Title: Effects of erythropoietin on memory-relevant neurocircuitry activity and recall in mood disorders

Running title: EPO: neural activity change in mood disorder

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Abstract

Objective: Erythropoietin (EPO) improves verbal memory and reverses sub-field hippocampal volume loss across depression and bipolar disorder. This study aimed to investigate with functional magnetic resonance imaging (fMRI) whether these effects were accompanied by *functional* changes in memory-relevant **neuro-circuits** in this cohort.

Method: Eighty-four patients with treatment-resistant unipolar depression who were moderately depressed or bipolar disorder in remission were randomized to eight weekly erythropoietin (40,000 IU) or saline 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 performed a picture encoding task followed by post-scan recall.

Results: Sixty-two patients had complete data (EPO: N=32, saline: N=30). EPO improved picture recall and increased encoding-related activity in dorsolateral prefrontal cortex (dlPFC) and temporo-parietal regions, but not in hippocampus. Recall correlated with activity in the identified dlPFC and temporo-parietal regions at baseline, and **change in recall correlated with activity change** in these regions from baseline to follow-up **across the entire cohort**. The effects of EPO were not correlated with change in mood, red blood cells, blood pressure or medication.

Conclusion: The findings highlight enhanced encoding-related dlPFC and temporo-parietal activity as **key neuronal underpinnings** of EPO-associated memory improvement.

Key words: Bipolar disorder, cognition disorders, randomized controlled trial, functional neuroimaging

Significant Outcomes

- The EPO-associated increase in dorsolateral prefrontal activity during picture encoding may reflect stronger strategic encoding in EPO-treated patients **given a general association between activity in this region and recall success.**
- The present findings highlight EPO as a candidate treatment for deficits in memory and neuroplasticity in mood disorders.
- The findings have implications beyond EPO as they provide novel evidence for a potential circuitry-based biomarker for pro-cognitive effects of treatments targeting cognition in mood disorders.

Limitations

- The study sample was heterogeneous, consisting of patients with treatment-resistant unipolar disorder who were moderately depressed and patients with bipolar disorder in partial or full remission.
- Participants received antidepressant or mood stabilizing treatment which may have non-specific effects on global brain activation.
- The therapeutic use of EPO in mood disorders is limited by its hematological effects and potential associated complications in non-anemic populations.

Introduction

There is no effective treatment for cognitive dysfunction in unipolar depression (UD) and bipolar disorder (BD) (1;2). Drug development is hampered by the absence of neural circuitry-based biomarker models for cognitive enhancement, and hence there is a lack of insight into whether candidate compounds target the neuronal origins of cognitive dysfunction (3). There is therefore a need for studies characterizing the neuronal mechanisms of novel candidate treatments with pro-cognitive effects to identify neurobiological targets of cognitive improvement.

Functional magnetic resonance imaging (fMRI) studies indicate that patients' memory deficits arise from aberrant neural activity in medial temporal regions including the hippocampus and medial and dorsolateral prefrontal cortex (PFC) during symptomatic phases (4-7) and after remission from mood episodes (8-10). Hippocampal *hypo*-activity has been observed during memory encoding and retrieval in UD (5-7;10) and during retrieval in BD (8), although two studies found no hippocampal activity change (7;11). Encoding-related hippocampal response correlates with recall accuracy in healthy but not in UD or BD individuals (4;5;8), which points to deficient hippocampal recruitment during memory formation in mood disorders. The majority of studies also report *hypo*-activity in the medial PFC (mPFC) and/or dorsolateral PFC (dlPFC) during memory encoding (4;6;9) and in the dlPFC during retrieval (6;8), although some found encoding-related dlPFC *hyper*-activity (8) or no PFC changes (5). This aberrant hippocampal and prefrontal activity in mood disorders is thought to reflect inefficient association and organization of information during encoding and retrieval processes (6).

