PROGRESS IN TRANSLATIONAL NEUROIMAGING: INTEGRATING PATHWAYS, SYSTEMS AND PHENOMENOLOGY IN NEUROLOGY AND PSYCHIATRY

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EDITORIAL
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Editorial: Progress in Translational Neuroimaging: Integrating Pathways, Systems, and Phenomenology in Neurology and Psychiatry

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Keywords: neuroimage analysis, phenomenology, diagnosis, psychiatry, nosology and classification of mental disorders

Editorial on the Research Topic

Progress in Translational Neuroimaging: Integrating Pathways, Systems, and Phenomenology in Neurology and Psychiatry

Classification is taxonomic nomenclature system established for the purposes of statistical analysis of the phenomena and in order to facilitate and uniform the language of professionals in different countries. Classification systems in medicine, hence in psychiatry are composed of nosological units. The latter as well as the relevant methods for their exploration are characterized with the criteria of validity, reliability, specificity, and sensitivity.

Whenever we introduce some term, method or assessment system, we should inquire whether it can capture the exact phenomenon *it is intended* to capture. In other words, when we introduce a term like "depression" or "depressiveness" as a symptom and develop relevant instruments to measure it (inventories and clinical interviews), we are supposed to ascertain that it measures specifically depression and not some other related, phenomenon, e.g. delusions. When two different assessment tools for depression happen to coincide in their score/interpretation, it is regarded as *convergent validity*, and whenever their results are discrepant it is considered as divergent or *discriminative validity*. Divergent validity should generally characterize phenomena which differ each from other and in this respect is supposed to assist differential diagnosis between them. In this context, a valid measure (e.g. test) for depression should have convergent validity with other test, established to assess depression and discriminative validity with tests, designed for assessment of paranoid delusions. In other fields of medicine, external criteria for validity are introduced, such as biological measures. No such criteria have been introduced so far in clinical psychiatry.

Psychiatric diagnosis is characterized with *relatively high level of reliability* (repeated clinical ratings from different clinicians) and problematic *validity* (lack of commonly used biological markers to reify the diagnosis).

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Over the past two decades there have been established mainly post-hoc correlations between the different types of clinical tools (cognitive assessment tests, interviews, and inventories) on one hand and imaging neuroscience. This far different kinds of measures represent mere statistical correlations with limited or no reference to the mechanism of disorder and therefore cannot be effectively translated and embodied into normative criteria, diagnostic standards, and clinical evaluation procedures (1). This undermines the reification of psychological clinical measures and mental disorders as diagnostic entities (2).

There has been raised a conceptual issue: What is the *subject of reification that procedures of translation may address*? Is it the clinical assessment inventory/test that is reified by means of functional MRI for example or the opposite?

This Research Topic has particular focus on whether and to what extent it is feasible to translate data from multimodal neuroimaging in terms of cross-validation against different trait and state measures, such as the Temperament and Character Inventory or Paranoid-Depressive Scale (Squarcina et al.) and PVSAT/PASAT (Iancheva et al.).

We assume that in this manner there is provided meaningful insight into the relevance of multimodal neuroimaging resources for both explanatory mechanisms of normal mental functions and brain dysfunctions which underlie mental disorders. In this fashion various papers contribute with neuroimaging findings to underpin the clinical measures of and the nosological entities.

In one of the presented papers in this Research Topic (Stoyanov et al.), the most powerful functional MRI pattern which allows discriminative validation of schizophrenia from affective disorders is comprised of BOLD signal corresponding to negative signature on depressive items response and positive signature to neutral items response from interest scale along with the signal associated with paranoid items. Essentially this means that neutral items are not neutral for patients with schizophrenia, but incorporated into their psychotic disturbance system.

In this Research Topic, we tried to consider all these above-mentioned critical issues by integrating studies on diverse pathways, neurosystems and phenomenology in both psychiatric and neurologic disorders. Specifically, 19 articles have been published on a variety of themes, being 11 of them original papers mostly implementing neuro-imaging approaches. Three studies focussed on schizophrenia, showing 1) instability of the default mode network (DMN) and impaired cognitive control when patients experience auditory verbal hallucinations (AVH), and 2-3) preliminary possibility to disentangle brain activation due to paranoia in schizophrenia and depression by using a self-evaluation scale. Three investigations explored mood disorders, reporting 1) mismatch between cerebral blood supply and oxygen metabolism in bipolar disorder, being a potential brain sign of energy homeostasis inefficiency, 2) no effect of the BDNF genotype on encoding-related neural activity in bipolar patients, with a superior memory performance in Met carriers, and 3) symptom improvement after 3-weeks of repeated transcranial magnetic stimulation (rTMS) therapy on the left dorsolateral prefrontal cortex (5 consecutive weekdays every week) in patients with unipolar depression. Three other reports detected fascinating results in different pathologies suggesting: 1) reduced interhemispheric functional connectivity in patients with obsessive-compulsive disorder (OCD), 2) activation in left Brodmann area (BA) 40 (particularly supramarginal gyrus) as a possible mechanism for diminishing fatigue impact on cognitive functioning in cognitively preserved patients with multiple sclerosis (MS), and 3) brain reorganization due to pre-/perinatal damage in left hemisphere (periventricular and cortico-subcortical lesions).

Interestingly, in healthy subjects the biochemical brain basis (phosphocreatine plus creatine -PCr+Cre-, glycerophosphocholine plus phosphocholine -GPC+PC-, and myo-inositol) of specific personality traits during adolescence were here presented with particular regards to self-directedness and self-transcendence along with the functional brain activations during memory paradigm performance after intensive learning particularly in both occipital and temporal regions.

Three single cases reported intriguing perspectives on delirious mania (due to mild encephalitis with reversible splenial lesion), schizophrenia (potential amelioration of eyeblink conditioning -EBC- after cerebellar transcranial direct current stimulation -tDCS-), and Alzheimer's disease (PSEN-1 gene mutation in an early-onset patient). Finally, five reviews described the state of the art of cognitive and neuroimaging correlates in PTSD, depression, substance abuse and eating disorders, debating their translational and clinical implications, and future directions whereas.

In conclusion, we believe this is a comprehensive and well composed Research Topic of Frontiers in Psychiatry pointing out key topics for translational psychiatric neurosciences and neuroimaging. In particular, this Research Topic corroborated the fact that nowadays, we need further research on the genetic, neurobiological, and cognitive bases of major psychiatric disorders in order to better delineate different domains charactering commonalities and dissimilarities across different spectrum and continuum diagnostic prototypes (3). This will ultimately help us to have an evolute idea of how human brain impairments affect neuropsychological functions, behavioral characteristics, general functioning, outcome, and disease trajectory of our patients (4). We finally, last but not least, thank the estimated authors who contributed to the issue to allow us to have the privilege to coordinate and guest edit such a prestigious issue.

AUTHOR CONTRIBUTIONS

Both authors have contributed on equal basis to this paper.

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Reduced Interhemispheric Functional Connectivity in Obsessive–Compulsive Disorder Patients

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Background: Neuroimaging studies have shown that the high synchrony of spontaneous neural activity in the homotopic regions between hemispheres is an important functional structural feature of normal human brains, and this feature is abnormal in the patients with various mental disorders. However, little is known about this feature in obsessive–compulsive disorder (OCD). This study aimed to further analyze the underlying neural mechanisms of OCD and to explore whether clinical characteristics are correlated with the alerted homotopic connectivity in patients with OCD.

Methods: Using voxel-mirrored homotopic connectivity (VMHC) during resting state, we compared 46 OCD patients and 46 healthy controls (HCs) matched for age, gender, and education level. A partial correlation analysis was used to investigate the relationship between altered VMHC and clinical characteristics in patients with OCD.

Results: Patients with OCD showed lower VMHC than HCs in fusiform gyrus/inferior occipital gyrus, lingual gyrus, postcentral gyrus/precentral gyrus, putamen, and orbital frontal gyrus. A significant positive correlation was observed between altered VMHC in the angular gyrus/middle occipital gyrus and illness duration in patients.

Conclusions: Interhemispheric functional imbalance may be an essential aspect of the pathophysiological mechanism of OCD, which is reflected not only in the cortico-striato-thalamo-cortical (CSTC) loop but also elsewhere in the brain.

Keywords: obsessive–compulsive disorder (OCD), r-fMRI, functional connectivity (FC), interhemispheric functional connectivity, homotopic connectivity, voxel-mirrored homotopic connectivity (VMHC)

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INTRODUCTION

Resting-state functional magnetic resonance imaging (r-fMRI) technology indirectly reflects the intrinsic, spontaneous neural activity of the brain and can be used to measure resting-state functional connectivity (RSFC) between brain regions directly (1). Voxel-mirrored homotopic connectivity (VMHC) is an R-fMRI analysis method proposed by Zuo XN in recent years (2). VMHC quantifies the RSFC between each voxel in one hemisphere and its mirrored counterpart in the other hemisphere (i.e., homotopic RSFC). R-fMRI studies have discovered the high synchronicity of spontaneous

activity between homotopic regions in healthy human brains, showing regional differences consistent with brain function levels (3, 4). Furthermore, a VMHC study using a large sample of healthy subjects (214 cases) demonstrated a robust homotopic RSFC architecture that exhibits regionally specific age- and sex-related changes across the lifespan (2). Therefore, high synchronicity of spontaneous neural activity between homotopic regions is considered an important feature of normal brain function.

Obsessive–compulsive disorder (OCD) is a common, typically chronic disorder marked by intrusive and disturbing thoughts (obsessions) and repetitive behaviors (compulsions) that the person feels driven to perform (5). The lifetime prevalence is about 1–3%. The patients understand that these compulsive symptoms are unreasonable, unnecessary, but they are unable to control or get rid of them, thus falling into anxiety and pain (6). Furthermore, OCD is characterized by intense emotional arousal and executive control impairments (7). These two mechanisms influence each other and are responsible for maintaining the obsessive–compulsive cycle (8). Although the exact pathophysiological mechanism of OCD is not fully understood, it is currently considered to be closely related to alterations in the cortico-striato-thalamo-cortical (CSTC) circuitry, which includes some main gray matter (GM) nodes such as the orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC), striatum, and thalamus (9, 10). The majority of previous OCD r-fMRI studies tended to use seed-based FC analyses with a focus on local abnormalities, especially within the fronto-striatal circuit. Recently, studies using the VMHC method explored altered homotopic RSFC in a variety of mental illnesses, such as depression, schizophrenia, sleep disorder, dementia, addiction barrier, bipolar disorders, and phobia (11–17). However, little is known about changes in the homotopic RSFC in OCD. Some early studies have suggested that OCD patients may have interhemispheric structural and functional abnormalities. Two studies on interhemispheric structural connectivity of the OCD patients found that abnormal corpus callosum (CC) morphology and fractional anisotropy (FA) (18, 19). Both increased and decreased FA values in the CC were reported in a meta-analysis of Diffusion tensor imaging (DTI) studies on OCD (20), which suggested that changes in the microstructure of the CC may be involved in the process of obsessions and compulsions (21). It is noteworthy that a neuropsychological study of OCD found that microstructural damage was significantly associated with cognitive performance in intra-hemispheric bundles but not in CC (22). Some Electroencephalography (EEG) studies found that compared with healthy controls (HCs), OCD patients had abnormal electrical activity on one side of the hemisphere (23–25) (left hemisphere or right hemisphere). A study of transcranial magnetic stimulation (TMS) found that stimulation of the right DLPFC resulted in the relief of OCD symptoms, while stimulation of the left DLPFC did not resolve (26). Additionally, a Positron emission tomography (PET) study found that left and right hemisphere DLPFC showed opposite perfusion responses in acute symptomatic OCD patients (27). Evidence from neurosurgery indicated that symptomatic improvements were observed in patients with OCD after right anterior capsulotomy, but not after left anterior capsulotomy (28, 29). Nonetheless, the deficits in these patients seem not to be related to a specific lateralized dysfunction of a particular hemisphere, but probably due to a functional inter-hemisphere imbalance (30). Although all of the above findings suggested that there may be a special interhemispheric functional effect in OCD patients, there is almost no R-fMRI study that specifically clarify clarifies what is the interhemispheric functional connectivity pattern of OCD patients compared to healthy controls.

In this study, we used R-fMRI combined with the VMHC approach to explore changes in homotopic connectivity in OCD patients. We compared the VMHC differences between OCD patients and HCs, and between treated and drug-naive OCD patients. The aims of this study were to verify that OCD patients had significant VMHC abnormalities (31) and to examine whether medical treatment affects the altered VMHC in OCD. Moreover, we expected to explore a relationship between altered VMHC values and the clinical characteristics of OCD patients.

MATERIALS AND METHODS

Participants

This study has been approved by the Ethics Committee of Kunming Medical University (ClinicalTrials.gov: NCT01298622). The researchers introduced all participants to the purpose, content, potential risks, and benefits of the study; the principle of voluntary participation; and the anonymity and confidentiality of the research. All participants signed informed consent.

A total of 49 OCD patients (including the outpatients and inpatients) were recruited from the First Affiliated Hospital of Kunming Medical University from October 2011 to December 2016. Inclusion criteria were as follows: a) comply with *Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition* (DSM-IV) criteria for OCD based on the Structured Clinical Interview; b) Yale–Brown Obsessive–Compulsive Scale (Y-BOCS) total score ≥16 points, and Hamilton Depression Rating Scale (HAMD) score <18 points; c) age ranges from 18 to 60 years old; d) preference for using the right hand; e) all the OCD patients' patients' obsessive–compulsive symptoms were not caused by another mental disorder or physical disease; f) exclude organic brain diseases and major physical illnesses; g) no metal implants in the body. When performing MRI scans, 25 of them are first-episode untreated patients; 24 had received psychiatric medication for more than 4 weeks. The vast majority of the drugs taken by 24 patients are SSRI (selective serotonin reuptake inhibitors) drugs. Of the 24 patients, 9 patients took sertraline, 5 patients took multiple drugs (two kinds of SSRI and venlafaxine or two kinds of SSRI and clomipramine), 3 patients took sertraline and fluoxetine, 3 patients took paroxetine, 2 patients took sertraline and paroxetine, and 2 patients took fluoxetine.

We also enrolled 46 healthy controls from society during the period from September 2011 to 2017. Entry criteria were as follows: a) age 18 to 60 years old; b) right-handed; c) no mental illness meeting the diagnostic criteria; d) no family history of mental illness; e) gender, age, handedness, and education years are matched with the OCD group; f) no metal implants in the body.

The obsessive–compulsive symptoms, depressive symptoms, and anxiety symptoms of the OCD group and the HC group

were evaluated using the Yale–Brown Obsessive Compulsive Scale (Y-BOCS), Hamilton Depression Rating Scale-17 items (HAMD-17), and Hamilton Anxiety Rating Scale (HAMA). The above evaluations were performed by two experienced clinical psychiatrists.

MRI Acquisition

MRI images were obtained using a Philips Achieva 3.0-T MRI scanner in the First Affiliated Hospital of Kunming Medical University. The participants were required to remain motionless and awake with their eyes closed. Soft earplugs and foam pads were used to reduce scanner noise and head motion. A gradient-echo sequence was also used to obtain high-resolution T1-weighted structural MRI images with the following parameters: time of repetition (TR)/time of echoing (TE) = 2,500/80 ms, slice thickness = 6 mm, field of vision (FOV) = AP (250 mm) × Right/left (RL) (193 mm) × Foot/head (FH) (142 mm), matrix size = 128 × 128, flip angle = 90°, slices = 16, gap = 2 mm, scan duration time = 45 s. Normal T1-weighted MRI scans were first performed to exclude obvious structural abnormalities. The resting-state functional images were acquired by using an echo-planar imaging (EPI) sequence with the following parameters: TR/TE = 2,200/35 ms, flip angle = 90°, FOV = 230 × 230 mm, matrix = 128 × 128, slice thickness = 3.0 mm without interlayer spacing, slices = 50, scan duration time = 17 min 40 s.

MRI Preprocessing

Functional magnetic resonance imaging (fMRI) data preprocessing were performed using the statistic parametric mapping software package (SPM12, http://www.fil.ion.ucl.ac.uk/spm) running in the Matlab 2012a (MathWorks, Natick, MA, USA) and in the Data Processing Assistant for Resting-State fMRI (DPARSF, http://rfmri.org/DPARSF) (32). The steps of preprocessing were as follows: a) format conversion: convert the Digital imaging and communications in medicine (DICOM) format of the original image data into Neuroimaging informatics technology initiative (NIFTI) format; b) removal of the first 10 time points; c) time correction; d) head motion correction, data removal of average head motion translation >2 mm and/or rotation >2° (excluding two untreated OCD subjects and one drug-treated OCD subject); e) linearly register each subject's T1 image to the corresponding functional image and then divide it into gray matter, white matter, and cerebrospinal fluid; f) removal of the influence due to covariates (24-head movement parameters, white matter signal, cerebrospinal fluid signal); g) Each of the abovementioned registered images was non-linearly registered to the MNI (Montreal Neurological Institute) standard space and resampled to a voxel size of 3 × 3 × 3 mm^3; h) the signal was linearly detrended and bandpass filtered at 0.01–0.08 Hz to reduce low-frequency drifts and high-frequency physiological noise (i.e., respiratory and cardiac) (33).

VMHC Calculation

Before using the Data processing & analysis for (resting-state) brain imaging (DPABI) software to calculate VMHC, a brain symmetry template was initially created to minimize the influence of geometric differences between the hemispheres on VMHC. Specifically, first, all 46 normalized T1 images of the healthy controls are averaged to create an average normalized T1 image; then, this average T1 image is re-averaged using its left and right mirrored versions to generate a particular group symmetric template. Then, this group symmetric template is applied to the 46 standardized images after the above preprocessing steps and then smoothed by a Gaussian kernel of 4-mm full width and half maximum (FWHM). VMHC is then calculated to obtain VMHC maps and zVMHC maps (Fisher *z*-transformation) for each subject. For each subject, VMHC was computed as Pearson correlation coefficient between each voxel's residual time series and that of a corresponding voxel in the opposite hemisphere as described in a previous study. Similarly, the OCD group was processed to obtain a group symmetric template and 46 zVMHC maps. More details about the VMHC method were given in the article (2).

Statistical Analysis

Based on the statistical module in the DPABI software, group differences on zVMHC maps between the patients and the controls were calculated by using two-sample *t* tests, after adjustment for age, gender, education, mean framewise displacement (mean FD), and medication status. Given that a prior study has suggested that RSFC could be affected by micromovements from volume to volume (34), we calculated the mean framewise displacement (FD) values for each subject, which can reflect the temporal derivative of the movement parameters. FD values were calculated for each item as described in a previous study (34). The threshold for significance was set at $p < 0.005$ (two-tailed) and 5,000 iterations corrected by the TFCE + PT (Permutation test with Threshold-Free Cluster Enhancement) methods in the PALM tool (PALM—Permutation Analysis of Linear Models) (35, 36). Then, we got a corrected T-map. To observe the clinical relevancies of VMHC, the voxel-wise Pearson correlation analysis was calculated between each patient's zVMHC map and clinical characteristics (Y-BOCS total score, Y-BOCS obsession score, Y-BOCS compulsion score, and illness duration) by using the abovementioned corrected T-map as a mask. Age, gender, mean FD, HAMD score, and HAMA score were applied as covariates of no interest. The threshold for significance was also set at $p < 0.005$ (two-tailed) and 5,000 iterations corrected by the TFCE + PT methods. Then, we extracted the mean zVMHC values of the brain regions exhibiting significant correlations between abnormal VMHC and clinical characteristics to get the scatter plot. Considering that SSRI may affect VMHC, two-sample *t* tests were used to compare differences in zVMHC maps between 23 treated and 23 drug-naïve OCD patients, controlling for age, gender, education, and mean FD. The threshold for significance was corrected for TFCE + PT at $p < 0.05$ (two-tailed).

RESULTS

Demographics and Clinical Characteristics

The data of three patients (two untreated OCD subjects and one drug-treated OCD subject) were excluded from the analyses due to excessive head movement. Hence, the final samples included 46 patients (23 untreated OCD subjects and 23 drug-treated OCD

subjects) and 46 controls. There were no statistical differences in gender, age, education level, and mean FD between 46 OCD and 46 HCs (see **Table 1**). Similarly, there were no statistical differences in gender, age, education level, obsessive–compulsive symptoms, depressive symptoms, anxiety symptoms, and mean FD between two patient groups (see **Table 1**).

VMHC Differences Between Groups

As shown in **Table 2** and **Figure 1**, compared to the controls, the OCD patients showed significantly decreased VMHC in the fusiform gyrus/inferior occipital gyrus ($t = -8.371$, $p < 0.005$), lingual gyrus ($t = -7.653$, $p < 0.005$), postcentral gyrus/precentral gyrus ($t = -7.701$, $p < 0.005$), putamen ($t = 4.321$, $p < 0.005$), and orbital frontal gyrus (OFC) ($t = 4.617$, $p < 0.005$). No regions showed increased VMHC in the patients relative to controls. Moreover, there were no significant differences in VMHC when comparing the medicated and unmedicated patient sub-groups.

Correlation Between Altered VMHC and Clinical Characteristics

The altered VMHC in the angular gyrus/middle occipital gyrus was found to be significantly positively correlated with disease duration ($R = 0.568$, $p < 0.05$, see **Table 3** and **Figures 2** and **3**). No other brain regions were found to have a significant correlation between VMHC values and symptom severity (Y-BOCS total score, Y-BOCS obsession score, and Y-BOCS compulsion score).

DISCUSSION

In this study, we found decreased VMHC within CSTC circuitry (putamen and OFC), the fusiform gyrus/inferior occipital gyrus, lingual gyrus, and postcentral gyrus/precentral gyrus in patients with OCD relative to controls. The altered VMHC was not correlated with the clinical severity of OCD symptoms in the patient group but had a significant positive correlation with disease duration. However, no brain regions showed significant differences in VMHC between the SSRI-treated and drug-naive patients.

Similar to this study, Wang et al. reported that patients with OCD had a lower VMHC in the CSTC circuitry (thalamus and OFC) than HCs, but no abnormal VMHC was found to be associated with the severity of clinical symptoms (after correction), nor was there a difference in VMHC between the SSRI-treated and drug-naive patients (37). However, inconsistent with this study, VMHC abnormalities in the fusiform gyrus/inferior occipital gyrus, lingual gyrus, and postcentral gyrus/precentral gyrus in OCD patients were not reported by Wang et al., which may be due to sample heterogeneity and analytical methods. For example, in this study, we calculated the group differences in VMHC between OCD patients and HCs based on whole brain voxels, while Wang et al. was based on the voxels that showed significant VMHC in any of the two groups (OCD patients and HCs) (37). To sum up, we found that OCD patients had significantly weaker homotopic RSFC than healthy controls, which is consistent with the findings in other various mental illnesses (11, 12, 14–17),

TABLE 1 | Demographic and clinical characteristics of participants.

Demographic data	OCD patients (46)	HCs (46)	t/χ^2 value	p value
Age (years)	30.39 ± 10.68	31.83 ± 10.27	–0.657	0.513[b]
Gender (male/female)	26/20	26/20	0.000	1.000[a]
Education (years)	12.70 ± 2.97	13.83 ± 3.47	–1.679	0.097[b]
Illness duration (months)	49.83 ± 51.54	NA	NA	NA
Y-BOCS total score	28.85 ± 6.56	10.00 ± 0.00	19.490	<0.001[b]
Y-BOCS obsession score	15.17 ± 3.83	5.00 ± 0.00	18.037	<0.001[b]
Y-BOCS compulsion score	13.89 ± 4.88	5.00 ± 0.00	12.364	<0.001[b]
HAMD score	10.20 ± 4.87	0.52 ± 0.78	13.315	<0.001[b]
HAMA score	10.35 ± 4.67	0.65 ± 0.71	13.916	<0.001[b]
Mean FD	0.096 ± 0.031	0.088 ± 0.025	1.262	0.210[b]
	Unmedicated OCD (23)	**Medicated OCD (23)**		
Age (years)	27.83 ± 10.53	32.96 ± 10.43	–1.660	0.104[b]
Gender (male/female)	11/12	15/8	1.415	0.234[a]
Education (years)	13.09 ± 3.09	12.30 ± 2.87	0.891	0.378[b]
Illness duration (months)	49.39 ± 60.18	50.26 ± 42.55	–0.057	0.955[b]
Y-BOCS total score	27.61 ± 6.16	30.09 ± 6.84	–1.291	0.204[b]
Y-BOCS obsession score	15.22 ± 4.12	15.13 ± 3.60	0.076	0.940[b]
Y-BOCS compulsion score	12.83 ± 4.74	14.96 ± 4.88	–1.502	0.140[b]
HAMD score	10.43 ± 5.00	9.96 ± 4.83	0.330	0.743[b]
HAMA score	10.00 ± 3.92	10.70 ± 5.39	–0.501	0.619[b]
Mean FD	0.093 ± 0.035	0.098 ± 0.028	–0.476	0.636[b]

Y-BOCS, Yale–Brown Obsessive–Compulsive Scale; HAMD, Hamilton Depression Rating Scale; HAMA, Hamilton Anxiety Scale; Mean FD, mean frame-wise displacement; HC, healthy controls; OCD, obsessive–compulsive disorder; NA, not available. [a]The p value for gender distribution was obtained by chi-square test. [b]The p values were obtained by two-sample t tests.

TABLE 2 | Regions showing significant differences in VMHC between OCD patients and HCs.

Region	BA	Peak MNI coordinates (*x*, *y*, *z*)	*t* value	Cluster size (voxel)
Fusiform Gyrus/Inferior Occipital Gyrus	19/37	± 39 –63 –15	–8.371	529
Postcentral Gyrus/ Precentral Gyrus	3	± 57 –9 33	–7.701	721
Lingual Gyrus	37	± 22 –54 –11	–7.653	445
Putamen	NA	± 15 12 –3	–4.321	146
Orbital Frontal Gyrus	11	± 9 42 –12	–4.617	50

VMHC, voxel-mirrored homotopic connectivity; BA, Brodmann area; MNI, Montreal Neurological Institute; NA, not available. Comparisons are adjusted for age, sex, education, and mean FD.

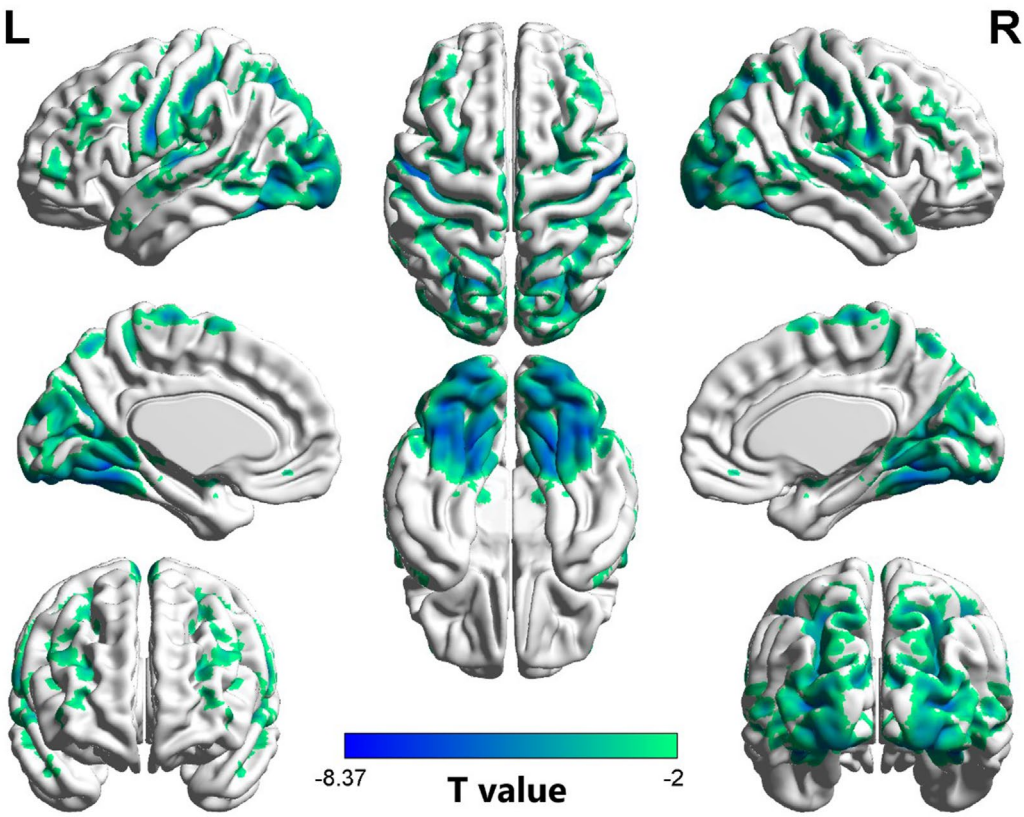

FIGURE 1 | Regions with decreased homotopic connectivity in obsessive–compulsive disorder (OCD) patients compared to healthy controls. L: left; R: right.

TABLE 3 | Regions showing significant correlations between VMHC value and illness duration in OCD patients.

Region	BA	Peak MNI coordinates (*x*, *y*, *z*)	*R* value	Cluster size (voxel)
Angular Gyrus/ Middle Occipital Gyrus	39	± 36 –63 30	0.568	9

meaning that homotopic RSFC abnormalities may be as critical pathophysiological features of mental illness as Rest state network (RSN) abnormalities are (38–41).

Although recent neuroimaging studies emphasize the abnormal structures and functions of the CSTC circuitry in OCD, these previous studies have not explored the VMHC changes in the CSTC circuitry. Therefore, the VMHC alterations in the striatum and OFC reported in this study may provide

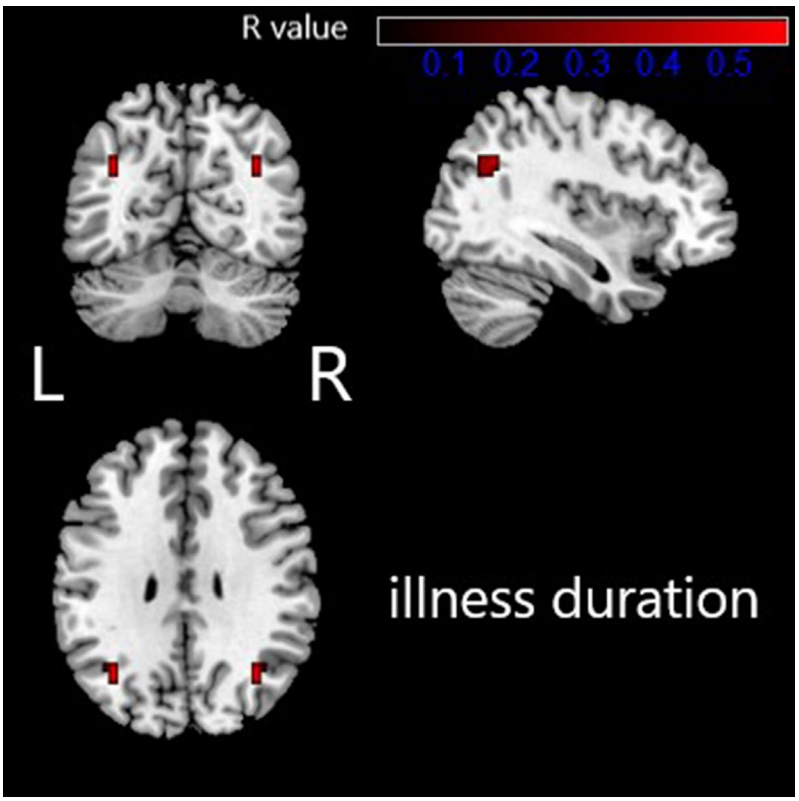

FIGURE 2 | Regions exhibiting significantly positive correlations between VMHC value and illness duration in OCD patients are presented as color overlays. The color bar represents *R* values. L: left; R: right.

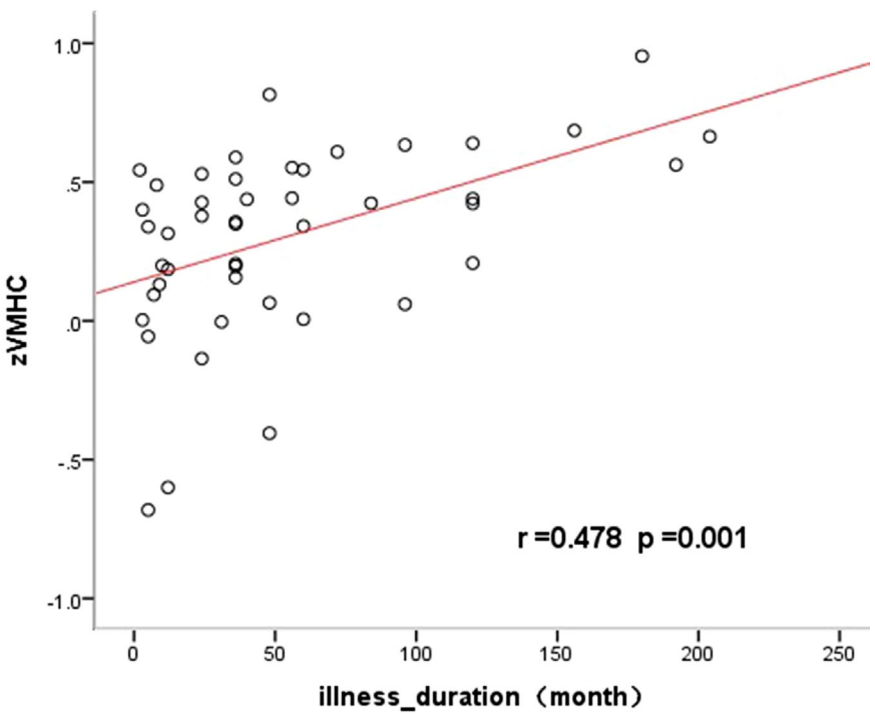

FIGURE 3 | Significantly positive correlations between the VMHC values and the illness duration in the angular gyrus/middle occipital gyrus in OCD.

new evidence for abnormalities in the CSTC circuitry in OCD. Recent studies have been identifying additional brain correlates associated with OCD symptomatology outside of CSTC circuitry (42), with these findings contributing to the generation of new hypotheses for the OCD pathogenesis (31, 43). Therefore, the VMHC alterations outside the CSTC loop found in this study seem to confirm that the pathophysiological mechanism of OCD may be not only related to the CSTC loop. A recent meta-analysis reported orbitofrontal and striatal dysfunction during executive control in OCD patients (44), as well as abnormalities in activation within precentral/postcentral and occipital lobe regions. In fact, these regions are also implicated in OCD during other tasks such as reward tasks (45, 46), psychomotor vigilance tasks (47), and emotional processing tasks (48–50). Therefore, based on the reduction of VMHC in the OFC, striatum, postcentral/precentral gyrus, and IOG/fusiform gyrus found in our study, we speculate that the abnormal VMHC may be related to cognitive/executive functional deficits and emotional processing impairment in OCD patients, an idea that should be tested directly in future research.

The CC is the main commissural fiber bundle mediating interhemispheric transfer (51), and broad reductions of homotopic connectivity after dissection of the CC (52) underscore the relevance of this structure for interhemispheric transfer. The CC has therefore been identified as an important structural basis of interhemispheric RSFC. A further supportive finding in the reviewed papers on OCD is the substantiation of microstructural abnormalities in the CC with strong evidence for increased and decreased FA (20, 53). Similarly, the results of our previous DTI studies based on the same OCD patients also support altered FA in the CC in OCD patients compared with HCs. Furthermore, the moderate correlations between VMHC and FA of the CC have been reported in patients with migraine and multiple sclerosis (54, 55). Therefore, these may suggest that reduced VMHC in OCD patients is based on obvious microstructural alterations of the CC, which should be further verified by implementing a correlation analysis between the altered VMHC and FA of the CC in OCD patients in future research.

To explore the effect of SSRI on the homotopic connectivity, we compared the group difference in VMHC between SSRI-treated and drug-naive OCD patients and found no differences in VMHC in any brain region. The findings were consistent with a recent similar study (37); these results might imply the limited effect of medication on regulating abnormal VMHC in OCD. However, as this is a cross-section study, further prospect study comparing the same group of patients before and after treatment is thus necessary to elucidate the exact effect of medication on VMHC in OCD patients.

The decreased VMHC in the angular gyrus/middle occipital gyrus was found to be positively correlated with the illness duration. This may be due to functional compensation during disease development. In fact, there is a lot of evidence that the duration of the disease can cause significant changes in brain structure and function in OCD patients. For example, illness duration has been found to be correlated with both hippocampus and left amygdala volume abnormalities in OCD (56). Furthermore, decreased left caudate nucleus–thalamus connectivity within the CSTC circuitry have been found to be positively correlated with the illness duration of OCD (57). Reduced connectivity in an emotion processing network spanning the left cerebellar lobule VI and the lingual gyrus has been reported to be correlated with illness duration (58). Changes in both the Regional Homogeneity (ReHo) within the OFC and the functional connectivity between the OFC and angular gyrus has been reported to be correlated negatively with OCD duration (59). However, since the results of the correlation analysis after multiple comparison correction showed that the cluster (9 voxels) was very small, the results from the present study should be interpreted with caution.

Up to now, this study is the second study to explore interhemispheric functional connectivity in OCD patients by using the VMHC method. The present study illustrates the interhemispheric functional imbalance in OCD patients, which should improve the understanding of OCD. In addition, the currently recommended method of TFCE + PT was used for multiple comparison corrections, which has been shown to control the false-positive rate to within 5% and to lead to the highest reproducibility when compared with other common thresholding methods (35).

LIMITATIONS

Some limitations should be taken into consideration. Firstly, the relationship between altered VMHC and FA of the CC was not assessed in the present study. Future studies using a multimodal imaging method, such as voxel-based morphometry (VBM) and DTI, would help identify the unknown structural basis for VMHC alterations. Secondly, neuropsychological data, especially cognitive and behavioral information, were not collected in our study. The relationship between deficits in VMHC and cognitive dysfunction should be investigated in future research. Thirdly, the VMHC results in our study were obtained during resting state, and therefore, a task-oriented functional MRI study could provide a complementary view. Fourthly, although a rough assessment in the study did not reveal a significant effect of drug therapy on VMHC, longitudinal studies may be needed to clarify the effect of the drug on VMHC. Finally, a symmetrical standard template was applied with smoothed imaging data to improve the functional correlations between mirrored regions in the study. In general, the human brain is not symmetrical. Although morphometric asymmetry could not account for the reduced VMHC (15), the effects of methodological symmetry should not be overlooked.

CONCLUSION

Interhemispheric functional imbalance, especially the imbalance in the CSTC circuit, is an essential aspect of the pathophysiological mechanism of OCD. Our results not only confirm that the CSTC circuit plays an important role in OCD, but also find that abnormal VMHC in areas other than the CSTC circuit is also involved in the pathophysiological mechanism of OCD.

DATA AVAILABILITY STATEMENT

All data sets generated for this study are included in the manuscript and supplemental documents.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the clinical trial guidelines of the Institutional Review Board of Kunming Medical University with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of Kunming Medical University.

AUTHOR CONTRIBUTIONS

KD analyzed the data and wrote the draft. TQ and FZ collected the imaging data. JX and ND helped to revise the draft and polished it. LJ collected clinical data. YC and XX gave guidance and helped edit the manuscript. All authors read and approved the final manuscript.

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The Optical-Coenaesthetic Disproportion Hypothesis of Feeding and Eating Disorders in the Light of Neuroscience

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This article builds on and extends the 'optical-coenaesthetic disproportion' (OCDisp) hypothesis of feeding and eating disorders (FEDs) matching data obtained through clinical research with laboratory evidence from neuroscience and neuropsychological studies. The OCDisp hypothesis, developed through the assessment in clinical setting of bodily experience using the IDentity and EAting (IDEA) disorder questionnaire, argues that in persons with FED the internal perception of one's embodied self (i.e., coenaesthesia) is deeply affected (their possibility to feel themselves is weakened or threatened by coenaesthopathic and emotional paroxysms; their bodily feelings are discontinuous over time), and as a compensation to it, these persons experience their own body as an object that is looked at by others. To FED persons, their body is principally given to them as an object 'to be seen.' The other's look serves as an optical prosthesis to cope with hypo- and dis-coenaesthesia and as a device through which persons with FED can define themselves and attenuate the anxiety produced by the conflicts between being-oneself and being-for-others. After describing the OCDisp hypothesis, we will gather evidence supporting it with neuroscience studies on FED. Our focus will be on data pointing to dampened multisensory integration of interoceptive and esteroceptive signals, demonstrating a predominance of the visual afferents toward signals arising within the body. In the final part of the article, we will show consistencies but also draw distinctions between our clinical hypothesis and neuroscience-based data and hypotheses and draft a potential agenda for translational research inspired by these.

Keywords: abnormal bodily phenomena, body-for-others, bodily self-consciousness, feeding and eating disorders, multisensory integration

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INTRODUCTION

Abnormal bodily phenomena are among the main experiential dimensions investigated in feeding and eating disorder (FED). Generally speaking, these include abnormal body image and anomalous bodily self-consciousness.

This evidence confirms what is being established by clinical research, namely, the 'optical-coenaesthetic disproportion' (OCDisp) hypothesis of FED (1–3). Coenaesthesia (deriving from Greek *koiné aesthesis*, common sensorium) is the internal perception of one's own body, the hub

of somatosensations, that is of sensations coming *from within one's body*. More in detail, it is the global experience in which all the single bodily sensations are synthesised, the crossroads of all interoceptive sensibility on which self-consciousness is grounded, including the feeling of existing, of being a self, and of being separated from the external world (4, 5). The OCDisp hypothesis argues that in persons with FED the coenaesthetic apprehension of oneself is troubled, and as a compensation to it, these persons experience their own body as an object that is looked at by others. This hypothesis was developed through the assessment in clinical setting of bodily experiences in persons with FED using the IDentity and EAting (IDEA) disorder questionnaire (6). The IDEA questionnaire assesses abnormalities in lived corporeality and personal identity. It consists of 23 items divided into four subscales: feeling oneself through the gaze of the other and defining oneself through the evaluation of the other, feeling oneself through objective measures, feeling extraneous from one's own body, and feeling oneself through starvation. This research allowed the identification of a specific pheno-phenotype (7) that expresses a gradient of vulnerability to FED along a continuum, rising from high-risk nonclinical subjects toward the clinical population of eating disorder patients and including obese patients (8–11). In a nutshell, to these persons, their body is principally given as an object "to be seen." The other's look serves as an optical prosthesis to cope with hypo- and dis-coenaesthesia and as a device through which persons with FED can define themselves and attenuate the anxiety produced by the conflicts between being-oneself and being-for- others. This article aims to match clinical data supporting the OCDisp hypothesis with evidence taken from laboratory (neuroscience and neuropsychological) studies on FED and, building on and extending these, draft a potential agenda for translational research inspired by the OCDisp hypothesis.

Both body image and bodily self-consciousness derive from complex integrative processes between different perceptual domains, which mainly include bodily signals coming from within the body (interoception) and esteroceptive stimuli (among which visual inputs are particularly relevant). Evidence obtained through research in the neurosciences suggests that body image and bodily self- consciousness are impaired in FED persons. In particular, dampened multisensory integration of interoceptive and esteroceptive signals, demonstrating predominance of the visual afferents toward signals arising from within the body, plays a major role in abnormal body and self experiences in persons with FED (12–22).

THE OPTICAL-COENAESTHETIC DISPROPORTION HYPOTHESIS OF FED

There are theoretical as well as clinical reasons to consider abnormal eating behaviours as epiphenomena of a more profound disorder of lived corporeality. Under normal conditions, bodily experience is combination of the way I feel myself from a first-person perspective and the way I experience myself through other sense modalities, one of the most important of which is sight.

Through sight, I see myself from a third-person perspective. Yet sight is also involved when I also experience myself as an object seen by others. This is a peculiar feature of FED psychopathology. There is empirical evidence that FED persons feel extraneous from their own body (6, 8, 11, 23); their possibility to feel themselves is weakened or threatened by coenaesthopathic and emotional paroxysms; their bodily feelings are discontinuous over time (23). Since their experience of their body from within is flawed or inconsistent, they cope with this by apprehending their body from without through the other's gaze. They experience their body as an object being looked at by another, rather than coenaesthetically or from a first-person perspective (24). What they seem to lack is the coenaesthetic apprehension of their own body as the more primitive and basic form of self-awareness (25). The way they feel looked at by the others is the principal mode to feel themselves and define their identity (6). Their body is principally given to them as an object "to be seen." We called this peculiar way of apprehending one's own body after Sartre (26) *body-for-others*—a body exposed and subjected to the other's gaze and thus reduced to its appearance.

As the first-person apprehension of one's body is based on coenaesthesia, whereas the third-person one is based on the sense of sight, we may call the dynamic balance between the apprehension of one's body through coenaesthesia and through the other's look the *optical-coenaesthetic proportion*—a prerequisite for constructing a safe and dependable sense of bodily self and personal identity. Under normal conditions, the constitution of our own body, and consequently of our own self and identity, depends on the dialectic integration between these two perspectives. In persons with FED, this dialectics breaks down. Particularly relevant to understanding a person with FED is to envision in the other's look a kind of visual prosthesis that helps him/her feel his/her own body. Feeling one's body as an object being looked at by another has a twofold effect: it makes FED people feel embarrassment and repulsion for their own body, but it also helps them recover a sense of selfhood, "unity," and "condensation" (27). This phenomenon is epitomised by the following micro-narratives: "The way I feel depends on the way I feel looked at by the others," "Sometimes I focalise myself through the gaze of the others," "For me it's very important to see myself through the eyes of the others," "Even if I think that the way the others evaluate me is wrong, I can't do without it" (6).

BODILY SELF-CONSCIOUSNESS: NEURAL BASES AND CONTROVERSIES

Several—and somehow controversial—constructs are available in neuroscience literature to depict the experience of the body, such as body image (BI) and bodily self-consciousness (BSC). Body image generally refers to inputs from the body (28). We endorse in this article the definition of BI (29). It encompasses a perceptual (body perception), an affective, and a cognitive domain (13). Each of these domains has distinct brain localisations: perceptual—posterior parietal cortex; affective—amygdala, insula and the prefrontal cortex; cognitive—parietal regions (30, 31). A more nuanced localisation of the cortical

areas specifically activated especially by visual body perception includes the extrastriate body area (the whole body and its components) and the fusiform body area (the configurational picturing of the whole body), as well cortical areas activated by face perception such as the fusiform face area, the occipital face area, and the posterior superior temporal sulcus (32). These systems constitute the detection network (33), while many other cortical regions are implied in the subsequent processing of the expressive, emotional, semantic, and cognitive features connected to body, face, and gaze.

A large part of research on the perceptual domain of BI is about its visual component (13, 34). Yet BI can be seen as the integration of egocentric signals coming from the body and allocentric inputs coming from the environment mediated by other sensory domains.

Also, online BI can be separated from offline BI (35). Online BI derives from egocentric signals coming from within the body itself, whereas offline BI is a more stable representation of the body, which may be assimilated to the long-term, memory stored BI (35, 36), comprehensive also of behaviours, attitudes, and body pertaining values.

Body image thus results from very complex integrative processes and dynamic interplay: perception and action, different perceptual domains (e.g., bodily signals coming from within the body and visual stimuli), egocentric and allocentric afferents, short- and long-term information, and online and offline inputs. To depict the integrative processing of egocentric online afferents and allocentric long-term stored information, the Bayesian error-prediction computational model is widely accepted (37): a reference-framework of body representation, memory-stored, aligns the incoming stimuli from different sensory systems according to the principles of predictive coding and free energy—where the first is the tendency to reduce the differences between predictions and incoming afferents (38), and the latter is the tendency to resist to possible disorder deriving from inconsistent matching (39). Notably, the incoming multisensory bodily signals represent the basic feature of selfhood. In this sense, the primary experience of the body is constitutive of self-consciousness; this implicit, prereflexive background is also designated as BSC.

Bodily self-consciousness is the multisensory integration of the afferents coming from within and from outside the body (esteroceptive inputs), including a) proprioceptive and vestibular signals indicating the position of the whole body and its components in the space; b) auditive and visual data concerning body shape and structure; and c) multisensory integration of bodily and esteroceptive stimuli, necessary to the development of peripersonal space, i.e., the space immediately surrounding our body, with diverse extension in correspondence of the trunk, face, and the arms. Further information contributes to the constitutive process of BSC: d) interoceptive afference regarding the physiological (homeostatic) condition of body organs and bodily functions (e.g., hunger or satiety) and e) the sense of agency regarding body actions (37).

The multisensory information is integrated in bimodal or multimodal neurons located mainly in parietal cortex, activated by afferents (visuotactile or auditive, proprioceptive, and kinestesic modalities) coming from primary sensory areas; the cortical areas involved in BSC include the premotor cortex, posterior parietal cortex, and particularly the intraparietal sulcus and the temporoparietal junction, the latter with functional connectivity with the right insula and the supplementary motor area (40). The insula has been recognised as a central hub for interoceptive signals (41). The BSC accounts for the basic features of selfhood: 1) self-identification, the immediate sense of ownership regarding the whole body and its components; 2) self-location, the sense of bodily position in the environmental space; 3) self-perspective, intended as the point of reference of our perceptual engagement in the world; 4) self-demarcation, since the peripersonal space draws the effectively lived self-boundaries; 5) self-agency, or the sense to be the operator of any motility engagement in the world (42–44).

A striking BSC characteristic is its plasticity (45). Long-lasting multisensory stimulation manipulating the spatiotemporal coherence of bodily signals alters BSC by reshaping peripersonal boundaries and inducing BSC for noncorporeal objects. The best known experimental paradigm to explore BSC plasticity is the rubber-hand illusion (RHI) where the probands' arm is hidden in a special machinery that consents to see only an artificial rubber hand. In RHI, there is a predominance of visual sensory afferents that reshape bodily (and subcomponents) limits and ownership. Prolonged tactile stimulation of the probands' real arm and—at the same time—the artificial hand may induce the illusion of ownership toward the fake hand (46).

NEUROLOGICAL UNDERPINNINGS OF FED: INTEGRATION AND DISINTEGRATION OF INTEROCEPTIVE AND ESTEROCEPTIVE INPUTS

A relatively small number of studies investigated body sensory networks in FED patients. The results of these studies can be summed up as follows: FED persons show diminished interoceptive/somatosensory signals, overreliance on visual afferents, and dampened multisensory integration of visual and interoceptive/somatosensory signals.

Diminished interoception. The interoceptive component of BSC is based on egocentric signals coming from the body (47). Interoception includes the physiological state of the entire body, concerning autonomic nervous system information about the condition of the body (e.g., taste, smell, hunger, thirst, and visceral sensation). Interoception is regarded as an essential component of subjective emotional experience in key theories of emotion including Damasio's (48, 49) somatic marker hypothesis that argues that perceiving changes in the bodily state (autonomic bodily signals) are the basis of our emotional experiences. People who have reduced interoceptive sensitivity have a correspondingly reduced experience of emotions. Patients with FED display impairment in haptic, proprioceptive, and interoceptive sensitivity demonstrating altered bottom-up processing of bodily signals (13, 33). Healthy subjects with BI disturbances (body dissatisfaction and uneasiness and BI avoidance) display the tendency to incorrectly estimate their

body measures when exposed to a virtual reality paradigm, for instance, when they evaluate virtual avatars with varying body massindexes (50). These impairments resulted evident in allocentric, third-person perspective (viewing the avatar body as they were other people who observed them).

Overreliance on visual afferents. Increased proneness to RHI in healthy people correlates with reduced interoceptive accuracy (51), demonstrating a predominance of the visual afferents toward signals arising within the body and, consequently, a greater BSC malleability. People with reduced interoceptive accuracy display a heightened tendency to self-objectivisation (the tendency to rely for self-identity upon mere body appearance), experiencing their own body from a third-person perspective (52). Increased proneness to RHI is also documented in anorexic (AN) patients (45) and in a group of FED patients (47); these patients display a more evident proprioceptive drift (a misconception of the position of the real finger) and a higher embodiment score (extension of ownership toward the fake arm). These findings put in evidence an overreliance to visual information that become dominant with respect to interoceptive signals. Zopf et al. (53) obtained similar findings in a group of AN patients, inversely related to the length of illness. Finally, Keizer et al. (54) documented that in AN patients exposure to virtual reality experiments reduces the tendency to overestimate their body size, suggesting a reshaping of BI induced by a modification of the BSC.

Dampened sensory integration. Some evidence for a dampened multisensory integration of visual and interoceptive/somatosensory signals has emerged from the functional magnetic resonance imaging study of brain resting states measuring functional connectivity of brain networks. Divergent procedures to acquire the signal (i.e., independent component analysis, box-seed investigation of predefined regions of interest, graph analysis), small patients sampling (with possible interindividual distribution), and clinical variability (including the co-occurrence of psychiatric comorbidities) may account for inconsistent findings. Nonetheless, some interesting evidences has emerged.

Underweight AN patients display decreased connectivity in the ventral visual network (left occipitotemporal junction, a region implied in short-term memory persistence of visual stimuli and body perception) (55). Recovered AN patients show a significant hypoconnectivity in the right middle frontal gyrus, involved in spatial working memory and also implied in the updating of spatial information. In both groups, hyperconnectivity in somatosensory network including the premotor areas was demonstrated. Impaired visuosomatosensory signals integration may be the root of altered body experience in AN patients. Decreased connectivity in the thalamoinsular subnetwork is suggestive of impaired interoception in AN patients (56).

Similarly, Phillipou et al. (57) in a sample of underweight AN patients highlighted hypoconnectivity between primary somatosensory and both motor cortical areas as secondary associative visual cortices. Lavagnino et al. (58) found in a small sample of unmedicated bulimic women decreased connectivity within the somatosensensory network and between the paracentral lobule and the right middle occipital gyrus, the right cuneus, and the posterior cingulate cortex (PCC), the latter invoked for self-specificity processing. This finding may substantiate the experience of extraneity referred by patients toward own's body. Defective connection between the paracentral lobule and the EBA may be referred to a multisensory defective integration (also, this finding displayed a negative correlation with interoceptive accuracy). Scaife et al. (59) found a set of decreased connectivity in recovered and restrictive AN patients, within the lateral visual area, the right temporal/temporal-occipital fusiform cortex (an area associated with face and body recognition), and finally in several cortical regions associated with interoceptive and somatosensory functions, documenting the impairment of body processing. The presence of brain networks anomalies in recovered patients can be considered a possible trait character or, alternatively, a sort of scar depending on the previous state of malnourishment.

THE ALLOCENTRIC LOCK HYPOTHESIS OF FED

Taken together, all these laboratory findings highlight the anomalous processing of bodily sensory afferents, the predominance of visual inputs, and the defective multisensory integration of the stimuli coming from within the body with the esteroceptive (visual) perceptions, indicating a defective constitution of the BSC in persons with FED. All this is nicely encapsulated in the allocentric lock hypothesis (ALH). There are several analogies between this and the OCDisp hypothesis, in particular the emphasis on the role of dampened multisensory integration of interoceptive and esteroceptive signals in the pathogenesis of anomalous bodily experiences in FED persons. Comparing these two hypotheses can help to amalgamate the clinical evidence gathered by the OCDisp hypothesis with data taken from the neurosciences captured by the ALH. Indeed, the ALH argues that FED symptoms are the outcome of a primary distorted experience of the body (19, 60) and more precisely consequences of the impairment of the process of integration between the egocentric experience of the body and the allocentric representation of it. If the process is impaired, the egocentric sensory inputs are no more able to update the contents of the allocentric representation of the body. The outcome of this impairment is that the subject is locked to the allocentric representation of his body, which primes the processing of any further body-related experience.

It is argued that there are two frames within which we gain access to our body: the egocentric and the allocentric. The egocentric frame (body as reference of first-person experience) is perceptive/experiential since it has its primary source on somatoperceptions and other sensory inputs such as tactile stimuli. It is field-open and unceasingly online since its inputs are constantly updated by new inputs. Thus, the kind of bodily experience it conveys is discontinuous over time. These are engrams of the present state of the body taking place within short-term memory processes. The allocentric frame (body as object in the physical world) is representational since it has its primary contents in somatorepresentations—abstract knowledge, beliefs, and attitudes related to body as an object of third-person experience. It is offline and in principle continuous over time if

its representations are not updated by inputs coming from the egocentric frame. It is encoded in long-term memory and located in the hippocampus and surrounding medial temporal lobe.

The egocentric frame corresponds to the unmediated, first-person experience of oneself as a spatiotemporal embodied agent—neither a representation of one's body, nor its perception as an external entity. It is the body experienced from within—how the body feels. The allocentric frame corresponds with the body seen from a third-person perspective as an entity existing in the outside world or perceived from without (e.g., when I look at myself in a mirror or remember my visual image). Sight is the sense modality through which I perceive my body from without as an object-body—how the body *looks*.

Under normal conditions, the egocentric experience of how our body feels is matched by an allocentric one. The interaction between egocentric and allocentric frames corresponds to the interaction between long- and short-term memory processes. Specifically, long-term memory involves the generation of allocentric representations, whereas short-term memory contains egocentric experiences driven by perception or by long-term memory. On one side, an egocentric experience of the body can influence an allocentric representation stored in long-term memory. On the other side, an allocentric representation of the body retrieved from long-term memory can influence egocentric sensory inputs, including body dimensions. If, for some reasons, the first process is impaired, the egocentric short-term sensory inputs are no more able to update the contents of the allocentric long-term representation of the body: the subject is locked to the allocentric representation of his body. This is what supposedly happens in persons with FED: their long-term offline allocentric body representation amounts to a negative self-image, driven by these patients' extreme sensibility to what they experience as the others' disapproving gaze and remarks, often eliciting in them feelings of shame and disgust related to their own body. This undesirable body representation is not updated by contrasting egocentric inputs driven by short-term online body perception; thus, the processing of any further body-related experience is impaired. The impossibility of updating the negative allocentric representation of the body locks the patient into an unsatisfying body.

