.82/0.98
systolic/diastolic, follow-up	123(18)/80 (11)	121(18)/74 (11)	0.71/0.14
Current Medication			
<i>Lithium, no. (%)</i>	9 (50)	5 (31.3)	0.27
<i>Anticonvulsants, no. (%)</i>	8 (50)	9 (50)	1
<i>Antidepressants, no. (%)</i>	9 (56)	9 (50)	0.72
<i>Antipsychotics, no. (%)</i>	4 (25)	6 (33)	0.60
<i>Benzodiazepines, no. (%)</i>	6 (37)	5 (27)	0.55
<i>Melatonin, no. (%)</i>	1 (6)	1 (5)	0.93
<i>Number of medications, Mean (SD)</i>	2.4 (1.1)	2.2 (.8)	0.56

**Table 2.** Peak cluster activation in regions of the *a priori* defined Volume Of Interest (VOI) spanning prefrontal and anterior cingulate cortex, the hippocampus-amygdala complex and fusiform gyrus showing (i) BOLD response in response to fearful and happy faces at baseline (main effect of task across all participants) and (ii) effects of EPO vs. saline on BOLD response to fearful vs. happy faces from baseline to follow-up.

Task and Region	Corrected p	Voxels	x	y	z
<b>Happy</b> ( <i>Main effect of task at baseline</i> )					
Left medial frontal gyrus	< 0.001	9495	-46	4	26
Right cerebellum	< 0.001	1464	38	-40	-30
Left cerebellum	< 0.001	1144	-38	-46	-28
<b>Fear</b> ( <i>Main effect of task at baseline</i> )					
Left medial frontal gyrus (BA 6)	< 0.001	1889	-34	-12	58
Right cerebellum	< 0.001	1322	40	-46	-28
Left cerebellum	< 0.001	1063	-44	-62	-26
Right cingulate gyrus (BA 32)	0.002	481	6	14	46
Right precentral gyrus (BA 6)	0.013	415	44	-2	54
Right inferior frontal gyrus (BA 44)	0.011	246	46	8	28
Left insula (BA 13)	0.024	88	-30	24	8
Right superior frontal gyrus (BA 6)	0.044	11	10	-12	68
<b>Happy &gt; Fear</b> ( <i>Main effect of task at baseline</i> )					
(No main effect of task)	-	-	-	-	-
<b>Fear &gt; Happy</b> ( <i>Main effect of task at baseline</i> )					
(No main effect of task)	-	-	-	-	-
<i>EPO &gt; saline</i>					
<b>Happy &gt; Fear</b> ( <i>Group x Time interaction</i> )					
Dorsal anterior cingulate cortex (BA 32)*	0.024	27	6	16	48
ACC ** Left ACC (BA 32)	0.031	31	-6	4	46
Amygdala- Putamen hippocampus**	0.031	8	-30	-10	-12
Cerebellum	30	0.009	20	-46	-16
Fusiform gyrus** Right fusiform gyrus (BA 37)	15	0.037	48	-44	-22

\*In the *a priori* defined Volume Of Interest (VOI) analysis. \*\*In the exploratory separate Regions of Interest (ROI) for the three regions included in the VOI. Abbreviations: ACC, anterior cingulate cortex.

**Figure 1.** Gender discrimination accuracy.

**Figure 2. A.** Region in the right superior frontal gyrus (SFG) showing activity increase to happy vs. fearful faces after EPO vs. saline treatment of patients with bipolar disorder. **B.** Extracted mean percent BOLD signal change to happy vs. fearful faces in the SFG cluster at baseline and follow-up. **C.** Correlation between change in SFG response and recognition of happy facial expressions in the EPO group from baseline to follow-up.

**Figure 3.** Exploratory Regions Of Interest (ROIs) showing increased response to happy vs. fearful faces in EPO vs. saline treated patients.

**Supplementary Figure S1.** Consort flow-chart.

**Supplementary Figure S2.** The a priori volume of interest (VOI).