Up-regulation of neurotrophins is a putative treatment target to restore neural network function within the hippocampus and PFC and thereby reverse cognitive deficits in mood disorders(12). The multifunctional trophic growth factor erythropoietin (EPO) may therefore represent a unique

therapeutic agent. Endogenous brain-derived EPO mediates neuroprotection and plays a key role in cognition (13) and systemically administered EPO has neuroprotective and neurotrophic actions and improves cognition in preclinical models of neuropsychiatric disease (14). The cognitive effects of EPO seem to derive from direct neurobiological actions as shown in fMRI studies by our group in which a single EPO administration enhanced memory-relevant hippocampal and prefrontal activity in healthy and depressed individuals without affecting red blood cells (15;16).

In two parallel randomized controlled efficacy trials, we demonstrated that eight weekly EPO (40,000 IU) vs. saline infusions produced mood-independent improvement of verbal memory in moderately depressed patients with treatment-resistant UD (17) and a trend towards improvement in partially remitted patients with BD (18). Structural MRI assessments of 69 patients from these trials with complete MRI data revealed that EPO reversed structural hippocampal volume loss within the cornu ammonis 1-3 and subiculum across these diagnostic groups, which correlated with patients' verbal memory improvement (19).

Aims of the study

The present exploratory fMRI study aims to build on these findings by determining if the effects of EPO were also accompanied by *functional* neural activity changes in a memory-relevant neural circuitry in this cohort. Since the EPO-associated cognitive improvement was not expected to be disease specific (15;17-21) we pooled fMRI data from the UD and BD patients from the two parallel EPO trials with identical designs (17;18). We hypothesized (I) that EPO would increase memory-related hippocampal activity, based on evidence for memory-related hippocampal hypo-activity in mood disorders, enhanced hippocampal response after a single EPO administration (15) and EPO-

associated structural hippocampal volume increase in this cohort (19), and (II) that EPO would enhance medial and/or dorsolateral prefrontal activity, given PFC hypo-activity in mood disorders and memory-related prefrontal activity increase after a single dose of EPO to healthy volunteers (15).

Materials and Methods

Study design

This exploratory fMRI study was conducted as part of two parallel phase 2 efficacy trials with a randomized, double-blind, placebo-controlled, parallel-group design that have been published in full (17;18;22).

Participants

Patients were recruited through Copenhagen Affective Disorders Clinic and advertisement on relevant websites and were screened with Schedules for Clinical Assessment in Neuropsychiatry (SCAN). Eligible patients had an ICD-10 diagnosis of treatment-resistant UD with moderate depressive symptoms (Hamilton Depression Rating Scale 17-items (HDRS-17) score ≥17) or of BD in partial remission (HDRS-17 and Young Mania Rating Scale (YMRS) scores ≤14). Patients were on stable medication from ≥2 weeks prior to trial start and medication was unchanged for the duration of the study. A detailed description of the screening, exclusion criteria, safety monitoring, and approvals, can be found in the primary outcome reports of the trials (17;18). The study was carried out in accordance with the latest version of the Declaration of Helsinki. After complete description of the study, written informed consent was obtained from all participants.

Randomization and masking

Block randomization was conducted with stratification for gender and age (< or ≥35 years). Outcome assessors were blinded to patients' treatment allocation throughout the trial and data analysis (for details see the primary outcome reports (17;18)). The Good Clinical Practice unit at Copenhagen University Hospital (www.gcp-enhed.dk/kbh) monitored that blinding was maintained.

Procedures

Participants received eight weekly intravenous infusions of EPO (Eprex; 40,000 IU; Janssen-Cilag) or saline (sodium chloride [NaCl] 0.9%). fMRI was performed at baseline and week 14 (6 weeks after treatment completion), at which time hematological parameters were expected to have normalized in the EPO group (19). Blood tests and blood pressure were taken on a weekly basis during the treatment and in week 14. Mood symptoms were measured at weeks 1, 5, 9, and 14 with the HDRS-17, YMRS, and Beck Depression Inventory (BDI).

Main fMRI study outcome

The main outcome of this exploratory fMRI study was differential change between EPO vs. saline groups in encoding-related neural activity and recall from baseline to follow-up.