CONCLUSIONS

Both clinical and laboratory findings show impaired BSC in persons with FED. This helps conceptualizing FED as disorders of embodiment rather than as merely disorders of eating and feeding behaviour. Neuroscientific data and clinical evidence confirm each other in drawing attention to three anomalous features of bodily self-experience: diminished or impaired interoception/coenaesthesia, increased esteroception *via* visual inputs, and abnormal integration between these two sources of bodily self-experience. In detail:

1) There is a general agreement in highlighting hypo- and dis-interoception/coenaesthesia in FED. Incidentally, coenaesthesia does not account for all BSC "because this entails a variegated network of integrated sensory modalities including egocentric/allocentric, online/offline, short-term/long-term stimuli. In the light of neuroscience studies, coenaesthesia (a key concept in phenomenological studies) can be redefined as egocentric, online, short-term somatoperception; this is diminished in persons with FED.
2) FED persons also show predominance of allocentric sources of BSC, namely, inputs from the visual domain. A peculiar source of allocentric visual inputs particularly relevant in establishing BSC in FED people is the body-for-others domain. This can be seen as an esteroceptive/allocentric/visual self prosthesis, compensating diminished coenaesthesia/interoception, but locking body perception in FED people to the gaze and evaluation of the others. The body-for-others is a candidate further dimension of BSC. There is clinical evidence for this, but laboratory (neuroscience and neuropsychological) evidence is not yet available.
3) Finally, BSC in FED people is also impaired in terms of anomalous integration between different frames of bodily self-experience (interoception/esteroception, egocentric/allocentric, coenaesthetic/visual, etc.). After our clinical research, we called this the OCDisp hypothesis. Further clinical and laboratory studies are needed to confirm this finding and expand this hypothesis.

AUTHOR CONTRIBUTIONS

GS contributed to the conceptualization of the optical-coenaesthetic disproportion hypothesis and manuscript draft. MB contributed to the narrative review of neuroscientific literature and revision of manuscript draft. MM contributed to the acquisition of neuroscientific literature, revision of manuscript draft and project management.

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Cross-Validation of Paranoid-Depressive Scale and Functional MRI: New Paradigm for Neuroscience Informed Clinical Psychopathology

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There is reported a study performed with a novel paradigm aiming at investigation of the translational validity of von Zerssen's paranoid-depression scale and its fMRI correlates in terms of focus on exploration of the results on the contrast between the Paranoid Specific (DP) blocks and the Depression Specific (DS) blocks. Patients with schizophrenia demonstrated significant activations in a number of regions (right angular gyrus, left posterior cingulate and precuneus, right transverse temporal gyrus) during responses to paranoia versus depression items which differ topologically from those found in patients with major depression (left middle cingulate and right superior temporal gyrus). The direct comparison between the groups, however, did not yield any residual activations after correction.

Keywords: functional MRI, depression, schizophrenia, validation, psychopathology

INTRODUCTION

Considering the status of psychiatry as a hybrid discipline which embraces both the natural sciences and the humanities (1, 2), we attempt to deliver a novel, experimentally fostered concept of *translational validity,* which is a non-conventional and instrumentalist approach to validation (3).

As discussed in earlier publications (4–6) clinical and neurobiological measures are considered valid for different reasons *inside* their own divergent domains. All disciplines concerned with mental health establish internal or *intra-correlative validity*, i.e. psychological scales are typically validated against other psychological measures, and neurobiological measures are validated with other neurobiological tests. Psychiatric assessment tools represent a circle of validation between first-person measures (self-evaluation inventories) and third-person perspective, the psychiatric interviews (7). What is still missing is the *inter-correlative* or *inter-disciplinary validity which entails consistent inter-domain translation*. In practical terms the lack of consistent inter-domain translation is undermining the validity of psychiatric classifications as well as the implementation of the research findings in clinical practice (8).

Furthermore, we consider of critical importance the notion of "state dependence" as contrasted with the traditional "state independence" of biomarkers (9). State dependence means in this context that certain correlations are directly relevant and may be specific to the current mental state, yet

not necessarily to the diagnosis in the medical sense. This is why the clinical and biological measures are beeing performed simultaneously in our paradigm (10).

The translation takes place on two levels according to the established psychometric vallidation standards: convergent and divergent (11). In first place the corresponding empirical measures are cross-validated on convergent level, e.g. depression clinical rating scale and blood oxygenation level dependent (BOLD) activation levels from functional magnetic resonance imaging (fMRI) during processing of neutral items in patient versus healthy control population (12). After that the construct is cross-validated in the same manner against another, presumably divergent clinical construct, e.g. paranoia. However discriminative power is not expected to be strong enough to underpin robust discrimination across nosological entities, but rather patterns of activation that may underpin the different psychopathological constructs and relevant measures.

In our previous studies we have tested the convergent validity by employing an fMRI paradigm using two types of visual stimuli—diagnostically specific (DS)—representing items from von Zerssen's depression subscale (13) and diagnostically neutral (DN)—from an interest scale. Thus, we have been able to demonstrated that in healthy controls, contrasting the two types of stimuli (DS vs DN) yielded no significant brain activations, and the correlation analyses did not find a relationship between brain activations and the total score of the DS statements. On the other hand, in depressed patients contrasting the DS to the DN stimuli produced significant activations in several brain regions, and there were positive correlations with the DS score in several activation clusters (12, 14). In this manner, we were able to confirm the sensitivity of the method (its ability to distinguish healthy controls from depressed patients), still we had to address its specificity (different patterns of brain activation behind different clinical constructs and respective measures). As it has already been stated, such patterns are not likely to trascend to the level of nosological specificity.

To handle this last issue and to test the divergent validity, we further developed our paradigm with the specific aim of investigating the translational validity of von Zerssen's paranoia-depression scale (13) and its fMRI correlates during their simultaneous implementation in patients with depression and schizophrenia.

METHODS

Subjects

We recruited 35 psychiatric patients with either a diagnosis of schizophrenia (n = 15, mean age 37.1 ± 12 years, 11 males), or depressive episode (n = 20, mean age 42.3 ± 12.1 years, 5 males) in the context of major depressive disorder (n = 10, mean age 39.9 ± 11.9 years, 4 males) or bipolar disorder (n = 10, mean age 44.8 ± 12.5 years, 2 males). Subjects were assessed by an experienced psychiatrist (ZA) using the general clinical interview and the structured Mini International Neuropsychiatric Interview (M.I.N.I 6.0) (15) as well as the Montgomery–Åsberg Depression Rating Scale (MADRS) (16) and the Positive and Negative Syndrome Scale (PANSS) (17).

Patients were excluded if they had a comorbid psychiatric disorder (such as anxiety, substance related disorder), major medical illness, neurological disease, history of head trauma with loss of consciousness, or metal implants not compatible with the MRI. All participants provided a written informed consent complying with the Declaration of Helsinki and the study was approved by the University's Ethics Committee.

MR Scanning

The scanning of the participants was performed on a 3T MRI system (GE Discovery 750w). The MR protocol included high resolution structural scan (Sag 3D T1 FSPGR sequence), with slice thickness 1 mm, matrix 256 × 256, TR (relaxation time) 7.2 ms, TE (echo time) 2.3, and flip angle 12°, and a functional scan (2D EPI sequence), with slice thickness 3 mm, matrix 64 × 64, TR 2000 msec, TE 30 msec, and flip angle 90°. Before each functional scan 5 dummy time series were acquired.

fMRI Stimuli and Procedure

We used a standard block-design with three different active conditions and one rest condition, with a total duration of 11 min and 44 s. Each active block lasted for 32 s and consisted of four text statements presented for 8 s each using NordicNeuroLab VisualSystem. For the Depression Specific (DS) blocks the statements were taken from the von Zerssen depression subscale ("I cry easily," "I am more sensitive to criticism than I was before"), while for the Paranoia Specific (DP) blocks they were taken from the paranoid subscale ("Other people constantly follow and control me"). As in our previous study (12), there were also Diagnostically Neutral (DN) blocks consisting of four statements from a questionnaire about general interests and likes (such as "I like to write books or plays," "I like to repair household appliances," etc.). Under each written statement four possible answers ("completely true," "mostly true," "somewhat true," "not true") and the respective four response buttons (upper left, lower left, lower right, upper right) were presented. There were four blocks of each type, alternating between the three active conditions (DS, DN, and DP) followed by a 20 s resting block with a fixation cross in the middle of the screen (DS__DN__DP__D). For the active conditions, the participants were instructed to read the statements carefully and to respond with a button press according to their level of agreement, and for the passive condition, to focus on the fixation cross without thinking of anything in particular.

fMRI Data Analysis

Data were analyzed using the SPM 12 (Statistical Paramertic Mapping, http://www.fil.ion.ucl.ac.uk/spm/) software running on MATLAB R2015 for Windows. The preprocessing included the following steps: i) realignment of the functional data for correction of head motion, ii) co-registration between the high-resolution anatomical image and the functional scans, iii) intra-individual estimation of spatial registration parameters based on the anatomical image, and iv) transformation of the co-registered functional data to standardized MNI (Montreal Neurological

Institute) space, followed by v) spatial smoothing with a 8 mm full-width-at-half-maximum Gaussian kernel.

First-level analysis was conducted using a general linear model (GLM) applied to the time series, convolved with a canonical hemodynamic response function. Nuisance covariates included the six rigid body motion parameters. T-contrasts were defined for the active vs passive conditions. The resulting individual contrast maps from each comparison were then used in a second-level random-effects analysis to test for differences between the two patient groups (schizophrenia > depression and depression > schizophrenia). Furthermore, ANOVA design was used to explore the three clinical diagnosis—schizophrenia, major depression, and bipolar depression. The level of significance was set to $p > 0.05$ FWE (Family Wise Error) corrected using an uncorrected cluster-forming threshold of $p < 0.001$, and gender was used as a covariate in all second-level analysis.

Following the logic of our study in terms of differentiating between the clinical diagnosis of schizophrenia and depression by means of simultaneous application of fMRI and a clinical assessment tool (e.g. von Zerssen paranoia-depression scale), we focused our exploration of the results on the contrast between the Paranoia Specific (DP) blocks and the Depression Specific (DS) blocks.

RESULTS

Demographic and Clinical Characteristics

The two patient groups did not differ significantly in their demographic and clinical characteristics (such as age, education, illness duration) except for the sex distribution, which was complying with the clinical reality, e.g. male prevalence in the schizophrenia group and the opposite for the depression group (**Table 1**).

The two depression subsamples—unipolar and bipolar—were not significantly different in their demographics as well as in their clinical features.

Comparative Analysis Across Schizophrenia and Depression Patients

The direct comparison of the DP > DS contrast between the two clinical populations produced multiple clusters of activation with significance level < 0.001 which did not survive above the 0.05 p-level after FWE correction. On the same inter-group level and prior to inclusion of gender as co-variate there was localized a cluster with sigin the right angular gyrus, with peak activation significance level $p = 0.036$, consistent with our findings on group level as described bellow. This cluster was above the level of significance after inclusion of gender as co-variate, which demonstrates the critical role of gender confound in such study design (discussed as limitation). On the group level (one sample *t*-test) the schizophrenic patients demonstrated residual activations in several clusters encompassing medial parietal and limbic structures (posterior cingulum and precuneus), as well as temporal and subcortical regions (for details see **Table 2**). The depressed patients, on the other hand, showed only two clusters with peak activations in middle cingulate and in superior temporal gyrus (**Table 2**). An illustration of these results is given in **Figure 1**.

Comparative Analysis Across Schizophrenia, Major Depression, and Bipolar Disorder

In addition, a one-way ANOVA model of the DP > DS contrast differentiated between the two depressed patients groups (unipolar and bipolar) and the schizophrenic group. Significant difference was found only between schizophrenic subjects and those with major depression in a single cluster located to the right pre/postcentral gyrus (201 voxels, $p = 0.05$ FWE) that is more activated in schizophrenia. The other between-group comparisons did not reach statistical significance. On the intragroup level, the schizophrenic subjects demonstrated residual activations almost identical to the ones revealed by the two-sample *t*-test, while the bipolar patients had only one cluster of greater activation located to the left middle cingulate gyrus (201 voxels, $p = 0.012$ FWE). No residual activations were found in major depression.

DISCUSSION

The main finding of our study maight be summarized as follows: patients with schizophrenia demonstrated significant activations in a number of regions (right angular gyrus, left posterior cingulate and precuneus, right transverse temporal gyrus) during responses to paranoia versus depression items (DP > DS contrast) which differ topologically from those found in patients with major depression (left middle cingulate and right superior temporal

TABLE 1 | Demographic and clinical characteristics of all participants.

	Schizophrenia patients (*n*=15)	Depressed patients (*n* = 20)	Statistical significance
Age (mean ± SD)	37.1 ± 12	42.3 ± 12.1	0.210[a]
Sex (M/F)	11/4	5/15	*0.005[b]
Education (secondary/higher)	10/5	11/9	0.486[b]
Age at onset (years)	27.7 ± 8.2	32.1 ± 10.9	0.267[a]
Illness duration (months)	110 ± 95	140 ± 93	0.406[a]
Episode duration (weeks)	9.1 ± 7.1	15.3 ± 10	0.307[a]

*SD, Standard Deviation. [a]Independent samples t-test, [b]χ^2-test, *p < 0.05.*

TABLE 2 | Clusters of significantly greater activations in schizophrenic and depressed patients when answering to psychosis items compared to depression items (DP > DS contrast).

Anatomical localization	Cluster size (voxels)	Peak MNI coordinates			p-value (FWE)
		x	y	z	
Schizophrenic patients					
Right angular gyrus, SMG	128	28	-46	36	0.004
Left posterior cingulus and precuneus	575	-6	-30	28	0.02
Right transverse temporal gyrus and anterior insula	3756	28	8	-10	0.05
Right caudate, thalamus	76	20	-12	22	0.05
Depressed patients					
Left middle cingulate gyrus	212	-8	-16	48	0.007
Right superior temporal gyrus	1243	42	-42	20	0.02

gyrus). The direct comparison between the groups, however, did not yield any residual activations after correction. The significance of these findings will be discussed in the following lines.

One of the clusters of activations, produced by the DP > DS contrast in schizophrenia, was in the area of the right angular gyrus, which belongs to the inferior parietal lobule system. It has been reported to be involved in semantic processing, social cognition, and reasoning as a cross-modal hub to converge multisensory information (18). Reversed assymetry in this region has been associated with schzophrenia (19) where abberant modulation/activation of the right angular gyrus was found as well (20).

Another cluster of residual activations in schizophrenic patients was stretching across the left posterior cingulate and precuneus which is implicated in autobiographical memory processing (21). It is assumed to contribute to episodic memory dysfunctions and abnormal functional connectivity that was found in schizophrenia (22, 23).

Amongst the significant clusters in our study, one was located in the right transverse temporal gyrus, or Heschl's convolution and anterior insula. The function of those regions is related to accoustic processing as an *Inner voice*, or internal subjective dialogue with oneself (24), as well as task-level control of focal attention (25), which are often disturbed in schizophrenia.

On the other hand, the significant activations in the group of depressed patients were located in left middle cingulate gyrus as well as right superior temporal gyrus which makes sense in the context of the functional role of those regions in depression (26, 27).

Our ANOVA findings are consistent with previously reported results (12) and may be explained with the activation of the motor cortex as patients with schzophrenia used more often their left hand to provide positive responses to paranoid items.

The limited inter-group contrast in our findings (not reaching statistical significance) might be explained by the discrepancy of psychometric or psychodiagnostic versus diagnostic i.e. nosological validity. Psychometric validity essentally covers validation of particular construct(s) by use of another method (here, translational validation of von Zerssen paranoia-depression scale with fMRI), and it appears to have been achieved in our model. However the nosological validity assumes the possibility to validate entire medical–psychiatric–diagnostic entity and it remains out of reach.

The items that compose diagnostic scales, however precise those may be in order to measure certain phenomenon, could create preconditions for terminological inaccuracy. The diagnostic validity of a psychological tool can trace out borders of a particular category, but this is not enough to make a diagnosis. Even formally precise psychometric tools as intelligence and cognitive assessment tests can be challenged when their results are viewed in a specific emotional and cultural context (28, 29).

Another possible explanation of the overlap between the activations related to the processing of paranoid and depressive items in both patient groups might lay in the clinical variations of depressive symptoms in affective disorders and schizophrenia. The background of rather "warm" affectivity, induced by melancholia and anxiety in the context of affective disorder and the "cold" affect in schizophrenic psychosis (30, 31), caused by blunted affect might be revealed on phenomenological level (during the clinical interview) but cannot be captured properly by brief clinical assessment tools widely employed in psychopathology.

In addition, negative or cognitive symptoms in schizophrenic patients may be mistakenly assessed by a psychological tool as depressive (pseudodepresive) and vice versa. Morover, about 25% to 50% of the patients with major depression have impairment of at least one cognitive sphere (32). The most common disturbances in cognitive functioning during a depressive episode are those of memory, attention, and the degree of processing of various incentive stimuli (33). This is to demonstrate that cognitive deficits can be seen as a central element in the course of a major depressive disorder, not just as secondary phenomena (31). In the same perspective, similar changes in cognitive functioning can be found in schizophrenic patients in the context of negative and cognitive symptoms, and it would be impossible for a psychological test to differentiate them on the level of nosological specificity.

To summarize it there are limitations concerning possible *nosological specificity* of evaluation measures in clinical psychiatry, as predicted in some earlier theoretical publications (5, 8).

Limitations

There are two major limitations which undermine generalizations from the current study.

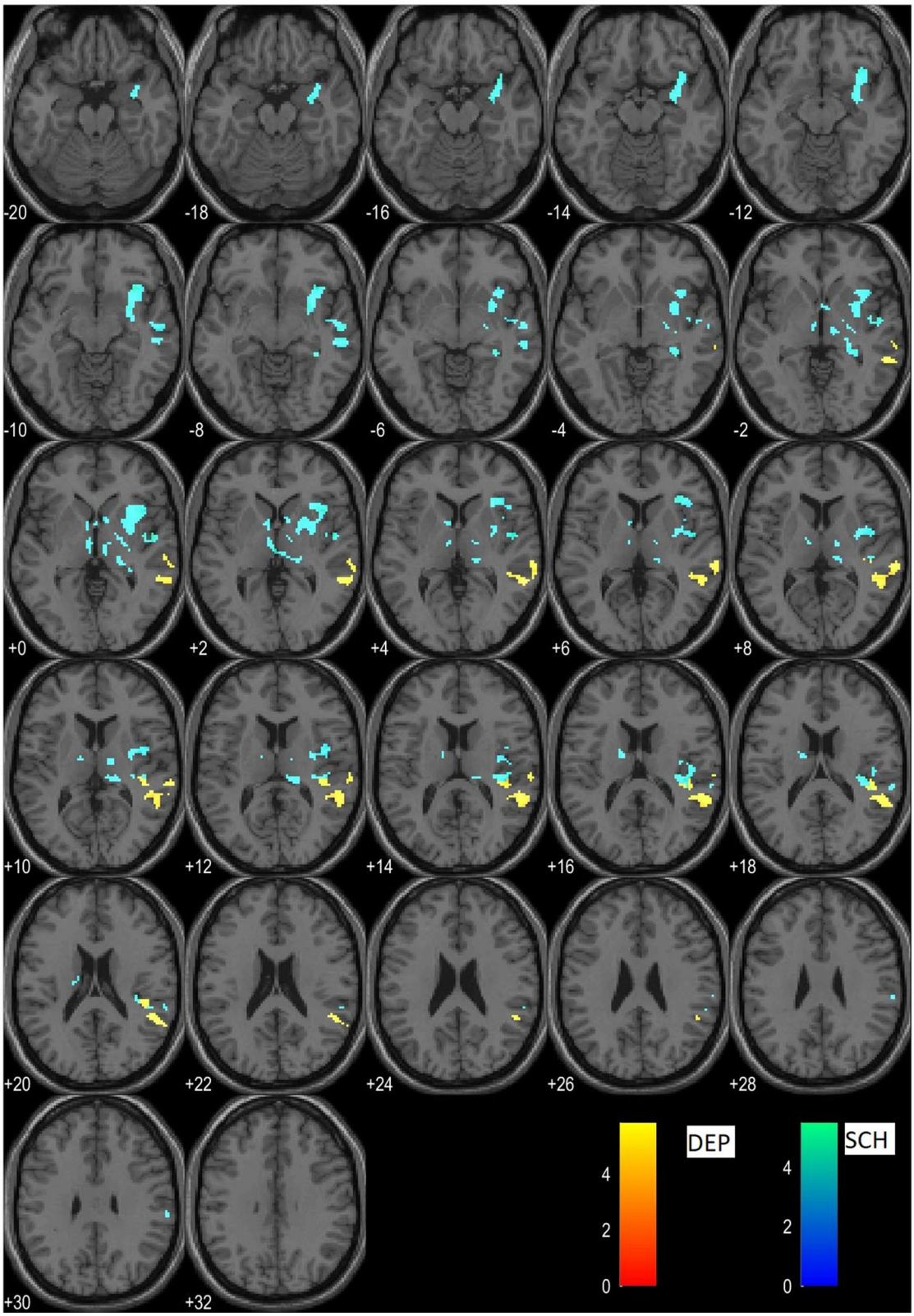

FIGURE 1 | Clusters of residual activations of the DP > DS contrast in schizophrenia (cyan) and in depression (yellow).

The first is concerned with the small sample size, especially when the sample is subdivided into clinical diagnostic groups.

The second is the innovative and non-conventional approach to the the experimental paradigm design, which presents an issue for comparison with other studies in the field.

The third limitation apparently concerns the gender structure of the sample. In future replication studies gender balance will be of critical importance in order to bolster the significance of the results.

From a more general perspective those limitations might be addressed by expanding the research in translational neuroimaging using similar approach aiming to identify the functional MRI substrate behind clinical self-evaluation measures.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee, Plovdiv Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DS has developed the conceptual rationale and delivered the text in the *Introduction* and a major part of the *Discussion*. SK has performed the statistical analysis and delivered the *Methods* and *Results* sections. ZA contributed to the interpretation of the *Results* and the *Discussion* sections. AS consulted the data analysis procedures and edited the manuscript. SB consulted the development of the paradigm and the data processing, and edited the paper and supervised the entire project. RP was involved in the empirical study and data processing.

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Neuroimaging Correlates of Depression—Implications to Clinical Practice

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The growth of the literature about neuroimaging of major depressive disorder (MDD) over the last several decades has contributed to the progress in recognizing precise brain areas, networks, and neurotransmitter processes related to depression. However, there are still doubts about the etiology and pathophysiology of depression that need answering. The authors did a nonsystematic review of the literature using PubMed database, with the following search terms: "major depressive disorder," "neuroimaging," "functional imaging," "magnetic resonance imaging," "functional magnetic resonance imaging," and "structural imaging," being selected the significant articles published on the topic. Anterior cingulate cortex, hippocampus, orbitomedial prefrontal cortex, amygdala basal ganglia, and the cerebellum were the main affected areas across the selected studies. These areas respond to particular neurotransmitter systems, neurochemicals, hormones, and other signal proteins; even more, the evidence supports a distorted frontolimbic mood regulatory pathway in MDD patients. Despite the positive findings, translation to treatment of MDD remains illusory. In conclusion, this article aims to be a critical review of the neuroimaging correlates of depression in clinical research with the purpose to improve clinical practice.

Keywords: major depressive disorder, major depression, magnetic resonance imaging, positron emission tomography, neuroimaging

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INTRODUCTION

Major depressive disorder (MDD) remains a critical disease that greatly impacts the global burden of disease (1). In the absence of biological markers, clinical-based methods continued to be the gold standard to diagnose this disorder (2). In clinical assessment, a nosological classification is used, according to international systems such as the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (3) and the *International Classification of Diseases, 10th Revision* (4). Efforts are continuously made in order to discover dependable biomarkers that clarify the neurobiological mechanisms of psychiatric disorders, identify populations at risk, and provide etiology-based treatments (5).

Studies involving imaging modalities such as structural magnetic resonance imaging (sMRI) and functional magnetic resonance imaging (fMRI) aim to outline brain irregularities accompanying MDD. Moreover, the knowledge provided by the neurobiological components resulting in the

pathogenesis of MDD can explain the existence of biomarkers for diagnosis, prognosis, and response prediction (5).

In this article, we expect to review the neuroimaging correlates of depression in clinical research. These neuroimaging correlates of depression are presented and discussed from a critical perspective.

METHODS

The authors performed a nonsystematic review of the literature using PubMed database, with the following search terms: "major depressive disorder," "neuroimaging," "functional imaging," "magnetic resonance imaging," "functional magnetic resonance imaging," and "structural imaging." The literature search was limited to the English language and limited to the dates up to January 2019. There were selected 28 significant articles published on the topic.

RESULTS

Neuroimaging Modalities in Depression

Table 1 contains the 26 articles that were selected and are divided by the type of neuroimaging modality and by brain regions studied in the respective article. One article used positron emission tomography (PET) and sMRI modalities, and the other were developed using only one modality. The imaging technic more focused on was fMRI (13 articles), followed by PET (eight articles) and sMRI (four articles).

Functional Magnetic Resonance Imaging

Resting-State Functional Magnetic Resonance Imaging

Major depressive disorder is characterized by depressed mood, anhedonia, and feelings of worthlessness; some of these alterations are related to the self, such as rumination (31–33) and autobiographical memory (34). Functional neuroimaging, in this case, fMRI, has achieved to isolate brain regions implicated in self-relation, for example, the anterior cingulate cortex (ACC), the medial prefrontal cortex (MPFC), the posterior cingulate cortex (PCC), dorsomedial thalamus, and the precuneus (35, 36).

The default-mode network (DMN) is composed by the lateral and medial parietal cortex, ventral and dorsal medial prefrontal cortices, and areas of the medial and lateral temporal cortices, (11) and is thought to be responsible for processing information related to survival instinct, as well as the capacity to plan the future, desires, and beliefs; all these tasks are related to the self (37–39), functions that are intertwined with the self.

Among the articles cited in fMRI, two demonstrated that subjects with MDD were prone to have an increased activity in the MPFC/ACC areas, as well as diminished activity in the PCC/precuneus and bilateral angular gyrus areas (10, 23).

The cognitive control network (CCN) is an entity responsible for attention-demanding cognitive tasks (40). The affective network (AN) is composed of regions of the ACC (41) responsible for processing emotions (41–46) and is crucial in fear, vigilance, and other emotional responses (43).

One of the articles selected showed increased connectivity in depression in the bilateral dorsomedial prefrontal cortex, which encompasses the DMN, AN, and CCN (10). Greicius et al. (47) show increased functional network connectivity in the thalamus, subgenual cingulate, the precuneus, and the orbitofrontal cortex (OFC) (7). The other two articles demonstrated a significant alteration in global brain networks focusing mainly in the DMN area and in the AN (19, 21).

Regarding effective connectivity of different brain areas, different from functional connectivity, effective connectivity is the effect one neuronal network employs on another network (48). Used an unusual method to measure that connectivity; they used spectral dynamic causal modeling (spDCM) (48). Using spDCM, found a decreased influence from the anterior insula to the middle frontal gyrus in medicated subjects with MDD. An important nexus between the anterior insula and amygdala was also found. A positive correlation between hippocampal node activation and the severity of depression was found, which confirms the relation that the right anterior insula has on depression pathophysiology (48). There was also a meaningful interconnection with activation in the right superior parietal lobule, the right precentral and postcentral gyrus, and in left precuneus (48, 49).

Task-Based Functional Magnetic Resonance Imaging

In depression, emotions tend to be perceived and processed erroneously; for example, good events tend to be assimilated and processed as negative or harmful to the person involved in them (50–54). It is also known that population with MDD has difficulty in recognizing and processing emotion in facial expression (sad vs. happy). The brain networks related to the identification of emotional facial expressions are the fusiform area in the ventral occipitotemporal cortex (55–57); the superior temporal sulcus (58); and the amygdala.

Sheline et al. (18) demonstrated in depressed patients a greater activation of the left amygdala during early stages. Besides, they also confirmed that after 8 weeks of antidepressant treatment (with selective serotonin reuptake inhibitor–sertraline 100 mg/d) amygdala activation decreased drastically (18).

Fu et al. (59) found that presentation of sad faces led to increased activation of the left hippocampus and mainly the amygdala and parahippocampal gyrus. They also reported the activation of the thalamus, dorsal cingulate gyrus, hypothalamus, ventral striatum, and insula. They also found that treatment with fluoxetine 20 mg/d led to a reduction of the response in the ventral striatum and thalamus (24).

In fMRI-TB, it is also possible to register disturbances in the DMN, Sheline et al. (11) report increased activity in the DMN in depressed subjects. In some proposed tasks, subjects would maintain or increase DMN activity, while control subjects would diminish DMN activity (11).

Translation is the dominion where it is possible to shift data across different subjects to enrich and perfect diagnosis and subsequent treatment, keeping in mind applicability in daily

TABLE 1 | Neuroimaging modalities in depression.

Rank	Article	Neuroimaging modality	Main brain areas studied
1	Deep brain stimulation for treatment-resistant depression (6)	PET	Subgenual cingulate region
2	Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus (7)	fMRI resting state (RS)	Subgenual cingulate region, thalamus
3	Subgenual prefrontal cortex abnormalities in mood disorders (8)	PET sMRI	Subgenual prefrontal cortex
4	Hippocampal atrophy in recurrent major depression (9)	sMRI	Hippocampus
5	Resting-state functional MRI in depression unmasks increased connectivity between networks *via* the dorsal nexus (10)	fMRI-RS	Cognitive control, the default mode, and affective networks
6	The default-mode network and self-referential processes in depression (11)	fMRI task based (TB)	Default-mode network
7	Depression duration, but not age, predicts hippocampal volume loss in medically healthy women with recurrent major depression (12)	sMRI	Hippocampus, amygdala
8	Failure to regulate: counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression (13)	fMRI-TB	Prefrontal cortex, amygdala
9	Untreated depression and hippocampal volume loss (14)	sMRI	Hippocampus
10	Subcallosal cingulate gyrus deep brain stimulation for treatment-resistant depression (15)	PET	Limbic and cortical regions
11	Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression (16)	PET	Frontostriatal networks
12	Increased amygdala and decreased dorsolateral prefrontal BOLD responses in unipolar depression: related and independent features (17)	fMRI-TB	Amygdala, dorsolateral prefrontal cortex
13	Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study (18)	fMRI-TB	Amygdala
14	Identifying major depression using whole-brain functional connectivity: a multivariate pattern analysis (19)	fMRI-RS	
15	Nucleus accumbens deep brain stimulation decreases ratings of depression and anxiety in treatment-resistant depression (20)	PET	Subgenual cingulate, prefrontal regions
16	Disrupted brain connectivity networks in drug-naive, first-episode MDD (21)	fMRI-RS	
17	Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes (22)	PET	Prefrontal cortex, anterior cingulate cortex, insula
18	Evidence of a dissociation pattern in resting-state default-mode network connectivity in first-episode, treatment-naive major depression patients. (23)	fMRI-RS	Anterior medial cortex, posterior medial cortex
19	Attenuation of the neural response to sad faces in major depression by antidepressant treatment: a prospective, event-related functional magnetic resonance imaging study (24)	fMRI-TB	Amygdala, striatum, frontoparietal cortex, pregenual cingulate cortex
20	Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy (MRS) (25)	MRS	Dorsomedial and dorsal anterolateral prefrontal cortices
21	Default-mode and task-positive network activity in MDD: implications for adaptive and maladaptive rumination (26)	fMRI-RS	Default-mode network, task-positive network, right frontoinsular cortex
22	A functional anatomical study of unipolar depression (27)	PET	Prefrontal cortex, amygdala
23	Can't shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals	fMRI-TBw	Amygdala
24	Cingulate function in depression: a potential predictor of treatment response (28)	PET	Rostral anterior cingulate region
25	Toward a neuroimaging treatment selection biomarker for MDD (29)	PET	Insula
26	A differential pattern of neural response toward sad versus happy facia expressions in MDD (30)	fMRI-TB	

clinical decisions. In psychiatry, it is much more complicated because of the heterogeneity and variability of clinical symptoms; for example, it is highly subjective to measure reliably the level of sadness or anhedonia in two or more people who suffer from MDD, which led to the necessity of devising a hypothesis of translation in depression (60). Stoyanov and colleagues (60) conceptualized an approach of translational cross-validation of psychiatric neurocognitive tests (Von Zerssen's Depression scale) with fMRI scans, expecting to find associations between these methods. Stoyanov and colleagues (61) operationalized this

concept, and in early findings, there is a weak correlation between the medial frontal cortex (MFC) and MDD subjects; activation in anterior thalamus, hippocampus, and parahippocampal gyrus, areas implicated in the pathophysiology of MDD, was reported as well.

Positron Emission Tomography

In PET, we are able to estimate brain functional degree on a regional scale. This is possible by quantifying the emission of positrons due to the half-life decay of the various radiopharmaceuticals. Different isotypes permit to evaluate different neurotransmitters receptors, hence its versatility (62).

Relating to the subgenual cingulate region, three articles revealed decreased brain activity/metabolism in patients with depression (8, 20, 28). Mayberg et al. (63) demonstrated that it was possible to diminish the intensity of depressive symptoms, in resistant MDD, through electrical stimulation of the subgenual cingulate white matter (6). Relating to limbic and cortical regions, including frontostriatal networks, two articles revealed altered metabolic activity in those areas after deep brain stimulation of the subcallosal cingulate gyrus/nucleus accumbens in subjects with refractory depressive disease (15, 16).

Two articles demonstrated increased microglial activation in patients with depression and identified that response to escitalopram or behavior therapy was predicted by insula activity level (22, 29).

Structural Magnetic Resonance Imaging

Four of the articles that evaluated hippocampal volume identified a volume reduction in patients with MDD (9, 12, 14, 64). Hippocampal volume reduction is observable in MDD (65, 66), mainly in the first episode (67). The cognitive decline observed in MDD over the various episodes of illness may be due to volume decrease in the hippocampus (68). Treatment with antidepressants may revert neurocognitive symptoms due to hippocampal volume increase (69). Arnone et al. (70) found that treatment with citalopram led to hippocampal volume increase after 8 weeks of treatment.

Kandilarova, (71) studying the volume of gray matter in affective disorders, found a decrease in gray matter volume, specifically in MDD. The main cluster affected (reduced gray matter) was the MFC and the ACC. The other relevant region was the OFC. Gray matter reduction in the ACC is probably related to the abnormalities found in cognitive and affective regulation, attention, problem solving, motivation, and decision making. Furthermore, the decrease in OFC gray matter explains the alterations in social and emotional behaviors and also in the processing of reward and punishment (71). Kong et al. (72) studied the impact of treatment of MDD with fluoxetine and reported volume increase mainly in the orbitofrontal and the dorsolateral cortices.

Sheline et al. (12) examined the amygdala, with a decreased bilateral amygdala core nuclei volume in patients with recurrent depression. Drevets et al. (8) demonstrated a subgenual prefrontal cortical volume reduction in subjects with depression.

Magnetic Resonance Spectroscopy

Shen et al. (25) through MRS examined the dorsomedial and dorsal anterolateral prefrontal cortices and concluded that in depressed patients the levels of glutamate, glutamine, and γ-aminobutyric acid were decreased.

CONCLUSION

In this article, we reviewed the neuroimaging correlates of depression, in various imaging modalities such as PET, MRI, fMRI, and MRS.

According to the studies we have reviewed, MDD influences major brain areas such as the DMN, AN, CCN, and amygdala and that these affected areas respond to medication, antidepressants.

When evaluating structural differences in brain areas in MDD, we find different variations through multiple brain regions. Nonetheless, evidence has been found that supports changes in gray matter volume in cortical and subcortical regions that might be associated with depressive states. These changes are present through the course of the illness.

The concept of translation is applied globally in medicine, except in psychiatry, mainly due to the heterogeneity of clinical symptoms. Small steps have been made in the field of translational neuroimaging. Nevertheless, they are promising. Despite advances in research on the genetic neuroimaging, psychoneuroimmunology, and multimodal imaging, further studies are necessary to confirm this concept in all these modalities, diminishing the gap between neuroscience and clinical psychiatry.

A significant weakness of the reviewed studies is that they generally have a small population, so we have to be careful in drawing conclusions to the general population. In addition, different studies focus on different brain areas, not being able to identify a pathognomonic finding of MDD. We also fail to conclude whether such differences represent a congenital structural anomaly, a result of the disease, or a compensatory adaptation.

The tendency to evaluate particular brain regions independently is an explicit limitation, as the various areas are interrelated. The circuit-based analysis will provide a foundation for behavioral process analysis. This will facilitate the identification and analysis of MDD and psychiatric symptoms, mostly subjective, but that is used in clinical practice.

More studies, with larger populations, and ideally focusing on circuits other than specific brain areas, will be necessary to draw further conclusions.

Studies including subjects not medicated might enlighten about MDD-related brain abnormalities, without possible unwanted effects that the medication might introduce.

AUTHOR CONTRIBUTIONS

All authors of this study had an active role in the manuscript and have thoroughly read the final manuscript.

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Changes in Global and Nodal Networks in Patients With Unipolar Depression After 3-Week Repeated Transcranial Magnetic Stimulation Treatment

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Objectives: Repeated transcranial magnetic stimulation (rTMS) therapy has been applied in depressive disorders, but its neurobiological effect has not been well understood. Changes in cortical source network after treatment need to be confirmed. The present study investigated the effect of 3-week rTMS therapy on the symptom severity and cortical source network in patients with unipolar depression.

Methods: Thirty-five patients with unipolar major depressive disorder participated in the study. High-frequency (10 Hz) rTMS was applied at the left dorsolateral prefrontal cortex during 3 weeks (five consecutive weekdays every week). Clinical symptoms were examined using the Hamilton Rating Scale for Depression and Anxiety. The resting state electroencephalography was recorded with 62 scalp channels before and after rTMS treatment.

Results: Clinical symptoms significantly improved after rTMS treatment in both the active ($p = 0.001$) and sham groups ($p = 0.002$). However, an increased cortical source network in global and nodal levels was observed only in the active group after a 3-week treatment.

Conclusions: The present study indicates that rTMS treatment leads to improved symptoms in patients with unipolar depression. Furthermore, treatment outcome of real effect was assured in changes of cortical source network.

Keywords: unipolar depression, repeated transcranial magnetic stimulation, electroencephalogram, cortical source network, brain stimulation

INTRODUCTION

Repeated transcranial magnetic stimulation (rTMS) has been proposed as an alternative treatment for depression (1, 2), and it has also been applied in other neuropsychiatric disorders (3–5). Basically, rTMS affects the neuronal polarization in the cytoplasmic membrane, which has significant impacts on the brain functions (6). Long-lasting effects on depression have been observed after applying high-frequency (10 to 20 Hz) rTMS at the dorsolateral prefrontal cortex (DLPFC) with multiple sessions in 10 to 15 consecutive days (7). Additionally, the primary effects of rTMS therapy have been

established in medication-resistant patients with major depressive disorder (MDD) (8). Therefore, rTMS may have a significant positive anti-depressive effect in patients with depression (9).

One previous study has demonstrated that rTMS therapy increases the connectivity of default mode regions, such as subgenual anterior cingulate cortex (sgACC), in depressive patients with traumatic brain injury (10). High-frequency stimulation applied over the left prefrontal cortex induces an enhanced theta–gamma coupling (11) and modulates the resting state functional connectivity between the DLPFC and the limbic lobe (12). In addition, accelerated high-frequency rTMS improves functional connectivity in the sgACC region in patients with treatment-resistant unipolar depression (13). Regional volume reduction of the sgACC has been demonstrated in depressive patients compared with bipolar disorder patients or healthy controls (14), and abnormal network homogeneity of default mode regions, such as precuneus and posterior cingulate cortex (PCC), has also been observed in patients with depression (15). However, the treatment effect of rTMS in patients with unipolar depression remains unclear how the clinically configured magnetic stimulation affects the cortical source region.

Electroencephalogram (EEG) has been considered a reliable approach to analyze the cortical network (16). The antidepressant effect is indicated of changes in neural network. (17). Furthermore, there is no study exploring changes of cortical source level network in patients with depression with 3-week treatment of rTMS. In this study, we investigated the effect of rTMS treatment on the cortical source network in patients with unipolar depression using EEG based on the graph theory. We hypothesized that patients with MDD would show significant changes in network measurements and symptom severity after rTMS treatment. The findings would help understand the neurobiological mechanisms underlying the effects of rTMS.

MATERIALS AND METHODS

Participants

Thirty-five patients with unipolar MDD participated in the study, and they were randomly classified into the active (19 participants: 4 men and 15 women) and sham (16 participants: 5 men and 11 women) groups. The age of all participants ranged between 18 and 65 years, and the mean age of participants was 33.53 ± 12.89 years in the active group and 35.00 ± 11.94 years in the sham group (**Table 1**). Drug information, education level, and frequency of physical activity are also shown in **Table 1**. The present study was conducted between February 2015 and November 2016. All participants were native Korean and were diagnosed with unipolar MDD according to the 4th edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-4*). There are very few differences in the diagnosis of the patient with depression between *DSM-4* and *DSM-5*. Clinically structured interviews were performed using the Mini-International Neuropsychiatric Interview (18) by a psychiatrist who was blind to the present study design. Ten participants were administered antidepressants during the study period (**Table 1**). The participants with other current and/or lifetime Axis I psychiatric disorders; history of epilepsy, spontaneous seizures, or brain surgery; substance use; or pregnancy were excluded from this study. Participants with contraindications for magnetic stimulation (e.g., cardiac pace makers, implanted medication pumps, or hearing aids consisting of metallic materials) were also excluded from this study. For safety purposes, all participants underwent a brief EEG session to screen for epileptiform EEG abnormalities before rTMS. The Institutional Review Board of Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea approved the study protocol (approval number: KC14DDSE0479). All participants provided written informed consent.

TABLE 1 | Demographic data of the participants in the present study.

Variables	ACTIVE rTMS (n = 19)	SHAM rTMS (n = 16)	t or χ^2
	Mean (standard deviation)		
Age	33.53 (12.89)	35.00 (11.94)	p = 0.730
Sex (n, male/female)	4/15	5/11	p = 0.700
Education	13.26 (1.66)	13.33 (2.69)	p = 0.926
Physical activity	2.32 (1.80)	2.13 (1.63)	p = 0.746
Medication			
Amitriptyline	3		
Escitalopram	1		
Fluoxetine	1	1	
Mirtazapine	1		
Paroxetine	1	2	
Sertraline	1	1	

Physical activity: 0: not at all, 1: one to two times a month, 2: three to four times a month, 3: one to two times a week, 4: three to four times a week, 5: not less than five times a week.

Clinical Assessments

Hamilton Rating Scales for Depression (HAM-D) and Anxiety (HAM-A)

The HAM-D and HAM-A were rated by a psychiatrist who was blind to the treatment groups. The HAM-D (19) and HAM-A (20) consist of 17 and 14 items, respectively.

rTMS Protocol

rTMS was conducted using a device named TAMAS (REMED, Daejeon, Korea) with a figure-of-eight-shaped coil. Before each rTMS session, the motor threshold (MT) was determined by stimulating the motor cortex with the lowest amount of energy required to produce five consecutive twitches of the right abductor pollicis brevis (APB) muscle. The stimulation was applied at 110% of the individual MT. The average stimulation intensity for all participants was 61.91 ± 21.06% of the maximal stimulator output. The stimulation was applied over the DLPFC, and the stimulation location was determined by moving the TMS coil 5 cm anterior to the optimal surface site for activation of the right APB muscle. The frequency of stimulation was set at 10 Hz for 5 s, with an intertrain interval of 25 s. Treatment sessions lasted for 30 min (60 trains) and included 3,000 pulses. Sham stimulation was performed using a sham coil, which elicited

no tactile sensation at the site of stimulation and induced no cortical stimulation, and thus provided only matched acoustic sensation. Each participant underwent 15 rTMS sessions on 15 consecutive weekdays.

Electrophysiological Measurement and Preprocess

Participants were seated in a comfortable chair in a sound-attenuated room. The resting-state EEG was recorded with eyes closed for 5 min. EEG data were recorded using a NeuroScan SynAmps amplifier (Compumedics USA, El Paso, TX, USA) with a head cap mounted with AgCl electrodes according to an extended international 10-20 system. We recorded EEG data from 62 scalp positions (FP1, FPZ, FP2, AF3, AF4, F7, F5, F3, F1, FZ, F2, F4, F6, F8, FT7, FC5, FC3, FC1, FCZ, FC2, FC4, FC6, FT8, T7, C5, C3, C1, CZ, C2, C4, C6, T8, TP7, CP5, CP3, CP1, CPZ, CP2, CP4, CP6, TP8, P7, P5, P3, P1, PZ, P2, P4, P6, P8, PO7, PO5, PO3, POZ, PO4, PO6, PO8, CB1, O1, OZ, O2, and CB2). Additional electrodes were placed above and below the left eye for vertical electrooculogram recording and at the outer canthus of each eye for horizontal electrooculogram recording. EEG data were recorded with a 1- to 100-Hz bandpass filter at a sampling rate of 1,000 Hz. The signals were referenced to both mastoids where the ground electrode was placed on the forehead. Impedance between the electrodes and scalp was maintained below 5 kΩ during the entire recording session. EEG data were preprocessed using Scan 4.5 software and Curry suite 7.0 (Compumedics USA, El Paso, TX, USA). The EEG data were bandpass filtered at 0.1 to 60 Hz. Gross artifacts, such as eye-related and muscle artifacts, were corrected using independent component analysis implemented with a multiple artifact rejection algorithm (21). After the removal of artifacts, the data were segmented into epochs with a duration of 10 s, and the epoch was rejected if it contained significant physiological artifacts (amplitude > 100 μV) at any sites over all electrodes. A total of 12 artifact-free epochs (2 min) were used for each subject for the source-level network analysis. It was demonstrated that the length of epoched EEG data (2 min) is sufficient for functional connectivity (22).

Source Localization

The source model, constructed from the Colin 27 standard template brain, consisted of 15,000 cortical vertices in both hemispheres. The three-layer (inner skull, outer skull, and the scalp) boundary element method (BEM) model for creating a lead field matrix was generated using the Open MEEG implemented in Brainstorm (https://neuroimage.usc.edu/brainstorm/) (23). A time series of source activity at the cortical vertex was evaluated using the weighted minimum-norm estimation method. After computing time series at each vertex, the representative signals of 68 region of interests (ROIs) based on the Desikan–Killiany Atlas (24) were estimated by principal component analysis. A time series of the cortical sources at each of the 68 ROIs was bandpass filtered (1–55 Hz) and divided into seven frequency bands [delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), low beta (12–18 Hz), mid beta (18–22 Hz), high beta (22–30 Hz), and gamma (30–55 Hz)].

Connectivity and Network Analysis

Phase locking value (PLV) based on the Hilbert transform was computed to evaluate functional connectivity between each pair of nodes in the whole brain (25). The value of PLV is related to the strength of the functional connection between two nodes. If PLV is approached to between two nodes, the strength of the functional connection is stronger than other pairs of nodes. A raw PLV matrix was used as an adjacency matrix for weighted network analysis.

Moreover, we computed various weighted network measures based on graph theory (26). We investigated the brain network using two different perspectives, i.e., "global level" and "nodal level." The global-level values represent the characteristics of a whole-brain network while the nodal-level values indicate the properties at each node (specific brain regions). We assessed a total of four different types of global-level network measures as follows: 1) strength, 2) clustering coefficient, 3) path length, and 4) efficiency. Additionally, the clustering coefficient and the efficiency at each node were evaluated for nodal-level analysis. All network measures were computed using the Brain Connectivity Toolbox (BCT, http://www.brain-connectivity-toolbox.net), an open Matlab source.

Statistical Analyses

Descriptive statistics were performed between the active and control groups using *t* test and chi-square test. A p value of less than 0.05 was considered statistically significant for two-tailed tests. The effects of rTMS treatment in each group were compared using the paired *t* tests. In the comparison of nodal levels, the *p* value was adjusted using the false discovery rate correction; the effect size was calculated using Cohen's *d*, and a Cohen's *d* value of >0.60 was considered significant.

RESULTS

Symptomatic differences were found between baseline and 3 weeks after rTMS treatment in both active and sham rTMS groups. In the active group, Hamilton depression and anxiety scores significantly decreased 3 weeks after treatment compared with the baseline (HAM-D: 21.00 ± 5.12 vs 15.47 ± 6.32, $p = 0.001$; HAM-A: 23.47 ± 7.38 vs 16.79 ± 6.88, $p = 0.001$; **Table 2**). In the sham group, Hamilton depression and anxiety scores also significantly decreased after 3 weeks compared with baseline (HAM-D: 19.31 ± 6.10 vs 15.38 ± 6.18, $p = 0.002$; HAM-A: 21.75 ± 8.01 vs 16.13 ± 7.33, $p < 0.001$; **Table 2**). Network analyses revealed significant differences between baseline and post-treatment in the active group. The global efficiency in delta frequency significantly increased after rTMS treatment (0.55 ± 0.06 vs 0.58 ± 0.07, $p = 0.044$). The global strength, clustering coefficient, and efficiency in theta frequency increased after rTMS treatment (strength: 34.58 ± 5.59 vs 37.87 ± 6.16, $p = 0.034$; clustering coefficient: 0.47 ± 0.10 vs 0.52 ± 0.11, $p = 0.043$; efficiency: 0.55 ± 0.07 vs 0.59 ± 0.08, $p = 0.025$; **Table 2**). The global strength, clustering coefficient, and efficiency in low-beta frequency increased after treatment (strength: 34.67 ± 5.98 vs 38.21 ± 6.27, $p =$

0.025; clustering coefficient: 0.46 ± 0.10 vs 0.52 ± 0.11, p = 0.038; efficiency: 0.55 ± 0.07 vs 0.59 ± 0.07, p = 0.021; **Table 2**). The global strength, clustering coefficient, and efficiency in mid-beta frequency increased after rTMS treatment (strength: 36.36 ± 6.15 vs 40.28 ± 5.75, p = 0.033; clustering coefficient: 0.49 ± 0.10 vs 0.56 ± 0.10, p = 0.033; efficiency: 0.57 ± 0.07 vs 0.62 ± 0.07, p = 0.037; **Table 2**). Nodal strength and clustering coefficient in mid-beta frequency significantly increased after treatment. In the nodal strength analysis, the right fusiform (38.91 ± 8.34 vs 45.10 ± 5.94, p = 0.049, Cohen's d = 0.85), left inferior temporal (38.59 ± 8.90 vs 44.18 ± 7.82, p = 0.049, Cohen's d = 0.67), right inferior temporal (41.13 ± 7.55 vs 45.97 ± 4.77, p = 0.049, Cohen's d = 0.77), right insula (39.84 ± 7.39 vs 44.94 ± 4.90, p = 0.049, Cohen's d = 0.74), left isthmus cingulate (40.03 ± 7.54 vs 44.98 ± 4.99, p = 0.049, Cohen's d = 0.77), right isthmus cingulate (39.99 ± 7.49 vs 44.89 ± 4.94, p = 0.049, Cohen's d = 0.77), left lateral orbitofrontal (40.15 ± 8.62 vs 46.13 ± 4.96, p = 0.049, Cohen's d = 0.85), right lateral orbitofrontal (40.93 ± 7.03 vs 45.98 ± 5.26, p = 0.049, Cohen's d = 0.81), left lingual (38.52 ± 7.28 vs 44.42 ± 7.15, p = 0.049, Cohen's d = 0.82), right lingual (38.86 ± 6.79 vs 44.45 ± 6.90, p = 0.049, Cohen's d = 0.82), left middle temporal (39.52 ± 8.31 vs 45.62 ± 7.10, p = 0.049, Cohen's d = 0.79), right middle temporal (40.68 ± 7.82 vs 45.64 ± 4.55, p = 0.049, Cohen's d = 0.77), left paracentral (42.04 ± 7.07 vs 46.78 ± 4.86, p = 0.049, Cohen's d = 0.78), right paracentral (42.17 ± 7.21 vs 47.07 ± 4.95, p = 0.049, Cohen's d = 0.79), left parahippocampal (42.14 ± 6.99 vs 46.87 ± 4.85, p = 0.049, Cohen's d = 0.79), right parahippocampal (42.07 ± 6.78 vs 46.65 ± 4.88, p = 0.049, Cohen's d = 0.77), right parsopercularis (41.98 ± 6.98 vs 46.50 ± 5.15, p = 0.049, Cohen's d = 0.74), right parstriangularis (40.88 ± 8.34 vs 45.98 ± 5.96, p = 0.049, Cohen's d = 0.70),

TABLE 2 | Comparison of psychometrics and values of global network between time interval.

Variables	ACTIVE rTMS (n = 19)		*p-value*	SHAM rTMS (n = 16)		*p-value*
	Baseline	Post		Baseline	Post	
	Mean (SD)			Mean (SD)		
Clinical measures						
HAM-D	21.00 (5.12)	15.47 (6.32)	**0.001**	19.31 (6.10)	15.38 (6.18)	**0.002**
HAM-A	23.47 (7.38)	16.79 (6.88)	**0.001**	21.75 (8.01)	16.13 (7.33)	**<0.001**
Delta						
Strength	34.75 (4.60)	37.22 (5.74)	0.065	36.14 (5.24)	34.90 (8.68)	0.615
CC	0.46 (0.07)	0.50 (0.10)	0.106	0.49 (0.08)	0.47 (0.14)	0.693
PL	2.28 (0.29)	2.21 (0.39)	0.346	2.25 (0.30)	2.35 (0.54)	0.48
Efficiency	0.55 (0.06)	0.58 (0.07)	**0.044**	0.56 (0.06)	0.55 (0.11)	0.573
Theta						
Strength	34.58 (5.59)	37.87 (6.16)	**0.034**	35.63 (4.77)	35.20 (7.74)	0.85
CC	0.47 (0.10)	0.52 (0.11)	**0.043**	0.49 (0.08)	0.48 (0.13)	0.875
PL	2.31 (0.37)	2.18 (0.40)	0.131	2.27 (0.28)	2.32 (0.50)	0.723
Efficiency	0.55 (0.07)	0.59 (0.08)	**0.025**	0.57 (0.07)	0.56 (0.10)	0.695
Alpha						
Strength	34.30 (5.96)	6.02 (1.38)	0.063	35.01 (5.87)	34.35 (7.31)	0.772
CC	0.46 (0.10)	0.51 (0.11)	0.081	0.47 (0.09)	0.46 (0.12)	0.891
PL	2.35 (0.42)	2.21 (0.42)	0.213	2.32 (0.32)	2.38 (0.50)	0.701
Efficiency	0.54 (0.08)	0.58 (0.08)	0.051	0.55 (0.07)	0.54 (0.09)	0.677
Low beta						
Strength	34.67 (5.98)	38.21 (6.27)	**0.025**	35.34 (6.37)	35.05 (6.46)	0.891
CC	0.46 (0.10)	0.52 (0.11)	**0.038**	0.47 (0.10)	0.48 (0.10)	0.882
PL	2.31 (0.36)	2.17 (0.42)	0.11	2.29 (0.36)	2.31 (0.43)	0.867
Efficiency	0.55 (0.07)	0.59 (0.07)	**0.021**	0.56 (0.07)	0.55 (0.08)	0.671
Mid beta						
Strength	36.36 (6.15)	40.28 (5.75)	**0.033**	39.55 (6.12)	38.70 (6.52)	0.757
CC	0.49 (0.10)	0.56 (0.10)	**0.033**	0.55 (0.10)	0.53 (0.10)	0.818
PL	2.18 (0.34)	2.01 (0.32)	0.083	2.04 (0.30)	2.11 (0.42)	0.659
Efficiency	0.57 (0.07)	0.62 (0.07)	**0.037**	0.61 (0.08)	0.59 (0.08)	0.685
High beta						
Strength	35.28 (6.88)	38.01 (4.83)	0.086	36.00 (5.06)	35.88 (6.77)	0.946
CC	0.47 (0.11)	0.51 (0.08)	0.113	0.48 (0.08)	0.49 (0.10)	0.811
PL	2.30 (0.43)	2.18 (0.33)	0.188	2.27 (0.26)	2.29 (0.44)	0.832
Efficiency	0.56 (0.08)	0.59 (0.05)	0.085	0.56 (0.06)	0.56 (0.08)	0.722
Gamma						
Strength	33.43 (5.74)	36.05 (5.46)	0.142	33.86 (5.07)	32.59 (7.04)	0.518
CC	0.44 (0.09)	0.48 (0.09)	0.183	0.44 (0.08)	0.43 (0.11)	0.689
PL	2.45 (0.40)	2.34 (0.40)	0.333	2.43 (0.28)	2.53 (0.49)	0.476
Efficiency	0.53 (0.07)	0.57 (0.06)	0.115	0.54 (0.06)	0.52 (0.08)	0.394

Bold, statistical significance at p < 0.05.

right pericalcarine (40.86 ± 6.50 vs 45.68 ± 5.47, p = 0.049, Cohen's d = 0.80), right postcentral (41.59 ± 8.12 vs 46.86 ± 6.27, p = 0.049, Cohen's d = 0.73), left posterior cingulate (43.09 ± 6.95 vs 47.75 ± 5.70, p = 0.049, Cohen's d = 0.73), and right posterior cingulate (43.10 ± 6.95 vs 47.73 ± 5.77, p = 0.049, Cohen's d = 0.72) regions significantly increased after rTMS treatment (**Figure 1** and **Table 3**). In nodal clustering coefficient analysis, the right fusiform (0.51 ± 0.12 vs 0.61 ± 0.09, p = 0.049, Cohen's d = 0.85), left inferior temporal (0.52 ± 0.12 vs 0.60 ± 0.11, p = 0.049, Cohen's d = 0.71), right inferior temporal (0.53 ± 0.12 vs 0.61 ± 0.08, p = 0.049, Cohen's d = 0.76), right insula (0.52 ± 0.12 vs 0.60 ± 0.08, p = 0.049, Cohen's d = 0.76), left isthmus cingulate (0.52 ± 0.12 vs 0.61 ± 0.08, p = 0.049, Cohen's d = 0.78), right isthmus cingulate (0.52 ± 0.12 vs 0.60 ± 0.08, p = 0.049, Cohen's d = 0.78), left lateral orbitofrontal (0.53 ± 0.13 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.83), right lateral orbitofrontal (0.53 ± 0.12 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.78), left lingual (0.51 ± 0.11 vs 0.60 ± 0.11, p = 0.049, Cohen's d = 0.78), right lingual (0.52 ± 0.10 vs 0.60 ± 0.11, p = 0.049, Cohen's d = 0.76), right medial orbitofrontal (0.53 ± 0.12 vs 0.61 ± 0.10, p = 0.049, Cohen's d = 0.72), left middle temporal (0.52 ± 0.12 vs 0.61 ± 0.10, p = 0.049, Cohen's d = 0.78), right middle temporal (0.53 ± 0.12 vs 0.61 ± 0.08, p = 0.049, Cohen's d = 0.78), left paracentral (0.54 ± 0.11 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.76), right paracentral (0.54 ± 0.12 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.77), left parahippocampal (0.54 ± 0.11 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.77), right parahippocampal (0.54 ± 0.11 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.76), left parsopercularis (0.53 ± 0.13 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.73), right parsopercularis (0.54 ± 0.11 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.73), right parsorbitalis (0.54 ± 0.12 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.72), right parstriangularis (0.53 ± 0.12 vs 0.61 ± 0.09, p = 0.049, Cohen's d = 0.74), left pericalcarine (0.54 ± 0.12 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.73), right pericalcarine (0.53 ± 0.11 vs 0.62 ± 0.09, p = 0.049, Cohen's d = 0.80), left postcentral (0.53 ± 0.12 vs 0.61 ± 0.10, p = 0.049, Cohen's d = 0.78), right postcentral (0.55 ± 0.12 vs 0.63 ± 0.10, p = 0.049, Cohen's d = 0.73), left posterior cingulate (0.55 ± 0.11 vs 0.63 ± 0.10, p = 0.049, Cohen's d = 0.72), right posterior cingulate (0.55 ± 0.12 vs 0.63 ± 0.10, p = 0.049, Cohen's d = 0.72), right precentral (0.53 ± 0.12 vs 0.61 ± 0.11, p = 0.049, Cohen's d = 0.70), left rostral anterior cingulate (0.53 ± 0.12 vs 0.61 ± 0.12, p = 0.049, Cohen's d = 0.67), and right rostral anterior cingulate (0.53 ± 0.12 vs 0.61 ± 0.12, p = 0.049, Cohen's d = 0.67) regions significantly increased after rTMS treatment (**Figure 1** and **Table 3**).