Explicit picture encoding task

An explicit picture encoding task was selected due to hippocampal engagement in encoding of visual scenes[1] and was similar to the task employed in our previous EPO studies (15;23). Pictures were matched for valence, arousal and complexity, and were presented in a blocked paradigm to maximize sensitivity for hippocampal blood-oxygen-level-dependent (BOLD) signal change (24). Six picture blocks (24 seconds each) were preceded by an instruction screen (two seconds) and interleaved with 24 seconds of fixation crosses, resulting in a task duration of four minutes, 50 seconds. Blocks consisted of six pictures presented serially for three seconds interleaved with a one-second fixation cross. Each block contained an equal number of pictures representing indoor and outdoor scenes. Participants were instructed to determine whether pictures showed 'indoor' or 'outdoor' scenes and to pay attention as they would be asked to recall the pictures. This was followed by a free recall test immediately after the scan. Alternate, matched versions of the task were administered at baseline and follow-up in a counterbalanced order to minimize effects of learning.

Control task

To investigate whether encoding-related neural responses were confounded by global effects of EPO on baseline cerebral blood flow, neural activation in the occipital cortex was assessed with a control visual stimulation paradigm: a flashing checkerboard (eight Hz) was presented in blocks of 14 seconds alternating with 14 seconds of a fixation cross for a total of six cycles (duration three minutes, eight seconds). Participants were instructed to lie with their eyes open during this time.

Magnetic resonance imaging

MRI data were collected at the Danish Research Centre for Magnetic Resonance with a 3 Tesla Siemens Trio MR scanner using an eight-channel head array coil. BOLD-sensitive fMRI used a T2*-weighted echo-planar imaging sequence with an echo time (TE) of 30 ms, repetition time (TR) of 2.49 ms and a low flip angle of 20° to minimize physiological noise (25). A total of 117 brain volumes were acquired in a single fMRI session, each consisting of 42 slices with three mm slice thickness and a field of view of 192 x 192 mm using a 64 x 64 grid. High-resolution 3D structural T1-weighted spin echo images were obtained after the first session of BOLD fMRI Inversion time (TI)=800, TE=3.93, TR=1540 ms, flip angle 9°; 256 x 256 FOV; 192 slices.

fMRI data analysis

We investigated hypothesis (I)— that EPO increases encoding-related hippocampal activity—by examining BOLD signal change within predefined hippocampal Regions of Interest (ROIs) obtained in standard space with mri3dX (http://www.idoimaging.com/program/160). Hypothesis (II)—that EPO increases medial and/or dorsolateral prefrontal response— was investigated with whole-brain exploratory analysis due to disparate findings regarding the particular PFC regions showing aberrant memory-related activity in mood disorders.

Functional MRI data processing was carried out using the FMRI Expert Analysis Tool Version 6.00, part of FMRIB's Software Library (www.fmrib.ox.ac.uk/fsl). Pre-processing included image realignment, non-brain removal, spatial normalization and spatial smoothing (Gaussian kernel, five mm full-width-half-maximum). The time series in each session were high pass-filtered (to maximum 0.008 Hz). The 'encoding' condition was modeled by convolving trials with a canonical hemodynamic response function (26). Mean percent BOLD signal change was extracted from the hippocampal ROIs

and (for the control task) an occipital cortex ROI activated by photic stimuli (27) and examined in the Statistical Package for Social Sciences (SPSS; version 22; IBM Corporation, Armonk, New York, United States) using repeated-measures analysis of variance (ANOVA) with time and group as independent variables and post-hoc t-tests to determine the origin of the between-group differences. Bonferroni correction was applied to adjust for the number of comparisons of signal change from the hippocampal ROIs.

For the whole-brain analysis that tested hypothesis (II), fixed-effects analyses were conducted for each participant to identify regions of encoding-related activity increase and decrease from baseline to follow-up. These parametric 'difference images' for each participant were then entered into the group-level analyses that employed a full mixed-effects approach (28). Given the exploratory nature of the whole-brain analysis, Z (Gaussianised T/F) statistic images were thresholded using clusters determined by a relatively liberal cluster-extent threshold of $Z>2.0$ and a corrected cluster significance threshold of $P=0.05$, consistent with our previous studies (15;29). A standard anatomical atlas (30) was used to localize the foci of peak cluster activation.

Statistical analyses of behavioral, demographic, and mood data

Speed and accuracy of picture categorization during fMRI and the number of recalled pictures after the scan 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. Change in mood symptoms were analyzed using ANOVA with time and group as independent variables. Medication status in EPO vs. saline groups was

examined using Pearson's Chi Square and Fisher's Exact tests. Hemoglobin 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 Pearson's correlations between (a) picture recall and BOLD response at *baseline* in areas affected by EPO and (b) the *change* from baseline to follow-up in recall and in BOLD response. Changes in memory and BOLD response were also correlated with changes in hemoglobin, blood pressure, and mood. Statistical analyses were performed in SPSS.

Results

Participant flow and characteristics

Of the 84 participants recruited between September 2009 and October 2012, one withdrew at baseline, seven had missing behavioral data, and 14 had incomplete MRI data. Of the 14 participants with incomplete MRI data, one (EPO) was not scanned due to a stiff back, seven were not scanned at follow-up (three EPO, four saline) because of mild claustrophobia (two), dropout (three), or feeling too overwhelmed (two), and imaging data was lost due to technical problems for six participants (two EPO, four saline) (for more details, see the CONSORT chart in Figure S1). Data was thus analyzed for 62 participants (EPO: N=32; Saline: N=30). There were no differences between the included patients and those with missing data in demographic variables, illness load or mood symptoms at baseline or follow-up ($P \geq 0.10$). EPO and saline groups were comparable on baseline characteristics and medication ($P \geq 0.3$) (Table 1). EPO was well tolerated and we observed no severe adverse events (17;18).

Behavioral results

Accuracy of picture categorization during fMRI scanning revealed no effects of EPO vs. saline ($P \geq 0.14$). Categorization speed showed a differential effect of EPO vs. saline over time ($F(1,57)=5.0$, $P=0.03$; t-tests: $P \geq 0.08$), driven by slowed responses in the saline group **at follow-up** (paired t-test, **baseline to follow-up**: t=-4.1, df=27, $P<0.001$) (Table in Supplementary Information).

Picture recall showed a general learning effect ($F(1,60)=48.4$, $P<0.001$). EPO significantly increased the number of pictures recalled compared to saline ($F(1,60)=8.4$, $P=0.01$; posthoc t-tests non-significant) (Supplementary Table and Figure 1). This effect of EPO was independent of diagnosis (UD: $F(1,26)=4.7$, $P=0.04$; BD: $F(1,32)=4.3$, $P=0.046$). The EPO-associated improvement in picture recall was not correlated with *structural* volume increase in the left hippocampal CA 1-3 and subiculum ($P \geq 0.5$).

fMRI results

Hippocampal ROIs

The hippocampi were significantly activated during picture encoding at baseline (left: t=5.2, df=61, $P<0.001$; right: t=4.3, df=61, $P<0.001$) but were unaffected by EPO ($P \geq 0.3$). **Exploratory post-hoc analyses in UD and BD groups separately also revealed no effect of EPO on hippocampal response in BD ($P \geq 0.2$). In UD, there was a strong trend towards differential hippocampal activity change between EPO and saline groups that was driven by lower baseline activity in the saline group ($F(1,26)=4.13$, $P=0.052$; t=-2.4, df=26, $P=0.03$).**

Whole-brain analyses

Picture encoding activated occipital, temporo-parietal, and frontal regions (for cluster maxima see Table 2; green clusters in Figure 2). Exploratory whole-brain analysis revealed that EPO vs. saline increased activity in bilateral dlPFC and left-side medial temporal and superior parietal regions (for cluster maxima see Table 2; see colour-coded clusters in Figure 2). These regions overlapped with the neural network identified as engaged in picture encoding in a whole-brain analysis at baseline. **For exploratory purposes, we examined the mean percent BOLD signal change in the identified clusters for the UD and BD groups, separately. This revealed similar significant effects of EPO vs. saline within bilateral dlPFC and left superior parietal regions in each diagnostic group (*P*≤0.02).**

Visual stimulation control experiment

There were no baseline differences between groups or effects of EPO vs. saline on neural response in the occipital ROI ($P \geq 0.12$).