DISCUSSION

The present study investigated the effects of 3-week rTMS treatment on the cortical source network in patients with unipolar depression. Significant improvement in symptom severity of depression and anxiety was found after treatment in both active and sham groups. However, increased strength,

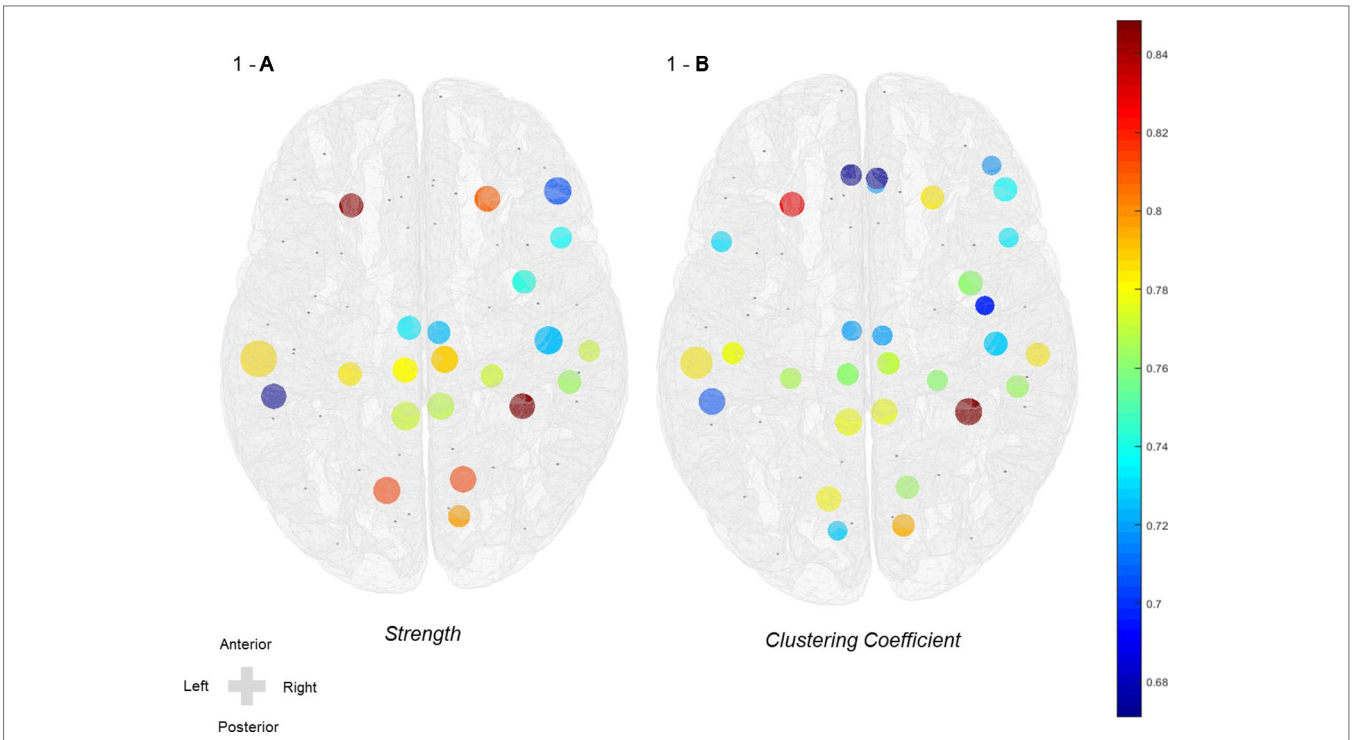

FIGURE 1 | Increased nodal strength and clustering coefficient of mid-beta frequency in the active group after rTMS treatment. **(A)** Nodal strength and **(B)** nodal clustering coefficient in mid-beta frequency. Color scale indicates effect size (Cohen's d value > 0.60).

TABLE 3 | In the active group, increased node values of strength and clustering coefficient in mid-beta frequency after 3 weeks of treatment.

Regions	Strength		Cohen's *d*	Clustering coefficient		Cohen's *d*	MNI coordinates		
	Baseline vs post mean (SD)	*p* value	Effect size	Baseline vs post mean (SD)	*p* value	Effect size	*x*	*y*	*z*
Fusiform R	38.91 (8.34) vs 45.10 (5.94)	0.049	0.85	0.51 (0.12) vs 0.61 (0.09)	0.049	0.85	−35.24	−14.92	23.53
Inferior temporal L	38.59 (8.90) vs 44.18 (7.82)	0.049	0.67	0.52 (0.12) vs 0.60 (0.11)	0.049	0.71	52.04	−11.39	24.42
Inferior temporal R	41.13 (7.55) vs 45.97 (4.77)	0.049	0.77	0.53 (0.12) vs 0.61 (0.08)	0.049	0.76	−51.76	−6.39	21.09
Insula R	39.84 (7.39) vs 44.94 (4.90)	0.049	0.74	0.52 (0.12) vs 0.60 (0.08)	0.049	0.76	−35.78	28.61	42.88
Isthmus cingulate L	40.03 (7.54) vs 44.98 (4.99)	0.049	0.77	0.52 (0.12) vs 0.61 (0.08)	0.049	0.78	5.78	−18.50	64.84
Isthmus cingulate R	39.99 (7.49) vs 44.89 (4.94)	0.049	0.77	0.52 (0.12) vs 0.60 (0.08)	0.049	0.78	−6.48	−15.01	65.35
Lateral orbitofrontal L	40.15 (8.62) vs 46.13 (4.96)	0.049	0.85	0.53 (0.13) vs 0.62 (0.09)	0.049	0.83	24.91	55.48	25.30
Lateral orbitofrontal R	40.93 (7.03) vs 45.98 (5.26)	0.049	0.81	0.53 (0.12) vs 0.62 (0.09)	0.049	0.78	−23.06	57.84	24.54
Lingual L	38.52 (7.28) vs 44.42 (7.15)	0.049	0.82	0.51 (0.11) vs 0.60 (0.11)	0.049	0.78	12.42	−44.54	42.23
Lingual R	38.86 (6.79) vs 44.45 (6.90)	0.049	0.82	0.52 (0.10) vs 0.60 (0.11)	0.049	0.76	−14.51	−40.68	43.61
Medial orbitofrontal R	–	–	–	0.53 (0.12) vs 0.61 (0.10)	0.049	0.72	−3.76	62.38	28.58
Middle temporal L	39.52 (8.31) vs 45.62 (7.10)	0.049	0.79	0.52 (0.12) vs 0.61 (0.10)	0.049	0.78	57.52	1.74	31.21
Middle temporal R	40.68 (7.82) vs 45.64 (4.55)	0.049	0.77	0.53 (0.12) vs 0.61 (0.08)	0.049	0.78	−58.64	4.38	30.23
Paracentral L	42.04 (7.07) vs 46.78 (4.86)	0.049	0.78	0.54 (0.11) vs 0.62 (0.09)	0.049	0.76	6.02	−2.21	103.46
Paracentral R	42.17 (7.21) vs 47.07 (4.95)	0.049	0.79	0.54 (0.12) vs 0.62 (0.09)	0.049	0.77	−7.93	1.43	103.05
Parahippocampal L	42.14 (6.99) vs 46.87 (4.85)	0.049	0.79	0.54 (0.11) vs 0.62 (0.09)	0.049	0.77	25.33	−3.60	25.34
Parahippocampal R	42.07 (6.78) vs 46.65 (4.88)	0.049	0.77	0.54 (0.11) vs 0.62 (0.09)	0.049	0.76	−24.49	−4.26	27.34
Parsopercularis L	–	–	–	0.53 (0.13) vs 0.62 (0.09)	0.049	0.73	48.91	42.83	59.06
Parsopercularis R	41.98 (6.98) vs 46.50 (5.15)	0.049	0.74	0.54 (0.11) vs 0.62 (0.09)	0.049	0.73	−48.83	44.11	57.72
Parsorbitalis R	–	–	–	0.54 (0.12) vs 0.62 (0.09)	0.049	0.72	−43.04	68.62	28.84
Parstriangularis R	40.88 (8.34) vs 45.98 (5.96)	0.049	0.70	0.53 (0.12) vs 0.61 (0.09)	0.049	0.74	−47.74	60.58	45.37
Pericalcarine L	–	–	–	0.54 (0.12) vs 0.62 (0.09)	0.049	0.73	9.33	−55.37	53.56
Pericalcarine R	40.86 (6.50) vs 45.68 (5.47)	0.049	0.80	0.53 (0.11) vs 0.62 (0.09)	0.049	0.80	−12.96	−53.46	54.79
Postcentral L	–	–	–	0.53 (0.12) vs 0.61 (0.10)	0.049	0.78	45.01	4.86	91.16
Postcentral R	41.59 (8.12) vs 46.86 (6.27)	0.049	0.73	0.55 (0.12) vs 0.63 (0.10)	0.049	0.73	−44.52	8.16	91.73
Posterior cingulate L	43.09 (6.95) vs 47.75 (5.70)	0.049	0.73	0.55 (0.11) vs 0.63 (0.10)	0.049	0.72	4.48	12.50	83.85
Posterior cingulate R	43.10 (6.95) vs 47.73 (5.77)	0.049	0.72	0.55 (0.12) vs 0.63 (0.10)	0.049	0.72	−5.75	11.02	84.87
Precentral R	–	–	–	0.53 (0.12) vs 0.61 (0.11)	0.049	0.70	−40.80	21.01	90.91
Rostral anterior cingulate L	–	–	–	0.53 (0.12) vs 0.61 (0.12)	0.049	0.67	4.80	65.47	44.84
Rostral anterior cingulate R	–	–	–	0.53 (0.12) vs 0.61 (0.12)	0.049	0.67	−3.95	64.33	46.63

L, left; R, right.
p value was adjusted by FDR correction.

clustering coefficient, and efficiency in the delta, theta, low-beta, and mid-beta frequency in the global level of the cortical source network were found only in the active group after rTMS treatment. Increased strength and clustering coefficient in mid-beta frequency in nodal levels were also observed after rTMS treatment in the active group.

The improvement in symptom severity of depression and anxiety is an essential clinical indication in the present study. The findings on the treatment effect in the active group could be reasonable; however, it should be clarified whether the significant effects in the active group were due to the actual effects of rTMS treatment. If our study includes the placebo effect, the authentic

neurophysiological changes induced by rTMS treatment could be observed in the active group. The small differences of clinical improvement between active and sham group could be explained for psychological expectancy effects to get a treatment (27, 28). All participants might have expected improvements of symptoms from their participation in the present study. Furthermore, a placebo effect could be induced due to daily visits to the hospital during the treatment period in the sham group.

In the global cortical source network, higher values of strength, clustering coefficient, and efficiency in the delta, theta, low beta, and mid beta were found in participants in the active group. Global efficiency in delta frequency could play a modulation role in suppression and amplification of neuronal population, which are implicated in the stabilization of neuronal network during the wakeful state (29, 30), and increased efficiency indicates increased concurrently connected information in the global network. In the global theta network, increased strength, clustering coefficient, and efficiency indicate enhanced dynamics of consciousness, body, and mind, which have a reciprocal relationship with the cooperation system of homeostasis (31). Depressive patients show a reduction in the beta band, which could be recovered after TMS treatment (32). Motor behavior control and executive function are also modulated by beta frequency network changes, which could be induced by functional improvement (32–35).

In the nodal network of the mid-beta band, strength, and clustering coefficient increased after TMS treatment. Previous studies have demonstrated that resting state network could be affected by TMS treatment (36, 37). The default mode network (DMN) involves PCC and parahippocampal gyrus (38), and strength and clustering coefficient of these regions increased after rTMS treatment in the present study. Increased clustering coefficient in the beta band after TMS has been also found in the cortical sensor level (39), but no studies have investigated the effect of rTMS treatment on the cortical source network in patients with unipolar depression. When both the node strength and the sum of weights of connected links increase, the nodal strength is associated with strong assemblies in the network (40); therefore, how neural network changes are related to the underlying treatment effect should be considered. The human brain network has three major functions, i.e., the executive control network (ECN), the salience network (SN), and the DMN (41, 42). In the present study, strength and clustering coefficient in the orbitofrontal region increased after TMS treatment. The frontal cortex, which is the brain area allocating the ECN, is associated with mood regulation in depressive patients (43). Inhibitory function in the orbitofrontal region is implicated in the maintenance of neuronal balance (44). Furthermore, ECN and DMN circuits have a cognitive engagement with episodic memory performance in patients with depression (45). The large parts of insula and ACC have allocated the crucial region of SN, which modulates the shift of a phase between ECN and DMN (46, 47). Therefore, the present study postulates that alterations in nodal network are caused by rTMS treatment and that the function of neural network could be improved in several core regions such as DMN, ECN, and SN.

CONCLUSIONS

In summary, our findings indicate that symptomatic improvement induced by rTMS is accompanied by the altered cortical source networks. The small differences in symptomatic changes between both groups prove that real effects of rTMS treatment on brain networks are an obvious biological evidence in patients with unipolar depression. However, the present study has several limitations. The sample size was small. In addition, the effects of rTMS need to be compared between unipolar depression and other psychiatric disorders.

DATA AVAILABILITY

All datasets generated for this study are included in the **Supplementary files**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Institutional Review Board of Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea approved the study protocol (approval number: KC14DDSE0479). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

K-IJ and J-HC contributed to the design of the research. K-IJ, J-HC and SL contributed to data collection. K-IJ, MS, and J-HC analyzed the data. K-IJ and H-JH interpreted the results and drafted, critically revised, and approved the version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2019.00686/full#supplementary-material

SUPPLEMENTARY TABLE 1 | The RAW data of the present study.

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Normal Cerebral Oxygen Consumption Despite Elevated Cerebral Blood Flow in Adolescents With Bipolar Disorder: Putative Neuroimaging Evidence of Anomalous Energy Metabolism

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Background: Regional cerebral blood flow (CBF) is reportedly altered in both adolescents and adults with bipolar disorder (BD). Whether these CBF differences are part of an overall imbalance in cerebral energy homeostasis remains unknown. Therefore, we examined global cerebral metabolic rate of oxygen consumption ($CMRO_2$) as a physiological index of brain metabolism in adolescents with and without BD.

Methods: One hundred and fifteen adolescents (mean age 17.3 ± 1.4 years), including 58 BD (type I, II, or not otherwise specified [NOS]) and 57 age-matched healthy controls (HCs) participated in this magnetic resonance imaging (MRI) study. Global estimates for venous blood oxygenation (Y_v) and grey matter CBF were measured using T2-relaxation-under-spin-tagging (TRUST) and arterial spin labeling (ASL) MRI, respectively. $CMRO_2$ was calculated using the Fick principle of arteriovenous difference to test for a group difference. We also examined $CMRO_2$ in relation to mood states (i.e. euthymic, depressed, or hypomanic/mixed).

Results: Although CBF was significantly higher in BD compared to HCs, there was no group difference in global $CMRO_2$, nor Y_v. Meanwhile, Y_v significantly decreased with age, and females tended to have greater CBF and $CMRO_2$ in comparison to males. Lastly, there was no significant association between $CMRO_2$ and mood states.

Conclusions: Our results indicate a potential mismatch between cerebral blood supply and oxygen metabolism in BD, suggesting inefficiency in energy homeostasis in the brain. Mapping $CMRO_2$ would provide the spatial resolution to investigate regional alterations in metabolism, particularly in the brain regions where CBF is increased.

Keywords: bipolar disorder, adolescent, cerebral metabolic rate of oxygen, $CMRO_2$, cerebral blood flow, venous oxygenation, TRUST MRI

INTRODUCTION

Bipolar disorder (BD) is a severe chronic mood disorder associated with an increased risk of developing premature cardiovascular disease (CVD) (1, 2). Indeed, CVD is the leading cause of mortality in BD, despite the increased risk of suicide and prevalence of other comorbidities (3, 4). In recent years, there has been increasing support for the notion regarding vascular pathology as an important neurobiological underpinning of BD (1, 5). This link between vascular pathology and BD is further evidenced by research showing alterations in cerebral blood flow (CBF) in individuals with BD (6). CBF is an important physiological parameter which reflects the supply of oxygen and glucose to the brain and is known to be associated with cardiometabolic risk factors (7). Although the human brain accounts for just 2% of total body mass, it consumes about 20% of the total energy (8). Thus, changes in blood supply may affect energy homeostasis in the brain that is crucial for normal neuronal functioning.

Most studies that have examined CBF in adults with BD have found decreased CBF in frontal and temporal brain regions in BD participants, particularly during depressive episodes, compared to healthy controls (HCs) (6, 9–11). In contrast, the sole study that has investigated CBF in adolescents with BD found region-specific elevation in CBF (12). These findings raise questions as to how alterations in cerebral hemodynamics affect energy homeostasis in the brain. Further research is needed to clarify the relationship between CBF changes and energy homeostasis in individuals with BD.

Multiple lines of evidence suggest that energy metabolism is disturbed in BD and is associated with mitochondrial dysfunction. Early studies using magnetic resonance spectroscopy (MRS) reported a reduction in phosphocreatine levels in the brains of BD patients, indicating inefficient adenosine triphosphate (ATP) synthesis (13, 14). Subsequent genetic studies found that BD is associated with mitochondrial DNA (mtDNA) mutations and polymorphisms (15, 16). In addition, post-mortem brain studies have shown abnormal mitochondrial morphology (17), reduction in mitochondrial protein function, and increased oxidative damage (18) in brains of patients with BD. Furthermore, studies have detected increased lactate concentration in the brain (19) and cerebrospinal fluid of individuals with BD (20), suggesting a shift from oxidative phosphorylation to glycolysis. Consistent with this, findings from positron emission tomography (PET) studies showing altered cerebral glucose metabolism in BD (17, 18) provide additional support for the view that abnormal energy metabolism is a key factor in the pathophysiology of BD.

Cerebral metabolic rate of oxygen consumption ($CMRO_2$) is a measurable index of oxygen utilization in the brain. It is defined as the amount of oxygen consumed per unit mass tissue and per unit time, and reflects oxygen demand in the brain. Regulation of oxygen metabolism is vital for normal neuronal functioning. Altered $CMRO_2$ is reported in studies on aging (21, 22) as well as in several disease states including Alzheimer's (23), Parkinson's (24), and multiple sclerosis (25).

$CMRO_2$ is related to the difference in oxygen saturation from artery to vein, known as the oxygen extraction fraction (26). Oxygenated blood that passes through the veins is the venous oxygenation (Y_v). Until recently, most studies have relied on PET combined with blood sampling to measure Y_v and calculate $CMRO_2$. The use of a radioactive tracer and the invasive nature likely limited its feasibility, especially in children and adolescents. This is reflected by a lack of studies examining brain physiology in youth.

The aim of the present study was to compare CBF, Y_V, and $CMRO_2$ in adolescents with and without BD. We used a validated T2-relaxation-under-spin-tagging (TRUST) magnetic resonance imaging (MRI) technique to noninvasively measure Y_v (27, 28). We also evaluated the relationship between CBF, Y_V, and $CMRO_2$ and mood states (i.e. euthymic, depressed, or hypomanic/mixed). We hypothesized that BD and HC participants will show differences in both CBF and the rate of oxygen utilization.

METHODS

Participants

One hundred and fifteen English-speaking adolescents between the ages of 13 and 20 years of both sexes and any race/ethnicity were included (58 BD, 57 HCs). BD participants who met criteria for BD [I, II, or not otherwise specified (NOS)] were recruited from a tertiary sub-specialty clinic at Sunnybrook Health Sciences Centre in Toronto, Ontario. HCs without any major mood or psychiatric disorders (i.e. no lifetime mood or psychotic disorders, no recent anxiety disorder or alcohol/drug dependence in the past 3 months) and no first- or second-degree family history of BD or psychotic disorder were recruited from the community. Participants were excluded if they met any of the following criteria: i) unable to provide informed consent; ii) existing cardiac condition, auto-immune illness, or inflammatory illness; iii) currently taking anti-inflammatory, anti-lipidemic, anti-hypertensive agents; iv) contraindications to MRI (e.g. cardiac pacemaker or other implanted device); v) neurological or cognitive impairment; and vi) infectious illness within the past 14 days. Written informed consent was obtained from both participants and their parent/guardian prior to any procedures. All procedures were approved by the research ethics board at Sunnybrook Health Sciences Centre. A semi-structured diagnostic interview was used to assess study requirements.

Clinical Procedures and Measures

Psychiatric diagnosis was determined using the Schedule for Affective Disorders and Schizophrenia for School Age Children, Present and Life Version (K-SADS-PL) (29). The K-SADS Depression Rating Scale (DEP-P) (30) and K-SADS Mania Rating Scale (MRS) (31) were used in place of the mood sections of the K-SADS-PL to assess current and lifetime symptoms of depression and mania. Symptom scores during the worst week in the past month were used as a measure of current mood state. Information provided by the participant, as well as their parent/guardian, was incorporated into the final summary score for each scale. BD-I and BD-II diagnosis was determined using the *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition* (*DSM-IV*), criteria (32). BD-NOS was operationally defined using

criteria outlined in the Course and Outcome of Bipolar Youth (COBY) study (33): i) two *DSM-IV* manic symptoms (three if only irritable mood is reported), ii) change in functioning, iii) mood and symptom duration of at least 4 h during a 24 h period, and iv) at least four cumulative 24 h periods of episodes that meet the mood, symptom, and functional change criteria over the participant's lifetime.

Overview of MRI Procedures

MRI was performed on a 3 T MR scanner (Philips Achieva, Philips Healthcare, Best, NL) using an eight-channel phased array RF head coil. The imaging protocol included T1-weighted images for anatomical registration, pseudo-continuous arterial spin labeling (PC-ASL) for CBF measurement, and TRUST for Y_v quantification.

Anatomical Imaging

Anatomical T1-weighted imaging was performed with high-resolution fast-field echo imaging [repetition time (TR)/echo time (TE)/inversion time (TI)] = 9.5/2.3/1,400 ms, field of view 240 × 191 mm, spatial resolution 0.94 × 1.17 × 1.2 mm, 256 × 164 × 140 matrix, scan duration 8:56 min:s). T1-weighted images were skull-stripped, co-registered to ASL space and standard space, normalized, and parcellated based on tissue type. All of the steps above were performed using Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) tools.

Global CBF

Gray matter (GM) CBF for the whole brain was measured using ASL. Phase contrast angiography scout images were acquired to visualize the position of the carotid and vertebral arteries that corresponded to the ASL labeling plane, and thereby facilitate ASL quality control. ASL data were acquired with the following parameters: single-shot two-dimensional echo-planar imaging (EPI) (TR/TE = 4,000/9.7 ms, 64 × 64 × 18 matrix, spatial resolution 3 × 3 × 5 mm), 1,650 ms labeling duration, post-label delay of 1,600 ms for the most inferior slice, 30 control-tag image pairs of unlabeled and labeled arterial blood water, and scan duration of 4:08 min:s.

Processing of ASL data was done using FSL tools (12). Images were first co-registered to a reference volume. Signal differences between consecutive control and tag images were obtained to measure the amount of perfused labeled arterial blood and thus provide relative CBF estimates. Signal in GM was optimized by removing images with excess head motion. Each individual's difference images were converted into a quantitative CBF map by relying on physiological and MR parameters, such as relaxation rates and transit times of the labeled arterial blood, to provide absolute units for the image intensity (ml/100 g/min) (34). A 5 mm smoothing kernel was applied to the CBF maps. GM global signal was reported based on mean CBF values extracted from GM masks that were segmented from the T1-weighted images and subsequently registered to ASL space.

Global Venous Oxygenation

Global cerebral venous oxygenation saturation, Y_v, was measured in the superior sagittal sinus (SSS) using the validated TRUST MRI technique (28, 35). First, a single oblique axial imaging slice was positioned parallel to the anterior-commissure posterior-commissure line 20 mm above the sinus confluence. TRUST MRI was performed with the following parameters: voxel size 3.44 × 3.44 × 5 mm^3, TR = 3,000 ms, TI = 1,022 ms, labeling thickness = 100 mm, gap = 22.5 mm, effective TE = 0, 40, 80, and 160 ms, and scan duration of 1:20 min:s.

TRUST data were processed using MATLAB scripts as described previously by Lu and colleagues (28, 35). First, a venous blood signal was extracted by pairwise subtraction of control and labeled images. A preliminary region-of-interest (ROI) mask was then manually drawn on the difference images to include the SSS. Within the ROI, four voxels showing the largest difference signals were selected for spatial averaging. A monoexponential function was used to fit the average venous blood signal as a function of TE and thereby obtain a T2 estimate, which was then converted to Y_v using a calibration curve. Hematocrit values for T2 calibration were based on laboratory reference values (LifeLabs, Ontario, Canada) accounting for both age and sex (36).

Global $CMRO_2$

Global $CMRO_2$ was calculated using the Fick principle in units of μmol O_2/100 g/min (26):

$$CMRO_2 = CBF * (Y_a - Y_v) * C_a$$

CBF values were extracted as the average CBF level within the grey mask on a per-participant basis. This single ROI served as a global CBF estimate. Y_v was also calculated on a per-participant basis, from within the SSS that drains a large proportion of the venous blood in the brain. Arterial oxygenation (Y_a) saturation, which is close to unity, was estimated to be 99% for all participants as it is minimally affected by age (21, 37). The value for C_a, which represents the amount of oxygen molecules that a unit volume of blood can carry, was based on physiological literature and assumed to be 833.7 μmol O_2/100 ml blood (38).

Statistical Analysis

All analyses were performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA). Comparison of demographic and clinical characteristics between groups was assessed using t-tests or chi-square tests, as appropriate. Group differences in global measures of CBF, Y_v, and $CMRO_2$ were investigated using analysis of covariance (ANCOVA), including age and sex as covariates, as these variables are known to have an effect on the physiological parameters (21, 22, 28). Pearson's correlation was used to investigate the associations between continuous variables. Significance was set at $p < 0.05$ for all analyses, and values are reported as means ± standard deviation (SD).

RESULTS

Demographic and Clinical Characteristics

Table 1 presents demographic and clinical characteristics. The majority of participants were female (57%) and Caucasian (70%) with a mean age of 17.3 ± 1.40. There were no significant age, race, or sex differences between groups. Mean body mass

index (BMI) was significantly higher in BD (24.04 ± 4.51 kg/m^2) than HCs (21.44 ± 2.79 kg/m^2; t = 3.73, p = 0.012, d = 0.69). As expected, there were multiple significant differences in clinical characteristics such as mood symptoms, medication, and lifetime history of cigarette smoking.

Global CBF

There was a significant effect of sex (t = 3.76, p < 0.001, d = 0.69). Overall, females had greater CBF in comparison to males (67.93 ± 12.82 vs. 60.08 ± 9.56 ml/100 g/min, respectively). The BD group had significantly greater global CBF in comparison to HCs, when controlling for age and sex (66.35 ± 12.18 vs. 62.64 ± 11.87 ml/100 g/min, respectively; F = 4.51, p = 0.036, η_p^2 = 0.039; **Figure 1A**). Sex was a significant covariate (F = 13.49, p < 0.001, η_p^2 = 0.11). There was no significant association between age and CBF (r = −0.15, p = 0.116; **Figure 1B**).

Global Venous Oxygenation

There was no significant difference in global Y_v between BD (70.22 ± 5.74%) and HC adolescents (69.54 ± 5.75%) after controlling for age and sex (F = 0.89, p = 0.349, η_p^2 = 0.008; **Figure 1C**). Age was a significant covariate (F = 4.90, p = 0.029, η_p^2 = 0.042). Since there was no significant difference between groups, the association between Y_v and age was examined using the whole adolescent population (N = 115). Correlation analysis revealed that age had a significant effect on global Y_v (r = −0.19, p = 0.045; **Figure 1D**). Specifically, there was an age-related decline of global Y_v at a rate of 0.77% per year. Furthermore, there was a significant positive correlation between Y_v and CBF values across subjects (r = 0.42, p < 0.001).

Global $CMRO_2$

There was no significant difference in global $CMRO_2$ between BD (156.27 ± 31.45 μmol/100 g/min) and HC adolescents (152.11 ± 33.74 μmol/100 g/min) when controlling for age and sex (F = 0.54, p = 0.47, η_p^2 = 0.005; **Figure 1E**). Sex was a significant covariate (F = 20.67, p < 0.001, η_p^2 = 0.157). Overall, female participants had higher $CMRO_2$ compared to males (165.30 ± 33.0 vs. 139.79 ± 25.73 μmol/100 g/min, respectively; t = 4.51, p < 0.001, d = 0.86). There was no significant association between age and $CMRO_2$ (r = 0.06, p = 0.50; **Figure 1F**).

Relationship With Mood States

Global CBF, Y_v, and $CMRO_2$ were not significantly correlated with the total mood scores for mania (MRS) or depression (DEP-P) (N = 115, p > 0.05). To assess if the participants' mood episode at the time of the MRI scan was associated with the cerebral metabolic measures, BD adolescents were further classified into three groups using their mood scores: presently hypomanic/mixed (MRS ≥12 with or without DRS ≥13; n = 23), presently depressed (DRS ≥13 and MRS ≤11; n = 17), and presently euthymic (DRS <12 and MRS ≤11; n = 18). Comparison of BD participants in the different mood states did not reveal any significant differences in the measurements for global Y_v, CBF, and $CMRO_2$ when controlling for age and sex (**Table 2**).

SENSITIVITY ANALYSIS

Smoking is a potential confounding variable that might influence cerebral hemodynamics and oxygen metabolism. Sensitivity analysis was therefore conducted to examine the effect of excluding the smokers (n = 7) in the BD group. Exclusion of these participants did not change the results for any of the above analyses.

A large number of BD participants were using various medication including second-generation antipsychotics (SGAs; 60%) and lithium (22%; **Table 1**). To examine the effect of medication on the MRI measures, ANCOVA analysis was rerun covarying for medication usage in addition to age and sex. Current SGA use was a marginally significant covariate of Y_v (F = *4.06, p = 0.046*) but not CBF or $CMRO_2$. Lithium use was not significantly associated with any of the three measures.

DISCUSSION

To the best of our knowledge, this is the first study to characterize cerebral oxygen metabolism in BD as well as in an adolescent population. We hypothesized that individuals with BD would show alterations in baseline $CMRO_2$ to reflect the change in CBF observed in prior studies (12, 39). Interestingly, our results demonstrate that although there is an increase in CBF among BD adolescents, the rate of global oxygen metabolism is unchanged. This imbalance between blood supply and oxygen consumption in the BD brain may reflect inefficiency in energy metabolism and the presence of some compensatory process to maintain $CMRO_2$. Alternative interpretations of this finding are discussed herein.

We did not observe a group effect of $CMRO_2$, which is to say that this largely global measure of oxygen consumption is not different between BD and HCs. The total GM CBF was significantly higher in BD compared to controls. This would in principle contribute to an increased $CMRO_2$ in BD, but this CBF group effect was a fairly modest effect size, whereas Y_v was not significantly different between groups. Therefore, since CBF and Y_v are used together to calculate $CMRO_2$, this helps to explain why there was no significant oxygen consumption group effect.

The relationship between CBF and $CMRO_2$ has been the topic of investigation for several decades. It is widely accepted that there is tight coupling between CBF and $CMRO_2$ at baseline (40). However, multiple studies have shown that there is an "uncoupling" between CBF and $CMRO_2$ during neuronal activation (41–44). Specifically, the increase in CBF during activation has been observed to be significantly greater than the increase in $CMRO_2$. In our study, although we were examining baseline physiology in the absence of neural stimulation, our results showed a significant increase in CBF without any changes in $CMRO_2$, which appears to be similar to this phenomenon. If BD adolescents indeed have altered coupling between CBF and $CMRO_2$ at baseline, it remains to be seen how this will affect CBF/$CMRO_2$ coupling during neural stimulation. Our finding of an increase in CBF in the absence of alterations in $CMRO_2$ can also be related to the phenomenon observed in hypercapnia

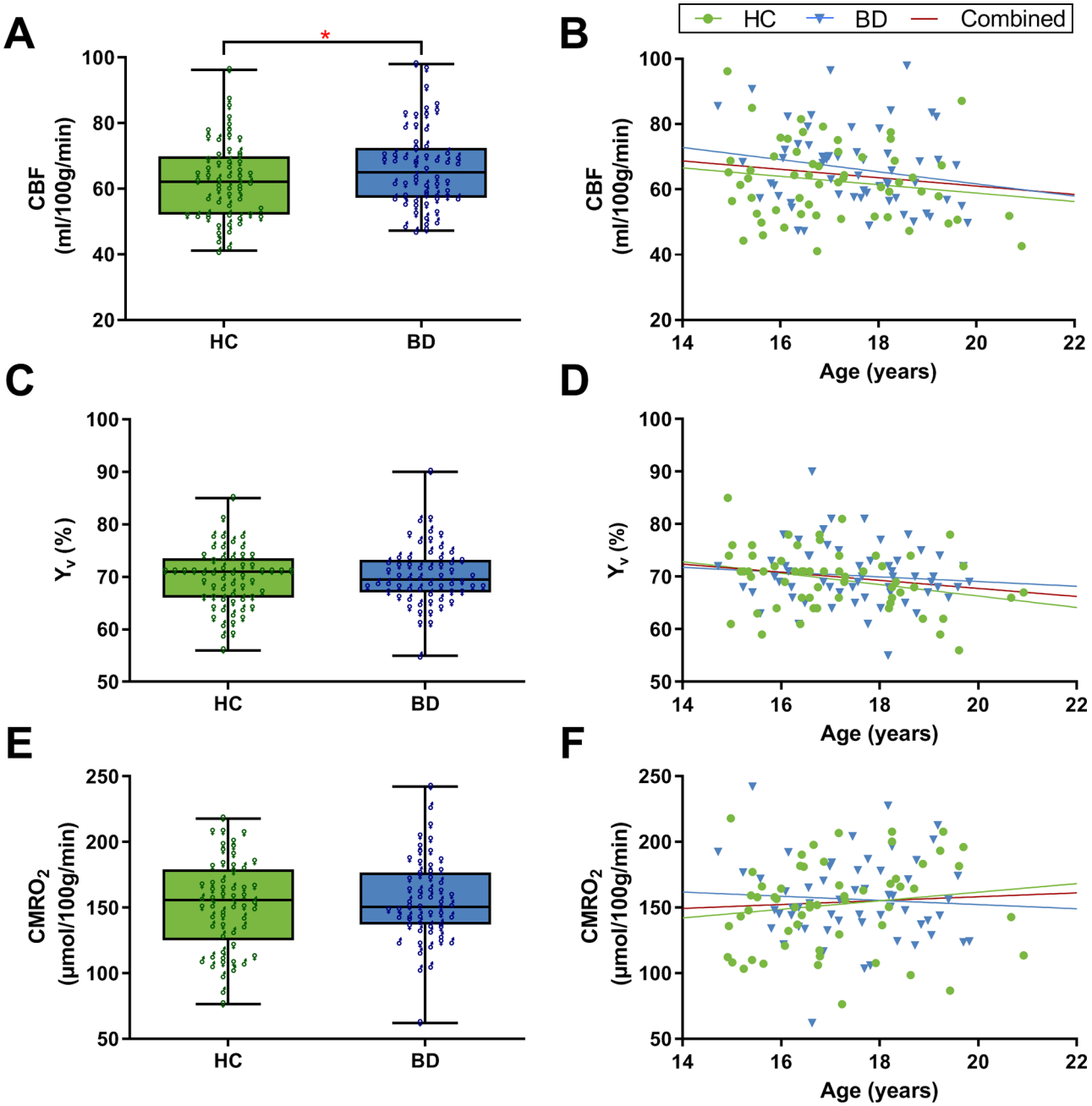

FIGURE 1 | Global grey matter cerebral blood flow (CBF), venous oxygenation (Y_v), and cerebral metabolic rate of oxygen ($CMRO_2$). **(A)** The bipolar disorder (BD) group had significantly greater CBF compared to healthy controls (HCs) ($F = 4.51$, $p = 0.036$). CBF was significantly greater in females compared to males ($t = 3.76$, $p < 0.001$). **(B)** There was no significant association between age and CBF. **(C)** Y_v did not significantly differ between BD and HCs. **(D)** Correlation analysis showed that age has a significant effect on Y_v; $r = -0.19$, $p = 0.045$. **(E)** $CMRO_2$ did not significantly differ between BD and HCs. Female participants overall had greater $CMRO_2$ compared to males ($t = 4.51$, $p < 0.001$). **(F)** There was no significant association between age and $CMRO_2$. Panels A, C, and E are box plots with superimposed data points showing distribution of values in BD (n = 58) and HCs (n = 57). Box = 25th and 75th percentiles; horizontal line = median, bars = min and max values; ♂ = male, ♀ = female. Panels B, D, and F are scatter plots showing the relationship between the physiological parameters and age (N = 115). Each marker represents data from one subject.

experiments where there is a hypercapnia-induced increase in CBF with negligible change in $CMRO_2$ (45, 46).

Another plausible interpretation of our results is that the increase in baseline CBF in BD might exist to serve functions other than oxygen metabolism. In addition to oxygen, the blood also carries and delivers glucose, the main source of energy for the brain. Similar to $CMRO_2$, the cerebral glucose metabolic rate of glucose (CMR_{glu}) represents another important index of neural

TABLE 1 | Demographic and clinical characteristics.[a]

Characteristics	Participants		Statistics	
	BD (N = 58)	HCs (N = 57)	T/χ^2	P-value
Sociodemographics				
Age, years	17.48 ± 1.25	17.05 ± 1.51	1.65	0.10
Age of BD onset, years	14.98 ± 2.33	—	—	—
Caucasian, n (%)	44(75.9)	36(63.2)	2.19	0.14
Sex (females), n (%)	32(55.2)	33(57.9)	0.09	0.77
SES, mean rank	56.80	59.22	1,583.50#	0.66
BD subtype, n (%)				
BD-I	18(31.0)	—	—	—
BD-II	19(32.8)	—	—	—
BD-NOS	21(36.2)	—	—	—
Clinical characteristics				
Smoking, n (%)	7(12.1)	0	7.33	0.007*
BMI	24.04 ± 4.51	21.44 ± 2.79	3.73	0.001*
History of depressive episodes, n (%)	47(81.0)	—	—	—
Current medication, n (%)				
SGA	35(60.3)	—	—	—
Lithium	13(22.4)	—	—	—
Antidepressant (SSRI)	6(10.3)	1(1.8)	3.71	0.054
Antidepressant (non-SSRI)	2(3.4)	0(0)	2.00	0.16
Stimulants	5(8.6)	3(5.3)	0.50	0.48
Clinical scores (±)				
Mania score—current	8.97 ± 10.24	0.12 ± .60	20.57	<0.001*
Mania score—lifetime	30.55 ± 10.98	0.63 ± 1.47	6.57	<0.001*
Depression score—current	15.45 ± 11.77	0.68 ± 1.81	17.45	<0.001*
Depression score—lifetime	30.62 ± 12.45	1.53 ± 2.64	9.44	<0.001*

*[a]Values are mean ± SD or frequency. BD, bipolar disorder; BD-I, bipolar I disorder; BD-II, bipolar II disorder; BD-NOS, bipolar disorder not otherwise specified; HCs, healthy controls; SES, Socioeconomic status; BMI, body mass index; SGA, second-generation antipsychotic; SSRI, selective serotonin reuptake inhibitor. #, Mann–Whitney U statistic, * = significant difference.*

TABLE 2 | CBF, Y_v, and $CMRO_2$ across mood states.[b]

Measure	BD hypomanic/ mixed (n = 23)	BD depressed (n = 17)	BD euthymic (n = 18)	Statistics		
				F	Partial η^2	p
CBF	67.37 (12.36)	63.53 (13.78)	67.73 (10.46)	0.81	0.030	0.45
Y_v	69.30 (5.20)	70.59 (6.97)	71.06 (5.26)	0.48	0.018	0.62
$CMRO_2$	163.66 (27.48)	146.25 (35.61)	156.29 (31.17)	1.01	0.037	0.37

[b]CBF in ml/100g/min, Y_v in $\%O_2$, $CMRO_2$ in µmol/100 g/min. Values are means ± SD. CBF, cerebral blood flow; Y_v, venous blood oxygenation; $CMRO_2$, cerebral metabolic rate of oxygen consumption.

function and energy homeostasis. However, unlike $CMRO_2$, it is widely accepted that there is a tight coupling between CMR_{glu} and CBF during neural activation. Indeed, in their seminal work, Fox and colleagues demonstrated that the increase is CBF (50%) during neural activation is associated with a similar increase in CMR_{glu} (51%) but a far more muted increase in $CMRO_2$ (5%) (42). Thus, it is possible that the increase in CBF observed in our BD participants might be related to alterations in CMR_{glu}.

Molecular studies examining mitochondrial function and oxidative stress provide strong evidence showing that energy metabolism is disturbed in BD (18, 47). In addition, studies have reported elevated lactate concentration in the brain (19) and cerebrospinal fluid (20) of individuals with BD. Furthermore, metabolomic analysis has found increased serum levels of pyruvate, the end product of glycolysis and the main fuel input for the citric acid cycle (CAC), in BD patients (48). Taken together, these findings suggest that there is a shift in metabolism from oxidative phosphorylation to the less-efficient glycolysis pathway in the brain of BD patients (49). Whereas the complete oxidation of glucose yields large amounts of energy in the form of ATP (30–36 ATP), glycolysis only produces 2 ATP (50). Thus, the brain of BD individuals may require more fuel in the form of glucose

to meet the high energy demands of the brain. The increase in CBF observed in our study might reflect a compensatory mechanism in place to increase glucose delivery to the brain of BD participants. This is in line with our hypothesis discussed earlier that the increase in CBF observed in BD participants may serve functions other than oxygen metabolism. Future studies that relate $CMRO_2$ with arterial lactate-to-glucose ratios in BD are warranted.

Although we did not observe a difference in global $CMRO_2$ between groups, it is possible that alterations in oxygen metabolism may exist and are localized to specific brain regions in BD. Multiple studies have demonstrated structural changes, more specifically GM volume reduction in specific regions of the brain (i.e. anterior cingulate cortex, middle frontal gyrus) of patients with mood disorders (51, 52). Furthermore, in our prior study examining CBF in BD adolescents, we found that CBF was significantly increased and localized in brain regions including the medial frontal and middle cingulate regions compared to HCs (12). Thus, it is can be expected that these brain regions that show structural changes and region-specific elevation in CBF will also have localized changes in energy metabolism. The current TRUST MRI acquisition provides only a global estimate of $CMRO_2$; thus, we are unable to provide insight on regional changes.

A recent study from our group identified that CBF is altered according to mood states in adolescents with BD (39). Therefore, in the present study, we similarly divided our BD participants into three groups, euthymic, depressed, or manic/hypomanic/mixed, to explore the possibility that $CMRO_2$ might also vary by mood state. We did not detect any significant differences in CBF, Y_v, or $CMRO_2$ between the three BD groups. As discussed earlier, it is likely that alterations in these physiological parameters may be localized rather than occur at a global level. Indeed, the mood state–related changes in CBF observed in the study mentioned were localized to certain brain regions (i.e. anterior cingulate cortex).

We observed an age-related decline in Y_v, which is a novel finding for this age group but is consistent with results from previous studies on normal adult aging (21, 28). This indicates that a greater fraction of oxygen is extracted by the brains of older individuals, which suggests alterations in oxygen demand with age. Interestingly, the rate of decline observed in our adolescent population is much greater (0.77% per year) than that reported in one study conducted on an older population (0.14% per year in ages 20–89 years) (21). This may be reflected by the fact that the developing brain undergoes extensive metabolic changes that persist until 16–18 years of age (53). In contrast to Y_v, we did not observe any significant association between age and CBF or age and $CMRO_2$. A number of studies have previously reported a decline in CBF with age (22, 54, 55). While our age range spanned early adolescence to young adulthood (14–21 years), in actuality, the vast majority of participants were in mid-adolescence (17.3 ± 1.4 years); as such, the age variance across participants was likely not sufficient to detect an effect of age on CBF. We observed a significant positive association between CBF and Y_v, which is consistent with prior findings in adults (56). Literature on age-related changes in $CMRO_2$ is inconsistent, with some studies reporting increased $CMRO_2$ with age (21, 22), while others have reported no change (57, 58) or even a decrease in $CMRO_2$ across the life span (37, 59).

We found that adolescent females have greater CBF and $CMRO_2$ compared to males. This finding is in line with recent studies conducted on older healthy cohorts (21, 22). Although it is not fully understood why such sex differences exist, a few hypotheses have been proposed. First, as females tend to have lower hematocrit values, it is thought that more CBF might be needed to carry an equivalent amount of oxygen as compared to males (21, 60). Others have attributed the higher baseline metabolic rate in women to the effect of hormones (i.e. estrogen) (61). In addition, differences in brain structure and size might also be a factor that reflects the observed sex-related differences.

There are some limitations to address in the present study. As described earlier, the TRUST MRI technique is limited to global estimations of Y_v and $CMRO_2$, as measurement is confined to a large terminal draining veins (i.e. SSS). Thus, we were unable to obtain information on any potential regional metabolic changes. Moreover, it is important to note that although our ROI for TRUST, the SSS, drains most of the cerebral cortex, not all of the cerebral blood is drained through this sinus (e.g. deep cerebral regions drain through the straight sinus instead). Therefore, Y_v might be underestimated in deeper cerebral regions. Second, our estimate of global CBF was based solely on ROI analysis of GM regions and does not account for CBF in white matter. Third, we did not measure Y_a using pulse oximetry for participants in this study and therefore estimated it to be a constant value of 99% based on physiological literature. The effect of this is likely to be minimal, as Y_a has been observed to be close to unity and minimally affected by age (21, 37). Similarly, we estimated hematocrit values (used for T2 calibration for TRUST) using laboratory reference values accounting for both age and sex instead of blood sampling to keep our study procedures completely noninvasive. Lastly, a large portion of our BD participants were using medication that might have impacted our findings. Indeed, medication use has previously been shown to influence CBF as well as cerebral metabolism (62–64).

CONCLUSION

In conclusion, this study demonstrates that cerebral oxygen utilization is not altered in adolescents with BD, despite the increased CBF observed in this population. Our results provide new insight on baseline energy homeostasis processes in BD. Furthermore, we found sex differences in that female adolescents overall have higher CBF and $CMRO_2$ than males. In addition, a rapid decrease of Y_v from adolescence to young adulthood was observed. Although the TRUST technique used herein offers several advantages that include noninvasiveness, speed (~1.5 min scan), and feasibility in children and adolescents, it is limited to global estimations of Y_v and $CMRO_2$. Research is currently underway to develop noninvasive MRI techniques for 3-D brain oxygenation mapping that permits Y_v quantification in both large sinuses as well as smaller cortical veins (65). Future studies that use these novel techniques are warranted to examine if there are any region-specific metabolic changes in BD.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available. We did not obtain consent from our participants for sharing of data when the study was conceived, so we cannot provide raw participant-level data.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Sunnybrook Health Sciences Centre Research Ethics Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SK performed data processing and analysis, interpreted results, and prepared the figures and manuscript. LF assisted with statistical analysis and revised the manuscript. AG helped with data processing and revised the manuscript. HL provided technical guidance and revised the manuscript. BG and BM contributed to study conception and experimental design and assisted with manuscript preparation.

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REVIEW
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Neuroimaging Studies in Patients With Mental Disorder and Co-occurring Substance Use Disorder: Summary of Findings

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Introduction: More than half of psychiatric patients have comorbid substance use disorder (dual diagnosis) and this rate, confirmed by many epidemiological studies, is substantially higher compared to general population. Combined operation of self-medication mechanisms, common etiological factors, and mutually causative influences most likely accounts for comorbidity, which, despite its clinical prevalence, remains underrepresented in psychiatric research, especially in terms of neuroimaging. The current paper attempts to review and discuss all existing methodologically sustainable structural and functional neuroimaging studies in comorbid subjects published in the last 20 years.

Methods: Performing a systematic PubMed/MEDLINE, Web of Science, and Cochrane databases search with predefined key-words and selection criteria, 43 structural and functional neuroimaging studies were analyzed.

Results: Although markedly inconsistent and confounded by a variety of sources, available data suggest that structural brain changes are slightly more pronounced, yet not qualitatively different in comorbid patients compared to non-comorbid ones. In schizophrenia (SZ) patients, somewhat greater gray matter reduction is seen in cingulate cortex, dorsolateral prefrontal and frontotemporal cortex, limbic structures (hippocampus), and basal ganglia (striatum). The magnitude of structural changes is positively correlated to duration and severity of substance use, but it is important to note that at least in the beginning of the disease, dual diagnosis subjects tend to show less brain abnormalities and better cognitive functioning than pure SZ ones suggesting lower preexisting neuropathological burden. When analysing neuroimaging findings in SZ and bipolar disorder subjects, dorsolateral prefrontal, cingular, and insular cortex emerge as common affected areas in both groups which might indicate a shared endophenotypic (i.e., transdiagnostic) disruption of brain networks involved in executive functioning, emotional processing, and social cognition, rendering affected individuals susceptible to both mental disorder and substance misuse. In patients with anxiety disorders and substance misuse, a common neuroimaging finding is reduced volume of limbic structures (n. accumbens, hippocampus and amygdala). Whether this is a neuropathological marker of common predisposition to specific behavioral symptoms and drug addiction or a result

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from neuroadaptation changes secondary to substance misuse is unknown. Future neuroimaging studies with larger samples, longitudinal design, and genetic subtyping are warranted to enhance current knowledge on comorbidity.

Keywords: neuroimaging studies, comorbidity, substance use disorders, mood disorders, anxiety disorders

INTRODUCTION

The co-occurring mental disorder and substance use disorder (SUD), a phenomenon also referred to as comorbidity or the older term *dual diagnosis* (DD) (1, 2), has been consistently replicated in a number of large epidemiological studies in the last three decades (3–10). Over 50% of psychiatric inpatients have a co-occurring SUD (11), and this rate is far bigger than what is found in general population (12) and predicted by a mere coincidence model (13). On the other hand, more than half of the individuals diagnosed with SUD meet the criteria for another mental disorder, the most common ones being anxiety, mood, personality, and schizophrenia/psychotic disorders (11). Dual-diagnosis subjects impose a serious challenge because of the higher severity of medical problems, social and familial burden, and the greater incidence of relapses related to both mental disorder and SUD (14).

Despite being conceptually criticized on multiple levels (15), the invariable presence of comorbidity in everyday practice has invoked a number of explanation attempts (5, 16). They may be broadly subdivided into [1] illness-mediated theories—an index disorder causes the secondary/comorbid condition; [2] theories of common causal factors—one or more independent etiological factors increase the risk for both disorders; [3] bidirectional theories—presence of mutually reciprocal causal influences between the comorbid disorders. Most epidemiological studies indicate that in terms of occurrence, the mental disorder has a temporal priority (4, 5, 13, 17, 18), thus lending credibility to the so-called "self-medication" hypothesis (19, 20), considering SUD as a secondary result of repeating substance use in an attempt to alleviate mental disorder symptoms. It is much more likely, however, that a combination of mechanisms acts for each pattern of comorbidity in each particular patient—for example, self-medication and bidirectional mechanisms are implicated in anxiety disorders–SUD association (21, 22), while in patients with schizophrenia and SUD, common neurobiological, neurodevelopmental, and genetic causal factors are intertwined with self-medication mechanisms (20, 23, 24).

Because in the last several decades psychiatry has built diagnostic categories resting exclusively on clinical symptoms (25), most neuroimaging studies have focused on brain structure or function in patients with particular diagnosis, comparing them with healthy controls. As a result, proportionately few studies have included comorbid patients (26), and the vast majority of them focus on schizophrenia and co-occurring SUD (27). Furthermore, at least to our knowledge, there are no studies comparing groups of DD subjects with different disorders (e.g., depression vs. anxiety disorder). However, neuroimaging research suggests shared neurobiological abnormalities in phenotypically and genetically related diagnoses such as schizophrenia and bipolar disorder (BD) (28–30), and data also coalesce around the hypothesis that different psychiatric illnesses entail pertuberations along the same neural circuits (31, 32). Taking this into account, the current article aims to review and discuss reported data with some focus on the possible cross-diagnostic validity of findings.

METHODS

PubMed/MEDLINE, Web of Science, and Cochrane databases were searched with the following keywords and word combinations: "Co-occurring disorders," "Comorbidity," "Dual diagnosis," "Magnetic resonance imaging" (MRI) and "functional Magnetic resonance imaging (fMRi)," "Schizophrenia and substance use disorder (SUD)," "Bipolar disorder and SUD," "Depression and SUD," "Anxiety disorder(s) and SUD". Besides that, in the process of analysis of the initially chosen publications, all appropriate papers indexed in the reference sections were inspected. Previous reviews focusing on similar topics were also taken into consideration as a cross-reference.

Articles were selected according to the following criteria: *a*) dated between January 1999 and July 2019; *b*) written in English; *c*) published in full text; *d*) using widely recognized and popular neuroimaging technique, e.g., MRI, PET etc.; *e*) performed in humans; *f*) including subjects meeting *International Statistical Classification of Diseases,-10th Revision*/*Diagnostic and Statistical Manual of mental disorders, Fourth Edition* (Fifth) (*DSM-IV*(*5*)) criteria for abuse of or dependence on at least one of the following substances: alcohol, cannabinoids, cocaine, hallucinogens, medicinal drugs (e.g., benzodiazepines), opioids, and stimulants (amphetamines, ecstasy).

RESULTS

The initial search made 91 hits, of which 53 were excluded based on selection criteria. In the process of reviewing, five more relevant publications emerged from reference literature and were included. Finally, 43 studies were chosen for participation in this review, and they are summarized on **Tables 1–4**.

Schizophrenia and SUD

Schizophrenia is by far the most prevalent diagnosis in neuroimaging research on comorbidity and with regards to type of substance misuse, the majority of studies have enrolled patients with alcohol, cannabis, or multiple SUDs abuse or dependence (26, 33, 34). As for the investigational tools, all but one of the studies employ MRI (VBM, RoI, DTI) and fMRI. For perspicuity reasons, structural and functional neuroimaging

studies are presented on separate tables in this review. In addition, based on the duration of illness and the age of the included population, structural neuroimaging studies for schizophrenia are subdivided into two separate tables: **Table 1** for studies including adolescent and young adult subjects with first episode or recent onset of the disease (up to 5 years) and **Table 2** for subjects with chronic schizophrenia lasting more than 5 years. This distinction was made for two reasons: first, this time interval was used in studies that include follow-up of patients with first-episode psychosis (41, 42). Second, studies of first-episode or recent-onset schizophrenia often include minimally treated or medication-naive subjects, which allows for better discrimination between structural changes imposed by substance use and those related to long-term antipsychotic treatment (35).

Other Mental Disorders

Only a few studies have included comorbid subjects with diagnosis other than schizophrenia (**Table 3**), and these are predominantly mood disorders (BD and major depression), anxiety and stress-related disorders, and also conditions typically occurring in childhood or adolescence. In terms of visualization method, five of the studies use structural MRI, and two are fMRI studies.

DISCUSSION

The current review tries to summarize and interprete the results of all methodologically consistent neuroimaging studies that have focused on DD patients and have been carried out over the past 20 years. Prior to discussing their findings and suggesting possible implications, several essential considerations have to be emphasized.

First, the most substantial limitations of all reviewed studies examining comorbid subjects are small sample sizes. With the exception of a few studies [e.g., (36, 45–46, 51, 54, 73)], most authors have included 8 to 25 comorbid patients in their samples with a corresponding number of controls. Furthermore, some authors have employed the same or significantly overlapping sample for several different articles (55–58, 62–63, 69) or used one sample for a structural and a functional neuroimaging study (64). As a consequence, the number of examined comorbid patients is highly insufficient to allow any definite conclusions, given the discrete size differences in compared anatomical structures. Such an inference is even truer for comorbid disorders other than schizophrenia, which are extremely underrepresented in the literature. Hence, future studies with much larger samples and more diverse diagnostic categories are warranted to confirm or reject the findings reported so far.