Associations between changes in BOLD fMRI and picture recall

Encoding-related hippocampal response at baseline showed no correlation with recall success ($P \geq 0.9$) and there was no correlation between changes in hippocampal activity and picture recall over time across the entire cohort ($P \geq 0.3$) (Figure 3A). In contrast, picture recall scaled linearly with encoding-related response in the identified clusters in the dlPFC (left: $r(60)=0.3$, $P=0.02$; right: $r(60)=0.3$, $P=0.03$), left medial temporal gyrus ($r(60)=0.3$, $P=0.04$) and left superior parietal gyrus ($r(60)=0.3$,

P=0.03), but not in the left dlPFC (BA 4) cluster (P=0.3), across the entire cohort. This linear relationship between individual recall performance and encoding activity remained significant after adjustment for mood symptoms (P≤0.03). Across the entire cohort, there was also a correlation between memory *improvement* and activity *increase* within the five neocortical clusters: dlPFC (left: r(60)=0.3, P=0.01, second left: r(60)=0.3, P=0.02; right: r(60)=0.3, P=0.01), and left-side medial temporal (r(60)=0.3, P=0.01) and superior parietal cortex (r(60)=0.3, P=0.047) (Figure 3B-F). These correlations prevailed after adjustment for mood symptoms (P≤0.03). Correlations between change in memory and BOLD response within the EPO group were non-significant (P≥0.5), possibly due to reduced statistical power with the lower sample size (EPO: N=32). The decreased picture categorization speed in saline-treated participants was unrelated to the EPO-associated memory improvement (P≥0.3) or activity changes in the identified clusters (P≥0.2).

No influence of hematocrit, blood pressure, mood or concomitant medication

EPO increased hemoglobin during treatment (23;24), but this effect tapered off and there was no difference between groups at the follow-up scan (P≥0.3). There was also no difference between groups in blood pressure at baseline or follow-up (systolic: P≥0.7; diastolic: P≥0.4). EPO-treated participants showed no correlation between changes in hemoglobin and neural response in the identified clusters (P≥0.2) or memory improvement (P≥0.6). There was also no significant correlation between change in blood pressure and neural responses (P≥0.06). EPO had no effect on mood (P≥0.2). There was a correlation between change in recall and mood in the EPO group (r(30)=-0.4, P=0.050). However, the effect of EPO vs. saline on picture recall remained significant in ANCOVA adjusted for mood change (P=0.01). Finally, the effects of EPO vs. saline on memory and neural activity prevailed in ANCOVA

adjusted for antipsychotic medication **(yes/no)**, lithium **(yes/no)**, and lithium dose ($P \leq 0.01$). Given the neurogenic effects of lithium, we conducted an exploratory analysis *restricted to lithium-treated patients*, which showed that the significant effects of EPO (N=9) vs. saline (N=9) on picture recall and neural activity in the identified clusters prevailed ($P \leq 0.046$). Further, post-hoc comparisons *within the EPO group* showed no differential change over time in neural response or picture recall between patients with (N=9) or without lithium (N=23) ($P \geq 0.2$).

Discussion

This exploratory fMRI study builds on our previous finding that EPO increases verbal memory and left hippocampal volume across UD and BD by elucidating the *functional* neural activity changes in this cohort. Compared with saline, eight weeks of EPO treatment had no effect on encoding-related hippocampal activity but enhanced bilateral dlPFC and left-side temporo-parietal response and improved picture recall. Across the entire cohort, picture recall correlated positively with encoding-related activity in the dlPFC and temporo-parietal regions at baseline, and **change in recall correlated** with activity change in these regions from baseline to follow-up. The observed effects of EPO were unrelated to change in mood, hemoglobin or blood pressure.