An additional holdback to data reliability is the low geographic, racial, and ethnocultural variety of reported studies—with only one exception of a study from Brazil (51), all the rest originate from Western Europe (37, 38, 41–47, 50, 52, 54, 60, 64, 67, 69), USA and Canada (36, 39, 40, 49, 53, 55–58, 61, 65, 66, 68, 70–78), and Australia (62, 63), with no studies from Asia, Africa, and most other parts of Europe and South America. Moreover, with regards to structural neuroimaging research in schizophrenia, there is an overall sex inequality of the examined populations with males constituting at least two-thirds of the DD subjects in more than half of the studies (36, 37, 39, 43–48, 50, 52–54, 61, 65) and representing the only studied group in the rest (38, 55–58, 62–64). While reflecting the clinicoepidemiological reality of more common SUDs in men including those with a co-occurring mental illness (79), this fact further obstructs the possibility of making definite conclusions given the existing evidence for gender influence on brain morphology in schizophrenia (80). Another restriction of the available data concerns the adolescent population with co-occurring SUD and schizophrenia. Apart from the few conducted studies involving very small patient groups with cannabis misuse only (47, 49, 53), some of the published articles do not even include patients meeting the threshold criteria for abuse and/or dependence (81, 82) and for this reason have not been taken into consideration in the present review. This fact renders any sound inferences regarding structural and functional brain changes in adolescent DD patients even more problematic than those for adults.

One very important consideration concerns diagnostic heterogeneity in the studied samples. Despite the strictly defined study protocols employed by authors striving to include "pure" DD subjects in their samples, there is still marked heterogeneity in both the substance use disorder and the co-occurring mental disorder. Some of the structural neuroimaging studies including those with largest samples have enrolled patients with polysubstance misuse (36, 39, 40, 43–46, 60), and even those focusing on a specific SUD—largely cannabis—feature subjects with additional current or past substance use disorder, most often alcohol or stimulants misuse [e.g (51, 54, 69)]. Combined drug use is an important confounding factor, which, according to some of the authors, may explain certain structural differences found in DD subjects such as striatal volume differences (54, 60). Furthermore, one of the major studies in terms of sample size compares schizophrenic patients with cannabis misuse against other substance misusing (e.g., not "clear") schizophrenic patients (45, 46). In one big study (54), the possible confounding effects of smoking, which has been reported in association with additional volume loss in schizophrenia (83), could not be separated, and this flaw is most likely also true for all the studies with adult SZ patients, although not explicitly stated by authors. As a result, the observed differences in brain morphology between comorbid and noncomorbid subjects could not be undoubtedly ascribed to the effects of a particular substance.

Substantial sample heterogeneity also exists for the co-occurring mental disorder. In fact, nearly all studies include patients with schizoaffective disorder (SAD) along with schizophrenics (SZ) in their samples (36, 37, 40, 43, 45, 46, 49, 53, 71). This shortcoming, resulting from both the inherent vagueness of SAD diagnosis and the categorical approach endorsed by structured diagnostic interviews (84), may as well bring some potentially positive implications. Specifically,

TABLE 1 | Structural neuroimaging findings in first-episode or recent-onset schizophrenic patients with SUD (duration of symptoms <5 years).

Authors	Type of study and sample size	Study population/diagnoses	Results
Scheler-Gilkey et al. (36)	Cross-sectional, MRI (qualitative assessment); N = 176	Male and female adults; SZ/SAD + AUD and SZ/SAD + SUD (n = 103) vs. SZ/SAD only (n = 73)	No difference on brain MRI
Cahn et al. (37)	Cross-sectional, MRI (RoI, VBM); N = 47	Male and female adults; recent-onset SZ/SFD/SAD + lifetime CUD (n = 27) vs. recent-onset SZ/SFD/SAD (n = 20)	No difference in brain MRI; reduced asymmetry of the lateral ventricles in the SZ/SFD/SAD + CUD-subgroup
Joyal et al. (38)	Cross-sectional, MRI (RoI); N = 64	Male adults; SZ + AUD (n = 19) vs. SZ only (n = 19) vs. HC (n = 26)	Significantly lower volume of cerebellar vermis in patients compared to controls, most expressed in DD patients. Anomalies in the posterior vermis in both SZ groups and in anterior vermis only in DD group
Szeszko et al. (39)	Cross-sectional, MRI (RoI); N = 107	Male and female adults; first-episode SZ + CUD ± SUD (n = 20) vs. first-episode SZ only (n = 31) vs. HC (n = 56)	SZ + CUD ± SUD patients had less anterior cingulate GM compared with the other two groups
Bangalore et al. (40)	Cross-sectional, MRI (RoI, VBM); N = 81	Male and female adults; first-episode SZ/SAD/SFD + lifetime CUD ± SUD (n = 15) vs. first-episode SZ/SAD/SFD only (n = 24) vs. HC (n = 42)	More prominent GM density and volume reduction in the right PCC in DD patients compared to SZ only group
Rais et al. (41, 42)	Cross sectional, 5 y longitudinal, MRI (VBM); N = 82	Male and female adults; first-episode SZ + CUD (n = 19) vs. first-episode SZ only (n = 32) vs. HC (n = 31)	Larger GM volume loss, greater lateral ventricle enlargement and more pronounced cortical thinning in ACC and DLPFC in the SZ + CUD group compared to SZ only
Wobrock et al. (43)	Cross-sectional, MRI (RoI); N = 41	Male and female adults; recent-onset SZ/SAD + lifetime SUD (n = 20) vs. recent-onset SZ/SAD only (n = 21)	No differences in the assessed morphology (superior temporal gyrus, amygdala-hippocampus complex and cingulum)
Ebdrup et al. (44)	Cross-sectional MRI (VBM); N = 91	Male and female adults; first-episode SZ + lifetime SUD (n = 9) vs. first-episode SZ only (n = 29) vs. HC (n = 43)	Significant hippocampal and caudate volume reductions in both SZ groups. Decrease in hippocampal volume more pronounced in SZ + lifetime SUD
Ho et al. (45) and Onwuameze et al. (46)	Cross-sectional, MRI (RoI); N = 235	Male and female adults; SZ/SAD + CUD ± SUD (n = 52) vs. SZ/SAD ± SUD (n = 183)	SZ/SAD + CUD ± SUD had smaller frontotemporal WM volumes than SZ/SAD ± SUD. Significant genotype-by-cannabis use interaction effects on WM volumes and on neurocognitive impairment
James et al. (47)	Cross-sectional, MRI (DTI, VBM); N = 60	Male and female adolescents (13–18 y); recent-onset SZ + CUD (n = 16) vs. recent-onset SZ only (n = 16) vs. HC (n = 28)	Diffuse reduction in GM and WM in SZ patients compared to HC; greater GM density loss in DD group in temporal fusiform gyrus, parahippocampal gyrus, ventral striatum, right middle temporal gyrus, insular cortex, precuneus, right paracingular gyrus, DLPFC, left postcentral gyrus, lateral occipital cortex and cerebellum; greater WM loss in DD group in brainstem, internal capsule, corona radiata, superior and inferior longitudinal fasciculus
Cohen et al. (48)	Cross-sectional, MRI (VBM, cortical pattern matching); N = 55	Male and female adults; first-episode SZ + CUD (n = 6) vs. first-episode SZ only (n = 13) vs. CUD only (n = 17) vs. HC (n = 19)	SZ (with and without CUD) had lower total cerebellar GM than HC; no difference between SZ with and without CUD
Kumra et al. (49)	Cross-sectional, MRI (RoI); N = 115	Male and female adolescents; HC (n = 51) vs. CUD (n = 16) vs. early-onset SZ/SAD/SZF (n = 35) vs. early-onset SZ/SAD/SZF + CUD (n = 13)	Decreased GM volume in the left superior parietal cortex in all three patient groups compared to HC, least expressed in DD. The latter had less GM in the left thalamus, compared to CUD and SZ
Schnell et al. (50)	Cross-sectional, MRI (DORTEL-VBM, RoI); N = 54	Male and female adults; first-episode SZ + lifetime CUD (n = 30) vs. first-episode SZ only (n = 24)	Less severe middle frontal gray matter deficits as well as cognitive impairments in DD group
Cunha et al. (51)	Cross-sectional, MRI (VBM, RoI); N = 200	Male and female adults; first-episode psychosis-FEP (non-affective or affective) as assessed by SCID-IV; FEP + CUD (n = 28) vs. FEP only (n = 78) vs. HC (n = 94)	GM deficits in hippocampus and parahippocampal gyrus and PFC as well as LV enlargement in FEP only group compared to DD group; better cognitive performance of DD group (equal to HC)
Malchow et al. (52)	Cross-sectional, MRI (RoI); N = 79	Male and female adults; first-episode SZ + CUD (n = 29) vs. SZ only (n = 20) vs. HC (n = 30)	Decreased volume of the left hippocampus, bilateral amygdala and caudate nucleus and increased midsagittal CC1 segment of the corpus callosum in all SZ subjects. DD patients with family history of SZ showed lower volumes of the bilateral caudate nucleus and increased midsagittal area of the CC2 subsegment of the corpus callosum compared to all other patients

(Continued)

TABLE 1 | Continued

Authors	Type of study and sample size	Study population/diagnoses	Results
Epstein et al. (53)	Cross-sectional, MRI (RoI); N = 134	Male and female adolescents; HC (n = 53) vs. CUD (n = 29) vs. early-onset SZ/SAD/SFD (n = 34) vs. early-onset SZ/SAD/SFD + CUD (n = 18)	Smaller surface area in the right caudal ACC in DD group compared to SZ and CUD groups; this finding significantly correlates with less efficient executive attention
Koenders et al. (54)	Cross-sectional, MRI (RoI); N = 197	Male adults; recent-onset SZ + CUD (n = 80) vs. resent-onset SZ only (n = 33) vs. HC (n = 84)	All SZ subjects had smaller volumes of most brain regions (amygdala, putamen, insula, parahippocampus, and fusiform gyrus) than HC. SZ + CUD had a larger volume of the putamen compared to SZ only, possibly explained by polysubstance use\

RoI, region(s) of interest; VBM, voxel-based morphometry; DTI, diffusion tensor imaging; SZ, schizophrenia; SAD, schizoaffective disorder; SFD, schizophreniform disorder; AUD, alcohol use disorder (abuse or dependence); AD, alcohol dependence; SUD, substance use disorder (abuse or dependence) including the following substances (varying dependent on study): cocaine, stimulants (amphetamines, ecstasy), hallucinogens, opioids, cannabinoids, alcohol, medicinal drugs; CUD, cannabis use disorder (abuse or dependence); DD, dual diagnosis; HC, healthy controls; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; VLPFC, ventrolateral prefrontal cortex; PFC, prefrontal cortex; LV, lateral ventricles; WM, white matter; GM, gray matter.

TABLE 2 | Structural neuroimaging findings in schizophrenic patients with illness duration >5 years and comorbid SUD.

Authors	Type of study and sample size	Study population/diagnoses	Results
Sullivan et al. (55)	Cross-sectional, MRI (RoI); N = 132	Male adults; SZ only (n = 27) vs. SZ + AD (n = 19) vs. AD only (n = 25) vs. HC (n = 61)	Ventricular enlargement in both SZ groups, greater in DD patients. Decreased cerebellar volume in DD and AD patients, not in SZ only ones
Sullivan et al. (56)	Cross-sectional, MRI (RoI); N = 122	Male adults; SZ only (n = 27), Vs. SZ + AD (n = 19) vs. AD only (n = 25) vs. HC (n = 51)	Volume deficits in pons in DD patients
Mathalon et al. (57)	Cross-sectional, MRI (RoI); N = 223	Male adults; SZ/SAD + lifetime AUD (n =35) vs. SZ/SAD only (n = 64) vs. AD only (n = 62) vs. HC (n = 62)	Greatest GM volume deficits in DD group, particularly in the prefrontal and anterior superior temporal brain regions
Deshmukh et al. (58)	Cross-sectional, MRI (RoI); N = 122	Male adults; SZ only (n = 27) vs. SZ + AD (n = 19) vs. AD only (n = 25) vs. + HC (n = 51)	Caudate, putamen, and nucleus accumbens showed different patterns of volume deficits in SZ and AD; no evidence for compounded deficits in DD group
Potvin et al. (59)	Cross-sectional, MRI (VBM); N = 38	Male and female adults; SZ + AUD/CUD (n = 12) vs. SZ only (n = 11) vs. HC (n = 15)	Increased gray matter density in the ventral striatum in DD compared with pure SZ
Schiffer et al. (60)	Cross-sectional, MRI (VBM); N = 51	Male adults; SZ + current or lifetime SUD (n = 12) vs. SZ only (n = 12) vs. SUD only (n = 13) vs. HC (n = 14)	GM matter losses in lateral orbitofrontal and temporal regions associated with SZ, and in medial orbitofrontal, ACC and frontopolar cortex with addiction. DD subjects had higher volume decreases in ACC, frontopolar and superior parietal regions and increased nonplanning impulsivity compared to SZ
Smith et al. (61)	Cross-sectional, MRI (HDBM-LD); N = 107	Male and female adults; SZ + past AUD (n = 16) vs. SZ only (n = 35) vs. HC (n = 56)	DD group had more severe shape abnormalities in the hippocampus, thalamus, striatum, and globus pallidus compared to SZ only group
Solowij et al. (62, 63)	Cross-sectional, MRI (RoI, semiautomatic method); N = 48	Male adults; SZ + CUD (n = 8) vs. SZ only (n = 9) vs. CUD only (n = 15) vs. HC (n = 16)	Significantly smaller cerebellar WM volume and hippocampal shape change in all patient groups compared to HC, most severely expressed in DD group
Gizewski et al. (64)	Cross-sectional, MRI (VBM); N = 48	Male adults; HC (n = 12) vs. AD (n = 12) vs. SZ + AD (n = 12) vs. SZ only (n = 12)	All SZ patients (AD and non-AD) had reduced GM volume in the left VLPFC compared to HC
Smith et al. (65)	Cross-sectional, MRI (HDBM-LD); N = 97	Male and female adults; HC (n = 44) vs. past CUD (n = 10) vs. SZ only (n = 28) vs. SZ + past CUD (n = 15)	Similar cannabis related shape differences (suggestive of localized volume loss) in the striatum, globus pallidus, and thalamus in past CUD and SZ + past CUD more pronounced in the latter. Significant cannabis related decrease in working memory across groups

RoI, region(s) of interest; VBM, voxel-based morphometry; HDBM-LD, large-deformation high-dimensional brain mapping; SZ, schizophrenia; SAD, schizoaffective disorder; SFD, schizophreniform disorder; AUD, alcohol use disorder (abuse or dependence); AD, alcohol dependence; SUD, substance use disorder (abuse or dependence) including the following substances (varying dependent on study): cocaine, stimulants (amphetamines, ecstasy), hallucinogens, opioids, cannabinoids, alcohol, medicinal drugs; CUD, cannabis use disorder (abuse or dependence); DD, dual diagnosis; HC, healthy controls; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; VLPFC, ventrolateral prefrontal cortex; PFC, prefrontal cortex; LV, lateral ventricles; WM, white matter; GM, gray matter.

TABLE 3 | Functional neuroimaging findings in schizophrenic patients with SUD.

Author	Type of study and sample size	Study population/ diagnoses	Results
Mancini-Marïe et al. (66)	Functional MRI (fMRI) during passive viewing of emotionally negative pictures; N = 23	Adults; SZ + SUD (n = 12) vs. SZ only (n = 11)	Heightened activity in the right medial prefrontal cortex, left medial prefrontal cortex, right orbitofrontal cortex, and left amygdala only in DD group who also showed higher subjectively rated experience
Joyal et al. (67)	fMRI during execution of a go/no-go task measuring response inhibition capacity; N = 36	Adult males; homicide offenders with SZ + APD + SUD (n = 12) vs, SZ only (n = 12) vs. HC (n = 12)	Substantially less activation of frontal basal cortices and higher activation in frontal motor, premotor and anterior cingulate regions in SZ + APD + SUD group compared to the other two groups; findings related to personality characteristics (antisocial behavior) and not to SZ
Potvin et al. (68)	fMRI during passive viewing of an emotional film excerpt with social content; N = 22	Adult males and females; SZ + SUD (AUD/CUD/ AUD + CUD) in the last 18 mo (n = 11) vs. SZ only (n = 11).	Increased activation in right superior parietal cortex and left medial prefrontal cortex in DD patients in comparison to SZ only group. The former also had higher subjective emotional experience on a self-report scale.
Løberg et al. (69)	fMRI during auditory listening task engaging verbal processing, attention and cognitive control; N = 26	Adult males and females; SZ + CUD (n = 13) vs. SZ only (n = 13)	Very similar activation patterns of both groups overall; slight difference in cortical activation dynamics of the default mode network and cognitive performance in favor of the SZ + CUD group
Bourque et al. (70)	fMRI during task for encoding and recognition of a series of positive and negative pictures; N = 49	Male adults; SZ + CUD (n = 14) vs. SZ only (n = 14) vs. HC (n = 21)	Recognition of positive and negative stimuli prominently impaired in SZ group compared to DD and HC. Emotional memory and prefrontal lobe functions preserved in DD in comparison to SZ patients
Thompson et al. (71)	[^{11}C] raclopride PET in two sessions: baseline and after receiving amphetamine; N = 26	Male and female adults; SZ/ SAD + SUD (n = 11) vs. HC (n = 15)	DD subjects displayed significant blunting of striatal DA release suggesting that in SUD-SZ patients, hypersensitivity of D2 receptors rather than excess presynaptic dopamine release is the predominant dopaminergic alteration
Gizewski et al. (64)	fMRI with mind reading task that involves empathy (cognitive and affective); N = 48	Male adults; HC (n = 12) vs. AD (n = 12) vs. SZ + AD (n = 12) vs. SZ only (n = 12)	All SZ patients (AD and non-AD) had decreased activity in left VLPFC; all clinical groups (as opposed to HC) had decreased activity in AIC; DD patients had more preserved social skills compared to SZ only patients

SZ, schizophrenia; APD, antisocial personality disorder; AUD, alcohol use disorder (abuse or dependence); CUD, cannabis use disorder; SUD, substance use disorder (abuse or dependence) including the following substances: cocaine, stimulants (amphetamines, ecstasy), cannabinoids, alcohol; HC, healthy controls; DD, dual diagnosis; VLPFC, ventrolateral prefrontal cortex; AIC, anterior insular cortex.

considering the low clinical utility and reliability of SAD diagnostic criteria (85), and the low temporal consistency of SAD diagnosis found in longitudinal studies (86, 87), it could be hypothesized that the reported neuroimaging findings for comorbid SAD patients also apply to bipolar patients with a co-occurring SUD. Furthermore, one study has even included *DSM-IV*–defined "affective psychosis" subjects, and this category is largely limited to bipolar patients (51). Of course, given the scarce neuroimaging investigation focusing solely on bipolar comorbid subjects, future studies with sufficient magnitude are needed to test the validity of this assumption.

Another major drawback that should be outlined is related to the status of substance use disorders in the studied patients—particularly whether these are lifetime diagnoses in patients currently in stable and long-term remission or active phase conditions. In this line of thought, while some studies with comorbid schizophrenia and co-occurring SUD, particularly alcohol and cannabis, have only included subjects with past abuse or dependence [e.g., (40, 59, 60, 62, 63, 69)], others have investigated patients with a short or even no prior remission [e.g., (37, 54, 58)]. This might represent a substantial confounding factor since a positive correlation between recency of substance use (especially alcohol) and greater volume deficit in some brain structures such as nucleus accumbens has been reported (57).

The last constraint discussed here concerns concomitant antipsychotic treatment, which has a definite correlation with certain structural effects on the brain (35, 88). As most of the SZ study samples have been exposed to this class of medications [e.g., Refs. (41, 42, 45, 46, 54–60, 69) and others], its effects must necessarily be taken into account when discussing the results. Moreover, some authors (45–47) have found more structural changes in SZ patients with long-term therapy such as dysmorphology and volume deficits in the thalamus and striatal and other basal ganglia, more pronounced in those treated with atypical antipsychotics. Antipsychotics-related overall decrease in total brain gray and white matter has also been reported (45, 46). Taken together, these data further limit the significance and validity of reported neuroimaging findings in DD and non-DD subjects.

With all the considerations emphasized so far, the results of structural and functional neuroimaging studies in DD patients will be analyzed below as follows:

TABLE 4 | Structural and functional neuroimaging findings in studies with nonschizophrenic patients with SUD.

Author	Type of study and sample size	Study population/diagnoses	Results
De Bellis et al. (72)	Cross-sectional, MRI (RoI); N = 42	Male and females adolescents and young adults (13–21 y); AUD + mental disorder* (n = 14) vs. HC (n = 28)	DD subjects had smaller PFC and PFC WM volumes compared with controls; significant sex-by-group effect in favor of males; PFC volume variables significantly correlated with measures of alcohol consumption
Woodward et al. (73)	Cross-sectional, MRI (RoI); N = 99	Male adults combat veterans (n = 99); PTSD + lifetime AUD vs. PTSD only vs. lifetime AUD only vs. HC	Smaller hippocampal volume in PTSD subjects more pronounced in DD group. PTSD was strongly associated with comorbid major depression
Hassel et al. (74)	fMRI with viewing of event-related paradigms of happy and fear faces; N = 30	Male and female adults: BD + SUD ± ED (n = 8) vs. BD only (n = 6) vs. HC (n = 16)	Reduced dorsal prefrontal-cortical activity to all faces and greater subcortical-striatal activity to happy and neutral faces in BD patients compared to controls; differences more expressed in DD group
Cornelius et al. (75)	BOLD fMRI using threat related amygdala reactivity paradigm; N = 6	Male and female adults; current CUD + current MDD (n = 6)	Amygdala reactivity inversely related to level of cannabis use suggesting an inhibitory effect of cannabinoids on amygdala function
Sameti et al. (76)	Cross-sectional, MRI (RoI, model-based segmentation PC software); N = 100	Male and female adults; past AUD + current or past mental disorder* (n = 52) vs. control group with past or current mental disorder (n = 48)	Minimal differences between groups in subcortical volumes (LV, thalamus, caudate, pallidum, putamen, hippocampus, amygdala, n. accumbens); in DD group, however, subcortical structures were smaller in those with vs. without current or past mental disorder
Nery et al. (77)	Cross-sectional, MRI (VBM); N = 67	Male and female adults; BD + past AUD (n = 21) vs. BD only (n = 21) vs. healthy controls (n = 25)	DD patients had smaller GM volumes in the left medial frontal and right anterior cingulate gyri compared to BD only group. The latter did not present GM volume differences compared to HC
Lippard et al. (78)	Longitudinal MRI, (VBM) with mean follow-up of 6 y; N = 30	Male and female adolescents and young adults with BD (n = 30)	Lower GM volume in prefrontal, insular, and temporopolar cortices were observed at baseline among adolescents with BD reporting subsequent alcohol and cannabis misuse compared to adolescents with BD who did not

**Mental disorder includes the following categories ranged according to the rate of occurrence: other substance abuse or dependence, major depressive disorder, conduct disorder, posttraumatic stress disorder, attention-deficit hyperactivity disorder, oppositional defiant disorder, generalized anxiety disorder, bipolar disorder, and antisocial personality disorder. Legend: RoI = region(s) of interest; VBM, voxel-based morphometry; PFC, prefrontal cortex; WM, white (cerebral) matter; LV, lateral ventricles; PTSD, posttraumatic stress disorder; MDD, major depressive disorder; AUD, alcohol use disorder (abuse or dependence); BD, bipolar disorder; DD, dual diagnosis; GM, gray matter; WM, white matter.*

Structural and Functional Neuroimaging Findings in Schizophrenia and SUD

As mentioned earlier, most of the studies have included predominantly cannabis, alcohol, and combined misuse subjects, and there is marked inconsistency across study findings, especially for cannabis use disorders (54). Whereas some studies detect no or insignificant differences between DD and non-DD patients (37, 43, 48, 58, 64), others suggest more severe structural changes in DD compared to non-DD patients (39–42, 44-47, 52, 60–63), and finally some authors find the opposite correlation (50, 51, 54). With regard to recent-onset or first-episode schizophrenia studies (i.e., with illness duration up to 5 years), reported findings consolidate around greater volume reduction on the expense of gray matter in DD subjects, with most commonly affected areas being anterior and posterior cingulate cortex (ACC, PCC) (39–42, 53), dorsolateral prefrontal cortex (DLPFC) (41, 42, 47), hippocampus (44, 52), striatum (47, 52), and frontotemporal cortex and cerebellum (39–42, 47). In addition, less cerebral white matter in frontotemporal cortex (45, 46) as well as diffuse subcortical areas (47) has been reported. None of these findings, however, is specific, and they represent only quantitative difference as compared to pure schizophrenia. Expectedly, in some studies, the magnitude of structural disruptions is positively correlated with neurocognitive impairment (45, 46, 53). Studies with chronic patients (i.e., with illness duration >5 years) also show slightly more pronounced volume loss in DD subjects affecting prefrontal, frontotemporal, parietal, and anterior cingulate cortical areas (57, 60), hippocampus (61–63), thalamus, striatum and globus pallidus (61, 65), cerebellar

gray and white matter (55, 62, 63), and pons (56). Again, no specificity of findings may be claimed as the same are often reported in literature both with regard to pure schizophrenia (89) and pure alcohol and cannabis misuse (90, 91). The most distinctive neurocognitive impairment pattern in this group of studies was reported by Schiffer et al. (2010) who found significantly greater impulsivity in DD subjects compared to pure SZ ones, while executive functioning deficits of both groups were on par.

As noted above, studies that fail to demonstrate differences between DD and non-DD groups also exist. For example, with respect to alcohol use disorders (AUDs), Gizewski et al. (2013) found similar gray matter volume decrease in the left VLPFC in long-term abstinent alcoholic and nonalcoholic SZ groups compared to healthy controls. Deshmukh et al. (2005) comparing pure chronic SZ patients versus chronic alcohol-dependent SZ ones with a varying duration of preceding abstinence demonstrated somewhat greater volume deficit in SZ in striatum (putamen) and n. accumbens with no evidence for a compounded structural deficit in DD subjects (58). In the same sample, however, other authors did demonstrate greater ventricular enlargement and cerebellar volume loss (55) and more pronounced gray matter deficits in prefrontal and superior temporal cortex (57) and pons (56) in DD group, with the latter structure not considered as directly affected by schizophrenia (26). In a mixed SUD sample of recent-onset schizophrenics, Wobrock et al. (2009) found no differences in superior temporal gyrus, amygdala-hippocampus complex, and cingulum between comorbid and noncomorbid subjects (43). In a similar group of SZ patients with and without co-occurring alcohol or cannabis use disorder, Potvin et al. (2007) also did not find significant structural brain differences between groups except for increased gray matter density in the ventral striatum for the DD group (59). Interestingly, a similar finding of increased dorsal striatum (putamen) was reported by Koenders et al. (2009) in a comparison of cannabis misuse versus noncannabis misuse schizophrenic patients (54). As deficits in other areas (limbic structures, anterior cingulate, orbitofrontal and fusiform gyrus, insula, thalamus, and caudate) were not detected, the findings of both studies could be related to striatal neuroadaptation changes emerging from repetitive drug use (54, 59). Further considering cannabis use disorders, Cohen et al. (2012) did not detect differences in comorbid and noncomorbid subjects in a first-episode schizophrenia sample in which both groups had lower total cerebellar gray matter than healthy controls (48). Similarly, investigating a group of adolescent early-onset schizophrenia subjects with and without co-occurring cannabis use disorder, Kumra et al. (2012) did not find additional volumetric deficit in DD patients compared to pure EOS and pure CUDs, while all three groups had smaller gray matter volume in the left parietal cortex than controls. DD patients had somewhat smaller left hypothalamus than pure SZ subjects, but for the left parietal cortical surface, the opposite relationship was observed, and it was the size of this area that showed significant positive association with results on a neurocognitive test for attention and working memory supporting its stronger implication in schizophrenia-related neuropathological processes (49).

Finally, studies that favor DD subjects in terms of severity of illness and associated neuroimaging findings will be discussed. In the first of them, with stringent inclusion criteria regarding schizophrenia, Schnell et al. (2012) found less severe gray matter deficits in the left DLPFC in first-episode patients with past cannabis use disorder versus pure schizophrenia ones. Moreover, this result was paired with superior cognitive performance in verbal and working memory tests in the DD group (50). Similarly, in a larger study employing less rigorous selection criteria and thus including both nonaffective- and affective-type first-episode psychosis, Cunha et al. (2012) reported milder gray matter deficits in hippocampus, parahippocampal gyrus, and prefrontal cortex; smaller lateral ventricles enlargement and better cognitive performance in patients with cannabis use disorders versus those without (51). These results are in striking controversy with data from Rais et al. (41, 42), Ho et al. (45) and Onwuameze et al. (46) reported above, which indicate both more significant gray and white matter deficits in a number of cortical structures and worse performance on neurocognitive tests in cannabis misusing SZ subjects. In fact, explanations exist for both adverse and beneficial effects on marijuana in schizophrenia population. Regarding the former, theories suggest neurotoxic effects of cannabis, which are either direct *via* disturbed control of the endogenous cannabinoid system on glutamate and γ-aminobutyric acid release and subsequent impairment in maturation of neural circuitries in adolescence (92) or an indirect including complex genotype-by-cannabis interactions that leads to brain morphologic changes. Supporting that, in the only study of its type, Ho et al. (45) found significant association between more severe frontotemporal white matter deficits in DD subjects and a particular genetic variant of the cannabinoid 1 receptor (CNR1). The alternative set of explanations generally regards DD patients as a specific subgroup that is intrinsically less vulnerable to schizophrenia than cannabis-naive patients, has better premorbid cognitive functions and social adjustment (93), and probably would not have developed psychotic symptoms without the effects of substance use. Such a hypothesis regarding not only marijuana, but SUDs in general, is supported by the available functional neuroimaging research. Nearly all studies of this type report better preserved functioning in areas associated with emotional processing—medial prefrontal cortex (66, 68, 70) and social cognition—ventrolateral prefrontal cortex and anterior insular cortex (64). In addition, data show that areas associated with verbal processing and attention (posterior cingulate cortex, inferior parietal lobe and precentral gyrus) and executive functioning (DLPFC) also show higher activity in DD patients (69, 70). In further support of the hypothesis that comorbid SZ subjects might represent a subgroup with less neurobiological abnormalities than noncomorbid SZ, Thompson et al. (71) in a recent [^{11}C] raclopride study hypothesized that a hypersensitivity of D2 receptors rather than excess presynaptic dopamine release is the predominant dopaminergic alteration in comorbid subjects. However, at least some preexisting neuropathological diathesis is seemingly necessary to reach psychotic state as witnessed by the study of Uhlmann et al. (2016) showing thinner prefrontal and temporal cortical areas and decreased hippocampal volume in methamphetamine-dependent patients with psychosis versus

those without (94). In fact, both sets of explanations are not necessarily mutually exclusive: as hypothesized by Cunha et al. (51), the exposure to cannabis or other substances may be a prerequisite for development of first episode of psychosis in an initially relatively "preserved" brain, but with repeating use, severe gray matter deficit occurs, which is accountable for worse clinical and cognitive presentations of dual-diagnosis patients reported in longitudinal studies.

Structural and Functional Neuroimaging in Patients With Diagnoses Other Than Schizophrenia

Although far more limited as compared to the comorbid schizophrenia research, available data are consistent with more severe neuroimaging changes in this population. Starting with BD, two structural and one fMRI studies demonstrate certain differences between comorbid and noncomorbid subjects. In the earliest of them, Hassel et al. (2009) showed abnormal pattern of brain activation in a small group of bipolar patients (n = 14) compared with controls (74). Using an event-related fMRI paradigm with happy, neutral, and sad faces, they found reduced dorsal prefrontal-cortical activity to all faces and greater subcortical-striatal activity to happy and neutral faces in all bipolar patients. Interestingly, decrease in prefrontal activity was more pronounced in comorbid patients, and authors have hypothesized that this phenomenon may be linked to stronger difficulties in integrating socioemotional information and, subsequently, emotion regulation. Moreover, similarly decreased DLPFC activity has been reported in substance abusers in decision making and facial matching tasks (95, 96). In a subsequent cross-sectional MRI study, Nery et al. (2011) found smaller gray matter volumes in the left medial frontal and right anterior cingulate gyri in a sample of bipolar patients with long-term remission AUD compared to pure bipolar ones (77). As these frontal lobe subareas are connected with other prefrontal areas and high-order association regions (orbitofrontal cortex, temporal and parietal lobe, and subcortical structures) and insofar prefrontal cortex plays an important role in the inhibitory control of inappropriate compulsive behaviors such as addiction (97), the authors suggested that the observed gray matter deficits are a structural correlate of the impaired "top-down" inhibitory control in prefrontal brain areas that distinguishes BD-AUD patients from BD patients without AUD (77). Supporting this assumption, in a recent study with longitudinal design, Lippard at al. (2017) reported lower baseline gray matter volume in prefrontal, insular, and temporopolar cortices in those adolescents who later developed alcohol and cannabis misuse, suggesting a possible endophenotype significance of these findings (78). Interestingly, sex-based difference in structural findings was also observed in that while decreased baseline gray matter volume in DLPFC was positively correlated with substance use problems in both females and males, lower orbitofrontal cortex and insular gray matter predicted substance use problems in females, whereas in males, these were associated with lower right prefrontal cortex gray matter volume. In addition to that, greater depressive symptoms at baseline were associated with greater substance use problems at follow-up, and depressive symptoms in females in particular were related to lower insular gray matter volume. Besides having a potential structural biomarker implication, this latter finding may also aid to see the popular explanatory theory of depression-SUD association as a manifestation of shared neurobiological vulnerability (98) in a new light. Further focusing on sex differences in brain structure, De Bellis et al. (72) in a sample of adolescents and young adult patients with alcohol and polysubstance misuse and an array of comorbid psychiatric diagnoses [i.e., mood, anxiety, and stress-related disorders; attention-deficit/hyperactivity disorder (ADHD); conduct and oppositional defiant disorders; and antisocial personality disorder] found smaller cerebellar gray matter volumes only in males. However, as this finding correlated substantially with a co-occurring diagnosis of ADHD, it was not regarded as associated with substance use. Such an association was found in fact, but with decreased gray and white matter volumes in prefrontal cortex, which was present in both sexes. The authors hypothesized that this finding might be either the result of direct or indirect detrimental effects of the substances on PFC development, a neurotoxic interference of the same with its maturation, or, alternatively, a reflection of inherent vulnerability for delayed PFC maturation subsequently enhancing the risk for poorer cognitive functioning and greater impulsivity and hence onset of substance misuse (72).

Other than BD, posttraumatic stress disorder (PTSD) has been most studied in nonpsychotic spectrum dual-diagnosis population. In a large study with 99 PTSD war veterans, a significant proportion of which had also comorbid depression, Woodward et al. (2006) found that past alcohol abuse or dependence has a significant inverse correlation with hippocampal volume (73). Although in nonalcoholic PTSD subjects the size of this structure was also reduced, the magnitude of the structural change was much smaller, suggesting that lower hippocampal volume might be a structural marker of shared neurobiological or genetic vulnerability to both alcoholism and PTSD (73). Further support for the close association between stress responses, limbic structures activity, and psychoactive substances was found in fMRI study by Cornelius et al. (2010) who investigated a small group of patients with comorbid cannabis dependence and depression (75). By means of threat-related amygdala reactivity paradigm, they showed that this structure known for its leading role in physiological and behavioral responses to stress and rich in CB1 receptors (99) displays reduced reactivity consistently correlating with the level of cannabis use. Such a finding supports the self-medication explanation theory for anxiety and substance use disorders comorbidity presented earlier. Additional evidence for implication of comorbid mood and anxiety disorders in structural brain changes in AUDs was presented by Sameti et al. (2011). By means of structural MRI comparison, these authors found in long-term abstinent alcoholics (LTAAs) smaller nucleus accumbens and hippocampus volumes in those LTAA individuals with a lifetime anxiety disorder than in those without (76). In addition to reduced n. accumbens, in alcohol-misusing patients with current anxiety disorder, a trend toward smaller putamen volumes was observed. Notably,

the same association of smaller hippocampus and amygdala volumes in LTAA was also detected in subjects with a lifetime externalizing disorder diagnosis (i.e., conduct disorder, defiant disorder, ADHD, and antisocial personality disorder). The authors hypothesized that both internalizing (i.e., mood and anxiety) and externalizing disorders are associated with disrupted hypothalamic-pituitary-adrenal (HPA) axis response to stress and with impaired interactions of the former with mesolimbic reward circuitry, but this deviation is a result of two opposite mechanisms—a hypersensitization of the HPA axis with subsequent neurotoxic hypercorticism in mood/anxiety disorders and an undersensitization with hypocorticism in externalizing disorders (76). As a consequence, vulnerability to abuse of drugs is increased in both groups—in an attempt to reduce negative psychological effects of stress in mood/anxiety disorders and as a way of stimulating reduced HPA reactivity in externalizing disorders (similar to thrill and adventure-seeking behavior typical for this group of subjects).

CONCLUSION

Definite conclusions would be substantially enhanced by future studies on comorbidity engaging much larger samples, endorsing more powerful longitudinal design, and enhanced by genetic polymorphism subtyping. Currently available data, although markedly inconsistent and confounded by a variety of sources (e.g., different study design, small and heterogenic samples, concomitant medications, smoking, etc.), support the assumption that in substance-misusing psychiatric patients structural brain changes are more pronounced, yet not qualitatively different from what is seen in noncomorbid subjects with psychotic and nonpsychotic diagnoses. In SZ patients, neuroimaging studies support the assumption for somewhat greater gray matter reduction in cingulate cortex (anterior and posterior), dorsolateral prefrontal and frontotemporal cortex, limbic structures (hippocampus), and basal ganglia (striatum). However, the magnitude of these structural changes is dependent on duration and severity of substance use, and at least in some of DD subjects, preexisting brain abnormalities are less pronounced than in pure SZ ones, which corresponds to better social and cognitive functioning and in general to lower neurodevelopmental and/or genetic pathological diathesis. As most studies on SZ also included schizoaffective diagnoses, thus probably enrolling a significant proportion of bipolar DD subjects, it is reasonable to compare their findings to what is reported by studies with "pure" bipolar patients. In doing so, the dorsolateral prefrontal, cingular, and insular cortices emerge as commonly affected areas in both SZ and BD. Taken together, these findings may implicate a shared endophenotypic (i.e., transdiagnostic) disruption of brain areas involved in executive functioning, emotional processing, and social cognition, which renders affected individuals susceptible to both mental disorder and substance misuse. Notably, gray matter loss in the anterior insula and dorsal part of the anterior cingular cortex has also been emphasized as a transdiagnostic finding in psychiatric patients in a nuber of recent studies (100, 101).

In comorbidity of anxiety and stress-related disorders (PTSD), as well as externalizing disorders with substance misuse, a common neuroimaging finding is the reduced volume of limbic structures (n. accumbens, hippocampus, amygdala). However, whether this reflects an underlying neuropathological characteristic predisposing to both specific behavioral symptoms and drug addiction or is a secondary effect of self-medication substance misuse on brain reward circuitry remains to be clarified.

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Functional Magnetic Resonance Imaging Correlations Between Fatigue and Cognitive Performance in Patients With Relapsing Remitting Multiple Sclerosis

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The correlation between fatigue and cognitive performance in multiple sclerosis (MS) is well reported, but the intimate mechanisms of the fatigue impact on cognition are not fully defined yet. The aim of this study is to investigate blood oxygen level–dependent (BOLD) activations in relapsing remitting MS (RRMS) patients with and without cognitive dysfunction and the impact of fatigue on cortical activations. Forty-two patients with RRMS were enrolled in the study. Cognitive functioning was assessed by the Symbol Digit Modalities Test (SDMT) and Paced Serial Addition Test (PASAT). A cutoff point of a total score of 55 on the SDMT was used to divide the patients into two groups: cognitively impaired (CI), SDMT score equal to or below 55 points, and cognitively preserved (CP), SMDT score above 55 points. Fatigue was assessed by the Modified Fatigue Impact Scale (MFIS). Participants were assessed with the Beck Depression Inventory (BDI) prior to inclusion in order to exclude major depressive episode. Functional Magnetic Resonance Imaging (fMRI) scanning was performed on a 3T MRI. The PVSAT (Paced Visual Serial Addition Test) paradigm was applied as a cognitive task. All functional data were analyzed with SPM12 and statistical analysis with SPSS 19.0. No statistically significant differences between CI and CP patients were found (p=0.953, p=0.322) in the MFIS and BDI score. Performance on the PASAT in CI patients was 34.07±13.721, for CP patients 46.42±11.453, and the SDMT performance in the CI patient group was 42.40±9.179, in the CP group 57.83±2.552. Between-group analysis revealed increased activations in left Brodmann area (BA) 40 in CP patients with several clusters located in the left supramarginal gyrus. Regression analysis showed increased BOLD signal in left BA 40, right BA 40, and left BA 6, associated with a higher score on MFIS. Stronger BOLD signal in left BA 31 was associated with a lower score on MFIS. Significance level was set to p<0.05, FWE (family-wise error) corrected. The differences in BOLD activations suggest the presence of cortical reorganization in our CP patients. The impact of fatigue on cortical activation during a cognitive task is demonstrated by inconformity of activated areas depending on the MFIS score. Our results suggest that activation in BA 40 may represent a mechanism for diminishing fatigue impact on cognitive functioning in CP patients.

Keywords: fMRI, Multiple Sclerosis, Cognition, Fatigue, MFIs

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INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS) characterized with widespread lesions in brain and spinal cord. It is prevalent in young adults and therefore with significant social burden (1). Research in the past 30 years has indicated that cognitive impairment affects between 40% and 70% of all MS patients. The MS-related cognitive dysfunction appears in various domains such as attention, information processing speed, processing efficiency, executive functioning, and working memory. These deficits affect many aspects of daily life, thus resulting in decreased quality of life (2). Fatigue is the most common symptom in MS and is reported in over 90% of patients (2, 3). Rudroff et al. in a recent review propose the following definition for fatigue: "The decrease in physical and/or mental performance that result from changes in central, psychological and/ or peripheral factors." The authors emphasize the conditional dependencies of all included factors, such as the task that is performed, the environmental conditions in which it is performed, and the physical and mental capacity of the individual (4). The assessment of fatigue is most often conducted with self-report questionnaires. They range from single-item scales such as a visual analogue scale (VAS) to multidimensional scales incorporating several dimensions of fatigue such as physical and mental. The Fatigue Severity Scale (FSS) and the Modified Fatigue Impact Scale (MFIS) are two multidimensional scales that are predominantly applied in studies with MS patients (4). The relationship between fatigue and cognitive performance in MS is well reported (3, 5, 6). One obstacle remains the difficulty to objectively differentiate cognitive fatigue from physical, and in addition, research has revealed little or no relationship between self-reported and objective measurements of fatigue in clinical populations (7). As a result, the intimate mechanisms of the fatigue impact on cognition are not fully defined yet. The combined assessment with neuropsychological testing and functional MRI (fMRI) has revealed an opportunity for investigating complex compensatory mechanisms involved in cognitive functioning. Translational validation of cognitive tests and the correlation with fatigue and mood in patients with relapsing remitting multiple sclerosis (RRMS) might be a stepping-stone towards better understanding of this intricate interplay between some of the most common symptoms of MS. The aim of this study is to investigate blood oxygen level–dependent (BOLD) activations in RRMS patients with and without cognitive dysfunction and the impact of fatigue on cortical activations.

MATERIALS AND METHODS

Participants

Forty-two patients diagnosed with RRMS according to McDonald's criteria (2017) were enrolled in the study (8). The following inclusion criteria were applied to all participants: remission phase of the disease (defined as a period of improvement or stable clinical condition for at least 3 months), age between 18 and 55 years, primary education and, treatment with first-line disease modifying therapies (interferon-beta or glatiramer acetate). Exclusion criteria were: treatment with corticosteroids 3 months prior to entering the study; exacerbation phase of MS; and known history of drug or alcohol abuse, psychiatric illness, and other chronic diseases. All patients underwent a standard neurological examination and were assessed by the Expanded Disability Status Scale (EDSS).

Cognitive function was assessed by the Symbol Digit Modalities Test (SDMT) and Paced Serial Addition Test 3' (PASAT). Neuropsychological evaluation was conducted within 24 h prior to the fMRI scanning. The participants were assessed during the same time period of the day, between 10 and 12 am, to eliminate significant circadian variations. Fatigue was assessed by the MFIS. All participants were evaluated by the Beck Depression Inventory (BDI) prior to inclusion. BDI version II, consisting of 21 questions, has a total score that varies from 0 to 63, where 0–10 is considered normal, 11–16 = mild mood disturbances, 17–20 = borderline clinical depression, 21–30 = moderate depression, 31–40 = severe depression, and over 40 = extreme depression. The MFIS consists of 21 questions, including three aspects of fatigue—physical, cognitive, and psychosocial. Total score ranges from 0 to 84, where 38 and over is considered MS-related fatigue syndrome. (9)

A cutoff point of a total score of 55 on the SDMT was used to divide the patients into two groups: cognitively impaired (CI), SDMT score equal to or below 55 points, and cognitively preserved (CP), SMDT score above 55 points, based on the predictive value of the SDMT score proven by Parmenter et al (10).

Participants gave written informed consent prior to any study procedures, and the study protocol was approved by the ethics committee of Medical University of Plovdiv.

fMRI Acquisition

The scanning of the participants was executed on a 3T MRI system (GE Discovery 750w) with a protocol including a structural scan (SagT1 3D BRAVO, slice thickness 1 mm, matrix 256 × 256, flip angle 12°) and a functional scan [2D echo planar imaging (EPI), slice thickness 3 mm, matrix 96 × 96, TR (relaxation time) 3,000 ms, TE (echo time) 30, flip angle 90°]. Before each functional scan, five dummy time series were acquired.

The PVSAT (Paced Visual Serial Addition Test) paradigm was applied as a cognitive task during fMRI (11). The PVSAT paradigm consists of two "on" conditions and one "off" condition and a total duration of 11 min 51 s during the fMRI scanning. All "on" blocks are composed of 21 random numbers presented for 3 s each. Before each "on" block, one of the two cues was presented, either "add" or "repeat," for 3 s. During the "add" condition, the participants were instructed to add each projected number with the previous. During the "repeat" condition, the participants were instructed to silently repeat once each presented number. There were four blocks of each type, alternating between add and repeat followed always by a 21 s "off" block representing a centrally located fixation cross, during which the participants were instructed to look at the cross without thinking of anything in particular (12).

fMRI Data Analysis

All functional data were analyzed with statistical parametric mapping (SPM12) software running on MATLAB R2017a for Windows. The preprocessing included the following steps: 1) realignment of the functional data in order to correct for head motion; 2) co-registration was conducted between the high-resolution structural image and the functional scans; 3) estimation of spatial registration parameters based on the anatomical image was performed. Consequently, transformation of all co-registered functional data was standardized to MNI (Montreal Neurological Institute) space; those steps were followed by spatial smoothing with a 6 mm full-width-at-half-maximum Gaussian kernel. First-level analysis was then specified, parameters estimated, and t-contrasts defined for all active conditions together and separately vs. the passive condition. The following five contrasts were obtained for each subject: (add+repeat>off), (add>off), (repeat>off), (add>repeat), and (repeat>add). The (add>off) contrast was considered as clinically informative and was used for assessment.

The resulting contrast maps were then used in a second-level random effects analysis to look for the between-group differences, CP vs. CI. The aim was to compare BOLD activations during (add>off) in both groups. The level of significance was set at $p < 0.05$, FWE (family-wise error) corrected. Regression analysis was used to assess positive and negative correlations between MFIS score and BOLD activations during the cognitive task.

Statistical Data Analysis

Demographic and clinical characteristics of the subjects were analyzed with SPSS 19.0 for Windows. Normality of distribution was assessed by means of the Kolmogorov–Smirnov test. Between-group analysis of normally distributed data was done by independent sample t-test.

RESULTS

Demographic, clinical, and cognitive data for all participants is presented in **Table 1**. Comparative statistics was performed between the two groups.

Between-group analysis revealed increased activations in left Brodmann area (BA) 40 in CP patients, with a significance level of $p < 0.001$. Analysis yielded several clusters located in the left supramarginal gyrus (BA 40) of a cluster size of 63 voxels, MNI coordinates −56 −36 32, p-value 0.563; a cluster with 24 voxels, MNI coordinates −28 −40 44, p-value 0.848; and a cluster of 22 voxels, MNI coordinates −62 −26 40, with a p-value of 0.863 (**Figure 1**).

Regression analysis yielded increased activations in left BA 40, right BA 40 (supramarginal gyrus), and left BA 6 (premotor cortex) in patients with a higher score on MFIS. Stronger BOLD activation in left BA 31 (posterior cingulate gyrus) was associated with a lower score on MFIS. Significance level was set to $p < 0.05$, FWE corrected (**Figures 2** and **3**).

DISCUSSION

The neuropsychological assessment of our subjects confirmed the predictive value of SDMT for cognitive dysfunction as established by Parmenter et al. This is evidenced by the significant difference in performance on the PASAT in both groups (10). Although the use of only one neuropsychological test to categorize our patients may be a limitation, we chose the statistically validated threshold introduced by Parmenter et al. since the SDMT is currently not corrected for age and education within the Bulgarian population. In recent articles, research in MS clearly supports the reliability and validity of the SMDT, and based on current evidence, the test is included as an indispensable method in the recommendations for cognitive screening and management in MS care by the Consortium of Multiple Sclerosis Centers (13, 14).

Interestingly, our study showed no statistical difference in regard to educational background between CP and CI patients. Considering the well-established theory of cognitive reserve, this may not be that unparalleled. According to Sumowski and Leavitt, the reserve against disease-related cognitive impairment consists of both genetic/heritable and environmental factors. Maximal lifetime brain growth (MLBG) is considered a main heritable factor (15). The protective effect of a larger MLBG is based on the "brain reserve" concept explained by Satz, where cognitive decline emerges when brain volume falls below a critical threshold; thus, people with larger MLBG can better withstand disease burden associated with loss of brain volume/brain atrophy without cognitive decline (16). The environmental factor at play is intellectual enrichment, which is largely a product of life experience and is not always linked to education. Educational attainment is often impacted by factors outside

TABLE 1 | Demographic, clinical and cognitive data for all participants.

Characteristics	CI (n=30)	CP (n=12)	P
Age (mean ± SD)	40.70 ± 7.7	36.92 ± 7.4	0.155
Education (mean ± SD)	13.20 ± 2.4	14.42 ± 2.7	0.157
Disease duration (mean ± SD)	10.13 ± 4.8	8.42 ± 5.3	0.313
EDSS (mean ± SD)	2.200 ± .65	1.625 ± .74	0.017
SDMT (mean ± SD)	42.40 ± 9.18	57.83 ± 2.56	0.000
PASAT (mean ± SD)	34.07 ± 13.72	46.42 ± 11.45	0.009
MFIS (mean ± SD)	12.43 ± 12.1	12.17 ± 15.91	0.953
BDI (mean ± SD)	4.30 ± 4.94	2.67 ± 4.29	0.322

CI, cognitively impaired; CP, cognitively preserved; EDSS, Expanded Disability Status Scale; SDMT, Symbol Digit Modalities Test; PASAT, Paced Serial Addition Test; MFIS, Modified Fatigue Impact Scale; BDI, Beck Depression Inventory.

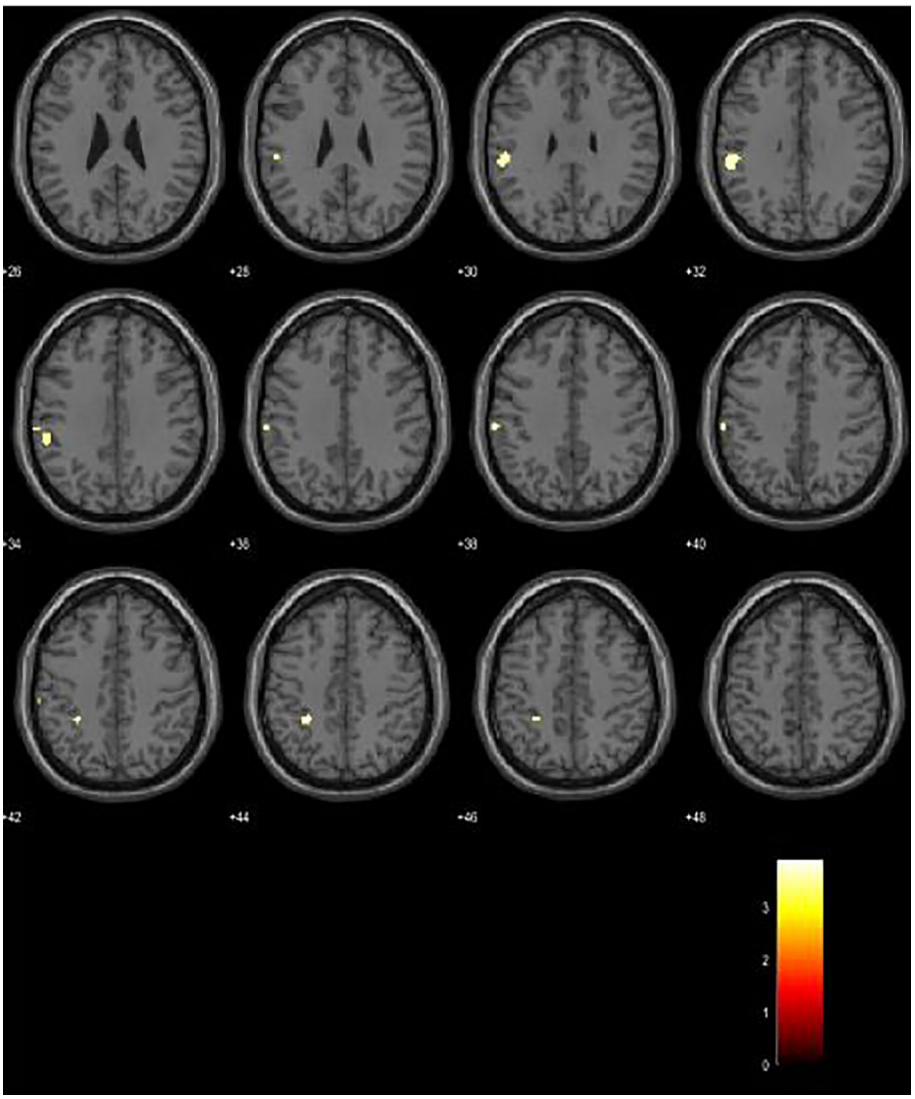

FIGURE 1 | Between-group analysis revealed increased BOLD signal in CP patients.

our control such as socioeconomic and parental educational status. Research conducted by Sumowski, Rocca, et al. in MS patients concluded that greater early life cognitive leisure protects MS patients from cognitive decline independently of MLBG and education (17).

Contrary to what we initially expected, our results showed no significant difference between CP and CI patients in the mean MFIS score. The mean score on the MFIS in patients was relatively low and did not reach the "clinically significant" cutoff point of 38, adopted by Flachenecker et al (9). However, the regression analysis revealed a notable inconformity in BOLD signal in relationship to the MFIS score. Activations in left and right supramarginal gyrus (BA 40) and left premotor cortex (BA 6) were associated with a higher score on the MFIS questionnaire; on the other hand, the left posterior cingulate gyrus (BA 31) was associated with a lower MFIS score. According to existing data resulting from studies investigating the correlation between fatigue and fMRI, the MFIS scale has not been used so widely in similar research. Most published studies evaluate fatigue with the FSS, self-reported cognitive fatigue by a VAS scale, or physical fatigue after induction with a motor task (18). This differentiation between fatigue domains is pragmatic considering some of the recommendations made by researchers in this field (4, 7). That may be one of the disadvantages of our study. On the other hand, the BOLD clusters revealed in our research are in line with some previous studies. DeLuca et al. investigated neural correlates of cognitive fatigue using fMRI in MS patients. Participants performed a modified version of the SDMT during fMRI acquisition, and cognitive fatigue was defined operationally as an increase in BOLD response across time. The authors hypothesized that patients would show a greater increase in cerebral activity on the cognitive task across time than healthy controls. Among areas with fatigue interaction were BA 40 and BA 19 (3). More

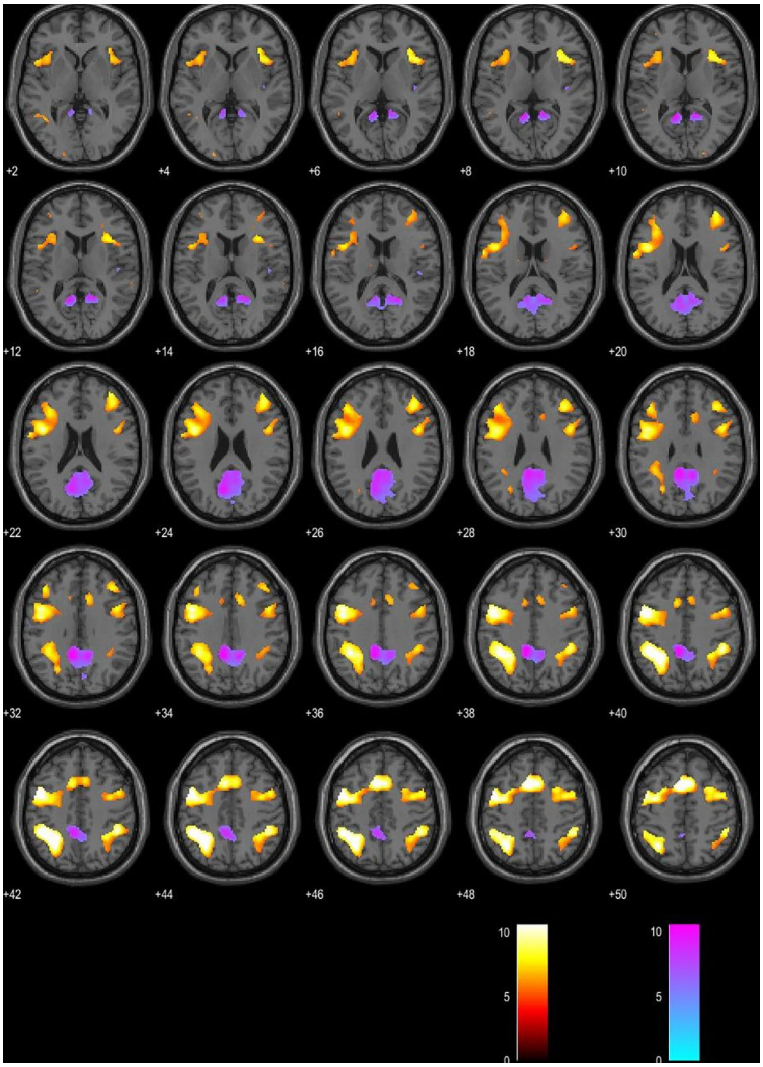

FIGURE 2 | Blood oxygen level–dependent (BOLD) signal associated with higher Modified Fatigue Impact Scale (MFIS) score is presented in the red color map. BOLD signal associated with lower MFIS score is presented in the blue color map. Presented in the axial plane.

recent research by Genova et al. used fMRI to examine where in the brain BOLD activity covaried with "state" fatigue assessed during a task designed to induce fatigue while in the scanner. The authors implemented a subtler approach to the definition of fatigue, where state fatigue refers to a temporary condition which can change over time; on the other hand, "trait" fatigue indicates the opposite. The latter was explored by diffusion tensor imaging (DTI) to investigate white matter integrity. During performance of this cognitively fatiguing task, BOLD activations in BA 6, BA 39, and BA 37 were associated with self-reported state fatigue evaluated by VAS in MS patients (19). It is suggested that depression, mood, and anxiety should be included as covariates when investigating MS-related fatigue. Depression affects a significant part of patients with MS during their life; Bakshi et al. found that depression is associated with MS-related fatigue and should be controlled for (4, 20, 21). In this regard, our fatigue evaluation is valid, since all patients were assessed by the BDI, and the mean score for both subgroups was presented within normal limits.