The absence of *functional* hippocampal correlates of the EPO-associated structural increase in the left hippocampal CA1-3 and subiculum (19) was unexpected. A potential explanation is that hippocampal activity during picture encoding may not be a sensitive assay of the functions performed by the left hippocampus. Indeed, there is evidence for specialization of the hippocampi, with the *left* hippocampus being preferentially activated during *verbal* memory retrieval and the *right* hippocampus being more engaged in *pictorial* memory processes(31). This could explain why the EPO-associated

left hippocampal volume increase correlated with *verbal* memory improvement *but not with* changes in picture-related left hippocampal activity or picture recall. The absence of EPO-associated effects on hippocampal activity also contrasts with the enhanced bilateral hippocampal response during picture retrieval after a single EPO administration to healthy volunteers (15). This discrepancy may be related to differences in the implemented memory paradigms (picture encoding vs. retrieval) or study populations (healthy volunteers vs. participants with mood disorders). Alternatively, the neural mechanisms of EPO treatment may change over time, with early enhancement of hippocampal encoding, which translates into stronger strategic top-down mechanisms and improved memory performance after long-term treatment.

The effects of EPO on encoding-related dlPFC response may counteract prefrontal hypo-activity during memory encoding in mood disorders (4;6;9). **Indeed, failure to recruit dlPFC during active task performance may be a common neural correlate for cognitive deficits in mood disorders (32;33).** Explicit memory training with use of visuospatial mnemonic strategies increases encoding-related activity in the medial and lateral PFC and in temporo-parietal regions across healthy individuals and patients with mild cognitive impairment (34;35). The effects of EPO on dlPFC and temporo-parietal responses may thus reflect strengthened visuospatial mnemonic processes. In keeping with this **interpretation**, dlPFC and temporo-parietal activity during picture encoding correlated positively with recall success **across the entire cohort**. Such neural effects of EPO may occur early in treatment, as demonstrated by increased prefrontal and temporo-parietal activity during picture encoding one week after a single dose of EPO to healthy volunteers (15). Modulation of memory-related dlPFC and temporo-parietal response may thus represent a sensitive circuitry-based biomarker model for memory improvement (3).

From a mechanistic perspective, direct neurobiological actions are likely to mediate the observed effects of EPO. EPO increased hematocrit during the treatment phase (17;18), but hematocrit was normalized at week 14 and we observed no correlation between changes in hemoglobin and neural response in the identified ROIs. We also observed no effects of EPO on blood pressure or correlations between blood pressure and BOLD response in the EPO group. The visual control task revealed no effects of EPO on occipital response to photic stimuli, suggesting that the observed effects of EPO were unrelated to any global hemodynamic changes. Finally, the EPO-associated improvement of picture recall showed no correlation with mood changes. Together these findings suggest that direct neurobiological actions such as increase in neuroplasticity and neurogenesis underlie the observed effects of EPO.

The large sample size of 62 participants ensured higher statistical sensitivity than previous prospective fMRI investigations of cognition treatments involving 20-30 participants (36;37). Further, the randomized, placebo-controlled design accommodated for effects of repeated scanning and learning. Finally, the longitudinal assessments rendered it possible to capture intra-individual changes in encoding-associated neural activity and memory in response to EPO versus saline. A limitation was that participants were medicated since this may have non-specific effects on global brain activation. However, the two groups showed no difference in medication status or baseline neural response within the identified ROIs, and the effects of EPO on neural activity change prevailed after adjustment for medication status and dose. The study sample included both UD and BD patients who are characterized by somewhat distinct pathogenic processes. Nevertheless, memory dysfunction and aberrant memory-related hippocampal and prefrontal responses occur across both disorders, and EPO improves memory across several neuropsychiatric illnesses (17;18;20;21). The UD and BD patients also differed with

respect to the depression severity **which increased the heterogeneity of the cohort**. However, they were equally distributed between the drug groups, and the effects of EPO on memory and neural activity prevailed after adjustment for mood symptoms. Cluster-extent based thresholding in fMRI analysis is problematic when the clusters span multiple anatomical regions due to liberal statistical thresholds (38). However, despite the relatively liberal cluster-extent based statistical threshold in our exploratory whole-brain analysis, the identified clusters were spatially specific (range: 511-816 voxels). Nevertheless, the present results should be regarded as *exploratory* in nature. Although the primary efficacy outcomes of the trials were analyzed with intention-to-treat analysis in the original trials (17;18), we explored the neural activity changes using complete data sets. **However,** the risk of bias is low since (i) almost all (90%) participants completed both fMRI scans and the main missing-data mechanism was technical difficulties, and (ii) comparisons between the included patients and those with missing data revealed no differences in demographic variables or mood symptoms at baseline or follow-up. Finally, the therapeutic use of EPO in mood disorders is limited by its hematological effects and potential associated complications in non-anemic populations. From a clinical perspective, an interesting alternative is therefore the modified non-hematopoietic EPO molecules **such as carbamylated EPO (CEPO) and asialo-EPO** that enhance neuroplasticity and cognition **through activation of similar neurobiological pathways** in preclinical models **(for review, see (39;40)). There may also be other pharmacological alternatives although the findings in the field are still preliminary (for review, see (41)).**

The present findings highlight enhanced encoding-related dlPFC and temporo-parietal response as **key neuronal underpinnings** of memory improvement in EPO-treated patients. These exploratory findings

have more general implications beyond EPO as they provide novel evidence for a potential circuitry-based biomarker for pro-cognitive effects of treatments for cognition dysfunction in mood disorders.

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Declaration of interest

KWM reports having received consultancy fees from Lundbeck. MV discloses consultancy fees from Eli Lilly, Lundbeck, Servier and Astra Zeneca. OBP is a member of the board of directors of the Elsass Foundation. CJH has received consultancy fees from P1vital Ltd, Lundbeck, Servier and Eli-Lilly, and

is a company director of Oxford Psychologists Ltd. 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. 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. LVK reports having been a consultant for Lundbeck and AstraZeneca within the last 3 years. HE, JM, EA and LR report no biomedical financial interests or potential conflicts of interest.

All statistical analyses were performed by KWM at Psychiatric Centre Copenhagen, Copenhagen University Hospital, Rigshospitalet and Danish Research Centre for Magnetic Resonance, email: Kamilla.miskowiak@regionh.dk

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Figure legends

Figure 1. Percent improvement in picture recall from individual baseline. This was calculated as the improvement from baseline to follow-up adjusting to each patient's individual starting point. For unadjusted recall scores, see Table 2. P-values for the results of the ANOVA of picture recall scores between groups are provided in Table 2. Error bars denote standard errors of the mean.

Figure legend 2. Lower part. Neural response during picture encoding across all participants at baseline across the brain (green). Clusters show increased response in the erythropoietin (EPO) vs. saline groups in the exploratory whole-brain analysis (yellow, red, light blue and dark blue). Images are thresholded at $Z>2.0$ and $P<0.05$, corrected for multiple comparisons at a cluster level. Upper part. Plot of mean percent blood-oxygen-level-dependent (BOLD) signal change during encoding within these regions of interest in the EPO (red bars) and saline (blue bars) groups at baseline and follow-up. Bars show the mean; error bars show the standard error. **Compared to saline, EPO treatment leads to a relative increase in BOLD signal change during picture encoding in these regions from baseline to follow-up.**

Figure legend 3. Linear relation between *change* in memory performance (total number of pictures recalled) and in encoding-related blood-oxygen-level-dependent (BOLD) response within the identified regions of interest showing an effect of erythropoietin (EPO) vs. saline in the exploratory whole-brain analyses (A-E) and in the bilateral hippocampi (F). Across the entire cohort, there was a positive correlation between change in encoding-related activity in bilateral dorsolateral prefrontal cortex (A-C), occipito-temporal (D) and superior parietal (E) regions and in memory performance (r=0.25-0.34,

$P<0.05$). Using the entire cohort, there was no correlation between change in hippocampal activity and memory performance (F; indicated with black trend line).

Figure S1 for supplementary material. CONSORT flow chart.