Between-group analysis comparing BOLD activations in CP vs. CI patients during the PVSAT cognitive paradigm revealed increased activity in our CP patient group, located in the left supramarginal gyrus (BA 40). Our results are in agreement with multiple conducted studies investigating cortical recruitment during cognitive tasks in patients with MS. Since the introduction of functional MRI as a method, it has been extensively applied in neuroscience to illuminate how cortical activation is altered after brain tissue injury (22). Staffen et al. conducted an earlier fMRI research in patients with RRMS with PVSAT paradigm. Compared to healthy controls, the patient group revealed additional cortical recruitment in left BA 6, 8, and 9 and right BA 39 (23). Mainero et al. investigated functional brain activity in patients with RRMS and controls during PASAT and a recall task. The authors observed that fMRI activity was greater in

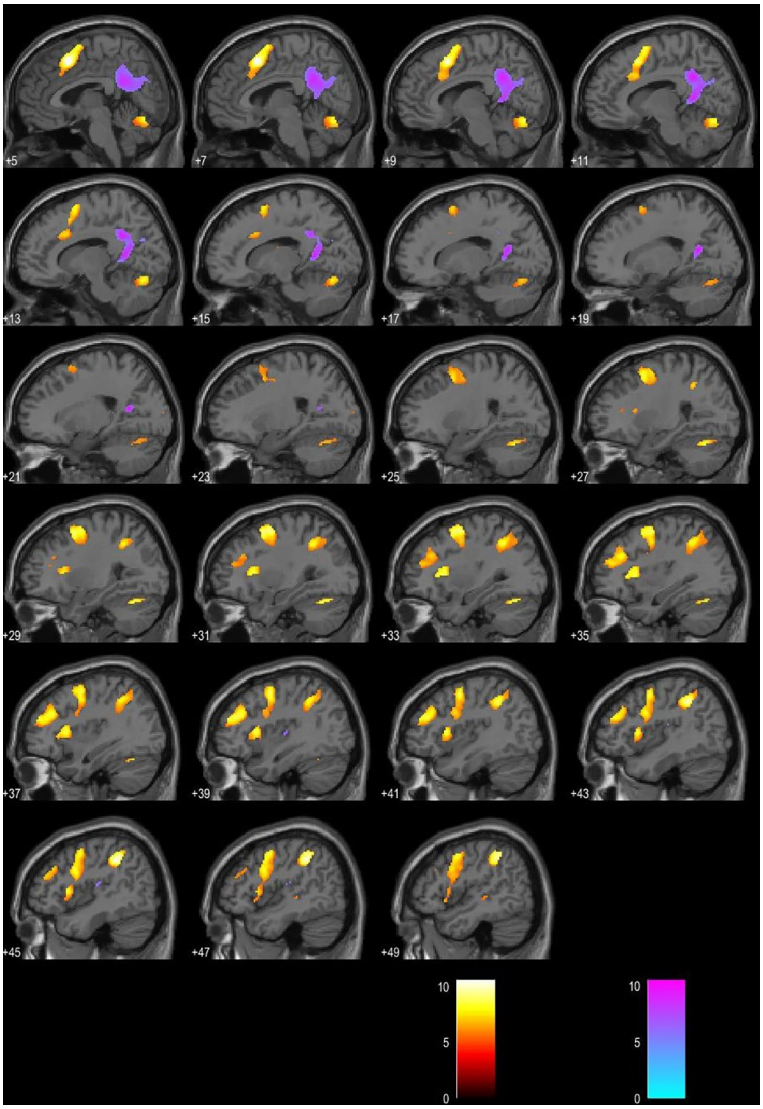

FIGURE 3 | Blood oxygen level–dependent (BOLD) signal associated with higher Modified Fatigue Impact Scale (MFIS) score is presented in the red color map. BOLD signal associated with lower MFIS score is presented in the blue color map. Presented in the sagittal plane.

patients with better cognitive function than those with worse cognitive performance and interpreted the data as evidence for compensatory brain reorganization (24). The indication that an increase of cortical activity is demonstrated in patients with preserved cognitive functions, and on the contrary, a decrease in cognitively impaired patients, is well established by several studies (25–27). This reorganization in brain activity is often referred to as proof of brain plasticity; consequently, the question arises whether this mechanism is adaptive or maladaptive. Therefore, researchers compel for longitudinal task-based and resting state fMRI studies with structural MRI data as covariates in order to understand this complicated MS-related cognitive dysfunction (28–31). In light of this, a major disadvantage of our study is the lack of structural MRI data for lesion load and brain atrophy in our subjects. Our study demonstrates that activation of BA 40 both represents a compensatory recruitment in CP patients and is associated with a higher MFIS score. We interpret this overlap as a possible mechanism for diminishing fatigue impact on cognitive functioning in CP patients. Further studies in this direction are necessary in order to understand how preserved cognitive functioning is affected by mood disturbances and fatigue.

Considering the vast concomitant symptoms in MS such as fatigue, anxiety, mood disturbances, and depression, we cannot deny their interdisciplinary nature. Despite the accumulation of data in that direction, these issues are still partly neglected in daily patient management and MS research. Feinstein et al. imply that psychiatrists and neuropsychologists should therefore play a much more prominent role in daily patient management (32). From a scientific point of view, translational neuroscience and its development is essentially a bridge between disciplines in medicine. As observed by Stoyanov, fMRI is an indispensable

tool in translational methodology, and by original definition, its purpose is to translate knowledge in different neuroscientific aspects (33, 34).

In conclusion, our study confirms the presence of cortical reorganization and additional cortical recruitment in patients with preserved cognitive function. The impact of fatigue on cortical activation during a cognitive task is demonstrated by inconformity of activated areas depending on the MFIS score. Our results suggest that activation in BA 40 may represent a mechanism for diminishing fatigue impact on cognitive functioning in CP patients.

Limitations

Using only one neuropsychological test for classifying our patients is a real limitation. The BICAMS (Brief International Cognitive Assessment of MS) is, however, not a validated battery for the Bulgarian population, and it has not been translated in the Bulgarian language. Because of this, we had to circumvent this obstacle as best we could, by incorporating SDMT and PASAT, two tests that are well established for the population we investigate. The cited study conducted by Parmenter et al. compares the SDMT score to the Minimal Assessment of Cognitive Function in MS (MACFIMS) results. Patients were considered cognitively impaired when performing one and a half standard deviations below controls on two or more MACFIMS variables, excluding the SDMT. The authors conclude that the Bayesian statistics showed that a total score of 55 or lower accurately categorized 72% of the patients with a sensitivity of 0.82, specificity of 0.60, positive predictive value of 0.71, and negative predictive value of 0.73. In consequence, we chose to rely on this statistically validated threshold of 55 on the SDMT since the test has not yet been validated and corrected for age and education within the Bulgarian population.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical University Plovdiv Ethical Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DI contributed substantially to the design of the work and performed all data acquisition, analysis, and interpretation. AT contributed substantially to the design of the work, performed statistical analysis and interpretation, and provided important feedback and revising. SM contributed to the design of the work, and provided technical methodology and revision. KT contributed to the design of the work, functional technical parameters, and data interpretation.

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ORIGINAL RESEARCH
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The Assertive Brain: Anterior Cingulate Phosphocreatine plus Creatine Levels Correlate With Self-Directedness in Healthy Adolescents

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Despite various advances in the study of the neurobiological underpinnings of personality traits, the specific neural correlates associated with character and temperament traits are not yet fully understood. Therefore, this study aims to fill this gap by exploring the biochemical basis of personality, explored with the temperament and character inventory (TCI), during brain development in a sample of adolescents. Twenty-six healthy adolescents (aged between 13 and 21 years; 17 males and 9 females) with behavioral and emotional problems underwent a TCI evaluation and a 3T single-voxel proton magnetic resonance spectroscopy (^{1}H MRS) acquisition of the anterior cingulate cortex (ACC). Absolute metabolite levels were estimated using LCModel: significant correlations between metabolite levels and selective TCI scales were identified. Specifically, phosphocreatine plus creatine (PCr+Cre) significantly correlated with self-directedness, positively, and with a self-transcendence (ST), negatively, while glycerophosphocholine plus phosphocholine (GPC+PC) and myo-inositol negatively correlated with ST. To the best of our knowledge, this is the first study reporting associations of brain metabolites with personality traits in adolescents. Therefore, our results represent a step forward for personality neuroscience within the study of biochemical systems and brain structures.

Keywords: magnetic resonance spectroscopy, temperament character inventory, adolescence, brain biochemistry, brain metabolism

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INTRODUCTION

The genetic and neural underpinnings of personality traits have gained increasing interest within the scientific community. Indeed, in the last decades, many studies have started to assess human personality from a scientific point of view, with the final aim of disentangling the neural basis of personality dimensions.

Interestingly, the majority of neuroimaging and behavioral studies employed the temperament and character inventory (TCI) (1) with the aim of investigating personality traits in normal subjects or in patients. The TCI is a well known personality inventory developed by Robert Cloninger, which models personality using seven psychobiological factors (1). It is composed of four temperament and three character scales. Temperament dimensions refer to the way each individual behaviorally responds to a specific class of stimuli, while character dimensions refer to self-concepts and inter-individual differences in goals and values, which may be associated to the functioning of higher cognitive systems. For details on the definition of each scale, please refer to a previous work of our group (2). Notably, twin studies reported that genetic factors have significant effects on temperament and on character dimensions, where the heritability does not show strong differences (3–5).

With regards to behavioral studies, evidence from our research group reported that selective temperament and character dimensions were associated with selective impairments in decision-making, during adolescence (6), magic ideations in twins (7) and proved to be useful for describing the development of personality in childhood (2). Additionally, a recent study carried out by Crescentini et al. (8) also reported that specific temperament and character traits might have protective effects on well being and psychosocial adjustment or explain emotional–behavioral difficulties in adolescents. Specifically, the study carried out by Brambilla et al. (7) found significant correlations between magical ideation and specific personality traits such as novelty seeking, cooperativeness, self-directedness, and self-transcendence in a sample of adult twins, mostly explained by genetic factors. In particular, self-directedness, a major proxy of psychological consciousness and confidence, and self-transcendence, an indication for spirituality and mysticism, are negatively and positively, respectively, associated with magical ideation.

From a neurobiological perspective, although the identification of specific brain deficits associated with personality traits is of great interest, the neurobiological bases of character and temperament dimensions are not yet fully understood. However, the available evidence reported some interesting results. Indeed, some studies highlighted the link between personality characteristics and connectivity areas in the brain (9) and white matter integrity (10, 11). Moreover, the review and meta-analysis carried out by Mincic (12) showed that the personality trait of negative emotionality was associated with selective deficits in brain regions within the cortico-limbic system, ultimately implying alterations in information communication and processing. Authors reported reduced gray matter volumes in the left medial orbitofrontal gyrus and rostral anterior cingulate cortex and increased volumes in the left amygdala and anterior parahippocampal gyrus in individuals who have predominant negative traits regarding emotions. This study's results further confirm the presence of morphological alterations associated with negative personality traits, as also reported by previous studies (13, 14).

Interestingly, the paucity of neuroimaging studies on personality traits is also present in regards to proton magnetic resonance spectroscopy (^{1}H MRS) investigations. ^{1}H MRS is the only technique that can access *in vivo* metabolite levels including N-acetylaspartate (NAA), phosphocreatine plus creatine (PCr+Cre), glycerophosphocholine plus phosphocholine (GPC+PC), and myo-inositol in localized brain areas (15, 16). The available *in vivo* ^{1}H MRS evidence on personality traits suggested that the individual variation in absolute brain metabolites levels may relate to specific aspects of personality functioning in healthy individuals. For example, lower PCr+Cre levels in the right precuneus were associated with agreeableness and extraversion, indicating a possible lower production of high-energy phosphate, PCr, correlating with these traits (17). Similarly, Kim et al. (18) explored the association between functional/structural alteration of the anterior cingulate cortex and harm avoidance traits. The authors showed that harm avoidance scores correlated negatively with glutamate concentrations and positively with GABA concentrations in anterior cingulate cortex, ultimately suggesting that glutamate and GABA concentrations in anterior cingulate cortex could underline the HA temperament trait.

In this context, this study aims at exploring, for the first time to the best of our knowledge, the biochemical basis of personality during brain development in a sample of adolescents with the final goal of teasing apart the biochemical system associated with personality traits. We hypothesized an association of PCr+Cre levels from the anterior cingulate cortex with selective personality traits in healthy individuals. This hypothesis derives by the evidence reported by previous studies in both healthy (19) and depressed (20) adolescents, which showed the key role of creatine's modulation in brain energy metabolism. Furthermore, since Kondo et al. (20) also found that creatine levels within the frontal lobe were inversely associated with depressive symptoms, we expect that PCr+Cre levels will be more likely be associated with SD, a personality trait consistently found associated with depression (21, 22).

MATERIALS AND METHODS

Participants

Twenty-six subjects (17 males; 9 females) took part to the study. The participants were selected within a cohort of adolescents, aged between 13 and 21 years (mean age ± 1 SD: 16.9 ± 1.7 years old), referred to the Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) "E.Medea" (Italy) between 2003 and 2008 because of behavioral or emotional problems such as anxiety and attentional deficit (see socio-demographic and clinical details in **Table 1**). Within the main cohort, we selected those subjects who did not meet criteria for a ICD-10 and DSM-IV diagnoses at the time of the study according to the Development and Well-Being Assessment (DAWBA; 23). Participants with reports of an IQ lower than 70 on their medical record, or diagnosed with a pervasive developmental disorder, severe hypoacusia or hypovision, severe linguistic comprehension deficit, central nervous system lesion, neurological condition, or a genetic syndrome, were excluded.

TABLE 1 | Socio-demographic and clinical variables of our sample.

N = 26 (9 females)	Minimum	Maximum	Mean	Std. deviation
Age	14.4	20.8	16.9	1.7
SES	20.0	80.0	50.5	14.6
Estimated IQ (based on vocabulary and block design)	82.5	127.5	101.7	10.9
CBCL-Int	34.0	73.0	55.1	9.9
CBCL-Ext	34.0	66.0	51.7	8.3
TCI-SD	6.0	24.0	17.1	4.9
TCI-ST	0.0	8.0	3.1	2.0
TCI-CO	9.0	24.0	19.0	3.4
TCI-NS	2.0	17.0	10.1	4.1
TCI-HA	1.0	17.0	8.8	4.3
TCI-RD	3.0	13.0	8.6	2.9
TCI-P	0.0	5.0	2.5	1.4

CBCL, child behavior checklist; SES, socio-economic status; estimated IQ, IQ estimated from the Vocabulary and Block Design subtests of the Wechsler Intelligence Scale for Children (WISC—13–15 years) and Wechsler Adult Intelligence Scale (WAIS—16 and older); TCI, Temperament and Character Inventory; Int, internalizing problems index; Ext, externalizing problems index; SD, self-directedness; ST, self-transcendence; CO, cooperativeness; NS, novelty seeking; HA, harm avoidance; RD, reward dependency; P, persistence. The means of the weighted scores were converted into standard scores.

Also, the children's parents filled the Child Behavior Checklist (CBCL 6-18) (24), which evaluates the behavior (see below for a full description—**Table 1** reports the results).

All children and their parents were Italian native speakers or were fluent in Italian. The study was approved by the Ethical committee of the IRCCS "E. Medea." All parents gave written informed consent.

Psycho-Cognitive Measures

Participants were assessed to exclude behavioral, emotional, psychiatric, or neurological disorders with the DAWBA (23). The DAWBA parent interview and the DAWBA interview for young people were administered to all participants. DAWBA is a tool allowing for a structured diagnosis according to both DSM-IV (25) and ICD-10 (26). The participants IQ was tested with the vocabulary and block design subtests of the Wechsler Intelligence Scale for Children (WISC—13–15 years) and Wechsler Adult Intelligence Scale (WAIS—16 and older). For each participant, the IQ was estimated converting the mean of the weighted scores into standard scores. Parental socio-economic status was also estimated.

Subjects were assessed with the Child Behavior Checklist (CBCL 6-18) (24), a questionnaire assessing social competences and behavioral problems in children from 6 to 18 years of age. The questionnaire is filled by one or both parents who evaluate the child behavior with reference to a period encompassing the past 6 months. The CBCL 6-18 is composed by eight syndrome scales based on factor analysis: anxious/depressed, withdrawn/depressed, somatic complaints, social problems, thought problems, attention problems, rule-breaking behavior, aggressive behavior, and six DSM-oriented scales: affective problems, anxiety problems, somatic problems, attention deficit/hyperactivity, and oppositional defiant behavior (24).

Each item consists in a statement describing a target behavior. The parents must indicate if a statement apply completely (score = 2), partially (score = 1), or does not apply (score = 0) to their children. For each syndrome and DSM-oriented scale, scores are calculated as the sum of the scores of each item in that scale. The internalizing problems index is obtained as the sum of the scores of the anxious/depressed, withdrawn/depressed, and somatic complaint scales. The externalizing problems index is given by the sum of the rule-breaking behavior and aggressive behavior scales. A total problem index is given by the sum of all scores and the scores assigned to the items of an additional scale measuring "other problems" (i.e., a scale whose items do not refer to any specific syndrome). The raw scores are then converted into T standard scores according to the child's age and gender. We used the internalizing and externalizing problems indexes as covariates for the regression analyses. In the present study, we administered the CBCL 6-18 Italian independent back translation authorized and approved by T. Achenbach.

The TCI (1) is a self-report questionnaire measuring the seven dimensions of temperament and character postulated according to the psychobiologic model of personality (1). The temperament scales are as follows. Each of the dimensions of the TCI, except persistence, is computed as the sum of scores on three to five subscales which measure correlated traits. Here, we used a shorter version with 125 items, previously validated on a large cohort of healthy individuals by our research group (27). The questions can be clustered into four sub-scales tapping each temperament component: novelty seeking (20 items), harm avoidance (20 items), reward dependence (15 items), persistence (5 items), and into three sub-scales tapping each character component: self-directedness (25 items), cooperativeness (25 items), and self-transcendence (15 items).

Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) Acquisition

Single-voxel *in vivo* ^{1}H MRS spectra were acquired using the point-resolved spectroscopy (PRESS) sequence on a 3T whole-body MR system (Philips Achieva, Philips, the Netherlands; TR = 3,000 ms, TE = 36 ms, voxel sixe 17 x 17 x 17 mm = 4.91 mm^3, 2,048 complex data points, spectral bandwidth of 2,000 Hz, 128 water suppressed, and 2 water unsuppressed averages). The ^{1}H MRS voxel was positioned in the anterior cingulate cortex as depicted in **Figure 1**. 3D T1-weighted

images (190 slices, TR = 8.2 ms, TE = 3.75 ms, flip angle = 8°, FOV = 240x240 mm, pixel dimension = 1x1x1 mm^3) were also acquired. The proportions of tissue content of gray matter, white matter, and cerebro-spinal fluid within the localized voxels were estimated using FSL and FreeSurfer (28; http://surfer.nmr.mgh.harvard.edu/).

Absolute metabolite levels were estimated using the linear combination model [LCModel, version 6.3-1 (29)] software with a simulated basis set, the unsuppressed water signal, and by incorporating the appropriate correction factors (T1 and T2 relations) (Gasparovic et al. (30)). The quantified metabolites included PCr+Cre, GPC+PC, myo-inositol, glutamate, and NAA and expressed in absolute levels with institutional units.

Correlations

Preliminary Pearson correlations and scatter plots were run in order to identify associations between metabolites levels and the TCI measures, to highlighting the direction of the relation between the predictors and the dependent variables. Correlations between the different measures of the metabolite levels (i.e., NAA, PCr+Cre, GPC+PC, myo-inositol, and glutamate), which were the variable that were used in the regressions as predictors, were computed for verifying the collinearity between predictors.

Regression Analysis

Analyses were conducted using SPSS version 23 (SPSS Inc, Chicago). Four independent linear regression models were calculated, using block entry method (SPSS default), with raw scores at each TCI-125 subscale as dependent variable. The metabolite levels of NAA, PCr+Cre, GPC+PC, myo-inositol, and glutamate were entered as independent variables, one at a time. So, individual models were run for each metabolite at a time. All the regressions models analyzed also included as covariates age and gender. Moreover, CBCL internalizing and externalizing indexes (T scores) were entered as additional covariates. This was done in order to account for the correlation that in some cases existed between TCI scores and those covariates (see **Table S1** in **Supplementary Materials**).

The alpha level (significance level) = 0.05 was adjusted in order to account for the number of model dividing it by the number of models N = 6. Only the p-values less or equal to 0.05/6 = 0.01 allowed for the rejection of the null hypothesis.

RESULTS

Descriptive Statistics

Subjects had a total IQ of 101.73 ± 10.88 (range 82.5–127.5) estimated with WISC or WAIS depending on age. The parental

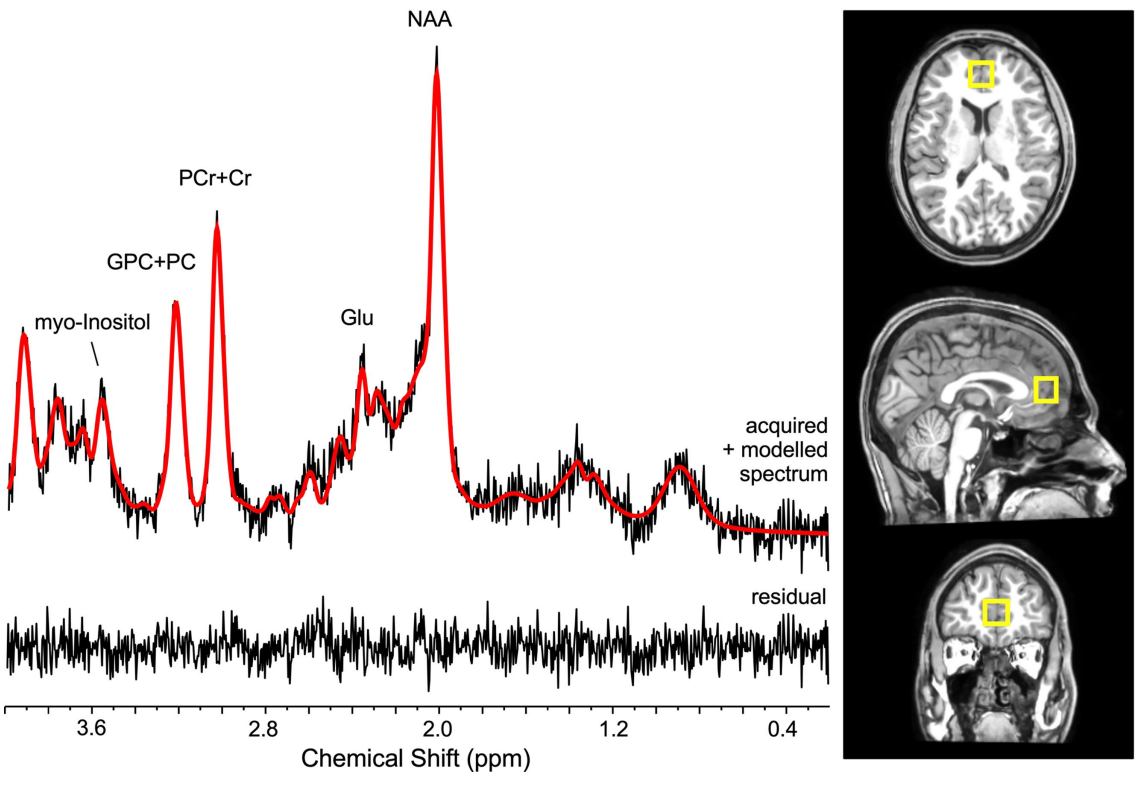

FIGURE 1 | Sample spectrum (left) and voxel positioning in the anterior cingulate cortex (right).

socio-economical status (SES) ranged between 20 and 80 (mean = 50.51; standard deviation = 14.55). The CBCL and TCI scores are reported in **Table 1**. **Table 2** reports the concentration levels of each metabolite. The number of normal, clinical, and subclinical subjects is reported in **Table S2**.

Regression and Correlation Analyses

The analysis showed significant correlations between i) NAA and PCr+Cr ($r = 0.38$, $p = 0.05$), ii) NAA and GPC+PC ($r = 0.49$, $p = 0.01$), iii) PCr+Cr and GPC+PC ($r = 0.78$, $p < 0.001$), iv) PCr+Cr and MYO ($r = 0.60$, $p < 0.001$), v) PCr+Cr and Glu ($r = 0.60$, $p < 0.001$), and vi) GPC+PC and MYO ($r = 0.63$, $p < 0.001$). Since most of the metabolites' concentrations correlated, we decided to perform separate analyses using one metabolite at a time as predictor.

The regression analyses revealed that the concentration of PCr + Cr predicted significantly the outcome at the TCI_SD. Moreover, PCr + CR could predict the outcome at the TCI_ST. Additionally, TCI_ST was also predicted by the concentration levels of GPC + PC and myo-inositol. The ACC PCr+Cre levels significantly predicted positively the SD and negatively the ST. The ST scores were also significantly predicted by the levels of GPC+PC and myo-inositol and, in both cases, the relation was inverse. Scatter plots for these quantities are represented in **Figure 2**, **Figure 3**, **Supplementary Figure 1**, and **Supplementary Figure 2**. Results from regression analysis are summarized in **Table 3**.

TABLE 2 | Metabolites levels measured using ^{1}H MRS.

	Mean (I.U.)	StDev (I.U.)	Min (I.U.)	Max (I.U.)
PCr+Cre	9.4	0.7	8.0	10.6
GPC+PC	2.1	0.3	1.6	2.9
Myo-inositol	6.6	1.0	4.9	8.6
Glutamate	14.3	1.0	12.2	16.1
NAA	10.4	0.9	8.8	12.1

StDev, 1 standard deviation; Min, minimum value; Max, maximum value. PCr+Cre, phosphocreatine plus creatine; GPC+PC, glycerophosphocholine plus phosphocholine; NAA, N-acetylaspartate; I.U., institutional units.

DISCUSSION

The investigation of personality traits in association with regional brain biochemistry, as explored in this study, might allow building prediction models, which identify specific biomarkers associated with inter-individual differences in personality traits.

In this study, we report significant associations of PCr+Cre, GPC+PC, and myo-inositol levels in the anterior cingulate cortex with specific and opposite personality traits. Specifically, PCr+Cre significantly predicts SD, positively, and ST, negatively, while GPC+PC and myo-inositol negatively predict ST. Although the correlation with TCI was only weak, age was used as covariate for ensuring to factor out any possible effect. In any case, the effect of a covariate on the results is proportional to the strength of its relation with the dependent variable. Since it has been reported that PCr+Cre plays a key role in brain energy homeostasis, increasing PCr+Cre levels may boost brain performance, as suggested by previous investigations in neurological (31) and healthy (32) conditions. Indeed, in these studies, PCr+Cre was found to have significant neuroprotective effects (31), and its biochemical levels have been shown to be involved during mental training (32). Additionally, oral Cre supplementation has been reported to have significant effects during calculation, in particular reducing mental fatigue and oxygen demand during the task (33) as well as to have positive effects on working memory and intelligence (34), further supporting its role in dynamically modulating brain energy capacity during cognitive performance. Importantly, it has been demonstrated that SD dimension of the TCI showed important correlations with other

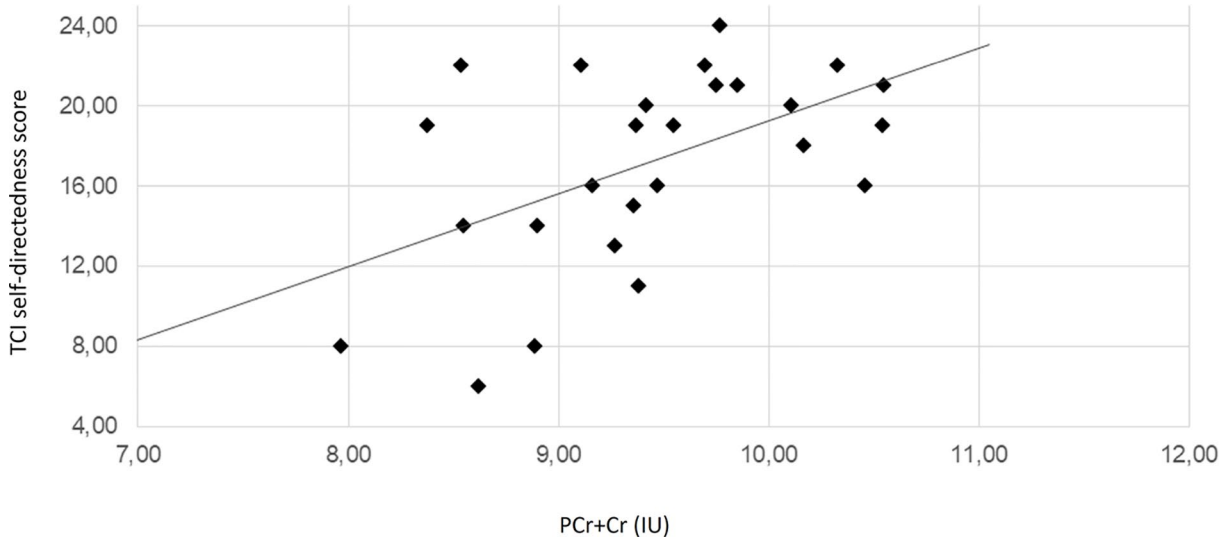

FIGURE 2 | Scatter plot depicting the relationship of PCr+Cre level and TCI self-directedness scores. The solid line represents the linear regression line. The correlation coefficient r is 0.52 (Pearson correlation, $p = 0.006$). IU, institutional units.

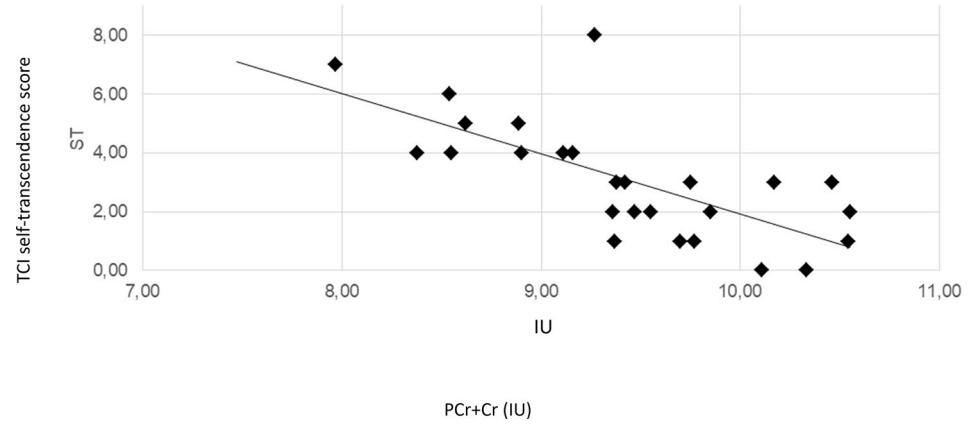

FIGURE 3 | Scatter plot depicting the relationship of PCr+Cre concentration and TCI self-transcendence scores. The solid line represents the linear regression line. The correlation coefficient r is −0.71 (Pearson correlation, $p < 0.001$). IU, institutional units.

personality models or questionnaires, including the five-factor model (FFM) (35) and the Big Five Questionnaire (BFQ) (36). Specifically, it has been reported that SD directly correlates with conscientiousness and extraversion, and inversely, with neuroticism, dimensions of the FFM, as well as positively correlated with conscientiousness, emotional stability, and dynamism dimensions of the BFQ (36) in healthy adult subjects. Therefore, the neuroimaging studies investigating the putative association between these FFM and BFG dimensions and brain deficits might be useful to further support our results. Indeed, one resting-state fMRI study reported that conscientiousness and extraversion predicted resting state functional connectivity in several brain areas, including the anterior cingulate cortex in adult subjects (37). Moreover, a structural MRI study also showed that prefrontal volumes were larger in adults with higher conscientiousness and smaller in those with higher neuroticism (38). Therefore, all together, these results seem to point toward the hypothesis that deficits in prefrontal regions including the anterior cingulate cortex, might be associated with specific personality traits.

Finally, our results also showed a negative association of GPC+PC and myo-inositol in the anterior cingulate cortex with ST in our group of adolescents. Also, in this case, our result is not surprising especially because it has been reported that anterior cingulate cortex is involved in decision making and deployment of cognitive control (39) as well as in carefulness, industriousness, and organization activities (37), which might not be directly associated with ST traits. Indeed, higher scores in ST might identify subjects with higher levels of creativity, spirituality, and mysticism (7), all activities that do not require the engagement of higher order cognitive regions such as the anterior cingulate cortex, ultimately suggesting that this region needs a lower metabolic availability in individuals who rank high in ST.

Limitations

Our findings should be considered in light of some methodological limitations, which could have potentially affected the reliability and generalizability of our results. First, the technique that we employed does not assess the brain biochemistry at whole brain level, but only within a restricted region of interest. This constrains the results' generalizability and the comparability to previous structural personality studies.

Secondly, each metabolite was entered as predictor, one at a time, in discrete simple regression models, instead of running comprehensive multiple regressions (i.e., including all the metabolites as independent variables contributing to the TCI's data distributions). The choice was mandatory because of the collinearity between the independent variables, most of which were correlating between each other. This grew the number of models that were tested, so increasing the risk for false discovery. Nonetheless, this risk was overcome by correcting the significance level for multiple comparisons (see paragraph 2.5 for details). Third, although the sample was relatively small, it was well selected for the absence of potentially confounding variables. Despite these limitations, the data were reasonably distributed across the sample and the conclusions were not driven by unbalances between the participants.

Conclusions

Despite these limitations, our results may represent a step forward for personality studies, as we began to discriminate the biochemical systems underpinning personality functioning. Furthermore, understanding the biological mechanisms building personality traits may provide new insight on the mechanisms of drug action, which may ultimately lead to more rapid and effective treatments of personality disorders. Indeed, since it has been also reported that frontal lobe PCr+Cre is a valid treatment target in adolescent depression (20) and SD has been suggested to be a general trait marker for depression (21, 22), it is plausible that interventions aiming at modulating PCr+Cre concentrations might be a new effective strategy for the treatment of either dysfunctional personality

TABLE 3 | Results of linear regression, performed using the forced block entry method.

	Character								Temperament											
	Self-directedness			Self-transcendence			Cooperativeness			Novelty seeking			Harm Avoidance			Reward Dependence			Persistence		
	R2 (Beta)	F	p	R2 (Beta)	F	p	R2 (Beta)	F	p	R2 (Beta)	F	p	R2 (Beta)	F	p	R2 (Beta)	F	p	R2 (Beta)	F	p
PCr+Cre	**0.61 (2.99)**	**8.9**	**0.01**	**0.58 (−1.96)**	**20.4**	**<0.001**	0.13 (0.69)	0.4	0.5	0.31 (−0.28)	0.07	0.8	0.33 (0.98)	0.7	0.4	0.18 (0.64)	0.5	0.5	0.11 (−0.11)	0.1	0.8
GPC+PC	0.50 (4. 22)	2.8	0.1	**0.40 (−3.40)**	**8.7**	**0.01**	0.13 (1.53)	0.4	0.5	0.31 (−0.74)	0.1	0.7	0.31 (−0.27)	0.0	0.9	0.16 (−0.32)	0.0	0.9	0.12 (−0.37)	0.1	0.7
Myo-inositol	0.47 (1.05)	1.5	0.2	**0.45 (−1.20)**	**11.0**	**<0.001**	0.13 (−0.39)	0.2	0.6	0.30(−0.17)	0.05	0.8	0.35 (0.90)	1.2	0.3	0.17 (0.32)	0.3	0.6	0.13 (0.22)	0.5	0.5
Glutamate	0.58 (−0.14)	6.9	0.02	0.29 (−0.84)	4.1	0.1	0.12 (0.34)	0.2	0.7	0.32 (0.63)	0.6	0.4	0.32 (0.39)	0.2	0.7	0.16 (0.07)	0.01	0.9	0.11 (−0.00)	0.0	1.0
NAA	0.54 (1.75)	4.7	0.04	0.22 (−0.62)	2.0	0.1	0.12 (0.31)	0.1	0.7	0.37 (−1–19)	2.3	0.1	0.31 (0.24)	0.1	0.8	0.17 (−0.41)	0.4	0.5	0.11 (−0.05)	0.0	0.9

Bold values are statistically significant (p = 0.01 after Bonferroni correction). PCr+Cre, phosphocreatine plus creatine; GPC+PC, glycerophosphocholine plus phosphocholine; NAA, N-acetylaspartate.

traits or depression. This is because it has been demonstrated that, even in healthy brains (19), increasing creatine levels following oral creatine supplementation modify brain energy metabolism, which has been found to be altered in various psychiatric illnesses, including depression and schizophrenia (19). Therefore, future ^{1}H MRS studies are warranted to further explore the role of brain chemistry on major psychiatric disorders. Finally, overall, our results suggest not only that personality traits are associated with specific biochemical circuits but also that this association is present already during adolescence, ultimately underlining the importance of investigating the relationship between personality traits and biological measures during the development. However, future larger studies are needed to better discriminate the biomarkers characterizing the different personality traits.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical committee of the IRCCS "E.Medea." Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

PB designed the study. LS and JS performed the MR analyses. GD, CaB, LS, JS, and PB performed the statistics. MN and PB supervised enrolment and evaluation of the subject sample. MMa, CaB, MG, and SP recruited and administered scales and interviews. MMa, BT, CaB, and FF contributed to data collection and eased accessibility to MR facilities. LS, CaB, and PB wrote the first version of the manuscript. GD and JS revised the earlier versions of the manuscript. All authors contributed to the writing and accepted the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2019.00763/full#supplementary-material

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An Individualized Approach to Neuroplasticity After Early Unilateral Brain Damage

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Introduction: Reorganization after early lesions in the developing brain has been an object of extensive scientific work, but even growing data from translational neuroscience studies in the last 20 years does not provide unified factors for prediction of type of reorganization and rehabilitation potential of patients with unilateral cerebral palsy (UCP) due to pre/perinatal insult.

Aim: To analyze the type of motor, language, and sensory brain reorganization in patients with right-sided cerebral palsy due to pre/perinatal isolated left-sided brain lesions taking into consideration the type (cortico-subcortical or periventricular) and extent (gray and white matter damage) of the lesion, etiology, comorbidity, and other postnatal factors that could have played a role in the complex process of brain plasticity.

Material and Methods: Eight patients with unilateral right cerebral palsy were included in the study. The individual data from fMRI of primary sensory, motor, and language representation were analyzed and compared with respective comprehensive etiological, clinical, and morphological data. Patients were examined clinically and psychologically, and investigated by structural and functional 3T GE scanner. A correlation between the type and extent of the lesion (involvement of cortical and subcortical structures), timing of lesion, type of reorganization (laterality index), and clinical and psychological outcome was done.

Results: Significant interindividual diversity was found in the patient group predominantly in the patterns of motor reorganization. Patients with small periventricular lesions have ipsilesional representation of primary motor, sensory, and word generation function. Patients with lesions involving left cortico-subcortical regions show various models of reorganization in all three modalities (ipsilesional, contralesional, and bilateral) and different clinical outcome that seem to be impossible for prediction. However, patients with UCP who demonstrate ipsilesional motor cortical activation have better motor functional capacity.

Conclusion: The type and size of the pre/perinatal lesion in left hemisphere could affect the natural potential of the young brain for reorganization and therefore the clinical outcome. Much larger sample and additional correlation with morphological data

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(volumetry, morphometry, tractography) is needed for determination of possible risk or protective factors that could play a role in the complex process of brain plasticity.

Keywords: pre/perinatal brain lesion, functional MRI, motor reorganization, sensory reorganization, language reorganization, dyslexia, functional capacity, predictive factors

INTRODUCTION

Translational neuroscience has developed over the past few decades as innovative field to bridge knowledge across disciplines in medicine and especially to translate data/knowledge from fundamental neurosciences (such as neurobiology) to explanation of human brain functions in health and disease (1).

Brain reorganization after early lesions in the developing brain has been an object of extensive scientific work, but even with the achievements from translational neuroscience in the last 20 years it is still not clarified which factors could predict type of reorganization and rehabilitation potential of patients with unilateral cerebral palsy (UCP) due to pre/perinatal insult. UCP implies an excellent model for studying brain plasticity. It comprises heterogenic conditions in terms of etiology, timing, morphology, clinical signs, and severity of impairments (2). Variety of unilateral brain lesions, acquired in the pre/perinatal period could lead to UCP with abnormal motor behavior as the core feature of this unprogressive condition. Lesions acquired during the first two trimesters of pregnancy interfere with the processes of neuronal migration, proliferation, and cortical organization, leading to cortical malformations and, thus, disturb the normal function of the affected area. Lesions that are acquired in the third trimester and in early postnatal life disrupt structures that are already formed, but also interfere with the processes of dendritic arborization, axonal sprouting, and myelination. The size and extent of the lesion in the hemisphere, as well as its type—periventricular lesion (PVL) or cortico-subcortical lesion (CSL)— could affect these processes in different ways (3). The brain maturation and reorganization might be additionally influenced by the factors leading to the initial insult like genetic conditions, infections, neonatal encephalopathy, and medical intervention, and also by postinsult events like early therapeutic intervention and epilepsy. Recent studies comment on the inability of the immature brain to follow the simple Kennard principle due to many events that shape different developmental trajectory (4). Taking all these reasons together, applying one unified model of brain reorganization in patients with UCP seems impossible, and three main models of reorganization—ipsilesional, contralesional, and bilateral have been widely discussed (5). Despite the growing number of studies in this area, it still remains difficult to predict individual remodeling. Functional MRI (fMRI) is a novel method, which is an excellent tool for studying brain reorganization in various brain functions (motor, sensory, language, cognition, etc.) using different tasks. Due to its noninvasive nature and good spatial and acceptable temporal resolution, it is widely used in studies of developmental disorders. UCP represents an appealing model for the study of brain plasticity for several main reasons:

- Lesion is acquired before 2 years of age, when reorganization potential of the contralesional hemisphere is significant due to the ongoing development of the normal projections (6).
- The study is conducted when reorganization is already completed (it's most intensive during 6 months after injury) and the type of reorganization is more or less fixed (7).
- Most of the lesions could be specified in time of appearance with high certainty depending on risk factors, prenatal imaging techniques, type of lesion, etc. (3).

Unfortunately, it remains very difficult to homogenize the group of studied patients so that a large sample study can be achieved for sufficient statistical results. UCP is a rare disorder with cumulative prevalence of 0.6 to 1 *per* 1,000 live births. The exact prevalence of CP is unknown in Bulgaria, but the largest Bulgarian study on CP included 143 patients with UCP out of CP sample of 521 patients (personal correspondence with Dr. Elena Rodopska, University Hospital "St. Naum," Sofia, Bulgaria). UCP is an umbrella term that includes several conditions that vary in etiology and morphology (2). With demographic variables added, it is almost impossible to obtain homogeneous group of patients. Moreover, the study samples are reduced due to absence of motivation or inability to perform the task inside the MRI. These challenges explain the relatively small sample number of patients with UCP that were included in fMRI studies in literature—between 3 and 25 (5).

The aim of this study is to analyze the type of motor, language, and sensory brain reorganization in patients with right-sided cerebral palsy due to pre/perinatal isolated left-sided brain lesions taking into consideration the type (cortico-subcortical or periventricular) and extent (gray and white matter damage) of the lesion, etiology, comorbidity, and other postnatal factors that could have played a role in the complex process of brain plasticity.

MATERIAL AND METHODS

Participants

The study was performed prospectively in the Complex of Translational Neuroscience, Medical University—Plovdiv for the period 2017–2019. Eight patients with diagnosis of right-sided cerebral palsy (three males, aged 13–15 years, and five females, aged 10–30 years) were included in the study.

The inclusion criteria were:

- Congenital right-sided hemiparesis with MACS level ≤3
- Mental age >7 years
- Unilateral left-sided brain lesion proved by a brain image

- Fulfilled informed consent (from either the patient or his parent if the patient is under the age of 18 years) for participation in the study

The exclusion criteria were:

- Patient and his parents' disagreement for participation in the study
- Impossibility to stay calm during fMRI scan
- Uncooperativeness of the patients or inability to perform the fMRI paradigms
- Presence of implants in the patients' body, which is contraindication for MRI

The patients were examined by clinical and psychological tests and investigated by structural 3T scan and fMRI tests.

The study design was approved by the Ethical Committee of Medical University—Plovdiv.

Procedures

Clinical and Psychological Examination

The medical interview with the patients included questions about familial risk factors, risk factors during pregnancy, delivery, and early postnatal period, time of diagnosis, laboratory, genetic, and imaging data, type and duration of rehabilitation, and comorbidities (epilepsy, cognitive deficits, visual and other sensory deficits). Clinical investigation (physical and neurologic examination) was done by either a specialist in pediatric neurology or adult neurologist.

Severity of movement difficulties was evaluated by the score on the Manual Ability Classification System (MACS) and the Modified Ashworth Scale (MAS). The MACS classifies a person's ability to handle objects in important daily activities across a five-point scale (level I—handle most objects easily; to level V—severely limited in their ability) (8). MAS further characterized the children by documenting severity of movement restriction due to spasticity across the elbow, wrist, fingers, and thumb (0—indicating no movement restriction, to 4—reflecting rigidity/severe contracture). The sensory examination included examination of touch, pain, joint position sense, stereognosis, and graphesthesia. Rehabilitation was classified as absent (−), rare—less than one course *per* month (+), moderate—one or more courses *per* month (++), and frequent—every day (+++). Time of onset of rehabilitation was registered for every patient. All participants received psychological evaluation by a psychologist with IQ (WISC-IV), as well as evaluation for dyslexia with DDE-2 battery, both adapted in Bulgarian language (9, 10).

Mri Procedure

Data Acquisition

Scanning of all patients was executed on a 3T MRI system—GE Discovery 750w with a protocol including a structural scan: SagT1 FSPGR BRAVO, slice thickness 1 mm, matrix 256 × 256, flip angle 12°. Additional AxFLAIR scan was performed for better qualification of lesions. The protocol for all functional scans contained 2D Echo planar imaging, slice thickness 3 mm, matrix 96 × 96, relaxation time 3,000 ms, echo time −30, and flip angle 90°. Before each functional scan, five dummy time series were acquired.

Experimental Paradigms

All patients were familiarized with the fMRI procedure through animated presentation. The experiment contained five paradigms, each implemented in block design: two active movement conditions—left (ML) and right (MR) hand finger tapping; two passive sensory conditions—left (SL) and right (SR) hand brushing; and a word generation paradigm. During one session, each task was performed for 30 sec and repeated 5 times after 30 sec of rest. Finger tapping task was performed with repetitive touching of first and second finger with frequency of approximately 1 Hz. Movements were directly observed by an experimenter. Hand brushing task was performed with gentle brushing of the back of each hand with frequency of 1 Hz by the same experimenter. The beginning of each active and passive block for the motor and sensory paradigm was presented on the screen in front of the patient with the word "Start" and "Stop," respectively. For the word generation task there were five different letters presented on the screen (one for each block of the task) and the patient was asked to think of as many words as possible, starting with the letter presented on the screen (silent generation of words). The total duration of the functional scan was 25 min. Paradigms were shown in a randomized order.

Analysis of Imaging Data

Preprocessing steps were carried out using custom routines available in SPM12 (Wellcome Department of Imaging Neuroscience, University College, London, UK, http://www.fil.ion.ucl.ac.uk/spm) . Images were corrected for head movements by realigning all images with the first image of the first session, and a mean image of the realigned volumes was created. To remove variance due to unwanted head movements that might have been task-related, images were unwrapped (11). The 3D-dataset was segmented in native space, using a unified segmentation approach (12). The segmented tissue maps were coregistered to the mean functional image from the first session. The crucial step of normalization capitalizes on the fact that chronic lesions are overwhelmingly classified as CSF during tissue segmentation (13). This tissue class is then used as the basis for an automatically generated lesion mask which in turn is used to implement a cost-function masking approach (14) during spatial normalization. These segmentation parameters were used to normalize the functional series to a final resolution of 2×2×2 mm. In the end, the images were spatially smoothed 8 mm full width at half-maximum (FWHM).

The model for first-level analysis was then specified with parameters estimated, and t-contrasts defined for active versus passive condition for all five experiments (Motor Right—MR, Motor Left—ML, Sensory Right—SR, Sensory Left—SL, Word Generation—WG). The level of significance was set at $p < 0.05$ familywise error corrected and cluster extent threshold of 10 voxels. Statistical results were presented using SPM extension Bspm view (http://www.bobspunt.com/software/bspmview/) .

Laterality index (LI) was calculated for motor and language representation using the commercially available tool LI (http://

www.medizin.uni tuebingen.de/kinder/en/research/neuroimaging/software/) . LI was obtained by computing LI = (nL − nR)/(nL + nR), where nL and nR are the number of activated voxels in left (LH) and right (RH) hemisphere, respectively (15). The absolute value 0.10 was used as threshold for definite lateralization (16). Patients with a positive index (LI > 0.10) were considered left-lateralized for language, while those with a negative index (LI < −0.10) were considered as right-lateralized. Values of |LI| = 0.10 represent a "bilateral" or uncertain activation. For calculation of LI for language representation, the total number of voxels in the gray matter was used, while LI for motor representation was calculated using the number of activated voxels only in the primary motor cortex (PMC).

The extent of injury of gray matter (GM) and white matter (WM) was classified according to maximum width of lesion as 1 = mild (<10 mm), 2 = moderate (10–20 mm), and 3 = severe (>20 mm) (17). Lesional volume was calculated and visualized using MRICron (https://www.nitrc.org/projects/mricron/).

Presumed timing of the lesions was judged by the criteria offered by (18) malformation of the cortical development (MCD) occurs during I and II trimester, while PVL and CSL during III trimester or perinatally. In addition, PVL could be approximately assigned to the period of 24–36 weeks of gestation, and CSL after 36 weeks of gestation (19).

Statistical Analysis of Behavioral and Imaging Data

Due to the small number of subjects, no statistical analysis on group level was done. Patients were divided in three main groups depending on type of the lesion, and comments were made on group and individual level, associated with various behavioral data.

Results

Demographic and Clinical Data With Risk Factors for Pre/Perinatal Brain Lesions

Five of the patients had radiological evidence of involvement of unilateral cortical and subcortical regions in the territory supplied by the left middle cerebral artery, further referred to as CSL lesions (P1–5), probable arterial infarction. Two had no cortical involvement but only unilateral PVL, presenting with mild enlargement of the frontal horn of the left lateral ventricle and periventricular T1 hypointensity and FLAIR hyperintensity—presumable venous infarctions or unilateral periventricular leukomalacia (P6 and P7). One had MCD—closed lip left-sided schizencephaly (P8) (**Figure 1**).

All five patients with CSL (MCA-infarction) had second and third degree of involvement of both cortex and adjacent white matter tracts including partially the Rolandic and the Broca area (**Table 1**). For the patient with MCD this involvement

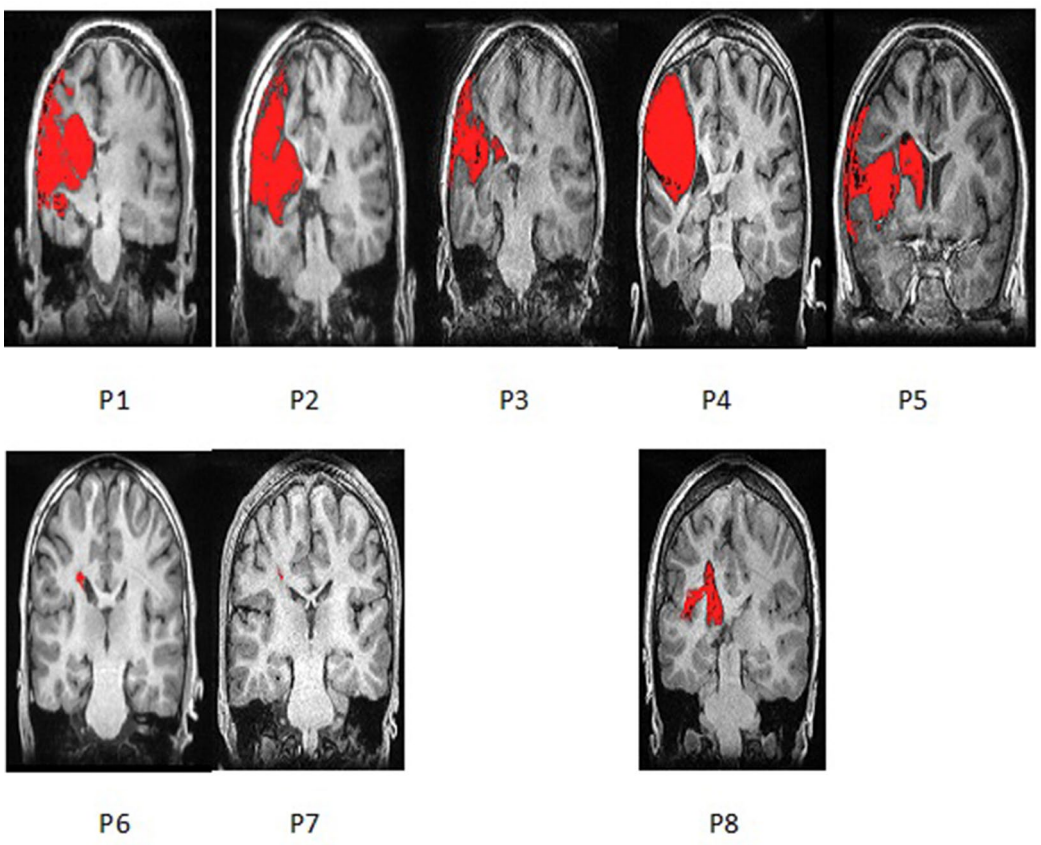

FIGURE 1 | Coronal section of T1 Structural MRI of all eight patients showing their brain lesions (lesion represented in red color). Images are presented *via* MRICron and therefore flipped—left side of the brain is on the left side of the image.

TABLE 1 | Demographic and clinical data of patients.

Subject	Age	Lesion	Extent of injury - classification	Extent of injury (mm^3)	Prenatal risk factors	Thrombophillya	Neonatal encephalopathy	Time of insult	Rehabilitation	MACS level	Sensory deficit	Epilepsy	IQ	Dyslexia
P1	26–30	LMCA infarction	3	26.72	Eclampsia	NA	No	Late III trimester	+	3	No	Yes	70	Yes
P2	16–20	LMCA infarction	3	42.59	No	NA	Yes—birth trauma	Birth	+	3	No	No	60	Yes
P3	10–15	LMCA infarction	2	16.88	No	NA	No	Late III trimester	+	2	No	Yes	80	Yes
P4	10–15	LMCA infarction	3	59.65	No	Yes	No	Late III trimester	–	1	No	Yes	89	Yes
P5	10–15	LMCA infarction	2	15.04	No	NA	Yes—cardiac arrest	Birth	+++	1	No	Yes	75	No
P6	10–15	PVL small leftlesion	1	0.73	No	NA	No	Early III trimester	++	1	No	No	98	Yes
P7	10–15	PVL small left lesion	1	1.21	Yes—eclampsia	Yes	No	Early III trimester	–	1	No	No	78	No
P8	21–25	MCDleft	1	9,18	Yes—bleeding	NA	Yes—breathing problem	II trimester	+	3	No	Yes	50	NA

F, female; M, male; LMCA, left middle cerebral artery; PVL, periventricular lesion; MCD, malformation of cortical development; NA, not available.

was of lesser extent (first degree). Both patients with PVL had involvement of the anterior portion of the internal capsule, defined as first-degree white matter injury. There was no cortical involvement for the patients with PVL.

Functional capacity measured by MACS was very good in both patients with PVL, poor in the patient with MCD, and variable in the patients with CSL. The same distribution was found for the degree of limb spasticity, measured by MAS.

No patient was found to have sensory deficits during clinical examination of touch, pain, joint position, stereognosis, and graphesthesia.

Five of six patients with cortical involvement (four CSL and the MCD) had epilepsy with partial or secondary generalized seizures originating from the left hemisphere. All of them were under a stable dose of anticonvulsive medication by the time of the study.

IQ varied from 50 to 90 (**Table 1**).

Four out of five patients with CSL and one out of two with PVL were dyslexic. One patient couldn't be evaluated for dyslexia, because of lack of cooperativeness.

The risk factors presumably involved in the brain damage causing the lesions were: risk pregnancy (found in three patients), prematurity (none of the tested patients), genetic thrombophilia factors (one patient), birth asphyxia and trauma (one patient). Timing of these factors was referred to occurrence of the lesion, i.e., severe birth asphyxia in P5 and finding of left porencephalic cyst leads to diagnosis of left middle cerebral artery infarction that we assumed as having occurred at birth. Maternal bleeding around 26th gestational week and finding of closed lip shizencephalic cleft could also suggest the time of insult.

Activation During Motor Task With the Impaired Hand

All eight patients completed finger tapping task successfully with the right hand. Mirror movements during the task were observed in P1 and P3.

During finger tapping with the impaired hand three types of activation in the PMC were found (**Figure 2**):

1. Predominant activation in preserved areas of left precentral gyrus—M1 (ipsilateral to the lesion) in four patients (two with PVL: P6 and P7, and two with CSL: P4 and P5)
2. Bilateral distribution of the activation in PMC—in two patients (one with CSL: P1, and the one with MCD: P8)
3. Activation only in the contralesional (right) precentral gyrus—in the other two patients with CSL (P2 and P3)

Additional extensive activation in various cortical regions in both hemispheres was observed in all patients, involving areas outside typical motor system representation.

A clear association was found between functional capacity of the patients and the type of reorganization: all four patients with MACS level I showed predominantly left-sided activation in the PMC, while those with MACS level II and III had either bilateral or right-sided activation. Patients with PVL showed strong ipsilesional activation, while patients with cortical involvement had all three patterns of activation in their PMC. As to extend of the lesion, P6 and P7 who had first degree of injury and no GM involvement showed ipsilesional activation, while the other patient with small lesion (<10 mm) but with cortical involvement showed bilateral activation with LI (−0.03). Patients with second degree of injury showed two completely different patterns—contra- or ipsilesional activation (P3 and P5), similar to P1, P2, and P4, who had the greatest extend of injury to both white and gray matter. Rehabilitation frequency didn't seem to affect the reorganization—P5 and P6 who showed ipsilesional activation had very frequent rehabilitation, unlike P 4 and P7 who had similar lesions and patterns of motor activation, but had barely conducted rehabilitation.

Activation During Motor Task—Nonimpaired Hand

Finger tapping paradigm with the left hand was performed successfully by all patients and no mirror movements were

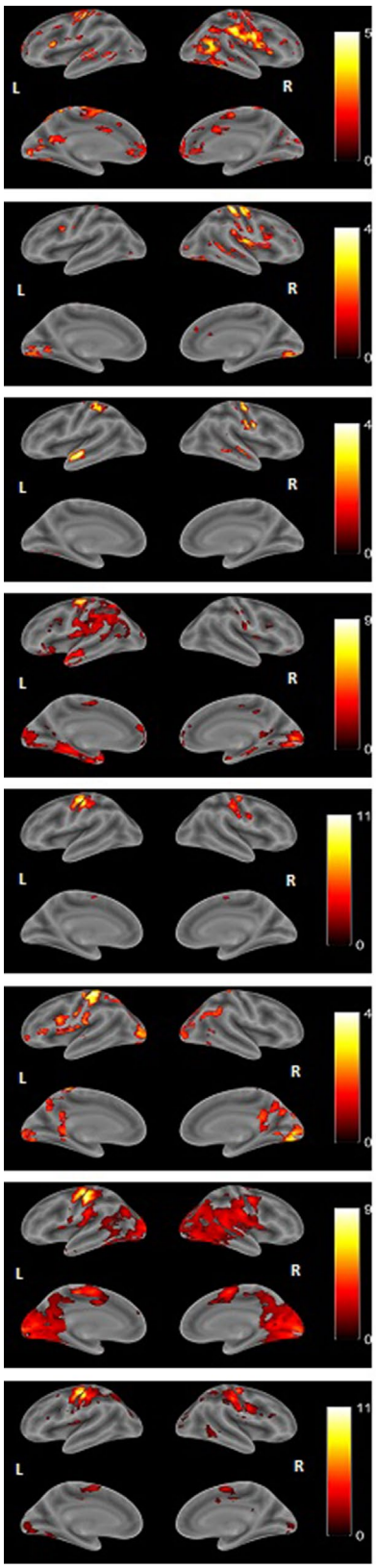

FIGURE 2 | Examples of render images of the three different patterns of activation during motor task with the impaired right hand (MR task). Color bar represents t-value.

observed during the task. Results were pretty consistent in all patients showing activation in their right PMC with different degrees (**Table 2**) and **Figure 3**. Unlike motor task with the impaired hand, additional activation was observed only in regions typically involved with motor processing, like basal ganglia bilaterally or contralateral cerebellum. No extensive activation of other cortical regions was found.

Activation During Language Task

CSL patients showed either right lateralization of cortical activation during the language task (P1, P4, P5), or bilateral one (P2, P3). The MCD (P8) patient showed bilateral activation during the language task. Left-sided predominance (typical for healthy controls) was found in the two PVL patients (P6, P7) (**Table 3** and **Figure 4**).

Dyslexic patients showed various patterns of activation: two with right lateralization, two bilateral, and one left lateralization. Patients who neither had epilepsy nor treatment showed language lateralization shifted to the left hemisphere (P6, P7) or bilateral (P2).

Sensory Task—Impaired Hand

Only one patient (P3) could not conduct hand brushing task because she could not endure the full length of the protocol. All patients with PVL and the patient with MCD had left postcentral gyrus activation, as well as the two CSL patients (P4 and P5) in whom the lesion did not encompass the entire left postcentral gyrus. Two other patients with CSL (P1 and P2) showed no activation in the primary sensory cortex; instead there was significant activation of posteriorly or anteriorly located regions. All four patients with CSL showed additional involvement of other areas outside the sensory cortex in both hemispheres. No one showed activation in the contralesional primary sensory cortex during hand brushing task on the impaired hand (SR).

Comparison with the behavioral data showed some association between the quality of motor function and the area of cortical sensory representation: participants with better motor performance (MACS level I) showing sensory reorganization in the left postcentral gyrus, while participants with worse MACS level (P1 and P2) showing shifting of the activation during SR outside the postcentral gyrus (**Table 4** and **Figure 5**).

Sensory Task—Nonimpaired Left Hand

Two patients (one PVL: P6, and one CSL: P1) showed no suprathreshold activation in either left or right primary sensory cortex during hand brushing of the nonimpaired hand. Two CSL patients (P2 and P4) and one MCD (P8) patient had only contralateral activation in right postcentral gyrus; one CSL (P5) and one PVL patient (P7) showed involvement of both primary sensory cortices (**Table 5**).

Discussion

UCP amounts up to 30% of all CPs and is an appealing model for the study of brain plasticity by fMRI because of coexistence of nonprogressive brain lesion in one hemisphere, and normal surrounding tissues in the same one, and non- or less affected

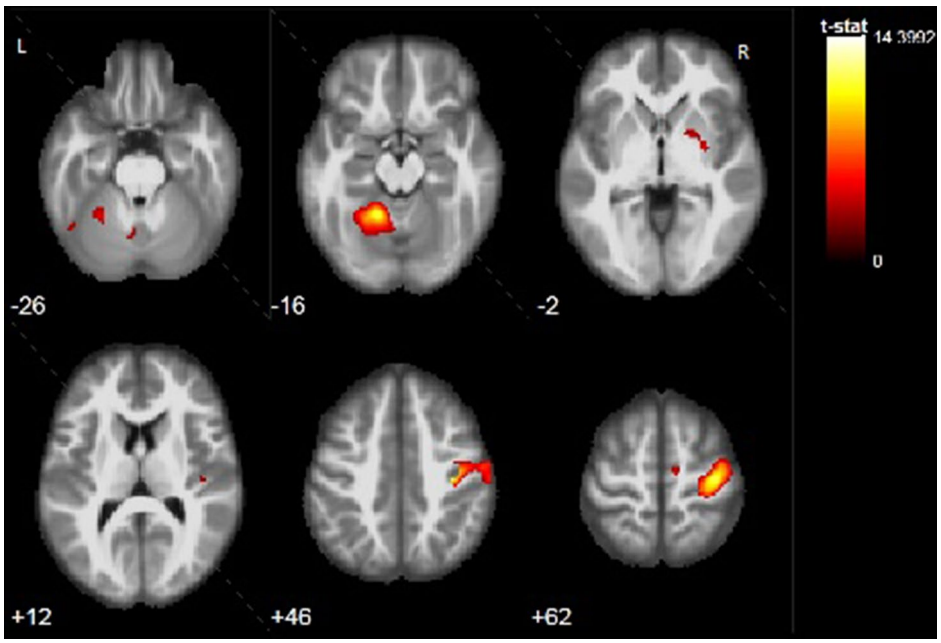

FIGURE 3 | Example of activation during motor task with the nonimpaired left hand (ML task) of P3. Activation is found in the right precentral gyrus, right putamen, and left cerebellar lobule IV, V, VIII, IX. Color bar represents t-value.

TABLE 2 | Activation in the PMC during finger tapping with the nonimpaired hand.

Subject	Lesion	ML: Left motor cortex activation					ML: Right motor cortex activation				
				MNI Coordinates					MNI Coordinates		
		Extent	t-value	x	y	z	Extent	t-value	x	y	z
P1	LMCA infarction	–	–	–	–	–	10,041	6,816	56	–8	46
P2	LMCA infarction	–	–	–	–	–	1,887	3,027	20	–32	74
P3	LMCA infarction	–	–	–	–	–	998	12,310	36	–16	50
P4	LMCA infarction	–	–	–	–	–	3,339	15,151	42	–18	56
P5	LMCA infarction	–	–	–	–	–	600	9,680	44	–22	56
P6	LPVL: small lesion	–	–	–	–	–	3,114	2,517	14	–26	86
P7	LPVL: small lesion	–	–	–	–	–	410	7,889	50	–14	52
P8	LMCD	–	–	–	–	–	1,587	13,104	34	–26	54

ML, motor left; LMCA, left middle cerebral artery; LPVL, left periventricular lesion; LMCD, left malformation of cortical development; MNI, Montreal Neurological Institute.

opposite hemisphere, which allows the compensation mechanisms for some functions. fMRI shows that the nondominant hemisphere could acquire dominance or codominance in motor, visual, auditory, and language functions after pre/perinatal brain lesions in dominant hemisphere (5).

However, patients with UCP have considerable variability in etiology, time of appearance, size, and location of the lesion, as well as functional capacity, making it difficult to obtain not only large homogeneous patient samples, but also a unified model of brain reorganization. Reported causes of UCP are periventricular white matter lesions, posthemorrhagic porencephalic lesions, infarcts in the middle cerebral artery, and brain malformations (20). Despite the great capacity for plasticity of immature brain and the proposed largely linear relationship between age at brain injury and functional outcome, great variability in outcome from early brain insult is observed, including poor recovery from early prenatal lesions (21).

In our study we applied an individual approach to present the model of reorganization of motor, sensory, and language functions in every patient, and then tried to analyze the established models of brain reorganization in accordance with the etiological type, size and location of the lesion, time of appearance of the lesion, or functional capacity of the patient. Due to the small group of patients, we assume more descriptive approach to our data. Analyzing the reorganization of all three domains within the same patients has not been considered in the previous studies, so we believe that it is the main strength of our study.

Motor Reorganization

Population studies of children with perinatally acquired unilateral lesions show they have better quality of life than those with bilateral lesions, which is in direct correlation with the better GMFCS (Gross Motor Function Classification System) level (22). Nevertheless those patients usually experience motor, sensory,

TABLE 3 | Activation in right and left hemisphere during the language task (WG).

Subject	Lesion	Dyslexia	Epilepsy	LI (whole brain)	Voxels (right)	Voxels (left)	Clusters (right)	Clusters (left)
P1	LMCA infarction	Yes	Yes	–0.127	23,995	13,986	21	44
P2	LMCA infarction	Yes	No	0.007	38,626	29,979	6	20
P3	LMCA infarction	Yes	Yes	0.082	18,979	16,892	23	44
P4	LMCA infarction	No	Yes	–0.364	36,632	17,031	36	64
P5	LMCA infarction	No	Yes	–0.144	34,436	19,733	12	44
P6	LPVL: small lesion	Yes	No	0.143	55,894	41,593	11	27
P7	LPVL: small lesion	No	No	0.232	44,034	36,726	14	37
P8	LMCD	NA	Yes	0.0426	44,979	36,852	12	20

LMCA, left middle cerebral artery; LPVL, left periventricular lesion; LMCD, left malformation of cortical development; NA, not available; LI, laterality index.
Lateralization to the right hemisphere:
Bilateral representation:
Lateralization to the left hemisphere:

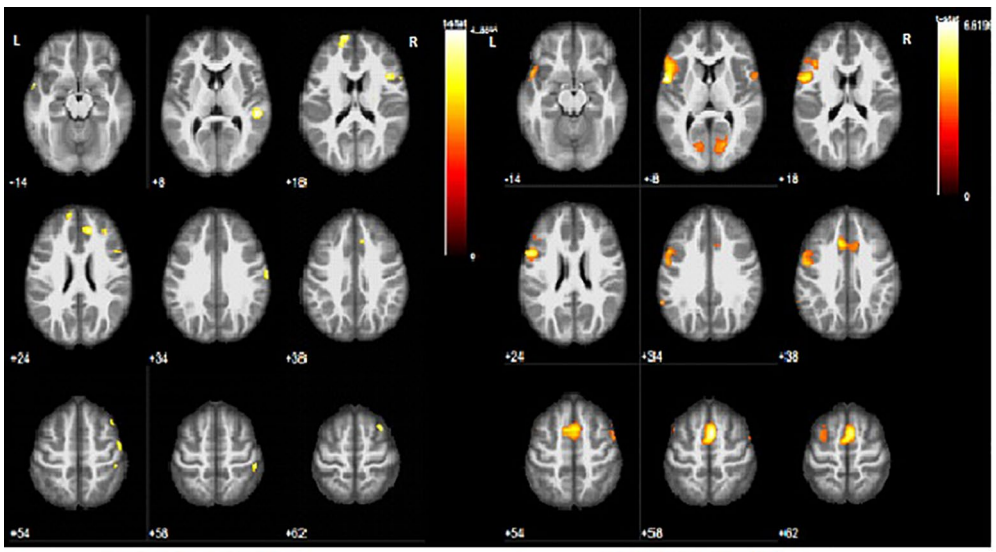

FIGURE 4 | Two types of activation during WG task. None of the patients shown on the figure was found to be dyslexic.

language, visiospatial, or executive difficulties, which interfere with their everyday life. Children with UCP could never achieve a normal hand function in contrast with language abilities with even minor lesion in the corticospinal tract leading to motor impairment (3).

Our results confirmed the variety of functional motor capacity of patients with pre/perinatally acquired unilateral left hemispheric lesions, demonstrating MACS level varying from I to III.

Models of Motor Reorganization

Three models of functional motor reorganization have been found in patients with UCP: only contralesional; only ipsilesional; and bilateral. A recent systematic review showed bilateral activation with stronger contralesional predominance to be the most common model for motor reorganization in UCP (5).

Cao et al. showed bilateral activation during paretic hand movement in all patients, but all of them have cortical lesions (MCDs or CSLs) (23). Similar results in patients with big cortical lesions are reported also by Staudt et al. and Vandermeeren et al. (24, 25). Bilateral activation was reported by Staudt et al. also in patients with small PVL, but in the premotor area (18).

In our study we chose to concentrate on the PMC as site of motor representation. We hypothesize that motor function could be a direct consequence of the number of active neurons left in their original place in the precentral gyrus.

Our study results showed only two out of eight patients had contralesional activation and another two patients bilateral activation. The remaining four patients showed ipsilesional activation which is the rarest model in literature (5). We suppose that methodological and other issues may have an effect on the variability of the models of reorganization, but probably the most important variable is the type and extent of the lesions.

TABLE 4 | Activation in the primary sensory cortex during SR task.

Subject	Lesion	SR: left sensory cortex activation					SR: right sensory cortex activation				
				MNI coordinates					MNI coordinates		
		Extent	t-value	x	y	z	Extent	t-value	x	y	z
P1	LMCA infarction	–	–	–	–	–	–	–	–	–	–
P2	LMCA infarction	–	–	–	–	–	–	–	–	–	–
P3	LMCA infarction	Not conducted					Not conducted				
P4	LMCA infarction	32	5,460,258	–36	–26	70	–	–	–	–	–
P5	LMCA infarction	2,067	781,183	–40	–26	60	–	–	–	–	–
P6	LPVL: small lesion	295	4,890,559	–52	–42	54	–	–	–	–	–
P7	LPVL: small lesion	1,477	6,491,543	–42	–22	58	–	–	–	–	–
P8	LMCD	2,212	1,417,368	–38	–34	60	–	–	–	–	–

SR, sensory right; LMCA, left middle cerebral artery; LPVL, left periventricular lesion; LMCD, left malformation of cortical development; MNI, Montreal Neurological Institute.
Activation outside left postcentral gyrus:
Activation in the left postcentral gyrus:

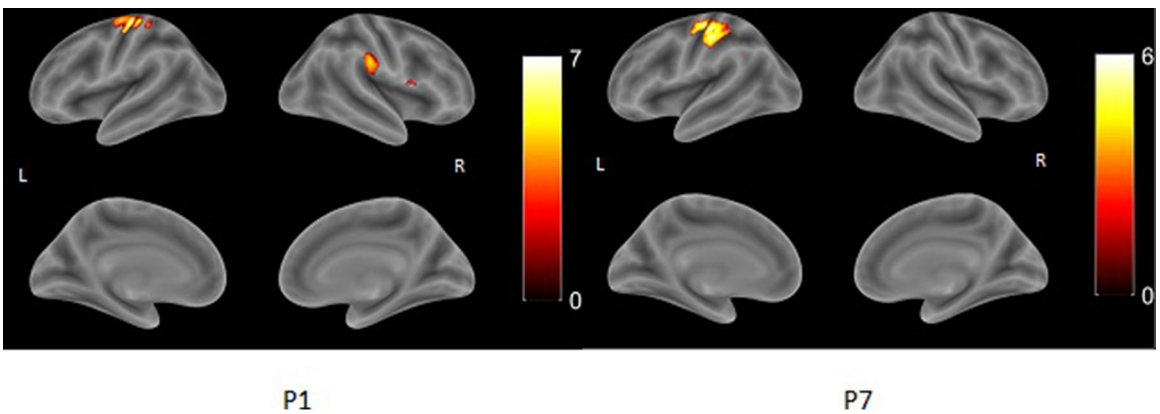

FIGURE 5 | Different patterns of cortical activation during sensory task on impaired right hand (SR). P1 has activation close to left postcentral gyrus, but not located in the gyrus, in contrast to P7 where the activation involves wide zone of the left postcentral gyrus. Color bar represents t-value.

TABLE 5 | Activation in primary sensory cortex during SL task.

Subject	Lesion	SL: left sensory cortex activation					SL: right sensory cortex activation				
				MNI coordinates					MNI coordinates		
		Extent	t-value	x	y	z	Extent	t-value	x	y	z
P1	LMCA infarction	–	–	–	–	–	–	–	–	–	–
P2	LMCA infarction	–	–	–	–	–	94	3,911,956	54	–18	54
P3	LMCA infarction	Not conducted					Not conducted				
P4	LMCA infarction	–	–	–	–	–	1,074	4,129	50	–18	40
P5	LMCA infarction	55	3,710	–62	–16	20	85	3,521	36	–36	66
P6	LPVL: small lesion	–	–	–	–	–	–	–	–	–	–
P7	LPVL: small lesion	1,573	5,771	–64	–26	24	3,626	5,413	46	–26	54
P8	LMCD	–	–	–	–	–	1,160	5,621	28	–32	68

SL, sensory left; LMCA, left middle cerebral artery; LPVL, left periventricular lesion; LMCD, left malformation of cortical development; MNI, Montreal Neurological Institute.

Models of Reorganization According to the Type, Location, and Size of the Lesion

Usually patients with lesions involving the left Rolandic area more often had bilateral and/or right predominant activation in M1. Activation of contralesional PMC occurs in patients with severe lesion and absent ipsilesional crossing corticospinal projections, which makes this motor cortex probably the only cortical motor area. Nevertheless, this is also a rare model of reorganization (review **Table 6**). Contralesional activation of motor cortex is suggested to be a result of preservation of the ipsilateral projections

TABLE 6 | Patterns of activation in the PMC during finger tapping task with the impaired hand (MR).

Subject	Lesion	MR—Left motor cortex activation					MR—Right motor cortex activation					LI	MACS
				MNI Coordinates					MNI Coordinates				
		Extent	t-value	x	y	z	Extent	t-value	X	y	z		
P1	LMCA infarction	11,408	5,845,313	−30	−26	54	11,408	55,427	56	−2	24	0	3
P2	LMCA infarction	206	2,538,333	−46	−2	30	2,284	4,886,111	30	−30	68	−0.83	3
P3	LMCA infarction	724	2,143,814	−16	−18	76	1,238	4,193,118	26	−22	72	−0.26	2
P4	LMCA infarction	25,934	880,033	−32	−18	60	None	None	None	None	None	1	1
P5	LMCA infarction	2,103	1,151,858	−32	−22	50	1,626	6,270,804	36	−14	68	0.12	1
P6	LPVL: small lesion	19,322	4,641,092	−32	−32	70	187	2,421,789	16	−34	74	0.82	1
P7	LPVL: small lesion	85	2,796,338	−60	−2	34	None	None	None	None	None	1	1
P8	LMCD	4,900	1,151,858	−32	−22	50	5,197	6,270,804	36	−14	68	−0.03	3

MR, motor right; LMCA, left middle cerebral artery; LPVL, left periventricular lesion; LMCD, left malformation of cortical development; MNI, Montreal Neurological Institute; LI, laterality index.

Ipsilesional activation:

Bilateral activation:

Contralesional activation:

from previous stages of development, or to axonal sprouting in "normal" crossed corticospinal axons from the unaffected hemisphere with new collateral branches re-crossing the midline to innervate motor neurons on the paretic side (3).

Our patients with CSL with extent of GM and WM injury: two or three showed different models of brain reorganization during motor task with impaired hand; two of five showed contralesional activation of motor cortex; one of five bilateral and two of five ipsilesional. P4 and P5 had large lesions that involve the Rolandic cortex, but not entirely and showed ipsilesional activation during the motor task. We presume that in these two patients the projections from the spared part of the Rolandic cortex played sufficient role in the motor control and didn't allow the contralesional projections to take over. It is known that the pattern of reorganization varies according to the extent of preservation of the motor area and its connections to the spinal cord (3). However, we could hypothesize that other individual factors like rehabilitation could also play a role in this type of reorganization. In P5, rehabilitation was started early (at 6 months of age) and was conducted daily for years, whereas in P4 no protective factor could be identified, but he was the one with genetically proven thrombophilia (mutation in PAI, ACE, and FV Leiden genes).

Results from patients without cortical involvement (P6 and P7 in our study) are consistent with the findings of Staudt et al. that patients with small PVL show strong ipsilesional activation (18).

Models of Reorganization and Time of Brain Injury

Previous studies have hypothesized that timing of the lesion is one of the best predictors for good motor performance and reorganization potential with better functional capacity achieved after early lesion (I, II, and early III trimester) (3). Our study, however, does not show consistency with this theory: P8 having an MCD (timing—II trimester) has severely impaired hand function with MACS level III, while P4 having CSL (timing—late III trimester) has almost normal hand function with MACS level I. However, our study population included only one patient with brain lesion occurring earlier than III trimester, like MCD, which doesn't allow definite conclusion on this matter.

Models of Reorganization and Motor Performance

Our results showed association between left-sided lateralization of the activation in M1 and better hand performance, which is in support of the thesis that normal or near-to-normal hand function seems possible only with preserved crossed corticospinal projections from the contralateral hemisphere. Similar results have been found in the TMS study of Holmström et al. with better performance on the Box and Blocks test and AHA (Assisting Hand Assessment) of children with ipsilesional motor projections, as well as in the study of Jang et al. (26, 27). Fiori et al. in a case report also discussed that it was not very likely for the intact contralesional hemisphere alone to be sufficient for a normal function of the ipsilateral hand in the presence of an early unilateral lesion in the opposite hemisphere (28).

Mackey et al. found correlation between preserved ipsilesional motor control and hand motor function and suggested it as a result of intact intracortical and interhemispheric inhibition (29). The other explanation of this correlation is the "crowding" theory and the effect of representation of motor function for both hands in one cortical region with impossibility of one motor cortex to be "enough" for both hands (27).

Based on our results we could also conclude that patients with UCP demonstrating ipsilesional activation of PMC during motor task have better motor functional capacity.

Sensory Reorganization

Unlike other studies none of our patients experienced sensory deficits, even those with cortical lesions involving big part of the postcentral gyrus.

Despite the variability in age, gender, clinical characteristics, level of sensory deficits, and type of lesion, patients with UCP demonstrated predominant compensation through ipsilesional reorganization of the sensory function (30, 31). Recent systematic

review revealed that almost all patients showed activation only in the ipsilesional hemisphere during sensory tasks and our results were in consistency with previously reported data (31–35). We found that reorganization in the sensory system occurred ipsilateral to the lesion independently on either its type or extent, or type of motor reorganization. Patients with damaged sensory cortex typically activate the intact portions of the postcentral gyrus with a somewhat more variable topography (36). Functionally, many of these patients show severe somatosensory deficits, which sometimes contrast with relatively spared motor abilities (36). However no one of our patients was found to have sensory deficits, despite the wide destruction of the primary sensory cortex in some patients.

It is interesting to discuss the possible relationship between sensory and motor functions and their cortical representations in patients with UCP.

According to our results, representation of primary motor and primary sensory function in different hemispheres is associated with worse hand function. This dissociation of lateralization is determined only by the type of motor representation, because sensory reorganization was found to be always ipsilesional. The possible explanation of this result has been commented in the discussion of motor reorganization, but other explanation of impaired motor function might be the dissociation of sensory input and motor output to different hemispheres. This has been suggested by several authors (30, 34). Bigger destruction of the primary sensory cortex with shifting of the sensory function to the neighboring cortical areas also leads to worsening of the motor function (P1 and P2). Both concepts (motor and sensory presentation in different hemispheres, and atypical ipsilesional sensory representation) may hamper the sensorimotor integration, which is important for skillful hand movements.

Considering the few number of studies and the small groups of patients (30–36), further research on the reorganization of the sensory system in UCP, especially in combination with the reorganization of the motor and language systems, will contribute to better understanding of brain neuroplasticity.

Language Reorganization

Children with pre- or perinatal brain injury of the dominant hemisphere could acquire age-appropriate language, in contrast to the aphasia following similar lesions in adulthood (37, 38). Recent studies support the hypothesis of the "dormant circuitry" available for language function that is inhibited in the nondominant hemisphere of healthy children, but which may be activated when primary regions in the dominant hemisphere become unavailable to exert inhibition (39–42). A shift of language production to the right hemisphere (zones that are homotopic to the original left language zones) has been registered in most children with congenital left hemispheric lesions by dichotic listening tests (43–45) and by fMRI (46–52). However, left hemisphere lesion does not obligatorily induce a shift of language representation to the right hemisphere (48, 50).

Several models of language reorganization in patients with right-sided UCP and left-sided brain lesions are described: bilateral activation with either ipsilesional or contralesional dominance; only ipsilesional activation; only contralesional activation. Bilateral activation with contralesional dominance prevails in more than a half of the investigated patients (5). Our results are in concordance with these data: three out of eight patients demonstrated bilateral activation; two of them suffering from large left MCA infarction and one with schizencephaly; three other showed contralesional (right-sided) activation, all of them with CSL and also large injury (extent of GM and WM lesion ≥2); and only 2 patients with small PVL had ipsilesional activation.

Patients with left MCA infarction, i.e., CSL, demonstrated contralesional or bilateral activation and none of them had ipsilesional activation which is probably the result of the destruction of their primary language cortex, while patients with small PVL and preserved language cortex had ipsilesional activation. Therefore the dominant contralesional activation occurred only in patients with large CSL (extent of injury ≥2), and not with PVL, which supported the thesis that both the site and extent of a left hemispheric lesion determine the capacity for reorganization. Knecht and Lidzba and Lidzba et al., like us, reported a prevalence of greater right hemispheric language dominance in cortical lesions compared to PVL (53, 54).

Raja Beharelle et al. suggested that language reorganization depended more on the type rather than the size of lesion (50). However, LI values were inversely correlated with severity of the lesion according to Chilosi et al.: in cortical and subcortical, but not in PVLs right hemisphere language dominance is significantly associated with more severe brain damage, and our results supported this conclusion (42).

According to some authors, lateralization of language areas should be regarded differentially according to the cortical regions: UCP patients with better language outcome show a functional organization for language that favors left over right activity in frontal brain regions and a bilateral pattern of activity in right and left temporal-parietal regions (50).

The association between language and intellect is also discussed. Some researchers consider that the greater the shift to the right of language functions, the lower the cognitive and expressive language scores (40, 42). However, we could not support these statements, because two of our patients with right-sided contralesional language activation had high IQ, 89 and 90, respectively, in contrast with the other two patients with bilateral activation with IQ, respectively, 50 and 60. According to our results the type of language reorganization does not predict the language outcome. Our study confirmed the thesis that atypical language lateralization (in terms of LIs) is not necessarily associated with impaired performance during experimental tasks (55–57).

Reorganization According to Dyslexia

The children with CP had poorer phonological processing abilities than controlled typical children (58, 59), and these abilities correlate with their reading skills (60, 61). Several studies point out that reading recognition and reading comprehension abilities are lower than verbal intelligence in patients with cerebral palsy, although there were some inconsistencies in the findings (62, 63). In accordance with these data, five out of seven of our patients had dyslexia and three of them were with normal intelligence (IQ above 70).

Language activation in adults with isolated dyslexia is slightly right lateralized, in contrast with typical readers with left-lateralized activation. This suggests that the activation in the right hemisphere

in isolated dyslexic individuals is likely to be the cause rather than the consequence of reading impairment (64). These speculations may be inferred to UCP patients with dyslexia, although the effect of the structural damage should not be underestimated. Accordingly, four out of five patients with dyslexia in our study had right hemispheric activation with or without left hemispheric activation during silent word generation task.

Four out of five patients with CSL and one out of two patients with PVL had dyslexia, i.e., it could be suggested that left hemispheric lesion, especially including left frontal cortex could result in dyslexia. The left inferior frontal gyrus is associated not only with articulation but also is involved in phonological processing (65). Activation in this area is positively correlated with reading ability (65).

Dyslexia correlates to some extent with motor reorganization and performance in our patients as three out of five dyslexic patients had worse MACS grades (grade 2 and 3) and contralesional or bilateral activation during the motor task, while the two patients without dyslexia had better MACS grades (grade 1) and ipsilesional motor activation.

These results require further studies to clarify the relationship between dyslexia and the type and size of the lesion in left hemisphere.

Limitations and Factors Influencing the Results

The strongest limitation of the language fMRI task was the impossibility to evaluate the exact execution of the task by the participants inside the MRI, although all the participants were asked to reproduce verbally the task after the experiment. Silent, but not vocal, word generation is really important to the experiment in order to avoid activation in motor areas involved in language production.

Many factors could influence the task performances, either related or unrelated directly to UCP. In terms of age, there is evidence in previous studies that left lateralization for language production gets stronger with age (66–68). This stronger shift to the left hemisphere occurs in healthy subjects in late childhood and adolescence and is independent of the region of interest used for calculation of LI (whole brain, prefrontal cortex, frontotemporal regions) (68). Our study population, however, contains patients between age 11 and 29 and these age effects should be minimal or finalized.

Epilepsy is a common comorbidity in patients with CP, as well as in our patient group (five of eight patients have epilepsy and are under medication). There are evidences that both epileptic activity and medication (especially carbamazepine) could influence cognition and cognitive and language representation in the brain (69–71). All five patients with epilepsy in our study had atypical language representation (bilateral or contralesional—in the right hemisphere), so it could be speculated that factors playing a role in "shifting" of verbal production in the right hemisphere could be a result not only from the lesion itself, but also from other factors like epileptiform activity or antiepileptic drugs. The possible effects of epileptic activity on language representation was discussed by Lidzba et al., and a suggestion was made that evaluation of language production in nonepileptogenic lesions is somehow more reliable due to the lack of confounding effect of epileptic activity (54). In larger sample study antiepileptic drugs and epileptiform activity could be evaluated as predictive or significant factors for language representation.

CONCLUSION

Despite the limitations of the study (small sample, different type of brain lesions, some confounding factors), several conclusions could be made:

- Patients with small PVLs have ipsilesional representation of primary motor, sensory, and word generation function. This, however, does not strictly correlate with better outcome, especially in terms of language and cognition—one of the patients has borderline IQ score and the other one is dyslexic, although with normal intelligence, but both had very good motor capacity and no sensory deficit.
- Patients with lesions involving left CSL regions show various models of reorganization in all three modalities (ipsilesional, contralesional, and bilateral) and different clinical outcomes that seem to be impossible for prediction. Anyway, there is a tendency of larger lesion being associated more frequently with motor and language shift to the contralesional hemisphere, and atypical location of primary sensory cortex. Patients with UCP who demonstrate ipsilesional motor cortical activation have better motor functional capacity.

As this is a pilot study with only eight patients, the conclusions made are exploratory. Much larger sample and additional correlation with morphological data (volumetry, morphometry, tractography) is needed for determination of possible risk or protective factors that could play a role in the complex process of brain plasticity. Despite the mentioned limitations of the study, it is the first one that explores brain plasticity in three modalities at the same time with comparison to anatomical and clinical data.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical committee of Medical University–Plovdiv. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Conceptualization: KG, IP, and II. Methodology: KG, IP, and II. Validation and formal analysis: KG, IP, and II. Investigation: KG, IP, II, ET, AP, and KV. Resources: KG, IP, II, ET, AP, and KV. Data curation: KG, IP, II, ET, AP, and KV. Original draft preparation: KG, IP, and II. Writing—review and editing: KG, IP, II, ET, AP, and KV. Visualization: IP, KG, and II. Project administration: KG, II, and KV.

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Cross-Validation of Functional MRI and Paranoid-Depressive Scale: Results From Multivariate Analysis

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Introduction: There exists over the past decades a constant debate driven by controversies in the validity of psychiatric diagnosis. This debate is grounded in queries about both the validity and evidence strength of clinical measures.

Materials and Methods: The objective of the study is to construct a bottom-up unsupervised machine learning approach, where the brain signatures identified by three principal components based on activations yielded from the three kinds of diagnostically relevant stimuli are used in order to produce cross-validation markers which may effectively predict the variance on the level of clinical populations and eventually delineate diagnostic and classification groups. The stimuli represent items from a paranoid-depressive self-evaluation scale, administered simultaneously with functional magnetic resonance imaging (fMRI).

Results: We have been able to separate the two investigated clinical entities – schizophrenia and recurrent depression by use of multivariate linear model and principal component analysis. Following the individual and group MLM, we identified the three brain patterns that summarized all the individual variabilities of the individual brain patterns.

Discussion: This is a confirmation of the possibility to achieve bottom-up classification of mental disorders, by use of the brain signatures relevant to clinical evaluation tests.

Keywords: validation, psychopathology, machine learning, functional MRI, classification

INTRODUCTION

There exists over the past decades a constant debate driven by controversies in the validity of psychiatric diagnosis (1). This debate is grounded in queries about both the validity and evidence strength of clinical measures and the relevant classification and nomenclature systems (2) and eventually lead into crisis of confidence in psychiatry as medical discipline.

Those queries refer to a large extent to missing cross-validation of the clinical evaluation tools with data and explanatory models from neuroscience (3) and might be summarized in the following caveats.

- Normative and validation standards in psychopathology are fragmented from basic neuroscience, which applies different validation standards and procedures, both on statistical and conceptual levels.
- Fundamentally psychiatric clinical measures are constituted from narratives of the patient (self-assessment scales), the informant, and the expert (clinical rating scales), which are essentially comprised of subjective introspective and inter-subjective Likert scale items (4).
- Diagnostic entities in clinical psychiatry are not defined by biological signatures of disease as in the other medical disciplines, but with combinations and/or comparisons of those evaluation scales.

In our previous studies we have attempted to demonstrate the convergent and discriminative construct validity of the Depression Scale (5) and the functional magnetic resonance imaging (fMRI) signal by simultaneous administration of the items from the clinical scale as stimuli (6, 7). In those studies, we have employed neutral items from interest scale as contrast stimuli under block paradigm design. The t-contrasts on the second level of between-group comparison between patients with depression and healthy controls demonstrated significant differences in the activation of various brain regions during diagnostically significant scale items processing, contrasted with the processing of diagnostically neutral ones, notably in the left middle frontal gyrus, among others.

This paradigm has been further expanded by inclusion of paranoid items from Paranoid Depressive Scale (PD-S) by Von Zerssen and schizophrenia patients in order to investigate the contrast across different nosological groups and respective clinical measures (8). This model has been defined in top-down manner, from the clinical definition (psychiatric interview) to the corresponding brain activation determined by fMRI, administered simultaneously with clinical assessment scale (PD-S). Although certain encouraging results appeared on within-group level, they did not cross the statistical significance threshold on the between-group analysis level. We assumed that several factors undermine the translation of the functional MRI results to clinical measures in our data set. On one hand these disease entities might be assumed as a continuum of manifestation of one and the same underlying neurodegenerative or neuro-progressive process, as it is supported with reported abnormalities in the grey matter volume in patients with depression detected with voxel based morphometry (9–12). On the other hand, the included diagnoses may well represent discrete entities and the small number of recruited patients might be considered as confound in this study. Other caveats concern the innovative and non-conventional approach to the experimental paradigm design, which presents an issue for comparison with other studies in the field and the gender structure of the sample (8).

One critical premise of that model for translational validation is an exemplar instrumentalist validation (3), however including more robust biological reference measures. This approach is based on the assumption that scientific knowledge is instrumental: basically, it can provide us with suitable information about some limited domain of phenomena, and it explains and solves problems associated with that domain. In our case it would be instrumental to discriminate two clinical measurement constructs (paranoia and depression) with an incremental external validity operation, such as fMRI without any claim that those can delineate diagnostic entities in the medical sense, i.e. real nosological entities.

However, the data collected in our study are multi-dimensional both in space with a large number of voxels and including multiple observations per variable and highly correlated. Therefore, we have decided to complement the more conventional two sample t-tests analysis with multivariate methods, namely multivariate linear model (MLM) (13). Multivariate analysis is widely used in studies with highly-dimensional data and multiple variance. Furthermore, the method measures the strength of the relationship amongst variables and summarizes data about the individual differences. These methods have already been successfully applied to datasets from neuroimaging (14, 15) and on rather limited scale in psychiatry (16, 17).

AIM

In this context the aim of the present study is to identify by means of multivariate analysis the underlying biological signatures comprised of brain signals which may explain the variance across clinical diagnostic measures, presented simultaneously with the acquisition of the fMRI signal, such as depression (DS) and paranoid (PS) scale scores, particularly incorporated within PD-S, and diagnostically neutral (DN) items from the same interest scale as employed in our previous studies. In this way we may foster the diagnostic validity of the clinical measures and disease entities in question.

The objective of the study is to construct a bottom-up unsupervised machine learning approach, where the brain signatures identified by three principal components based on activations yielded from the three kinds of stimuli (DS, PS, and DN) are used in order to produce cross-validation markers which may effectively predict the variance on the level of clinical populations and eventually delineate diagnostic and classification groups.

METHODS

Subjects

We recruited 30 adult psychiatric patients with either a diagnosis of schizophrenia (n = 16, mean age 36.4 ± 12.5 y, 10 males), or depressive episode (n = 14, mean age 45.3 ± 12.5 y, five males). Subjects were assessed by an experienced psychiatrist using a comprehensive clinical interview and the structured Mini International Neuropsychiatric Interview (M.I.N.I 6.0) (18) as well as the Montgomery-Åsberg Depression Rating Scale (MADRS) (19) and the Positive and Negative Syndrome Scale (PANSS) (20). Diagnosis was based on the clinical interview, the assessment scales, and the available information from past psychiatric examinations, as well as from relatives/caregivers.

Inclusion criteria for the schizophrenic group were the following: 1) Diagnosis of Schizophrenia according to *DSM-IV TR* 2) Age 18 to 65 years. 3) PANSS total score at least 60. For the depression group subjects had to comply with the *DSM-IV TR* criteria for depressive episode (either in the context of major depressive or bipolar disorder), with MADRS score at least 20 and age between 18 and 65 years.

Patients were excluded if they had a comorbid psychiatric disorder (such as anxiety, substance related disorder), major medical illness, neurological disease, history of head trauma with loss of consciousness, or metal implants not compatible with the MRI. All participants provided a written informed consent complying with the Declaration of Helsinki and the study was approved by the university's ethics committee.

Data Acquisition

Patients were scanned on a 3T MRI system (GE Discovery 750w), starting with a high resolution structural scan (Sag 3D T1 FSPGR sequence), slice thickness 1 mm, matrix 256x256, relaxation time (TR) 7.2 ms, echo time (TE) 2.3, and flip angle 12°, followed by a functional scan (2D EPI sequence), with slice thickness 3 mm, matrix 64 × 64, TR 2,000 ms, TE 30 ms, and flip angle 90°.

Paradigm

The paradigm consisted of three different active conditions and one rest condition, with a total duration of 11 min and 44 s presented in a classic block design. Each active block lasted for 32 s and contained four text statements of 8 s. The statements of the Depression Specific (DS) and the Paranoia Specific (PS) blocks were taken from the von Zerssen depression and paranoia subscales accordingly. As in our previous study (7), there were also Diagnostically Neutral (DN) blocks consisting of four statements from a questionnaire about general interests and likes. Under each written statement four possible answers ("completely true," "mostly true," "somewhat true," "not true") and the respective four response buttons (upper left, lower left, lower right, upper right) were presented. In total there were four blocks of each type, and they were alternating between the three active conditions. After each active block a 20 s resting block followed with a fixation cross in the middle of the screen (DS:_DN:_PS:_DS:_).

Image Processing

The SPM 12 software (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm/) was used for the processing the functional data. The images were realigned, co-registered with the structural ones, normalized to Montreal Neurological Institute space, and smoothed with a 8 mm full-width-at-half-maximum Gaussian kernel. A general linear model was defined and the F-contrast on all three conditions was derived. The F-contrast map of each participant was used in the following analysis.

Multivariate Analysis

MLM is a method that is applied on the highly-dimensional data and creates a reduced set of features of the original data with minimal loss. The advantages of this method are threefold. First, unlike other dimension reduction methods such as principal component analysis (PCA), MLM takes into account information coming from the data (Y) and the information (contextual, experimental, behavioral, etc.) encoded in design matrix (X).

Second, MLM is specially adapted to fMRI data in particular taking into account temporal autocorrelation of the noise. Third, as MLM takes into account noise, it can be embedded into statistical framework for making inferences. We choose MLM because it is the most suited for fMRI data. The MLM analysis is implemented in the SPM toolbox Multivariate Methods for fMRI (https://github.com/LREN-CHUV/MLM).

We went through the following steps in our analysis:

1) First, we performed an MLM analysis for each individual (**Figure 1**, Individual Level MLM). The individual MLM analyses identify for each participant the brain patterns that explain most of the changes in the fMRI activity and that are most correlated with the clinical conditions (PS, DS, and DN).

   Our paradigm, as described earlier, was represented in a design matrix X which encoded three types of stimuli (PS, DS, and DN). Nuisance covariates included the six rigid body motion parameters were also added to the design matrix.

   According to MLM algorithm, for each subject i (i=1..s) we calculate the principal components of matrix $Z_i = \left(X_i^{'}\Sigma_i X_i\right)^{-1/2} X_i^{'}Y_i$, where X_i is a design matrix [time by covariates (three conditions and nuisance covariates)], Y_i is a data matrix (time by voxel), $X_i^{'}Y_i$ is their complex correlation normalized with $\left(X_i^{'}\Sigma_i X_i\right)^{-1/2}$, Σ_i represents the temporal covariance matrix of the data. For each matrix Z_i we search the decomposition $Z_i = U_i\Lambda_i V_i^{'}$, where U_i model parameters eigenvectors, Λ_i diagonal matrix of eigenvalues, V_i spatial eigenvectors. The model parameters eigenvectors are referred as clinical loadings and the spatial eigenvectors are referred as eigenimages. To consider only three active conditions (PS, DS, and DN), the space of interest for MLM analysis was defined by an F-contrast encompassing these condition, as mentioned earlier. As a result, we obtained three eigenimages for each subject that are used at the next step.
2) Second, to summarize the information from the individual MLMs, we then performed a second MLM analysis (**Figure 1**, Group Level MLM) using the brain patterns from the previous step while removing the confounding effects of age and gender.

   Thus, at this step we build the matrix $Z_G = \left(X_G^{'}\Sigma_G X_G\right)^{-1/2} X_G^{'}Y_G$ where X_G is the design matrix [subjects by covariates (diagnostic groups, age, gender)], and Y_G=[V_1,V_2,…,Vs] is a concatenation of eigenimages (number of active conditions by voxel) of each subject. We decompose matrix $Z_G = U_G\Lambda_G V_G^{'}$. The V_G identify the most consistent brain pattern across individuals in terms of variance explained, while to quantify individual differences we use the subject loadings U_G (i.e. the contribution of each subject to the main brain pattern).

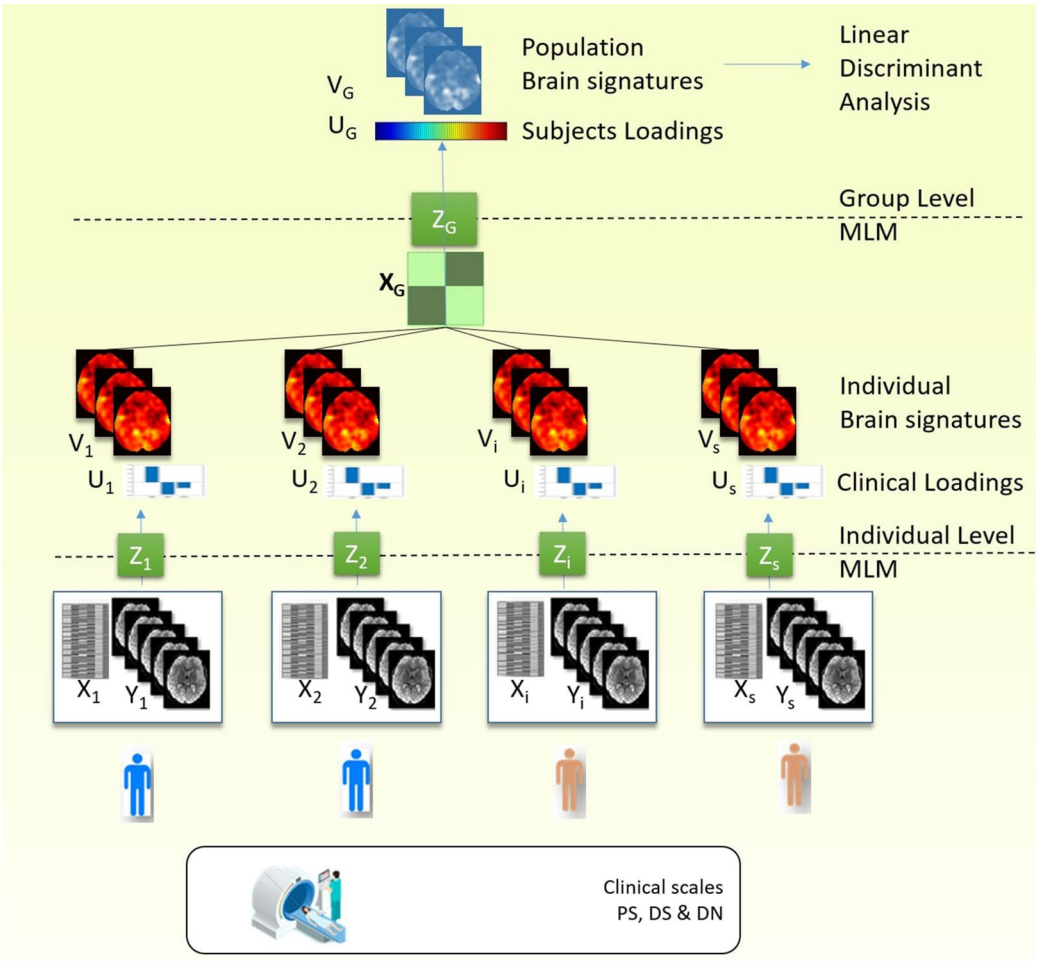

FIGURE 1 | Procedure stages: 1) Individual MLM: MLM decomposed covariance matrix between the fMRI data and the design matrix which contained the clinical scale. As a result, we obtained three components (or clinical loadings) and three brain signatures (or eigenimages). 2) Group MLM: The individual eigenimages obtained from the previous step for each subject are aggregated in the group analysis, and MLM analysis is performed on the covariance matrix between eigenimages and the design matrix which contained the diagnostic label and confounding variables (gender and age). As a result we obtained group level brain signatures (or eigenimages) and the subject loadings that discriminate between the diagnostic groups. 3) To test the predictive ability of the brain signature we use linear discriminant analysis and the subject loadings to classify the individuals in two diagnostic groups and test the accuracy rates using k-fold cross-validation.

3) In the last step we applied a linear discriminant analysis classifier (LDA in Statistics and Machine learning toolbox, version 11.0, Matlab R2016b) on each of the three subject loadings. The purpose of this final step is to test if the brain signatures can accurately discriminate the two clinical entities. Statistical significance of the final results, meaning the ability to discriminate diagnostic groups using unthresholded brain signatures was ensured by the use of linear discriminant analysis and k-fold cross-validation. We report the accuracy of classification with receiver operating characteristic (ROC) curves.

Figure 1 describes the schematic of our approach for discovering the brain signatures. To identify the brain signatures, we use multivariate method both at individual and group/population levels.

RESULTS

Demographic and Clinical Characteristics

The two patient groups did not differ significantly in their demographic and clinical characteristics (**Table 1**).

MLM Results

The individual MLMs showed a consistent profile across the different participants (see **Figure 2**, Clinical Loadings to the right side). In all the subjects, the first component that explained most of the variance corresponds to positive loading for the DS and DN and negative loadings for PS. The second component, shows a positive loading for DS and PS and negative loadings for DN, finally the last component shows a positive loading for PS and DN and a negative loading for DS.

TABLE 1 | Demographic and clinical characteristics of the samples.

	Schizophrenia patients (n = 16)	Depressed patients (n = 14)	Statistical significance
Age (mean ± SD)	36.4 ± 12.5	45.3 ± 12.5	0.064[a]
Sex (M/F)	10/6	5/9	0.143[b]
Education (secondary/higher)	11/5	8/6	0.452[b]
Age at onset (years)	28.5 ± 7.7	35.9 ± 11.2	0.099[a]
Illness duration (months)	93.8 ± 84.6	145.0 ± 86.0	0.200[a]
Episode duration (weeks)	8.6 ± 6.3	11.7 ± 9.4	0.419[a]

SD, standard deviation. [a] Independent samples t-test, [b] χ^2 test.

Following the individual and group MLM, we identified the three brain patterns that summarized all the individual variabilities of the individual brain patterns (see **Figure 2**). The first brain signature shows positive pattern that covers visual parietal, motor cortices and it also expands to the frontal lobes. The second brain signature was mostly characterized by a positive pattern in the temporal and negative pattern in the frontal and parietal lobes. Finally, the third signature had mainly medial temporal and mid-frontal contributions for the positive and negative signature respectively.

Figure 3 (left) represents the accuracy of the linear discriminant analysis on subjects' loadings for three signatures. The signatures were taken both for positive and negative patterns without thresholding. The accuracy was measured using k-fold cross validation with $k = 2$ and repeated 100 times to estimate the medians and 25th and 75th percentiles of its distribution. The median accuracy was respectively 0.67, 0.83, 0.90 for the first, second, and third signatures respectively. The performance of the classifier for each signature is measured with the ROC curves using schizophrenic group as reference (**Figure 3**, right).

DISCUSSION

In this current research, we have been able to separate the two investigated clinical entities—schizophrenia and depression by use of brain signatures derived from a task related fMRI where the paradigm comprised of answering to a self-assessment scale. This is a confirmation of the possibility to achieve bottom-up classification of mental disorders, by use of the brain signatures relevant to clinical evaluation tests.

However, there are several methodological issues to be discussed. On one hand, the small sample size might have influenced the results. On the other hand, the paradigm used was designed to discriminate between schizophrenia and depression by means of a contrast of the BOLD signal acquired during the depression and paranoia items processing but as we know both from clinical practice and psychiatric research these two domains may overlap (21). Symptoms of depression are often seen in schizophrenia and PCA of the PANSS items has revealed an anxio-depressive component highly correlated with other depression scales Hamilton depression rating scale (HAM-D), Calgary Depression Scale for Schizophrenia (CDSS) (16). Moreover, by means of PCA of ten frequently used negative symptom scales and structural brain imaging, Chuang et al. were able to find distinct correlation between the components and the white and gray matter volumes of different regions in a group of patients with schizophrenia and depression respectively (22).

Despite these limitations our study is adding to the growing body of evidence that multivariate approaches can be reliably used for distinguishing major psychiatric disorders by their respective brain signatures. For example, patterns derived from structural MRI have been used to discriminate between schizophrenia and healthy controls with high specificity and sensitivity ranging from 80% to 90%, and a bit less than 90% for schizophrenia versus bipolar disorder, as well as around 80% when compared to major depression (23–26).

Connectivity measures have also been used to distinguish between schizophrenia and healthy controls or depressed patients with an accuracy rate of 70% to 80% (27, 28). In a multisite study on fMRI (obtained under resting or different cognitive and emotional tasks), Orban et al. were able to achieve a discrimination rate of schizophrenia patients versus healthy controls as high as 84% (29). Thus, our accuracy rate of 67% to 90% is comparable to the results stated in most of the published literature to date. However, surprisingly the first two components that explain most of the variance did not necessarily led to the highest accuracy. This highlights again the limitation of psychiatric diagnostic entities. Put simply, there is a lot of variance due to biological processes although related to the disease that do not entirely correspond to the diagnostic groups. The first two brain signatures presents high contribution of the sensory cortices (motor or visuals), the third signature shows brain patterns with high loads in the temporal, parietal, and frontal regions. Unlike previous methods our two step hierarchical approach using semi unsupervised method allows to uncover these underlying biological processes and to identify the ones predictive of the diagnostic groups.

Moreover, what distinguishes our research from similar classification studies in the field is that our paradigm is based on the application of clinically relevant evaluation tools (in this case the PD-S) not just resting state or tasks that are irrelevant to the everyday patient assessment. In this way, our approach has the potential to practically bridge the gap between neuroscience and bedside care. We believe that the current research represents an advancement of the theoretical concept of the translational validation supporting it with further empirical results (25).

In contrast to our previous study where classical contrasting of the BOLD-signal elicited by the processing of the paranoia or depression items has failed to reveal statistically significant differences between the two clinical samples (despite the apparent differences), here by means of PCA and MLM we have achieved a meaningful distinction on the group level in a bottom-up fashion. This is in support of the further use of these

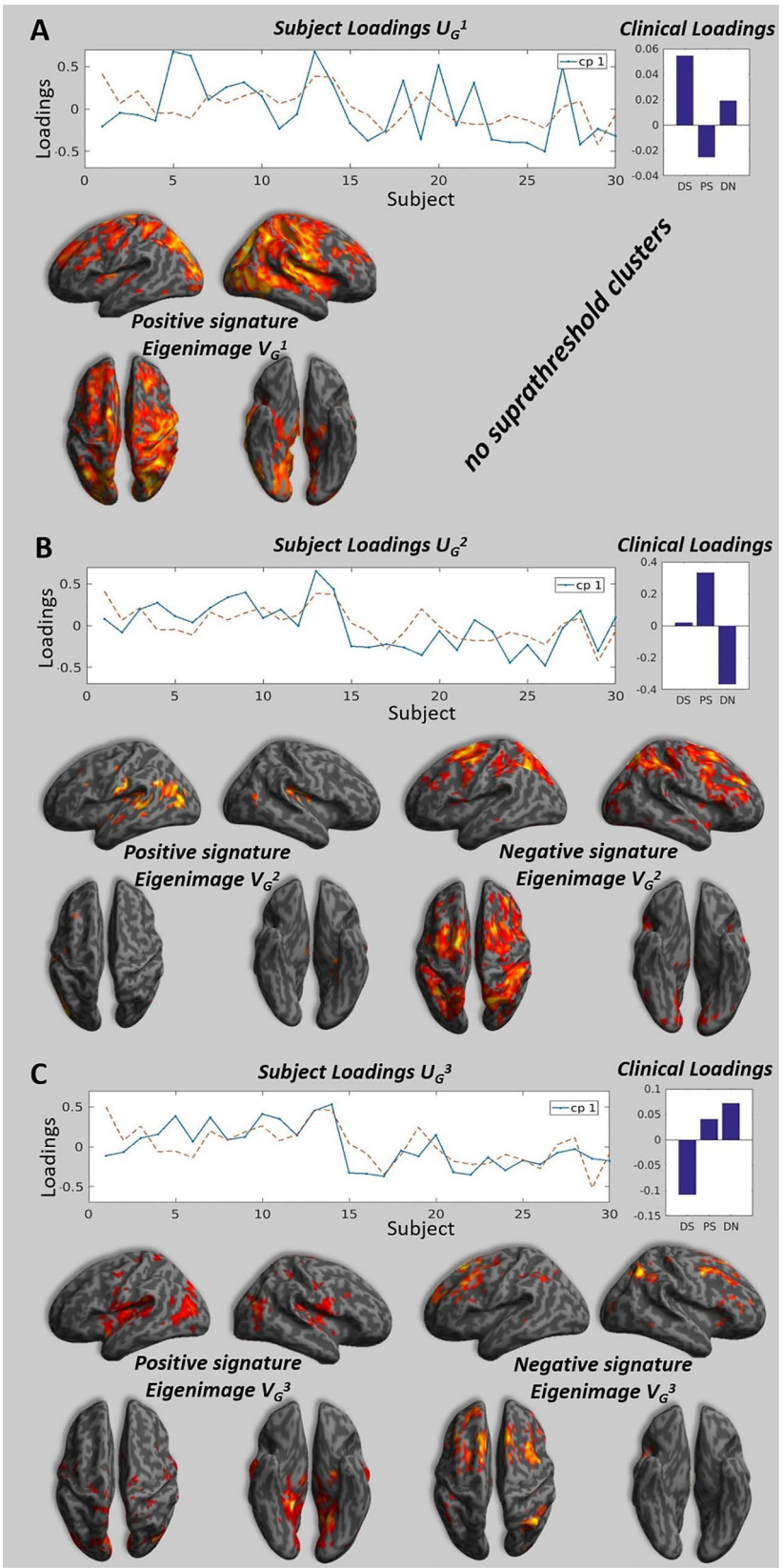

FIGURE 2 | Continued

FIGURE 2 | Brain signatures and subject loadings across all participants. Panels **A**, **B**, and **C** show the subject loadings for the first, second and third components, the corresponding signature and the clinical loadings. The subject loadings are shown as the solid blue line on the graph, the dotted line represents the projection of subject loadings in the design space (the units are arbitrary). The signatures represent the correlation between the subjects loadings and the value at each voxels. We project the strength of this correlation measure by a T-test on a 3D brain for illustration purposes, please note that the only valid test is the multivariate test that take into account all the voxels in the brain (see Kherif 2002 for details) and all the voxels with the appropriate weighting are taken into consideration when performing classification. The clinical loadings are the averaged clinical loadings of each subject calculated at the individual level MLM and weighted by the subject loading obtained at the group level MLM. **(A)** The highest peaks ($T > 3.40$, $p < 0.001$, uncorrected) for the positive pattern were located in the parietal cortex, precuneus, inferior occipital cortex, thalamus, interior cingulate gyrus, postcentral gyrus. There were no voxels significantly different from zero at the same threshold for negative pattern. **(B)** The highest peaks ($T > 1.7$, $p < 0.05$, uncorrected) for the positive pattern were located in the central operculum, superior temporal gyrus, and left hippocampus. The highest peaks ($T > 1.7$, $p < 0.05$) for the negative pattern were located in the superior frontal gyrus, middle frontal gyrus, angular gyrus. **(C)** The highest peaks ($T > 1.7$, $p < 0.05$, uncorrected) for the positive pattern were located in the lingual gyrus, precuneus, planum temporale, hippocampus, and insula. The highest peaks ($T > 1.7$, $p < 0.05$) for the negative pattern were located in the middle frontal gyrus, superior frontal gyrus, and angular gyrus.

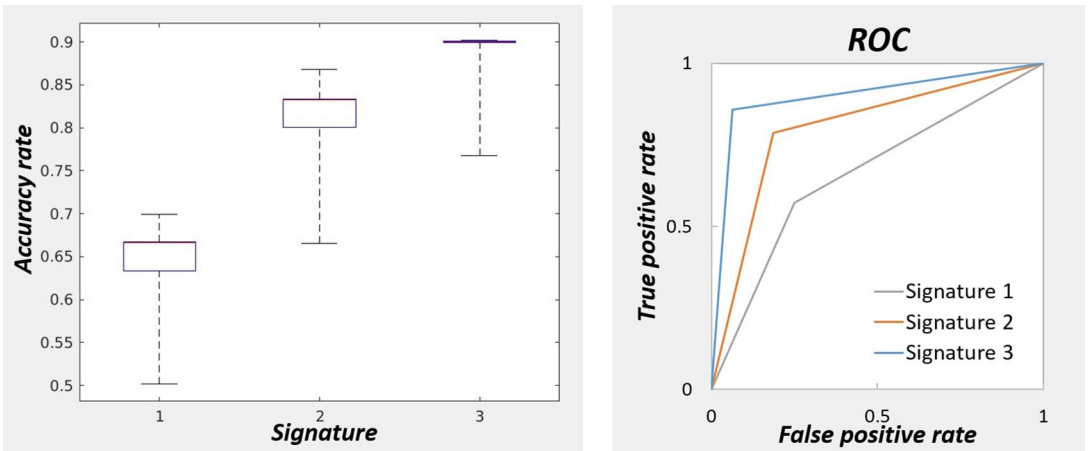

FIGURE 3 | On the left: Accuracy of the classifier for three signatures for predicting the diagnostic labels. The accuracies were obtained by cross-validation repeated 100 times to obtain the percentiles. The highest accuracy was obtained with the brain signature 3. On the right: Performance measurement of the classifiers for three signatures with receiver operating characteristic curve.

techniques as they might better reflect the complexity of both the neuroimaging data as such and the respective diagnostic classes.

CONCLUSION

This paper is supposed to complement our previous publications (6–8) which used conventional approach for top-down cross-validation of clinical self-evaluation diagnostic scale and fMRI, with rather limited results. Here, we demonstrate that by use of the items from the same clinical scale as fMRI stimuli and the means of machine learning it is possible to discover the brain signatures behind different psychiatric diagnostic classes and respective clinical measures.

This approach may potentially encourage in future re-validation of both psychiatric classifications and methods of assessment based on more robust neuro-biological evidence.

DATA AVAILABILITY STATEMENT

The data are made available to public on the following address: https://doi.org/10.5281/zenodo.3497072.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee, Plovdiv Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DS has formulated the concept behind the study as exposed in the introduction. FK has produced the methods and results. SK delivered the discussion. RP has been reponsible for the data management and statistical analysis. AL and JB performed second level statistical analysis and generated the figures in the article.

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The BDNF Val66Met Polymorphism Has No Effect on Encoding-Related Hippocampal Response But Influences Recall in Remitted Patients With Bipolar Disorder

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Background: Cognitive impairments in bipolar disorder (BD) such as memory deficits are associated with poor functional outcomes and it has been suggested that the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism contributes to individual variability in memory function in BD. The current study investigated the relationship between the BDNF Val66Met polymorphism, neural activity during a picture-encoding task, and subsequent memory recall.

Methods: A total of 70 patients with BD grouped according to genotype [ValVal or Met carriers (MetVal/MetMet)] underwent fMRI while performing a picture-encoding task. Memory for the encoded pictures was tested with a subsequent free recall memory task.

Results: There was no difference between the ValVal homozygotes and Met carriers in the involvement of hypothesized memory encoding regions i.e. hippocampus and dorsal prefrontal cortex (dPFC). However, an exploratory whole-brain analysis showed greater encoding-related lateral occipital cortex activity in Met carriers. Behaviorally, Met carriers also showed better free recall of the encoded pictures.

Conclusions: We found no effect of the BDNF genotype on encoding-related hippocampal and dPFC activity in BD, although Met carriers showed superior memory performance after the scan, which could be related to more efficient perceptual processing during encoding.

Keywords: hippocampus, cognitive impairment, affective disorders, fMRI, BDNF val66met genotype

BACKGROUND

Cognitive impairments in bipolar disorder (BD) are associated with reduced functional capacity and poor prognosis (1). Specifically, patients' verbal memory and executive function are among the strongest predictors for vocational capacity (2). The pattern of cognitive impairment in BD is heterogeneous, as some patients remain cognitively intact while up to 60% present with selective or global impairments (1). It is likely that genetic factors play a role in this heterogeneity, but the contribution of risk genotypes to the cognitive impairments in BD is poorly understood. It is important to gain a better understanding of these relationships and the factors that contribute to the cognitive heterogeneity seen in BD in order to offer personalized treatments and identify new therapeutic targets.

Several candidate vulnerability marker genes believed to confer risk of cognitive deficits in psychiatric disorders have been explored using cognitive assessments and imaging genetics (3). These include the Val66Met single nucleotide polymorphism in the gene encoding brain-derived neurotrophic factor (BDNF) and its relationship with memory function. In the Val66Met polymorphism, a valine to methionine substitution at codon 66 results in a switch from guanine to adenine at position 196 in the pro-region of the BDNF gene, leading to reduced activity-dependent BDNF secretion and potential associated changes in hippocampal functions such as episodic memory (4, 5).

There is some evidence suggesting that the Val66Met polymorphism affects cognition and neural processing, although findings from existing studies are mixed (6–10). A systematic review of studies using clinical and healthy populations found that 23 of 63 studies showed a significant relationship between memory and Val66Met carrier status (11). For example, Egan et al. (10) found reduced free recall memory for an auditory short story in Met carriers, which is known to rely on the hippocampus, although this effect of genotype was not present on a second measure of free recall [California Verbal Learning Test (CVLT)]. Some studies have also reported relationships between Val66Met and aspects of memory that are known to be less dependent of the hippocampus such as recognition memory (12, 13). However, in a study using a similar task to the task used in the current study, Dodds et al. (6) presented healthy participants with scene pictures during encoding and asked participants to make indoor/outdoor judgments of the pictures as they were presented. Subsequently, participants completed a recognition memory test indicating whether the presented pictures were old or new. This study found no differences in memory performance or neural activity during encoding between BDNF Val66Met genotypes among these healthy individuals. However, subsequent studies using similar paradigms have found both reduced (9) and increased (8) MTL activity during memory encoding, and hence, inconsistencies in studies with healthy participants remain.

Given the albeit mixed evidence for impact of BDNF genotype on memory performance and related neural processing across healthy and psychiatric populations, it is pertinent to consider that BDNF genotype might play a role in the cognitive heterogeneity seen in BD but only few studies have investigated the relationship between memory and Val66Met genotype in BD. One study showed that Met carriers with BD displayed reduced verbal memory performance (and smaller hippocampal volumes) compared to both healthy and depressed Met carriers, suggesting a specific disadvantage for Met carrier BD patients on memory function (14). Another study using a more general screening for cognitive function including memory did not find an association with Val66Met status (15). To our knowledge, no studies have investigated the neural underpinnings of the association between BDNF Val66Met genotype and memory performance in BD, which may provide a more sensitive measure of the relationship between genotype, memory function, and neural mechanisms.

In this study, we investigated the effects of BDNF genotype on encoding-related hippocampal and prefrontal cortex (PFC) activity and memory retrieval in a strategic picture-encoding task in 70 patients with BD in full or partial remission. We used a picture encoding task identical to Dodds et al. (6) but used a free recall memory task to assess memory performance instead of a recognition task. Free recall of complex scenes relies heavily on the hippocampus (16) and also the PFC (17, 18) and these neural mechanisms appear to be specific for free recall over for instance item memory (19–21). Further, studies have indicated a possible advantage of Met carriers in terms of PFC function and working memory (22–24). For instance, one study showed that over-expression of BDNF in mice resulted in decreased working memory function (23). Also, altered PFC activity has been associated with cognitive deficits in BD (25, 26). Hence, we hypothesized that i) Met carriers would show reduced hippocampal and/or increased dorsal PFC (dPFC) activity during picture encoding and ii) this altered activity would be associated with impaired free recall for the encoded pictures.

MATERIALS AND METHODS

Participants

Seventy BD patients in partial or full remission were included in the study. Baseline data for the patients was obtained from patients' participation in two randomized intervention studies investigating cognitive function in BD (27, 28). Participants were between 18 and 65 years of age (mean ± SD; 37 ± 10) and had normal or corrected-to-normal vision. All participants were screened with Schedules for Clinical Assessment in Neuropsychiatry (SCAN) to confirm diagnosis of BD. Participants were also rated on the Hamilton Depression Rating Scale [HDRS-17; (29)] and Young Mania Scale [YMRS; (30)] to confirm that they were in partial or full remission (HDRS and YMRS scores ≤14). The original studies were approved by the local ethics committee, Danish Data Agency, and Danish Medicines Agency, and written consent was obtained from all participants prior to beginning of the study. For further details on the recruitment and screening processes, please see previous studies (27, 28).

Genotyping

Genomic DNA was extracted from blood samples with the Maxwell Blood DNA purification kit (Promega, Madison, WI, USA) in accordance with the manufacturer's protocol and the samples were genotyped using the Illumina Infinium PsychArrayBeadChip (Illumina, San Diego, CA, USA).

Magnetic Resonance Imaging

fMRI data was collected at the Danish Research Centre for Magnetic Resonance with an eight-channel head coil on a 3T Siemens Trio MR scanner (Siemens, Erlangen, Germany). Blood oxygen level-dependent T2*-weighted functional images were acquired using echo-planar imaging (EPI) with the following parameters: repetition time (TR), 2490 ms; echo time, 30 ms; slice thickness, 3 mm; field of view (FOV), 192 × 192 mm using a 64 × 64 grid, flip angle 20°. A total of 117 volumes were acquired in a single fMRI session and each volume consisted of 42 slices. A field-map was recorded to allow distortion correction of the acquired EPI images. Participants also underwent a high-resolution structural scan where a T1-weighted 3D structural image was acquired to co-register with the functional images, with the following parameters: TI = 800, TE = 3.93, TR = 1540 ms, flip angle 9°, 1 × 1 × 1 mm voxel size, 256×256 FOV, and 192 slices.

fMRI Data Analysis

Pre-Processing

Pre-processing was carried out with the fMRI Expert Analysis Tool (FEAT; version 6.0) in FMRIB's software library (FSL; www.fmrib.ox.ac.uk/fsl). Standard pre-processing steps included non-brain removal, realignment, correction for B0 field distortions, slice time correction, and spatial normalization of functional and structural images to a Montreal Neurologic Institute (MNI) template. The normalized functional images were spatially smoothed using an isotropic Gaussian smoothing kernel with a full width at half maximum (FWHM) of 5 mm. The time series in each session was high pass filtered (to max. 0.008 Hz).

Statistical Analysis

At the first level, picture encoding and rest events were modelled as boxcar functions and convolved with a canonical hemodynamic response function. A picture encoding BOLD contrast was computed by subtracting non-encoding periods from the picture encoding events.

At group level, we assessed differences in the picture-encoding contrast task between the two genotype groups. The group-level statistical estimation was carried out using nonparametric permutation tests with the FSL Randomise tool (http://www.fmrib.ox.ac.uk/fsl/randomise/index.html, 31) using default settings and 5000 permutations. This method was used as it has been shown that permutation testing produces nominal results for clusterwise inference, while parametric statistical methods failed to do so (32). ValVal and Met carrier genotype groups were contrasted for each of the pre-determined regions of interest (ROIs). The ROIs were constructed on the MNI template and included bilateral hippocampi and the dPFC. The hippocampal ROI was anatomically defined, while the dPFC ROI was a spherical ROI based on a previous paper investigating dPFC activity during encoding and subsequent free recall (33) (**Figure 1**). The bilateral hippocampus was defined using cortical maps thresholded at 30% provided by the Harvard-Oxford cortical structure atlas implemented in FSLView (34). In addition to these ROI analyses, we conducted an exploratory whole-brain analysis applying a brain mask to investigate whether genotype groups' encoding-related activity differed in any other (unforeseen) regions. For analyses at the group level, demographic variables including age, sex, years of education, and clinical variables (HDRS and YMRS) were modelled as regressors of no interest. The resulting data were assessed using Threshold-Free Cluster Enhancement (TFCE) to identify potential clusters (35).

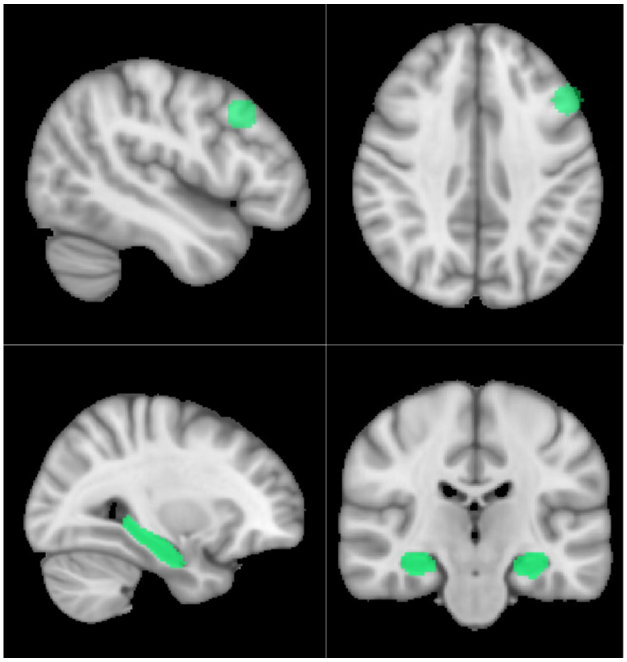

FIGURE 1 | Visualization of the dPFC (above) and hippocampal (below) ROIs.

Finally, we extracted the encoding-related BOLD signal change from the predefined ROIs and any additional regions showing differential response between genotype groups and correlated these values with participants subsequent recall score.

Picture Encoding and Retrieval Task

Stimuli pictures consisted of images of complex scenes collected from the International Affective Picture System [IAPS; (36)]. Several studies have demonstrated that encoding of such complex images is related to hippocampal activity (9, 37). All images were matched for valence, arousal, and complexity. Participants received instructions on the screen prior to the beginning of the task. During fMRI, participants viewed six 24-s picture blocks, each block consisting of six pictures. Each picture was presented on the screen for 3 s followed by an inter-stimulus interval (ISI) of 1 s in a pseudo-randomized order. Each picture block was followed by a short rest in which participants saw a fixation cross presented in the middle of the screen for 24 s. The total duration of the task was approximately 5 min. Participants were instructed to pay careful attention to the presented pictures, as they would be required to complete a subsequent memory task. Hence, the task comprised a strategic memory-encoding component. Participants were also instructed to make indoor-outdoor judgments for each picture, ensuring that participants paid attention to the task. Each picture block contained an equal number of indoor and outdoor scenes.

Immediately following the scanning session, participants completed a free recall test of the pictures, in which participants

were instructed to tell the experimenter about as many pictures they could remember and describe each picture in detail. One point was awarded for each remembered picture, yielding a total free recall score.

Statistical Analysis

Analyses of behavioral data and extracted mean percent signal change from the identified clusters in the FSL Randomise analysis were performed using SPSS (version 22; IBM Corporation).

As only a small number (N = 4) of participants carried the MetMet genotype, ValMet and MetMet carriers were pooled together in a Met-carrier (MetCar) group (N = 26) and compared to ValVal homozygotes (N = 43). Performance on the picture memory task was calculated as the number of correctly recalled images on the free recall test. For each participant, a raw score was calculated with a score of 1 being given for each remembered picture. The total number of false positives was also recorded. ValVal vs MetCar genotypes were compared for demographic and clinical variables and performance on memory tests using independent samples t-tests (two-tailed) and χ^2. The relationship between neural activity in ROIs/significant clusters and memory performance was assessed with Pearson's correlations and Fisher's r to z transformation. Likewise, the relationship between clinical measures, age, years of education, memory recall, and neural activity was assessed with Pearson's correlations. Interaction effects between genotype and total number of medications on memory recall and encoding-related activity was assessed with a univariate ANOVA.

For all tests, it was ensured that assumptions were met, and where assumptions were violated, data were either square-root transformed to fit a Gaussian distribution or analyzed with non-parametric tests (Mann-Whitney U). The alpha-level was set at P = 0.05.

RESULTS

Of the 70 participants recruited for the study, two participants had missing behavioral data and were omitted from fMRI analyses, yielding N = 68 subjects (ValVal: N = 42; ValMet/MetMet: N = 26) for analysis. There were no differences between the ValVal and the ValMet/MetMet genotype groups in demographic or clinical variables including age, years in education, age at onset, illness duration, number of hospitalisations, mood symptoms, or medication status (P > 0.05; **Table 1**).

Functional MRI Analysis

Main Effect of Task

To confirm that the task was associated with the expected activity in visual areas and the hypothesized ROIs, we carried out a non-parametric one-sample t-test across all subjects showing that picture encoding was associated with activity in a large network of brain regions, including the dorsal PFC, occipital, parietal, and temporal regions (see **Figure 2**; network shown in green). Across all participants, picture encoding vs. baseline was also associated with increased activity in the predefined ROIs: the bilateral hippocampi and the dPFC (TFCE corrected P < 0.05).

Effect of Genotype

In contrast to our hypothesis, there were no significant effects of genotype on encoding-related neural activity in the bilateral hippocampus, or in the dPFC at P = 0.05 (TFCE) in the ROI analyses. An exploratory whole-brain analysis showed that Met carriers displayed greater activity in a cluster located to the lateral occipital (LO) cortex compared to participants with the ValVal genotype (peak coordinate: x = –58 y = –66, z = –8; **Table 2**; **Figure 2**).

Relationship Between Neural Activity and Memory Performance

Across all subjects, there was no statistically significant correlation between hippocampal activity (mean % signal change) and

TABLE 1 | Means (SD) for demographic and clinical variables, picture memory task performance, and significance levels for differences between the ValVal and the ValMet/MetMet groups in remitted patients with bipolar disorder.

Variable	Genotype		P-value
	ValVal (N = 43)	ValMet/MetMet (N = 27)	
Age, mean (SD)	36.2 (10.3)	37.6 (10.7)	0.58
Years of education, mean (SD)	15.5 (3.2)	15.0 (3.4)	0.51
Age at onset	20.0 (9.3)	21.15 (9.6)	0.67
BD type, no type 1 (%)	18 (48.6)	13 (61.9)	0.33
Illness duration, mean (SD)	16.8 (8.9)	16.8 (11.4)	0.98
No of depressive episodes, mean (SD)	6.1 (5.9)	7.3 (8.7)	0.55
No of manic episodes mean (SD)	8.7 (8.5)	7.4 (10.8)	0.61
No of hospitalisations, mean (SD)	2.7(0.8)	2.7 (0.8)	0.78
Gender, no women (%)	60.0	56.5	0.99
HDRS-17 baseline, mean (SD)	7.6 (5.1)	7.9 (4.8)	0.75
YMRS baseline, mean (SD)	2.1 (2.4)	2.5 (2.4)	0.33
Picture Memory score, mean (SD)	8.0 (5.3)	10.3 (5.0)	0.05
Medication			
Lithium n (%)	17 (45.9)	11 (55.0)	
Anticonvulsants, n (%)	23 (62.2)	11 (55.0)	0.60
Antidepressants, n (%)	14 (37.8)	8 (40.0)	0.87
Antipsychotics, n (%)	14 (37.8)	6 (30.0)	0.51
Benzodiazepines, n (%)	10 (27.0)	5 (25.0)	0.87
Melatonin, n (%)	1 (2.7)	2 (10.0)	0.24
No of medications, means (SD)	2.16 (0.8)	2.10 (1.0)	0.80

HDRS-17, Hamilton Depression Rating Scale 17 items; YMRS, Young Mania Rating Scale; SD, standard deviation.

TABLE 2 | Significant clusters for the picture encoding task. Cluster peak and local maximum is represented with x, y, z MNI coordinates and Threshold-Free Cluster Enhancement corrected P-values.

Task and Region	Side	Cluster TFCE P	Cluster size (voxels)	x	y	z
Picture encoding						
Occipital cortex	L	P < 0.002	192	–58	–66	–8

L, left; MNI, Montreal Neurologic Institute; R, right; TFCE, Threshold-Free Cluster Enhancement.

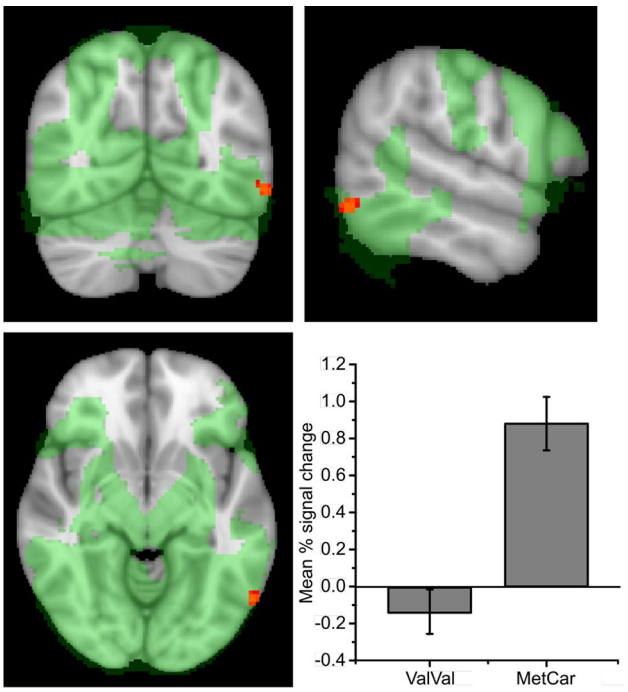

FIGURE 2 | Encoding activity during encoding across all participating remitted patients with bipolar disorder, showing activity in a large network (marked in green). Cluster shows encoding activity for ValMet/MetMet > ValVal (-56 -64 -10). Effects displayed on an MNI template and thresholded at P > 0.05 (TFCE whole-brain). Error bars show SE. Plot shows mean percent blood-oxygen dependent (BOLD) signal change during encoding within the significant cluster. Error bars show standard error (SE).

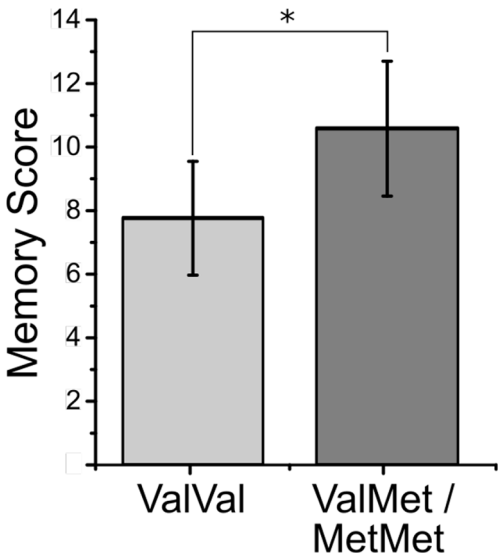

FIGURE 3 | Mean memory score on the picture memory task for the ValVal and the ValMet/MetMet groups in remitted patients with bipolar disorder. Error bars represent SE. Significance of asterisk is at the 0.05 level (exact p-value is P = 0.02).

memory recall, r(68) = 0.07, P = 0.56. There was also no statistically significant difference in this correlation between the ValVal (r = -0.07, P = 0.65) and the Met carrier (r = 0.05, P = 0.80) groups, Z = -0.47, P = 0.64. In the dPFC, there was no statistically significant correlation between memory recall and mean % signal change, r(68) = 0.10, P = 0.42, although when comparing the correlations for the two genotypes, these were significantly different, Z = 2.33, P = 0.02, which was due to a positive correlation between mean % signal change in the dPFC and memory for ValVal [r(42) = 0.29, P = 0.06], while this correlation was negative for Met carriers, r(24) = -0.31, P = 0.13. We also investigated the relationship between memory recall and the activity in the identified significant cluster in the occipital lobe across all subjects but this correlation was also non-significant $r_s(68) = 0.17$, P = 0.17. Furthermore, there was no difference in correlation between activity in the significant cluster and memory recall between the two groups, Z = 0.65, P = 0.51.

Behavioral Analyses

Picture recall after the scan: An independent samples t-test carried out on the square root-transformed data showed that unexpectedly, Met carriers displayed a superior picture memory performance (higher number of correctly recalled pictures) in comparison with Val homozygotes [mean ± SD, Met carriers: 10.3 ± 5.0, Val homozygotes: 8.0 ± 5.3; t(67) = 2.35, P = 0.02, d = 0.61; **Figure 3**]. There were no differences between the two groups in the number of false positives (i.e., "made up" pictures) (U = 437.0, P = 0.71, $\eta^2 = 0.002$). There were no significant correlations between picture recall and sub-syndromal mania or depression symptoms, age, and years of education in either of the two groups or across the entire sample (P ≥ 0.20). Use of antidepressants, lithium, anticonvulsants, antipsychotics, and diazepines was not associated with memory recall (P ≥ 0.13). There was no significant interaction effect between val66met BDNF genotype and total number of medications on memory recall (P = 0.23). The main effect of medication was also non-significant (P = 0.27).

Associations Between Mood, Medication and BOLD Response

To investigate whether associations with mood or medication status could explain our obtained fMRI results, we carried out the following analyses: Across the entire sample, subsyndromal depression symptoms showed a negative correlation with mean % signal change in the bilateral hippocampus *(HDRS; r = -0.25, P = 0.04)*. This effect was due to a strong relationship between HDRS scores and hippocampal activity within the ValVal group (r = -0.49, P < 0.001), while there was no significant relationship between HDRS scores and hippocampal activity in the Met carrier group alone (r = 0.28, P = 0.17). For the dPFC and the identified cluster in the LO cortex, there were no significant relationships between mean % signal change in these regions during encoding and HDRS or YMRS scores across the entire sample (P's > 0.20) or within the genotype groups (P's > 0.19).

Use of antidepressants, lithium, anticonvulsants, antipsychotics, and diazepines was not associated with mean percent BOLD signal change in the hippocampal or dPFC ROIs or in the identified LO cortex cluster (P's ≥ 0.05). Assessing the effects of the total number of medications on neural activity during encoding, there was no

significant interaction between val66met BDNF genotype and total number of medications for encoding-related activity in the dPFC or hippocampal ROIs, or in the identified LO cortex cluster ($P > 0.05$).

Sensitivity Analyses

To rule out the effects of subsyndromal symptoms in the current analyses, we conducted a sensitivity analysis only including the participants in full remission (HDRS < 7; YMRS < 7), yielding a total of 34 datasets for analysis.

Like in the full dataset, participants that were carriers of the Met allele showed numerically better memory performance ($M = 10.17$, $SD = 4.51$) compared to ValVal homozygotes ($M = 7.62$, $SD = 4.40$), although this effect did not reach significance for this subset of participants, $t(31) = 1.59$, $P = 0.12$. Consistent with the analyses conducted on all participants, for fMRI analyses, there was no significant effect of genotype on activity during encoding in the bilateral hippocampal ROI or the dPFC. An exploratory whole-brain analysis did also not show any significant effect of genotype on activity during encoding.

DISCUSSION

We investigated for the first time the effects of BDNF Val66Met genotype on encoding-related hippocampal response and memory performance in partially or fully remitted patients with BD. In contrast to our hypothesis, we found no impact of this BDNF genotype on hippocampal (or dorsal PFC response) during picture encoding. However, an exploratory whole-brain analysis showed that Met carriers displayed greater encoding-related neural activity in the LO cortex compared to ValVal homozygotes. At a behavioral level, Met carriers also displayed superior picture recall following the encoding session in the scanner compared to the ValVal homozygotes.

The lack of an association between BDNF genotype and hippocampal activity during encoding is in accordance with a previous study on healthy volunteers using an identical picture encoding task (6) but contrasts with other similar studies showing either no difference (9) or increased (8) MTL activity during encoding of complex scenes in healthy subjects. The lack of a healthy control group in this study to serve as a baseline challenges the interpretation of the current findings in relation to existing studies in healthy volunteers and hence comprises a limitation of the current study. Nevertheless, investigating the relationship between BDNF genotype, memory, and neural mechanisms in BD alone can still be used to identify potential mechanisms underlying the heterogeneity in cognitive impairment across BD patients. In this context, our results do not suggest that differences in hippocampal processing during memory encoding between Met carriers and ValVal homozygotes contribute to cognitive heterogeneity in BD.

In the exploratory whole-brain analysis, we observed an effect of BDNF Val66Met genotype on neural processing in a cluster located to the LO cortex, with Met carriers showing greater activity during encoding in this region compared to ValVal homozygotes, indicating a potential difference in visual processing that is dependent on genotype. The lateral occipital cortex is known to be involved in visual processing and object perception and recognition (38) and has specifically been implicated in identifying object shapes (39). Why encoding-related processing in this area should be greater in Met carriers than ValVal homozygotes is not clear, but this finding is consistent with the observed better memory recall in the Met carrier group, although we did not observe a significant relationship between the mean percent BOLD signal change extracted from the identified cluster and subsequent memory performance.

Surprisingly, we observed better picture recall in BD Met carriers than in ValVal homozygotes. This was in contrast to our hypothesis that Met carriers would show reduced memory performance and also contradicts the majority of studies reporting a relationship between BDNF genotype and memory performance, where higher Met load is often associated with poorer memory in both healthy volunteers (9, 11, 12) and BD (14, 40). In terms of comparison with results from studies using healthy volunteers, we expected that potential effects of Val66Met BDNF genotype would be exacerbated in BD. However, it is possible that residual BD symptoms might instead blunt potential differences due to BDNF genotype. Another possibility is that different methods of memory assessment explain the divergent findings; we used a free recall task of complex scenes whereas others investigating the relationship between BDNF genotype and memory in BD have used other tasks such as the CVLT (41, 42). Hence, it is possible that subtle differences in the mechanisms supporting different memory types might have played a role in the divergent findings, where Met carriers may be impaired compared to ValVal homozygotes on a verbal learning task but not a visual learning task, which is consistent with our finding showing increased activity during encoding in the LO cortex known to play a role in visual processing.

Also, some studies have suggested that there might be a possible advantage of Met carriers in terms of PFC function and working memory (22). Free recall is dependent on both PFC processing and working memory function and we did see a significant positive correlation between dPFC activity during encoding and subsequent memory for the Met carrier group that was not present in the ValVal group. Hence, it is possible that Met carriers more efficiently recruited the dPFC during encoding.

Another point is that the previous studies showing better performance for ValVal homozygotes compared to Met carriers in psychiatric populations have often used samples including both remitted and currently depressed patients (e.g. 15). Such samples may show reduced performance on cognitive tasks due to patients' affective symptoms (43), which might interact with Met load. A strength of the current study was the inclusion of a relatively homogeneous sample of remitted or partially remitted BD patients, which limited confounding effects of affective symptoms compared to previous studies. However, this study did include patients in both partial and full remission, and hence, sub-syndromal mood symptoms could have had an impact on memory performance in this study too. In this context, we found a negative correlation between residual depression symptoms and hippocampal activity during encoding across the entire sample, suggesting that patients with more depressive symptoms recruited the hippocampus less efficiently during learning. This

observation is consistent with the state-related impairments in learning and memory seen in depression. Interestingly, this association was more pronounced in the ValVal homozygotes who are believed to have more activity-dependent hippocampal BDNF trafficking and greater encoding-related hippocampal response when healthy (9).

To further address the potential significance of residual depressive symptoms, we carried out sensitivity analyses only including patients in full remission and consistent with the main analyses, we found no significant effect of genotype on activity during encoding in the predefined dPFC or hippocampal ROIs. For the sensitivity analyses, the whole-brain analyses did not yield any significant effect of genotype. However it should be noted that the sample size was dramatically reduced in these analyses (N = 33).

For the main analyses, we used a relatively large sample size (N = 68) for assessment of differences between the genotype groups in encoding-related neural activity. In comparison, other studies used between 22 and 58 participants (6, 8, 9, 14, 42). Due to a small number of participants with the MetMet genotype (N = 4), we decided to not compare patients with the MetMet and ValMet genotypes. We were thus unable to explore a potential association between Met load and clinical, cognitive and neural responses as done in previous studies (6, 44). Indeed, this could have brought more clarity to the current results showing a weak advantage for Met-carriers on the free recall task. Further, as all participants received psychotropic medication it cannot be excluded that this has influenced the present result. Finally, as mentioned previously, having a healthy control group to serve as a baseline and comparison with previous studies using healthy subjects would have been an advantage.

In conclusion, we found no association between BDNF val66met genotype and hippocampal or dorsal PFC activity during picture encoding, although Met carriers showed an unexpected greater picture recall after the scan. Exploratory whole-brain analysis revealed larger lateral occipital cortex response in Met carriers, which might reflect increased visual processing during picture encoding in this group, although activity in this region did not correlate with subsequent memory performance. Instead, Met carriers might show more efficient processing in the dPFC during encoding compared to ValVal carriers. The absence of effects of BDNF val66met genotype on memory-related hippocampal response in our BD sample may be due to the effects of subsyndromal symptoms overriding more subtle effects of BDNF val66met genotype since we allowed subsyndromal symptoms in our sample in the interest of generalizability. Specifically, future studies should use even larger samples (n > 70), include a healthy control group, and investigate fully remitted BD patients (with scores <7 on HDRS and YMRS) or healthy, non-medicated participants at high risk for BD (first-degree relatives) to assess potential dose-dependent effects of Met load on neural and cognitive measures of learning and memory in BD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by De Videnskabsetiske Komitéer, Region Hovedstaden. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KM designed the original studies together with LK, MV, and HS. KM was responsible for carrying out the data collection. LH and JM analyzed the data under the supervision of KM. LH and KM wrote the manuscript. All authors approved the final version of the manuscript for submission.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
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A Clinical Case of Patient Carrying Rare Pathological PSEN1 Gene Mutation (L424V) Demonstrates the Phenotypic Heterogenity of Early Onset Familial AD

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Dementia comprises several neurodegenerative disorders with similar neuropsychiatric features and Alzheimer's disease (AD) is the most common of them. Genetic factors are strongly implicated into its etiology especially for early-onset cases (EOAD) occuring before the age of 65. About 10% of these are inherited in autosomal dominant fashion *via* pathogenic polymorphisms in three genes — APP, PSEN-1, and PSEN-2. Despite genotypic clarity, however, phenotypic variability exists with different symptom constellations observed in patients with identical mutations. Below, we present a case of a 39-year-old male with a family history for early onset dementia who was referred to our department with anamnesis for abrupt behavioral change 7 months prior to hospitalization — noticeable slowing of speech and reactivity, impaired occupational functioning and irritability, followed by aphasic symptoms and transient episodes of disorientation. He was followed up for 2 years and manifested rapidly progressing cognitive decline with further deterioration of speech, apraxia, acalculia, ataxia, and subsequently bradykinesia and tremor. Based on the clinical and neuroimaging findings (severe cortical atrophy), familial EOAD was suspected and a whole exome sequence (WES) analysis was performed. It identified a heterozygous missense variant Leu424Val (g.71074C > G) in PSEN-1 gene considered to be pathogenic, and only reported once until now in a Spanish patient in 2009. Despite genotype identity however, distinct phenotypic presentations were observed in the two affected subjects, with different neuroimaging findings, and the presence and absence of seizures in the Spanish and Bulgarian case, respectively. Besides, myoclonus and spastic paraparesis considered "typical" EOAD clinical features were absent. Age of symptom onset was consistent with two of the reported mutations affecting 424 codon of PSEN-1 gene and significantly earlier than the other two implying that factors influencing activity of PSEN-1 pathological forms are yet to be clarified. Furthermore, our patient had co-occurring lupus erythematosus (LE) and we suggest that this condition might be etiologically linked to the PSEN-1 mutation. In addition to illustrating the symptomatic heterogeneity of PSEN-1 caused EOAD, our study confirms that in patients presenting

with early cognitive deterioration and family history for dementia, WES can be especially informative and should be considered as a first-line examination.

Keywords: phenotypic heterogeneity, whole exome sequencing, PSEN1 mutation, genetic inheritance, early onset Alzheimer's disease (EOAD)

BACKGROUND

Several neurodegenerative disorders are grouped under the umbrella term dementia (1) and these include Alzheimer's disease (AD) comprising more than 60% of cases, vascular dementia (VD), dementia with Lewy bodies (DLB), and frontotemporal dementias group (FTD) (2). Their symptoms often overlap (3) and precise diagnosis is difficult but may be boosted by several approaches, including genetic testing. (4).

Genetic background of dementia syndrome is widely recognized (5). It may present as a complex condition with many genes of small effect contributing to illness risk or, more rarely, as an autosomal dominant disease running in families (6). Such forms are almost exclusively found among early onset dementia (EOD) patients, i.e., diagnosed under the age of 65. With prevalence exceeding 40.0% of patients for some dementia subtypes (7), EOD is evidently not sporadic (8), and the genetic underpinnings of AD and FTD have been most extensively studied (9).

Early-onset AD (EOAD) represents 5.5% all AD cases (10) and in 10% of the affected, a familial model of inheritance is seen (11). Its clinical course is markedly accelerated (12) and myoclonus in the early stages may be common (13). To date, three major EOAD causative genes are recognized (14): mutations in Presenilin-1 (*PSEN-1,* 14q24.2*)* are identified in up to 50% of patients (15), abnormal forms of Amyloid Precursor Protein gene (*APP,* 21q21.3) are found in 15-22% (16), and Presenilin-2 (*PSEN-2,* 1q42.13) pathological variants account for less than 2.5% (17). So far, 51 pathological forms have been reported for *APP*, 220 for *PSEN-1*, and 16 for *PSEN-2* (18). All but a few of them are involved in the pathogenesis of AD through the process of formation of amyloid-β protein built plaques, which are a histopathologic hallmark of the disease and a central component of the "amyloid cascade hypothesis" for AD (19).

Several phenotypic clues point to the involvement of a particular gene. While spastic paraparesis and "cotton wool plaques" (20) are associated with a *PSEN-1* mutations, cerebral amyloid angiopathy with cerebral haemorrhages is typical for *APP* ones (21) and families with *PSEN-2* caused AD are primarily of Volga-German origin and with later onset of symptoms (22). However, recent studies show that clinical phenotype is not rigidly linked to aetiological genotype and may be modified by a number of factors such as previous experience, cognitive activity and epigenetic mechanisms (23). *PSEN-1* mutations are a good example of that given the remarkable heterogeneity in neuropsychiatric features among affected individuals whose symptoms are often nearly identical to those seen in FTD or DLB (24).

Below, we describe a clinical case of an EOAD patient carrying a rare form of *PSEN-1* mutation who was followed-up for 2 years after disease onset. His clinical presentation exemplifies the large symptomatic variability of EOAD dementia even in its aetiologically clarified monogenic forms.

CASE PRESENTATION

The subject first presented for consultation in October 2016 (aged 39) at the Department of Psychiatry of Pleven University Hospital with a history of abrupt behavioral change 7 months prior to hospitalization. Slowing of speech and responsivity as well as impaired occupational functioning were noticed first (the patient was working as a coupier in a small casino at that time). Several months after that, mild dysarthria and difficulties finding the right words (i.e., dysnomic manifestations) occurred, followed by emotianal lability with frequent and brief episodes of sadness (crying) or irritability with verbal outbusrsts. Transient episodes of disorientation and perplexity appeared after that. Relevant medical history included head trauma without loss of consciousness in 2014 and a skin form of lupus erythematodes since 2007, currently in remission. These conditions were not considered as causally related to presenting symptoms. Premorbid functioning was appropriate to social background and academic level (high school) and what attracted attention was family history showing that the patient's father had been diagnosed with dementia syndrome started at about 44 years of age and lead to death in a psychiatric institution 4 years after that. Unfortunately, available medical records were limited, with CT data for cortical atrophy described as an instrumental finding and rapidly progressing severe memory disturbance and dyspraxia followed by avolitional-apathy syndrome pointed out as clinical features.

During a short hospital stay, CT scan and a battery of cognitive tests were performed showing moderate cortical atrophy in par with a moderately severe dementia syndrome (MMSE score of 14) presenting with significant short-term memory impairment, problems with concentration, and sustained attention during cognitive tasks, substantially damaged abstract thinking with reduced ability to form and understand concepts, difficulties with verbalization of his own mental experiences. Constructional dypraxia was prominent together with writing problems (dysgraphia) while biographical memory was relatively spared. Laboratory check-up including CBT, renal and liver biochemistry, serum glucose, lipid profile, vitamin B12, folate and TSH levels, urine test as well as immunological examination for luetic and HIV infections did not reveal deviations. EEG examination was not judged to be indicated since patient did not show epileptic activity. Cerebrospinal fluid (CSF) biomarkers test and PET were not done because these diagnostic approaches are still with

limited availability in Bulgaria. Dementia syndrome was set as a working diagnosis, treatment with combined medication containig Ω-3 fatty acids, Vitamin B12, folic acid, Vitamin E, Gingko biloba extract, and phosphatidilserine was initiated, and he was referred to a neurologist. The latter arranged an MRI scan in November 2016 confirming the CT finding of enlarged brain convexity sulci predominantly in the temporal and frontal regions (**Figures 1** and **2**).

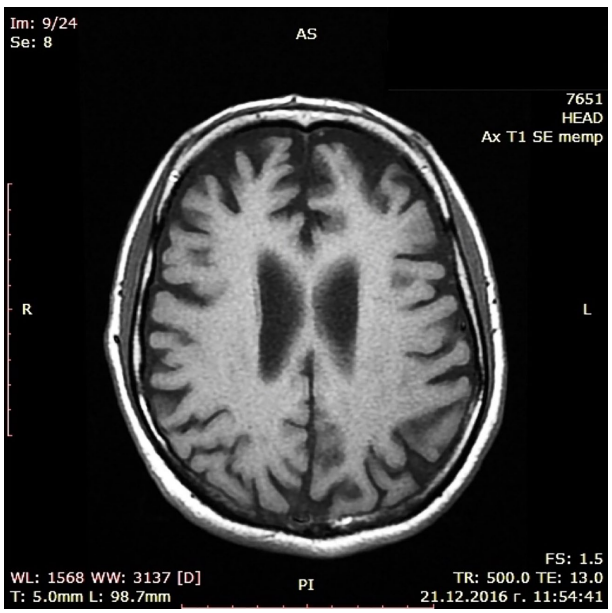

FIGURE 1 | MRI scan (transversal).

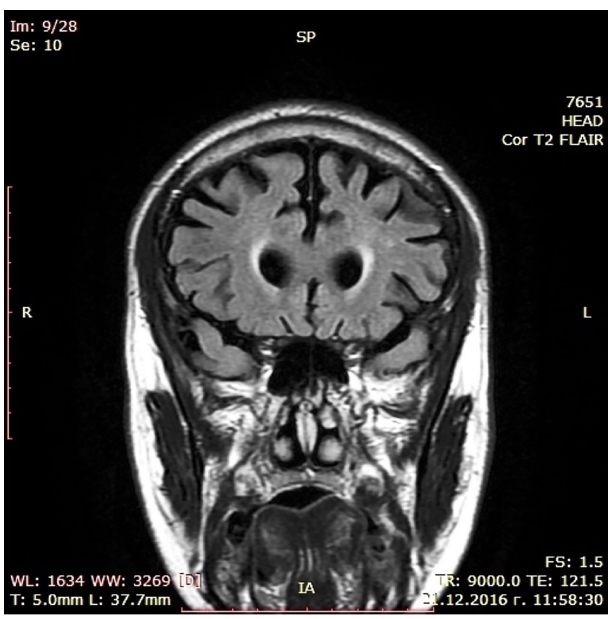

FIGURE 2 | MRI scan (coronal).

Besides, a vertigo syndrome was noted along with tinnitus, mild horizontal nystagmus, and positive Romberg sign. Vinpocetine 10-mg TID and memantine 5-mg BID were added to treatment.

Subsequently, the subject was followed-up every 6 months and showed rapid cognitive deterioration reflected in MMSE scores—from 11 in March 2017 to 3 in March 2018 (**Figure 3**) with progressive global aphasia, acalculia, apraxia, and bradykinesia. Ataxia and gait disturbances occurred in this period and episodes of spontaneous verbal and motor excitement were reported, sometimes accompanied by verbal aggression outbursts. Mild hand tremor and parkionsonian like facial expression were noticed during the neurological examination in October 2017. In March 2018, the tremor became severe, resulting in total loss of capacity for self-care. From the summer of 2018 onward, the patient was already bed-ridden with persisting coarse hand tremor increasing in intentional movements and almost missing at rest. Both global aphasia and visual agnosia were extremely expressed.

Based on clinical presentation and family history, an early onset familial AD was set as primary diagnosis and a complete blood sample for genetic testing was obtained after consent from caregivers in November 2018. Unfortunately, subject died soon after that from complicated respiratory infection, and a pathological examination could not be performed. Whole exome sequence (WES) analysis for 21 targeted genes associated with an array of inheritable neurodegenerative disorders was executed *via* a specially designed diagnostic panel (https://blueprintgenetics.com/tests/panels/neurology/dementia-panel/). It identified a heterozygous missense variant (g.71074C > G) Leu424Val in *PSEN-1* gene and this finding, confirmed by bidirectional Sanger sequencing, was judged to be pathogenic, i.e., causing clinical symptoms. To our best knowledge, the Leu424Val mutation has been reported in literature only once until now in 2009 (25) but the course and pattern of associated neuropsychiatric symptoms were different.

DISCUSSION

The presented case report describes a rare pathogenic variant of *PSEN-1* gene carried by a male subject with symptoms of

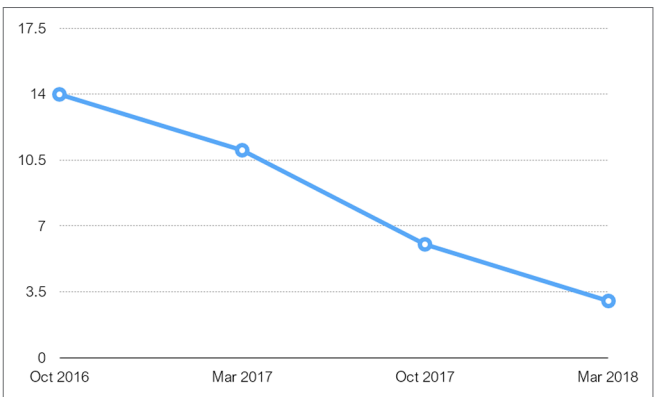

FIGURE 3 | Table charting MMSE scores over period of 17 months from first test done on initial admission.

cognitive decline started at about 38 years of age and with a family history of early onset dementia.

The *PSEN-1* gene (14q24.2) encodes presenilin-1, which is part of γ-secretase—a transmembrane protein complex responsible for the proteolytic cleavage of amyloid precursor protein (26). Mutations in *APP*, *PSEN-1*, and *PSEN-2* genes produce the same pathogenic activity, precisely an increase in the ratio of amyloid $\beta 1_{42}$ amino acid isoform to $\beta 1_{40}$ amino acid isoform levels (27), resulting in aggregation of the peptide into oligomers and ultimately amyloid fibriles forming plaques (28). This same mechanism has been confirmed for the *PSEN-1* Leu424Val (g.71074C > G) variant in a functional study by Sun et al. from 2017 (29).

According to AD and FTD Database (http://www.molgen.ua.ac.be/ADMutation) (g.71074C > G) Leu424Val mutation has been reported previously only once in 2009 by Robles et al. in Spain (25), in a female with early onset FTD. At the age of 26, she manifested symptoms of anorexia nervosa, at the age of 30, memory and attention deficit occurred, and at 34, abnormal behavior with impulsivity, aggression, and dysexecutive disorder were present. At 36, she showed aphasia, stereotyped behavior, hyperreflexia, grasping reflex, urinary incontinence, myoclonus, and seizures. Brain CT and SPECT showed diffuse cortico-subcortical atrophy (**Figure 4**) and fronto-temporo-parietal hypoperfusion. An identical mutation has also been reported by Ryman et al. (30) in an AD patient with age of onset 26 years, but it is most likely that it refers to the abovementioned case.

Notably, four other variants affecting the same PSEN-1 codon/amino acid - Leu424Arg (g.71075T > G), Leu424His (g.71075T > A), Leu424Phe (g.71074C > T) and Leu424Pro (g.73685864T > C) - have been reported in association with early onset familial AD (31)—**Table 1**. The first one has been originally described by Kowalska et al. (32, 33) in a Polish pedigree from the region of Poznan with familial AD with age of onset of 30-35 years, fast progression and typical clinical presentation. Interestingly, 10 years later the same mutation was replicated in a 38 year old Dutch male with familial AD but with much more diverse phenotype observed in the pedigree, including spastic paraparesis, frontal executive functional impairment, gait disturbances, ataxia, epilepsy, and hallucinations (34). Finally, this genotype was confirmed in 2014 in another patient from Poland—a 36 years old woman with positive family history for early dementia and atypical presentation initially suggestive of Huntington disease and later of familial prion disease (35)—**Table 1**. The Leu424His (g.71075T > A) variant was announced in 2005 by two teams—as an atypical AD in a 39 year old female patient from Poland (36) and in three subjects from a French family with autosomal-dominant AD in whom the onset of symptoms were between 38 and 42 years of age (37). Again, identical genotype produced distinct phenotypic manifestation. Specifically, marked clinical atypism was noted in the Polish case with symptoms suggestive of FTD (social disinhibition, personality change) and DLB (visual and auditory hallucinations, stereotypic behaviors, rigid-akinetic movement disorder) observed. In 2006, the Leu424Phe (g.71074C > T) mutation was identified in a Bulgarian pedigree with later onset atypical familal AD phenotype associated with significant behavioral abnormalities and white matter changes on neuroimaging (38) and has not been replicated as yet. The last and most recent abnormal variant—Leu424Pro (g.73685864T > C) —has been described in 2019 by Guven et al. (39) in a 51 years old Turkish male proband with EOAD demonstrated by deterioration of short-term memory and visuospatial skills with insidious beginning and slow progression. For understandable reasons, no replication has been reported so far.

Drawing a comparison between our case and the one described by Robles et al. (25), brings several interesting facts to the fore. First and most interesting, as in Leu424Arg (g.71075T > G) and Leu424His (g.71075T > A) variants, an identical polymorphism is associated with two quite distinct phenotypic presentations—in the Spanish patient, initial attentional deficit, behavioral deviations, and executive dysfunction lasting several years were observed, followed by somewhat milder impairment of memory and speech, while prominent and papidly progressing primary disturbance in episodic memory and speech were initially seen in our patient subsequently supervened by marked parkinsonian symptoms. Second, there is a difference in neuroimaging findings: a predominantly frontotemporal cortical atrophy was observed in our case and diffuse cortico-subcortical atrophy was reported by Robles et al. (25). Third, seizures and myoclonus were present in the patient from Spain, while no incidence of such has been observed in our patient. Besides that, age of onset of cognitive symptoms in the Spanish patient was earlier—between 30 and 34 years vs. approx. 38–39 years in our case. What the two subjects have in common is the abrupt occurrence of quite similar symptoms of irritability, despair, and verbal outbursts prior to manifestation of severe cognitive decline. In addition, in both subjects gate disturbances were noted.

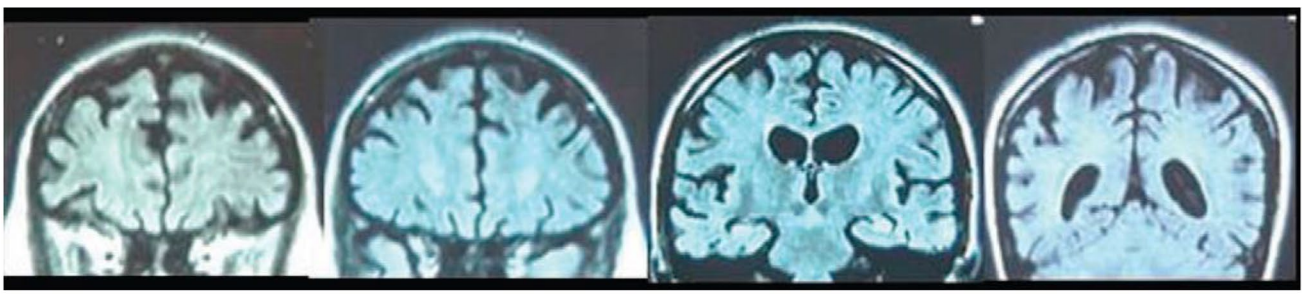

FIGURE 4 | Coronal MRI slices (anterior to posterior) from a 35 years old female patient carrying L424V *PSEN-1* mutation. From A. Robles et al. (2009). American Journal of Alzheimer's disease and othe dementias, 24(1); p.41 copyright © 2019. Reprinted by Permission of SAGE Publications, Inc.

TABLE 1 | Currently known pathological mutations at position 424 of the PSEN-1 gene with presenting phenotype in affected individuals/families.

Mutation	Proband	Symptom course	Neuroimaging/Biomarkers/ Neuropathology
Leu424Arg (g.71075T > G)	Case 1: 30 year old Polish male. Five family members with EOD and myoclonic jerks, all died within 4-8 years of disease onset.	Progressive memory and language impairment started at the age of 30. Sporadic myoclonic jerks and mild left hemiparesis a year later.	Moderate general brain atrophy with enlargement of ventricles and subarachnoid spaces (MRI, CT); photopenic focus in left occipital lobe and lowered brain-cerebellum ratio (SPECT). Neuropathological examination not done.
	Case 2: 38 year old Dutch male. Mother with cognitive decline and gait difficulties at 35, died at 39 with severe dementia, epilepsy and hallucinations. Grandfather with similar symptoms died in mid-30s.	Initially: incipient memory problems, bradyphrenia, slurred speech, spastic and ataxic gate, MMSE score 24/30. Later: dementia, spastic paraparesis, frontal executive dysfunction mimicking familial CJD and FTD.	Bilateral posterior parietofrontal atrophy, no white matter abnormalities (MRI); CSF biomarkers positive for AD (↑p-tau and total tau, ↓ amyloid $\beta1_{42}$ concentration). Neuropathological examination not done.
	Case 3: 34 years old Polish female with a positive family history for EOD, personality changes and involuntary movements. F++ather died at 37, his sister—at 40 with such symptoms.	Behavioral and personality changes (apathy and aggression) and cognitive decline (MMSE 18/30), severe involuntary movements—grimacing, smacking, and lips puckering, choreic movements in the upper and lower extremities, gait problems. Seizures occurred several months later.	Moderate general atrophy with ventricles and subarachnoid space enlargement (CT); Generalized diffuse slowing and a decrease in reactivity of the basic rhythm (EEG). Neuropathological examination not done.
Leu424His (g.71075T > A)	Case 1: 39 years old Polish female with unknown family history;	Symptoms at 39 years: depression, anxiety, personality changes and social inhibition. Soon after that—visual and auditory hallucinations and stereotyped behaviors; 1.5 year after that: deficit in short-term memory, attention and executive functions, MMSE 17/30, EPS (bradykinesia, rigidity) and bilateral primitive reflexes, rapid progression of symptoms.	Generalized cerebral atrophy (MRI); Diffuse cerebral hypoperfusion (SPECT). Neuropathological examination not done.
	Case 2: Three individuals from a French EOD family.	Onset of dementia symptoms between 38 and 42 years. Further details not provided.	N.A. Neuropathological examination not done.
Leu424Phe (g.71074C > T)	Two cases in a Bulgarian family with five other members affected by behavioral abnormalities and dementia.	Patient 1—depression at 54 years of age followed by dementia and grand mal epilepsy; Patient 2—memory loss, aggression, delusions, and hallucinations at age 60, followed by development of dementia.	Severe brain atrophy with white-matter changes. Neuropathological examination not done.
Leu424Val (g.71074C > G)	Case 1: 36 years old Spanish female with family history for late onset dementia (> 80 years) of maternal grandmother.	At 30: attentional deficit, forgetfulness and dysexecutive symptoms (difficulties driving etc.) At 34: irritability, despair, verbal aggression outbursts, impulsive behavior, sporadic suicide ideation, persisting memory impairment. At 35: aberrant behavior, bradylalia, dysnomia, mixed dysphasia, acalculia, ideatory apraxia, catatonic state, and ECT. Postural dystonia on neurological exam, followed by status epilepticus.	Generalized cortico-subcortical atrophy more pronounced in dorsal prefrontal, posterior parietal, and anterior temporal regions; hippocampus was proportional to the rest of the temporal lobe (CT and MRI). Diffuse slowing without other specific features (EEG). Second CT (17 months after the first): broadening of ventricles and Silvian fissures (i.e., progrssive atrophy); Extensive hypoperfusion affecting frontal, temporal and parietal lobes (SPECT). Neuropathological examination not done.
		At 36: stereotyped vocalizations and laughing, mydriatic hyporeflective pupils, grasping reflex, slight trunk flexion, urine incontinence, myoclonic jerks, several generalized motor seizures.	

(Continued)

TABLE 1 | Continued

Mutation	Proband	Symptom course	Neuroimaging/biomarkers
	Case 2: 39 years old male whose father had rapidly progressing dementia started at 44 years with severe memory problems, dyspraxia, avolition (died in 1985 aged 48).	At 38: mild slowing of psychomotorics and responsivity. At 39: bradyphrenia, slurred speech, dysnomic signs, emotional ability with transient episodes of sadness and irritability, followed by visuospatial difficulties and progressive short-term memory and attention impairment (MMSE 14/30), dyspraxia and dysgraphia. Vertigo, tinnitus and horizontal nystagmus noted in neurological exam. At 40: global aphasia, acalculia, apraxia, extreme bradykinesia, mild hand tremor, Parkinsonian facial expression, ataxina, gait disturbances, episodic verbal aggression outbursts. At 40: severe hand tremor almost absent at rest, extremely expressed aphasia and visual agnosia, bed-ridden (dies at 41 from complicated respiratory infection).	Cortical atrophy predominantly in temporal and frontal cortex (CT and MRI). Neuropathological examination not done.
Leu424Pro (g.73685864T > C)	51-year-old male patient from Turkey whose uncle had dementia and died at appr. 40 years of age. Proband's father has died from cancer aged 45 with preceding minor cognitive impairment.	Initial symptoms: deterioration of short-term memory and visuospatial abilities started at the age of 47. No significant neurological sings. MMSE score: 22/30. Currently followed-up, demonstrates benign course of the disease, no rapid progression.	Prominent medial temporal lobe atrophy and global cortical atrophy (MRI); Hypometabolism in parietal areas, the precuneus, and the posterior cingulate cortex (PET); Decreased amyloid level in CSF. Neuropathological examination not done.

If we are to compare the phenotypic expression of our patient with the majority of other *PSEN-1* mutations reported in literature, the most important notion here is that this is not a "typical" *PSEN-1* polymorphism, i.e., such as presenting with spastic paraparesis. Another substantial distinction is the absence of myoclonus, while the extrapyramidal symptoms, ataxia and gait disturbances we observed are in line with other reports in literature (34, 40–42). So is the age of onset—shortly after mid-30s—which is in accordance with data with the other two mutations in the same codon in exon 12 of the gene—Leu424Arg (33–35) and Leu424His (36, 37) but in striking non-compliance with Leu424Phe and Leu424Pro variants associated with significantly later onset of symptoms (> 55 years and > 47 years, respectively) in the affected individuals (38, 39). The last two cases, supplemented by the fact that our patient's father also did show later occurrence of symptoms (44 years) challenges the suggestion that age of AD onset is strongly determined by the position of the mutation of *PSEN-1* gene (43) but rather depend on intramolecular or intermolecular interactions of PSEN-1 with other proteins (39). At the same time, however, they support the observation of later occurrence of symptoms in mutations after codon 200 compared to those before it which has been stated in literature also as type 2 vs. type 1 pathology in EOAD (13). Besides, the purported modifying role of apoliprotein E4 status on age of onset of familial AD (44) is also questioned by the case subject who did not carry high risk E4 alelle and yet symptoms occurred five years earlier compared to his father. Clearly, much more identified and investigated EOAD pedigrees are needed in the future to confirm or refute these inferences.

Another point that should be emphasized is the co-occuring lupus erythematosus (LE) with predominant skin manifestations diagnosed in 2007. In our opinion, the presense of this conditon verified by an in-hospital histological examination several months prior to our first encounter is very interesting and suggests a possible pathogenic link between AD and LE *via* altered *PSEN-1* function. This assumption is supported by at least two lines of evidence. First, emerging data show that in addition to AD, γ-secretase is also involved in organ-specific (e.g., multiple sclerosis) as well as in generalized (e.g., systemic lupus erythematodes) autoimune diseases *via* the B-cell maturation antigen (BCMA) which is a part of the BAFF-APRIL receptor ligand system controlling activation and survival of B-lyphocytes (45). Second, γ-secretase operates several substrates including the Notch receptor protein (46) which is a part of Notch signalling pathway regulating cell proliferation but also implicated in innate immunity and inflamation, including rheumatioid arthritis and systemic lupus (47). Given the current lack of data supporting statistically significant co-occurrence of EOAD and LE, their combination in the presented case should be regarded as purely random. Yet, we consider screening of known *PSEN-1* mutation carriers for laboratory or clinical symptoms of

autoimune diseases scientifically meaningful and recommend it to all researchers in the field.

A certain limitation of our exploration is the paucity of information concerning precise characteristics and chronology of the clinical manifestation of our patient's father, as well as the lack of neuropathological examination of patient himself which would have probably revealed the characteristics and localization of amyloid plaques and amyloid angiopathy and the eventual presence of Lewy bodies. Besides, the subject's 18 years old daughter who currently does not manifest any neuropsychiatric symptoms has not been genetically tested yet due to non-provision of consent. Furthermore, the patient's sister (aged 45) from the first marriage of his father (see family tree on **Figure 5**) could not be found and examined and the last information for her showing absence of cognitive impairment dates back from several years ago.

What underlies phenotypic heterogeneity in *PSEN-1* mutations, once again confirmed by the presented case, remains to be clarified. A number of factors combining to modulate pathogenic polymorphisms have been discussed in literature, for example interaction between mutated PS-1 and its protein partners in γ-secretase (48), brain ischaemia (49), D-amino acid oxidase activator (DAOA) an other genes (50), education (44), racial and ethnic background (24). Obviously, future genetic, molecular, and bioinformatics studies are needed to address this issue.

CONCLUSION

In addition to further supporting the vast phenotypic variability produced by genetically identical missense mutations in the *PSEN-1* gene, our study confirms that in EOAD with positive family history, WES can be especially informative. In our view, it should be considered as a first-line examination given the ease of genome-wide assessment, the high diagnostic value, the accuracy of mutation detection, and the relatively affordable price of DNA extraction. Easily accessible online bioinformatics tools now simplify and expedite data review relevant to symptoms. Genetic mutations in AD have large prognostic value and, possibly, treatment implications in the future. Moreover, with informed consent provided, they enable longitudinal re-examination and new findings.

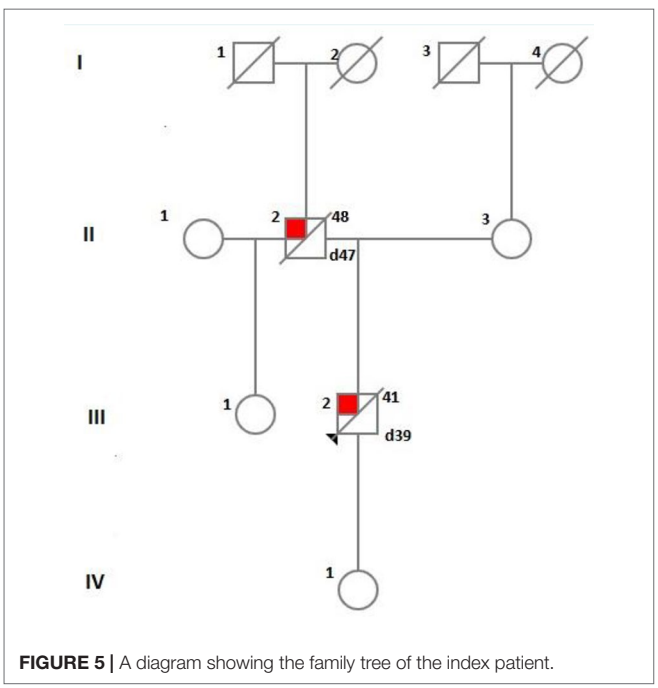

FIGURE 5 | A diagram showing the family tree of the index patient.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of Medical University Pleven, Bulgaria. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

KS: initial and subsequent patient psychiatric examination, obtaining and sending patient DNA sample, literature review and manuscript preparation. MSt: subsequent (follow-up) psychiatric examination, manuscript preparation. PC: subsequent (follow-up) psychiatric examination, manuscript preparation. LI: initial and subsequent neurological examination. MSw: literature review, manuscript preparation. The publication has been supported by a research grant from Medical University Pleven, Bulgaria.

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Current Challenges in Translational and Clinical fMRI and Future Directions

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Translational neuroscience is an important field that brings together clinical praxis with neuroscience methods. In this review article, the focus will be on functional neuroimaging (fMRI) and its applicability in clinical fMRI studies. In the light of the "replication crisis," three aspects will be critically discussed: First, the fMRI signal itself, second, current fMRI praxis, and, third, the next generation of analysis strategies. Current attempts such as resting-state fMRI, meta-analyses, and machine learning will be discussed with their advantages and potential pitfalls and disadvantages. One major concern is that the fMRI signal shows substantial within- and between-subject variability, which affects the reliability of both task-related, but in particularly resting-state fMRI studies. Furthermore, the lack of standardized acquisition and analysis methods hinders the further development of clinical relevant approaches. However, meta-analyses and machine-learning approaches may help to overcome current shortcomings in the methods by identifying new, and yet hidden relationships, and may help to build new models on disorder mechanisms. Furthermore, better control of parameters that may have an influence on the fMRI signal and that can easily be controlled for, like blood pressure, heart rate, diet, time of day, might improve reliability substantially.

Keywords: fMRI—functional magnetic resonance imaging, BOLD (blood oxygenation level dependent) signal, reliability, clinical fMRI, psychiatry

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INTRODUCTION

Translational neuroscience is an important branch within the broad field of neuroscience. In the context of this opinion article, translational neuroscience will be seen as the attempt of bridging neuroscience, neuroimaging, and clinics for improving our understanding of symptoms and disorders, and for better diagnostics and treatments (1, 2).

This is not a new attempt. Neuroimaging, and in particular functional magnetic resonance imaging (fMRI), has been considered as a revolutionary tool for exploring the healthy and the diseased brain for more than two decades (3, 4). Consequently, since fMRI entered the scene in the early 1990s, it had seen an enthusiastic phase over the first two decades. However, after this period, neuroimaging—like almost any other psychological and medical sciences—was overrun by the replication crisis. Recent studies have estimated the reproducibility of psychological studies to be

39% or less and indicated a severe limitation of neuroimaging (fMRI) study reliability (5–9). Furthermore, the neurophysiological mechanisms behind the BOLD/fMRI signal are only partly understood, which makes it difficult to generalise results or to use it on an individual level for diagnostic purposes. Thereby impeding the impact of highly needed neuroscience studies on theoretical and methodological progress, and, last but not least, the clinical application of fMRI.

In the following, this article will critically discuss current strategies and developments within the field of neuroimaging and tries to indicate possible future directions.

The Replication Crisis and Its Consequences

The neuroimaging research community has taken the "replication crisis" very seriously, like through the ReproNim initiative (10), and the Organisation of Human Brain Mapping (OHBM) announced in 2016 a new replication award, and put reproducibility high up on their agenda with several new best practice and data sharing initiatives (see, e.g., http://www.ohbmbrainmappingblog.com).

Jointly, psychology and neuroimaging suffer substantially from a lack of statistical power, meaning that the sample sizes are typically too small, and effect sizes are too low (11). This has not only been perceived as a critical challenge among scientists but has recently also received public attention. On the other hand, clinical applications require reliable single-case examinations but not group studies that may reliably show the general population effect but may vanish the information on interindividual and intraindividual variability. Consequently, the lack of information about the "naturally" occurring variability hinders the successful development of translational and clinical applications. Already in 2006, Paul Matthews and coworkers critically discussed the applicability of clinical fMRI for other applications than neurosurgical mapping (12). Although they wrote down their opinion more than a decade ago, it appears like that the development of clinical fMRI is in a "resting state," as recently pointed out by O'Connor and Zeffiro (13). Presurgical mapping is still the only reliable and widely used clinical application of fMRI. The critical question is, why haven't we yet achieved a breakthrough in clinical fMRI?

CURRENT STATUS

This article will critically discuss three aspects that are relevant to consider in the context of clinical fMRI: First, the fMRI signal itself, second, current fMRI praxis, and, third, the next generation of analysis strategies.

The Bold Signal Perturbation

One of the major knowledge gaps in the field is the assumption that the fMRI signal, i.e., the underlying BOLD effect (BOLD = blood oxygenation level dependent), is *sufficiently* reliable and stable, where "sufficiently" has never been defined yet. It is of crucial importance to keep in mind that the BOLD signal represents only an indirect measure of neuronal activity, through a cascade of physiological processes, called neurovascular coupling. Consequently, the observed variability of the BOLD signal does not necessarily justify the conclusion that the underlying neuronal activity shows variability to the same degree. Scientifically speaking, the BOLD signal is a physiological response that only indirectly reflects neuronal activity, and which is easily and directly influenced by blood pressure, blood oxygenation, or any other parameters that have an effect on the vascular system, which in turn affect the balloon effect that generates the BOLD signal (14, 15). The corresponding balloon model became the most influential and mostly used model in fMRI research (15–17). It is a neurophysiological model that describes the neuronal and vascular mechanisms that cause the BOLD signal given a neuronal activity. It rests on the assumption that the BOLD signal is caused by changes in the blood volume, blood flow, and the oxygen extraction rate. It is widely accepted that these are the main parameters that determine the strength of the BOLD signal. The balloon model and its corresponding hemodynamic response function is, for example, an integral part of several analysis models of fMRI data, but also for measures of functional and effective connectivity, like dynamic causal modelling (DCM) (18).

However, it is less studied, how susceptible the BOLD signal is to endogenous and exogenous influences and individual variability of the underlying mechanisms. Hence, it might occur that a change in the BOLD signal is detected while the *true* neuronal activity and connectivity remains unchanged. It is known that hormones (like cortisol), blood pressures, body mass index, time of the day (circadian rhythm), time of the year, sleep duration, and age influence blood volume, blood flow, and other vascular parameter, and hence the BOLD signal (19–23). Whether the individual variability of these parameters has a significant influence on the BOLD signal is largely unknown. To give another example, using magnetic resonance spectroscopy (MRS), it has been shown that the individually varying concentration of the inhibitory neurotransmitter GABA is reflected in the amplitude and shape of the BOLD signal (24). Complementary, comparable effects have been shown for the excitatory neurotransmitter glutamate (25, 26). The list of those endogenous parameters can be continued, including parameter that may predominantly affect neuronal signal transmission or vascular processes. In other words, the BOLD signal is most likely not stable within and not necessarily comparable between subjects. These factors are just additional sources of variability of the fMRI signal that comes in addition to all other sources of noise that are affecting the measurement, like other environmental factors, thermal noise, noise of the measurement system itself, movements of the subjects, daylight length, temperature, and whether, to name a few, that may affect brain functions but also the stability of the MR system (23, 27, 28).

Current fMRI Methods

Current Clinical Applications of fMRI

As outlined above, the only routinely used clinical application of fMRI is the presurgical mapping (see **Table 1**). This is mostly done in patients with brain tumours or epilepsy, since these diseases may cause substantial displacement of brain functions,

TABLE 1 | Schematic overview on reliability and current clinical applications of the different fMRI techniques.

	Reliability	Current clinical applications
Task-related fMRI: Localisation of functions	**Single subjects:** Reasonable good	Presurgical mapping; Occasionally single case studies
	Typical paradigms: Motor, Language, Memory	
	Group studies: Good; Various tasks are used	Psychiatry, Neurology
Task-related fMRI: Strength of activation	**Single subjects:** Poor - Limited -Depends on task, instructions & attention	Limited applicability
	Group studies: Limited - Good; Various Tasks	Psychiatry, Neurology
Resting-state fMRI	**Single subjects:** Poor – Limited - Good Reliability depends mainly on analysis methods, i.e. different measures show different reliability, but it depends also on scan length, instruction, etc.	Limited applicability
	Group studies: Limited – Good Reliability depends mainly on analysis methods, i.e. different measures show different reliability, but it depends also on scan length, instruction, etc.	Psychiatry, Neurology

and functional mapping with fMRI may help surgeons to localize important areas despite their unusual neuroanatomical localization (29). However, most of those clinical applications are task-related fMRI with simple paradigms, and the first reports date far back to the beginning of fMRI (30, 31).

One of the most common application is the localization of language areas and their lateralization, which is an essential information in the treatment of patients with epilepsy (32–38). In most occasions, this clinical application of fMRI shows comparable results as the invasive WADA test but might deviate in cases with atypical language dominance (39).

Good experience also exists for paradigms probing the localization of motor, sensory, and memory functions, which are often not only used for localization but also for predicting outcome (40–44). Nowadays, clinical fMRI is often combined with diffusion tensor imaging for localizing relevant fibre tracts (42).

In contrast to the presurgical mapping where it is sufficient enough to localize a function, any application in psychiatry, for instance, needs to focus on the strength of activations. Accordingly, there exists no routinely used clinical application of fMRI outside of the field of presurgical mapping due to the lack of sufficient reliability in the measurement of *individual* activation strength—for example task, instruction, and different levels of attention may influence reliability (45, 46). *Group studies*, by contrast, show a much higher reliability in detecting deviations in activation strength (47). Therefore, almost exclusively all fMRI studies in psychiatry have explored cohorts of patients (see **Table 1**). However, one possible way to circumvent this lack of reliability on the individual level has been recently suggested by Paek et al. in connection with a study on dementia by proposing repeated (baseline) measurements of the patients (48).

Another problem in clinical fMRI in psychiatry is the heterogeneity of patient populations. The disorders are often spectrum disorders with a continuum that ranges from normality to pathological (49), but also that varies between various symptoms and diagnosis, like between schizoaffective disorder, schizophrenia, bipolar disorder (50). Furthermore, the disorders often manifest in varies subtypes, and different studies may use different diagnostic criteria. Consequently, imaging results often differ substantially even on the level of group studies (51).

Is Resting-State fMRI the Solution?

While the first two decades of fMRI were mostly dominated by task-related fMRI, i.e., fMRI acquisitions while research subjects performed an active task, like a working memory, attention, or language task, the more recent years have seen an alternative approach, which is called "resting-state" fMRI (rs-fMRI). Here, research subjects are just scanned over a certain period without any concrete, active task—they are presumably "at rest." Surprisingly, the measured BOLD signal that is measured during such an rs-fMRI examination is not random but fluctuates in a spatially and temporally systematic manner (52, 53). It has been shown that even in the absence of a concrete task, certain brain areas are forming networks through characteristic correlated fluctuations of the BOLD signal, called *resting-state* or *intrinsic networks*. These network patterns can be detected by focusing on low frequent (<0.01Hz) fluctuations of the BOLD signal, since these fluctuations propagate through the underlying neuronal network structures, indicating an information exchange within the networks even in the absence of a concrete task. It has been further shown that these networks are very similar across individuals (52, 53). They are therefore assumed to reflect some fundamental —trait- or biomarker-like —brain processes.

From resting-state data, it is possible to identify neuronal networks that show in their spatial organization a striking similarity with those networks that have been identified through task-related fMRI (54). These networks are often identifiable also on single subject levels, but depends on the method that is used for extracting the information (46). Since this discovery, there has been a tremendously interest in resting-state fMRI and the examination of the related intrinsic-brain networks and their dynamics (17, 55–62). One of the most investigated networks in this respect is the "default mode network" (DMN) (61, 63). The DMN network is related to processes, like mind wandering, intrinsically focused attention, daydreaming, etc. Interestingly, there is a counterpart to the DMN, which has been described under different names in the literature. Here, it will be called the "extrinsic mode network" (EMN) and represents a network for extrinsically focused attention (61).

One reason why rs-fMRI became such a popular tool in the field of neuroimaging is that it may allow studying cognitive functions even in the absence of a task, which would be an intriguing possibility for doing clinical fMRI, especially in cases

where patients are severely affected like after a stroke or traumatic brain injury. Clinical applications of rs-fMRI are based on the assumption of certain interindividual and intraindividual stability of resting-state networks in healthy individuals to draw conclusions from observed deviations in patients. In order to increase comparability and to limit variability in data acquisition between studies, first sets of guidelines for standardized protocols have been developed (e.g., like the "Alzheimer's disease neuroimaging initiative" (ADNI); http://adni.loni.usc.edu), and other initiatives are following their example and have started similar undertakings.

However, one major disadvantage of rs-fMRI is that rs-fMRI studies still vary in their acquisition methods and whether they are conducted on a 1.5T, 3T, or 7T MR. The typical approach in rs-fMRI is to do an fMRI scan of several minutes duration with a repetition time (TR) of typically 1–3 s. But between studies and labs, there are already at least three different types of instructions, asking the research subjects to either close their eyes, keep them open, or to fixate on a fixation cross. Although the differences between these three possible instructions are moderate, they are still measurable (64). Interestingly, the most reliable results for most but not all examined networks were achieved when subjects fixated on a fixation cross. It is, however, difficult to control how well an individual followed that instruction as eye-tracking devices or eye cameras are typically not installed inside of an MR scanner and especially not in clinical MR scanners. Furthermore, different TR times may also cause varying results, since periodic signals like heart rate variability or respiration rate might affect results differently (65, 66). Another factor that varies between different studies and also influences the results is the duration of the resting-state examination that roughly varies between a few minutes and up to 12 min and more. The reliability of specific rs-fMRI seems to improve with scan durations, and acceptable good reliability for both intrasession and intersession rs-fMRI might be around 12 min (67).

Furthermore, there are also still no standards of how rs-fMRI data should be analysed. Previous studies have applied a wide spectrum of rs-fMRI analysis strategies, with varying levels of reliability (46, 68). But progress has been made in standardizing some of the procedures for achieving across-site comparability (69). It is, however, beyond the scope of this article to review all the different methods, but, just as an example, it has been shown that different methods do have different reliability, like measures of the static functional connectivity networks against the temporal dynamics of these networks (70).

In addition, rs-fMRI studies are based on the assumption of the inherent stability of the underlying resting-state networks across time and individuals. In other words, one assumes a low intrasubject and intersubject variability with high sensitivity to clinical deviations. This assumption has, however, never been thoroughly tested and might not be justifiable. There are only sparse and inconsistent reports that resting-states are indeed resting-traits (71), while the majority of reports point out that intraindividual variation can depend on environmental and psychological effects (72–74). Another source of variability is the time of the day and time of the year. In an effortful longitudinal study of a single subject over 3.5 years, Choe et al. could show that there were systematic variations with a "significant linear trend, annual periodicity, and persistence" (75). Others have found that resting-state activity varies with the circadian rhythm (76), sleep duration (22), prior events (72), or mood (73). But also the metabolic state of hunger against satiety has a measurable effect on various resting-state measures (77–79)—and the list of factors influencing rs-fMRI and/or the BOLD signal could be continued endlessly.

In summary, while acquisition methods and analysis strategies can be standardized, it will become challenging to control for additional endogenous and exogenous factors in a daily clinical routine. Although all mentioned factors might only have a moderate effect on resting-state measures, in the light of clinical applications, they may be in the same range that differentiates between patients and healthy controls. It is therefore questionable whether rs-fMRI will ever make it into a clinical tool. One might speculate, whether the reliability of task-related fMRI, with concrete tasks that requires focusing the attention, might be more superior and more suitable for clinical application (36, 45, 80).

Table 1 gives a schematic overview on reliability and current clinical applications of task-related and resting-state fMRI, separated for both single subject and group-level studies.

Next-Generation Data Analyses

Are Meta-Analyses the Solution?

In the light of increasing computational power, cloud computing, and open-access databases with thousands of datasets, meta-analyses became increasingly popular. Meta-analyses are a suitable tool for examining general network structures for a given cognitive task, and which areas, on average, show deviating effects in large patient populations. They may become important cornerstones for building new and more fine-grained models for various disorders.

But, like any emerging new method, the methods behind meta-analyses of large datasets are still under development and standards needs to be established (81). This implies that meta-analyses are not necessarily comparable and may suffer from the publication bias (82). This has been a known issue for decades since it has been noted that meta-analyses and randomized control studies may show different results (83), but methods are under development that control for potential biases (84). Furthermore, pure meta-analyses may not be the most sufficient way to go, since they often provide us only with very general common-sense solutions, that do not go much beyond to those functional lesion maps that already have been drawn in the first half of the last century (85, 86). As a side note, already then, the posterior cingulate cortex has been associated with self-awareness, which is nowadays called the "default mode network." Or, another example, there is a striking functional and structural similarity between Kleist maps, based on brain lesions and injuries, and the meta-analysis of neuroimaging data on language functions (87).

In summary, meta-analyses are important contributors in revising and updating our understanding of the structural and functional organization of cognitive functions, and how structure and function interact. Focusing either only on lesions or only on

fMRI results may not be sufficient enough for building new and more integrative and holistic theories of brain functions and sources of brain disorders. One way of achieving this is, for example, joining structural lesion maps with results from functional imaging within one multivariate analysis (88–90).

Is Machine Learning the Solution?

Over the most recent years, meta-analyses have been supplemented with machine- and deep-learning methods that can extract (partly hidden) information out of the data and may be able to detect a pattern that is not observable otherwise. The main characteristic that differentiates deep learning from other classifier approaches, e.g., for identifying subpopulations in a multimodal data space, is that features are learned automatically and do need a feature selection as a preceding step, which removes subjectivity and substantially improves accuracy (91). Deep learning has shown superior performance in detecting cross-modality relations and has attracted a substantial amount of attention among researchers from various fields. Furthermore, it has been nominated as one of the "10 breakthrough technologies" by MIT Technology Reviews (https://tinyurl.com/zx82sg5). Another advantage of deep-learning methods are their depth and breadth in model building, which may uncover hidden relations between factors that are of relevance for future clinical applications of fMRI in psychiatry (92–94).

However, one potential problem with machine-learning approaches might be, however, the problem of overfitting which may compromise generalization of the results (95). Overfitting means that the algorithm finds a solution that perfectly parameterises the given dataset but may fail to classify new data correctly. One reason for overfitting is the use of too-small sample sizes as training data (96). But the field of machine learning has been hit by the replication crisis, as well (97). This is most like caused by insufficiently shared code and (training) data. Accordingly, the use of machine- and deep-learning methods is only justifiable in combination with large-scale open-access databases and open-source software.

In summary, machine-learning approaches are a promising move toward new discoveries of hidden relationships. Once reliable patterns have been identified and validated across different databases, one could expect that these approaches will bring us much closer to clinical applications of single-subject fMRI, as they may allow identifying fingerprints for certain disorders. But, it is still a long way to go until we will see clinical fMRI for diagnostic purposes, since, despite promising progress, the most recent developments are still in their infancies.

DISCUSSION: THE NEXT STEPS IN TRANSLATIONAL NEUROSCIENCE

Translation neuroscience is a rapidly expanding field. If one takes the term literally, it means translating one concept from one domain or scientific discipline into another. In the field of neuroscience, it actually could be thought both ways, either translating a clinical concept into something that is measurable with neuroscientific methods and, the other way around, translating results from neuroscience into clinical praxis. The first way would lead to a better understanding of neurobiological underpinnings of a disorder, while the other way focuses more on the benefits of the patients. However, the issues raised above have to be taken into account for any translational research, whether it is for explorative purpose or diagnostics. The replication crisis might have triggered a new way of think and further attempts to exploring underlying mechanisms. Especially the recent years has seen an increasing interest in exploring all kind of endogenous and exogenous factors that might influence not only brain functions but also the mechanisms that generate the BOLD signal. Some of them have been discussed above, but the list of influencing factors is far from being complete. Moreover, it should highlight that there are indeed several processes that can affect the physiological and vascular basis of the BOLD signal but not necessarily the underlying neuronal mechanisms and activations, like the current blood pressure. Other factors, by contrast, might have a systematic effect on brain functions but do not have anything to do with the neurological or psychiatric disorder that should be examined, like the current phase of the circadian rhythm or the time point of the last meal. Or other factors are purely technical, like temperature, technical noise of the MR system. The influence of these factors might be boosted by the combination with nonstandardized fMRI acquisitions, different instructions (e.g., in rs-fMRI eyes open, eyes closed), nonstandardized analysis methods, less suitable algorithms. While there is an increasing number of reports recommending larger-sample sizes ($n > 100$) for improving reliability (98), other attempts are needed to improve single-subject fMRI (99), which are compulsory for pushing forward clinical fMRI.

Concerning the aspect of larger sample-sizes, this is mandatory for basic research of neuronal correlates of neurological and psychiatric disorders. Those studies are needed for building models and testing hypothesis of the source of a disorder and its progression. This needs a clear conceptualization of what neurobiological or cognitive components may cause the disorder, and how they can be measured with, for example, neuroimaging. While in some disorders, this might be a rather trivial endeavour, psychiatric disorders are often lacking such a definite relationship. However, machine-learning and big-data approaches may help uncovering hidden relationships and are promising strategies in current research applications (94). An often seen problem within fMRI studies is the huge overlap of results across different cognitive tasks and domains. As pointed out by Hugdahl and coworkers, the fronto-parietal attention network, aka EMN, is virtually activated every time the attention is focused on an extrinsic task (61). Such an unspecific but the fMRI results dominating activation pattern is difficult to interpret in the light of psychiatric disorders where problems may arise in one particular cognitive domain but not in others. Group studies and meta-analyses may provide the sufficient power to study also subtle effects within the EMN that may relate to psychiatric disorders, but the back translation into diagnostic attempts are difficult to achieve. However, one might also have to rethink the

concept of certain disorders. Many psychiatric disorders are nowadays formulated and specified as spectrum disorders, forming a gradient from healthy to severely affected with perhaps varying probabilities of certain comorbidities, with unclear aetiologies. Accordingly, it is less likely that one is able to identify one single spot in the brain or one single deviation in a biomarker that causes this particular set of symptoms. Furthermore, there are often unclear and not directly related functional-structural relationships. For example, functional differences seen in rs-fMRI data from patients with depression (100) may not directly correspond to deviations seen in structural data (101). Accordingly, the cognitive and neurobiological models of psychiatric disorders may take into account that the spectrum of a disorder has multiple sources and that structural and functional causes may or may not depend on each other. Unfortunately and despite uncountable neuroimaging studies and meta-analyses, neuroimaging results have yet not been used for redefining and specifying diagnostic categories, as, for example, specified by the DSM5 (4).

Concerning the translation from neuroimaging results in clinical praxis, i.e., using fMRI for diagnostics, this is an even more difficult attempt. As outlined earlier, clinical fMRI is routinely used only for presurgical mapping but not for diagnostic purposes per se, and in particular not for diagnostic purposes in psychiatric disorders. The fMRI signal is too easily affected by many different endogenous and exogenous factors that are difficult to control. Even with standardized acquisition and analysis protocols, substantial and clinically irrelevant variations in individual fMRI results will be still present. Moreover, these variations are most likely at the same magnitude as the deviation from the mean that one would expect in a patient. This problem is further amplified by the fact that many disorders are spectrum disorders with gradual deviations. However, advanced machine-learning approaches that have been applied to large databases achieved, for example, for the classification of autism spectrum disorders already accuracies of 70% (102) to 90% (103). Interestingly, both studies used the same dataset but different algorithms, indicating that the selection of the algorithms can bias the results. Furthermore, there has not been reached a consensus yet, which algorithms are superior or recommended for fMRI data in general or for the classification of specific disorders in particular. But further methodological progress and better standardizations can be expected in the near future.

Besides using fMRI for diagnostics and classification of patients, there have also been attempts at using fMRI for the development of drugs and validating of drug effects (104). However, also this field of translational neuroscience still suffers from the replication crisis, publication bias, and the lack of standard acquisition and analysis methods. Hence, there is currently only very limited applicability of fMRI for this purpose, as well.

CONCLUSION

In conclusion, developments and progress have been and will be made in all domains, covered by this article. The replication crisis has pushed the development of new strategies, like the ReproNim initiative, that will help to standardize acquisition and analysis pipelines. Furthermore, the increased computational power and the continually growing number of available open-access databases with large sample-sizes and longitudinal data will allow the generation of "norm"-databases that can describe the distribution and interindividual variability of cognitive functions and network structures. Longitudinal data that are increasingly available will also give a better picture of disease progression. Machine-learning approaches will become better and more reliable in identifying disorders from multiple sources. All these approaches may lead to redefinitions of symptoms and may give a clearer picture of the causes of various spectrum disorders. Whether this will finally lead to clinical fMRI as a diagnostic tool is difficult to predict, since the variability of the BOLD signal is still an unresolved issue. In light of that, rs-fMRI currently does not appear as a tool that shows sufficient reliability and stability within and between subjects. The most reliable way of conducting rs-fMRI might be in combination with a fixation task and at least 12-min scan duration. By contrast, task-related fMRI that require focused attention of the subject might have better reliability and hence predictive value on the single-0subject level but would require a careful selection of clinically relevant paradigms. Here, better theoretical models have to be developed for translating clinical concepts into meaningful fMRI paradigms. Furthermore, it would be beneficial to acquire the data (in particular rs-fMRI) approximately at the same time of the day, and a sleeping and diet protocol could explain further, but irrelevant variability. Meta-analyses, in turn, might help in identifying precisely clinical concepts. In essence, after almost three decades, fMRI has generated substantial new insights into neurological and psychiatric disorders. It has produced a vast amount of data and triggered the development of new methods both for data acquisition and data analysis. Although the reliability of fMRI is still limited and hinders its use for diagnostic purposes in a daily clinical routine, the field of translational neuroscience is continuously moving toward more standardized, more reliable, and more clinical relevant applications of fMRI.

In essence, it is not unlikely to expect that clinical fMRI will at one point go beyond its current presurgical application and toward more diagnostic applications. This will be achieved by improved and standardized methods, better understanding of the neurovascular-coupling mechanisms, and revised models of psychiatric disorders.

AUTHOR CONTRIBUTIONS

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Adult Mild Encephalitis With Reversible Splenial Lesion Associated With Delirious Mania: A Case Report

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Bellani M, Zanette G, Zovetti N, Barillari M, Del Piccolo L and Brambilla P (2020) Adult Mild Encephalitis With Reversible Splenial Lesion Associated With Delirious Mania: A Case Report. Front. Psychiatry 11:79. doi: 10.3389/fpsyt.2020.00079

Mild encephalitis with reversible splenial lesion is a rare clinic-radiological entity presenting with neurological and neuropsychiatric symptoms associated with cerebral lesion/s. Delirious mania is a severe psychiatric syndrome characterized by acute onset of delirium, excitement, and psychosis with a high mortality rate. In this paper, we present a case report of mild encephalitis with reversible splenial lesion clinically presenting as delirious mania and evolving into life-threatening multi-organ failure. The patient was treated with aripiprazole and benzodiazepine with poor effect and, after 4 days, the patient's condition significantly worsened requiring transfer to the intensive care unit where deep sedation with propofol was started. Our findings are in contrast with the traditional literature description of self-resolving and harmless mild encephalitis with reversible splenial lesion. Moreover, rapid clinical recovery and the progressive improvement of psychiatric symptoms after deep sedation with propofol in this case—considering propofol's neuroprotective and anti-inflammatory effects—supports the notion of propofol-mediated deep sedation for the treatment of severe manic symptoms associated with life-threatening conditions. Little is known about neural markers of the manic state, and the corpus callosum has been described to be involved in bipolar disorder. Abnormalities in this structure may represent a marker of vulnerability for this disorder.

Keywords: encephalitis, manic state, neuroimaging, deep sedation, propofol

BACKGROUND

Mild encephalitis with reversible splenial lesions (MERS) is a rare clinic-radiological entity, first identified in 2004 (1), defined by the presence of clinical neurological and neuropsychiatric symptoms associated with a single lesion in the midline of the splenium of the corpus callosum (SCC) (MERS type I) and, in some cases lesions with similar radiological aspects in the white matter of the cerebral hemispheres (MERS type II) (2). According to the literature, MERS seems to be more common in

children and young adults (3), presenting with disturbances of consciousness, seizures (more common in children) and headache (more common in adults) (2). The MERS diagnostic criteria are, according to Hoshino et al. (4): (i) clinical onset associated with neuropsychiatric symptoms, such as impaired consciousness within 1 week after fever onset; (ii) complete recovery without sequelae, mostly within 10 days after the onset of neuropsychiatric symptoms; (iii) high-signal intensity lesion in the SCC; (iv) involvement of the entire corpus callosum and bilateral cerebral white matter with symmetrical pattern may also occur; and (iv) lesion disappearing within 1 week, with no residual signal changes or atrophy. For MERS, typical magnetic resonance imaging markers are: (i) high signal intensity in T2 weighted images; (ii) decreased apparent diffusion coefficient (ADC) value of the lesion; and (iii) hyper-isointense signals on T1 weighted images (2). Furthermore, Tsuji et al. (5) report similar radiological features in a patient without neurological signs (5). Previous studies identified that MERS could be triggered by infections, such as influenza virus, rotavirus, mumps virus, mycoplasma pneumonia, and legionella pneumonia (2), and adverse drug reactions (ADR), particularly in patients with malignant neuroleptic syndrome (6–8), lithium intoxication (9), or antiepileptic drug withdrawal (10, 11). To our knowledge, the present case is only the second case (10) of MERS associated with mania and the first presenting with delirious mania.

Delirious mania is a severe psychiatric syndrome characterized by acute onset of delirium, excitement, and psychosis. It was initially described by Calmeil in 1832 as an "uncommon but life-threatening psychosis with extreme hyperactivity and mounting fear fading to stuporous exhaustion" (12). Different terms have been used to name delirious mania, e.g., lethal catatonia and malignant catatonia. It is a rare syndrome, possibly underestimated with several authors suggesting that as many as 15%–20% of all acutely manic patients show signs of delirium (12). Patients with this syndrome experience significant morbidity and mortality risk (12–14) if not quickly treated. The syndrome is often accompanied by signs of organ failure which are often unable to be managed in an ordinary psychiatric unit. Delirious mania is marked by acute onset of excitement, grandiosity, emotional lability, delusions, and insomnia (typical features of mania), and disorientation and altered consciousness (typical features of delirium). Bond (15) outlined six criteria that may distinguish delirious mania: (i) acute onset; (ii) presence of hypomania or mania; (iii) developing signs and symptoms of delirium; (iv) history of mania or depression; (v) family history of affective disorder; and (vi) responsivity to treatment for mania. Furthermore, patients may show a typical resistance to common pharmacological treatments at usual doses. Karmacharya et al. (12) suggested that the definitive treatment for this condition is electroconvulsive therapy (ECT) and when ECT is not available, high-dose benzodiazepines should be used. There is presently, however, no clear consensus on which clinical features are associated with delirious mania and which treatments are effective (14). Additionally, deep sedation in an intensive care unit may represent an option, especially in the acute phase of the disorder. Few articles describe the use of deep sedation as a treatment for refractory mania or delirious mania (16–18).

CASE REPORT

A 37-year-old man, with a history of schizoid personality disorder and previous brief psychotic episodes, was admitted to the Psychiatric Department (AOUI Verona) for a rapid onset of psychomotor agitation associated with delusional ideation, confusion, aggressive behavior, and mood elevation with dysphoria. These symptoms apparently started 5 days before, rapidly worsening during the 12 h before admission. The patient was on stable treatment with aripiprazole 10 mg daily and 2 weeks before the described episode the dose of aripiprazole was reduced to 7.5 mg daily. Combined antipsychotic and benzodiazepine treatment was immediately started with poor effect, making physical restraint necessary.

During the first days the patient developed physical alterations such as high blood pressure, tachycardia, elevation of body temperature (BT) and significant elevation of creatine phosphokinase (CPK). Levels of ammonia, antinuclear antibodies (ANA), (extractable nuclear antigens (ENA), anti-DNA autoantibodies, C3, C4, erythrocyte sedimentation rate (ESR) and procalcitonin did not reveal any alteration. The only altered inflammation index found was C-Reactive Protein (CRP), which reached levels of 50 mg/L. Blood culture and uroculture were negative.

On day 3, a brain computed tomography (CT) and an MRI examination were performed in the Radiology Department of G.B Rossi Hospital, Verona, with a 1.5 T Symphony Maestro Class scanner (Siemens, Germany, Enlargen). The Brain CT was normal, while MRI scan (**Figures 1A–E**) revealed an ovoid area of hyperintensity on T2-weighted and FLAIR images in the central part of the SCC. On diffusion weighted images the lesion showed high intensity of the signal with a low ADC value in comparison to the other components of the corpus callosum. After intravenous injection of paramagnetic contrast agent (Gadovist, 7 ml) no enhancement was detected. The radiological findings were therefore consistent with a MERS diagnosis even if the whole clinical picture seemed to be more severe than those reported nowadays in the literature.

On day 5, organ failure signs (blood pressure 170/110 mm /Hg; heart rate 136 bpm; BT 38.7°C; CPK 10.535 U/L) suggested the possible presence of Neuroleptic Malignant Syndrome (NMS). The patient was transferred to ICU, where deep sedation (midazolam, propofol) was started, allowing an adequate treatment of concomitant organic disorders. During the following days, the hypothesis of NMS was ruled out, and intensive hydration and urine alkalizing administration progressively reduced indexes of muscular damage (the rise of which could be ascribed to agitation and/or ADR). On day 15, the patient was afebrile, hemodynamically stable, awake but delusional, and not fully oriented. Psychomotor agitation was absent, and the patient was moved back to the psychiatric unit. A follow-up MRI performed 2 weeks after the first scan showed the complete vanishing of the lesion in the SCC, both in T2-weighted and in particular DWI sequences (**Figure 1F**). During following days, the patient became progressively more oriented and less delirious until day 32, when the patient was discharged on stable treatment with olanzapine and valproic acid.

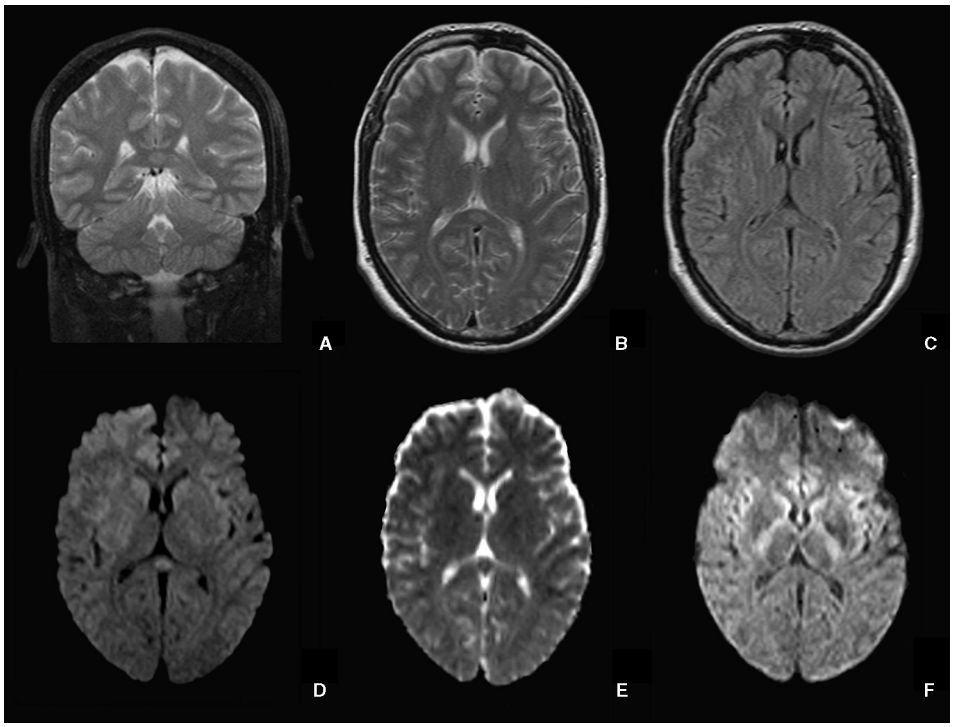

FIGURE 1 | Transient focal lesion in the SCC. Coronal and axial T2-weighted images **(A, B)** and axial FLAIR **(C)** showed an oval hyperintense focal lesion in the SCC. On axial DWI **(D)** the lesion is hyperintense with low values on the ADC map **(E)**. After 2 weeks, the lesion is no longer detectable on DWI **(F)**.

DISCUSSION

The case presented here may be, to our knowledge, the first report of an overlap of two rare conditions: MERS and delirious mania. According to the clinical and radiological findings, we have considered several differential diagnoses, such as infection-related MERS, acute disseminated encephalomyelitis (ADEM) clinical onset, paraneoplastic syndrome, metabolic disorder, NMS, and ADR. The clinical examinations and investigations performed during the hospitalization ruled out the majority of these diagnoses: infection indexes were negative, the splenial lesion disappeared in 10 days thus excluding ADEM, and the other clinical examinations and investigations were not consistent with paraneoplastic syndrome, metabolic disorder (such as acute porphyria or severe acute hyponatremia) or NMS, however, a specific diagnosis was not reached. An interesting hypothesis, needing further studies to be confirmed, is that the known anti-inflammatory (19–21), neuroprotective (22, 23) (possibly due to antioxidant activity) and immunomodulatory (23) effects of propofol could have contributed to MERS rapid resolution and clinical improvement. Although MERS pathophysiology is not completely understood, some hypotheses claim oxidative stress and intramyelinic oedema as two possible mechanisms (24, 25). Therefore, antioxidant and anti-inflammatory effects could possibly represent a focused treatment for MERS syndromes. Moreover, little is known about specific radiological features of the manic state, and the corpus callosum has been described to be largely involved in bipolar disorder (BD) and schizophrenia (26–29) possibly representing a marker of vulnerability for these disorders (30, 31).

Kobata et al. and Bulakasi et al. (32, 33) reported patients developing a reversible lesion of the SCC following rotavirus infection and influenza-associated encephalitis/encephalopathy manifesting confusion, agitation, and disorientation. In all the described cases, patients recovered in 4 to 9 days, and SCC lesions self-resolved without any life-threatening consequence. Furthermore, the authors speculated that cytotoxic edema was the major factor in the development of the encephalopathy and SCC lesion suggesting that an infarction would be otherwise irreversible and not consistent with the rapid self-recovery. In the report of Merizalde et al. (10) a bipolar patient developed a reversible lesion in the SCC due to the interruption of a lithium and oxcarbazepine based therapy. However, the patient described in our case developed a similar symptomatology even though no lithium/oxcarbazepine therapy was abruptly interrupted. Similarly, in the case report of Maeda and colleagues (34) a patient diagnosed with a major depressive disorder suffered from a focal lesion in the SCC subsequent to the administration of antiepileptic medication. Notably, as previously mentioned and speculated by other authors (32, 33), Maeda and colleagues suggested that reversible SCC lesions are probably caused by cytotoxic edemas. All the presented cases suggest that reversible lesions of the SCC can emerge from multiple causes (e.g. rotavirus infection, lithium withdrawal, administration of epileptic drugs) potentially giving rise to similar symptoms. The common

symptomatology seems to be confusion and agitation self-resolving in 2 weeks with a good prognosis (10). However, in our report, the patient's clinical condition diverged from most cases present in the literature, requiring intensive care and deep sedation. This finding is especially important because, in contrast with our findings, in all the previously mentioned reports patients' clinical conditions recovered quickly following a good prognosis in accordance with Hoshino's guidelines (4).

Concerning BD and mania, structural and diffusion MRI studies have widely reported abnormalities in volume, signal intensity, and microstructure of the corpus callosum, suggesting altered inter-hemispheric connectivity as a possible marker of illness, potentially leading to cognitive and emotional deficits (35). Our group found significantly increased ADC values in the anterior body and SCC in a sample of BD compared to healthy controls (31), suggesting microstructural anomalies specifically in the right hemisphere, and in another work, we applied Tract-Based Spatial Statistics (TBSS) in samples of BD and schizophrenia patients, confirming that fractional anisotropy (FA) is decreased in the fronto-temporal and callosal networks of these patients (36). Moreover, our group has also shown that alterations of the corpus callosum and impaired brain interhemispheric communication are involved in the pathophysiology and cognitive deficits present in BD (27, 28).

Recently a large tractography study confirmed low FA in white matter tracts, including the corpus callosum, with more severe biological abnormality in the subgroup of patients with psychosis. This provided additional evidence for the interhemispheric disconnectivity theory of BD, first described by Pettigrew and Miller (37). These authors considered slow interhemispheric switching as a marker of BD, and suggested that the right hemisphere is predominantly involved in depression and the left in mania. More generally, the disconnectivity hypothesis suggests that psychotic illnesses arise not from regionally specific focal pathophysiology in the brain, but rather from impaired integration between neuroanatomical regions (38). This impairment may be due to damage of axonal membranes or to axonal demyelination (39). According to the evidence, brain white matter and in particular the corpus callosum is considered a marker of vulnerability in patients with psychotic BD. Notably, other studies also found a generalized reduction in mean FA in the SCC, left cingulum, and in the anterior part of the left arcuate fasciculus in patients with BD, ultimately affecting interhemispheric communication (40). Future studies correlating MRI data with cognitive and clinical assessments are warranted to understand the specific functional correlates of these white matter deficits in BD (41).

CONCLUSION

We describe a rare case of delirious mania associated with reversible splenial lesion. The clinical-radiological features are consistent with Hoshino's diagnostic criteria for MERS (4). MERS is considered a self-resolving clinic-radiological syndrome that is not a life-threatening condition (2). This is in contrast to our report of delirious mania associated with a reversible splenial lesion. Delirious mania is in fact itself a life-threatening condition (12–14) and the above described organ failure signs were consistent with this interpretation. Moreover, this case supports the limited evidence (17, 18) for propofol-mediated deep sedation to treat life-threatening mania. This is also one of the few published reports describing possible radiological correlates of acute mania with MRI performed during the acute episode (10, 30, 31). Notably, Blumberg and colleagues studied acute mania and found a hyperactivation of the left caudate and anterior cingulate cortex, supporting the disconnection proposed by Pettigrew and Miller (37, 42). More evidence is now needed to: (i) better identify neural markers of acute mania; (ii) the role of the corpus callosum in the pathogenesis of BD; and (iii) a possible hemispheric specialization involved in manic symptoms. The opportunity of performing MRI scanning during the acute phase of a delirious mania has given us the possibility of achieving valuable data for further research in this field, allowing the connection between specific clinical signs and MRI imaging in a rare condition. The specific interaction between a localized lesion in the SCC, the evident clinical signs and symptoms, and the patient's psychiatric background remain unclear, and this report raises more questions than it answers. More work is needed to improve knowledge on the pathophysiology of mania.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The patient described in this case report signed an informed consent to allow possible publication for research purpose of his anonymized clinical and radiological data.

AUTHOR CONTRIBUTIONS

MBe was in charge of patient's clinical management and designed the case report and wrote the paper with GZ. MBa worked on the MRI data. PB and LP reviewed the main text. NZ contributed to revision process.

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BRIEF RESEARCH REPORT
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An fMRI Study of Adult Brain Cortical Activation Following Intensive Learning

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Functional imaging techniques, fMRI in particular, has given the possibility to investigate non-invasively the cognitive processes in healthy populations and different disorders concerning neuro-psychiatry, thus unfolding the concepts guiding diagnosis and patient management. Different brain structures seem to support different types of cognitive functions in particular learning and memory thus the neurobiological explanation of the retrieval of information is associated with knowledge of brain plasticity, memory circuits, synaptic neurotransmission and the modulation of glial cells. Consistent with fMRI investigations of memory systems we tested the dependability of a memory paradigm using heterogeneous memory stimuli in order to find the neurobiological basis that correlates with memory task performance. Our study resulted with statistical significant differences in brain activations across the block design contrasts in both occipital and temporal regions in 29 mentally healthy students during a memory paradigm performance after intensive learning. As functional magnetic resonance imaging has become an important and reliable tool for investigation of brain anatomy and its function in health and disease, it becomes clear that further research of neurobiological basis of cognitive and memory domains can clarify different diagnostic prototypes and thus explain the human brain impairments in neuropsychological patients, since these are characterized by various cognitive dysfunctions.

Keywords: functional MRI, memory paradigm, brain activation, intensive learning, cognition

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INTRODUCTION

Functional imaging techniques, fMRI in particular, has given the possibility to investigate non-invasively the cognitive processes not only in healthy populations, but in different disorders concerning neuro-psychiatry, thus unfolding the concepts guiding diagnosis and patient management (1, 2). In this sense, the original definition of translational neuroscience suggests that fundamental neurobiological knowledge can explain the brain functioning in healthy and pathological conditions (3).

Human brain seems to be a dynamic structure, which is continuously changing in response to experiences (4, 5). The application of different stimuli to the brain during experimental studies and

the evaluation of functional imaging results support the increasing evidence of activity-dependent neuroplastic changes of the human brain across the life-span (6, 7). Studying the cognitive functions and memory systems in particular may uncover the specific processes associated with aging and brain development (8).

The present study aimed to investigate the cortical brain activations in response to a memory paradigm performance during a functional MRI scan after intensive learning in healthy individuals.

MATERIALS AND METHODS

Subjects

We conducted an fMRI scanning of 29 mentally healthy, right handed students of Bulgarian origin (15 males and 14 females with a mean age of 20.0 ± 2.0). The handedness was determined using the Edinburgh handedness inventory (9). In order to examine the brain functioning after intensive learning, the experimental procedure was applied just after one month summer examination session with a duration of average 8 h intensive studying per day and mean exams final grade of 5.50.

Exclusion criteria included signs or a history of first degree relative with mental retardation, somatic disorders with neurological components; developmental, learning or psychotic disorders (schizophrenia, affective disorders, etc.) under DSM-IV (10); an identifiable neurological disorder (seizure disorder, head injury, multiple sclerosis etc.); a history of drug or alcohol abuse.

The study was carried out under the recommendations and protocol approval by the committee of "Research Complex for Translational Neuroscience" of Medical University of Plovdiv, Bulgaria. We obtained a written informed consent from all participants in accordance with ethical principles of the Declaration of Helsinki.

Experimental Procedure

The experimental procedure included a scan with a 3T MRI system—GE Discovery 750w with a protocol of structural scan—Sag 3D T1 F-BRAVO FSPGR, slice thickness 1 mm, matrix 256 × 256, flip angle 10° and standard block design functional scan—2D EPI, slice thickness 3 mm, matrix 64 × 64, TR (repetition time)—3,000 msec, TE (echo time)—30, flip angle 90°.

Using a standard block design with total duration of 4 min, we defined four consecutive sets of "on" and "off" blocks. Participants were first presented with four consecutive pictures of the same theme—landscapes, portraits, anatomical images of internal organs and geometric figures denoted by specific mismatching nouns (seasons, personal names, internal organs, figures) (**fixation—F part**) with a duration of 3 s, followed by three of the pictures with questions (**recall—R part**) for memory evaluation (What was the word under this picture? Four possible answers.), for each of which the response time was 6 s. Subjects responded by pressing one of four possible buttons (two in each hand). The total duration of the active "on" blocks was 30 s. A rest—"off" block of 30 s followed each of the "on" blocks (**Figure 1**). The subjects were instructed to read carefully the questions and answer with a button press during the active conditions and to look at the central fixation cross without thinking of anything in particular for the resting conditions.

fMRI Data Analysis

Functional MRI data analysis was done with SPM 12 (Statistical Parametric Mapping) software running on MATLAB R2015 for Windows. The preprocessing included five steps as follows: 1) realignment of the functional data for correction for head motion, 2) co-registration between the high-resolution anatomical image and the functional scans, 3) intra-individual estimation of spatial registration parameters based on the anatomical image, 4) transformation of the co-registered functional data to standardized MNI (Montreal Neurological Institute) space, followed by 5) spatial smoothing with a 6mm full-width-at-half-maximum Gaussian kernel.

We specified the first level analysis model, estimated and defined the parameters and t-contrasts for both active conditions vs. the passive condition (F > off, R > off), the first active condition vs. the second (F > R) and the opposite (R > F). The resulting contrast maps from each contrast and for each subject were then used in a second level random effects analysis for within group and between groups effects (males vs. females). Statistical significant level was set at $P < 0.05$ Family Wise Error (FWE) corrected.

RESULTS

fMRI Results

Our study resulted with statistical significant differences in brain activations across the block design contrasts in both occipital and temporal regions in males and females for two of the contrast maps—F > R and R > F.

Within both genders, the F > R contrast resulted in statistical significance of brain activations. We found significant clusters with peak activations in males in the region of left superior occipital gyrus (MNI coordinates −12, −88, 16) expanding to the right caudate nucleus (20, 16, 12) and left middle temporal gyrus (−54, −32, −2) (**Table 1**, **Figure 2**).

The clusters of female participants located in the right middle occipital gyrus (34, −80, 12), right putamen (18, 12, −2) and left caudate nucleus (−16, 14, 0) showed significant residual activations as a result of the F > R contrast (**Table 2**, **Figure 2**).

Significant BOLD-signal differences were found in the R > F contrast with clusters in left postcentral gyrus and left supramarginal gyrus (−58, −20, 30) in males (**Table 1**, **Figure 2**). Females showed peak activation of the R > F contrast in the right precuneus (14, −58, 30) and right supramarginal gyrus (58, −24, 28) (**Table 2**, **Figure 2**).

Correction for multiple comparisons failed in finding significant residual activations for the F > R and R > F contrasts when applied in between-group analysis of males vs. females.

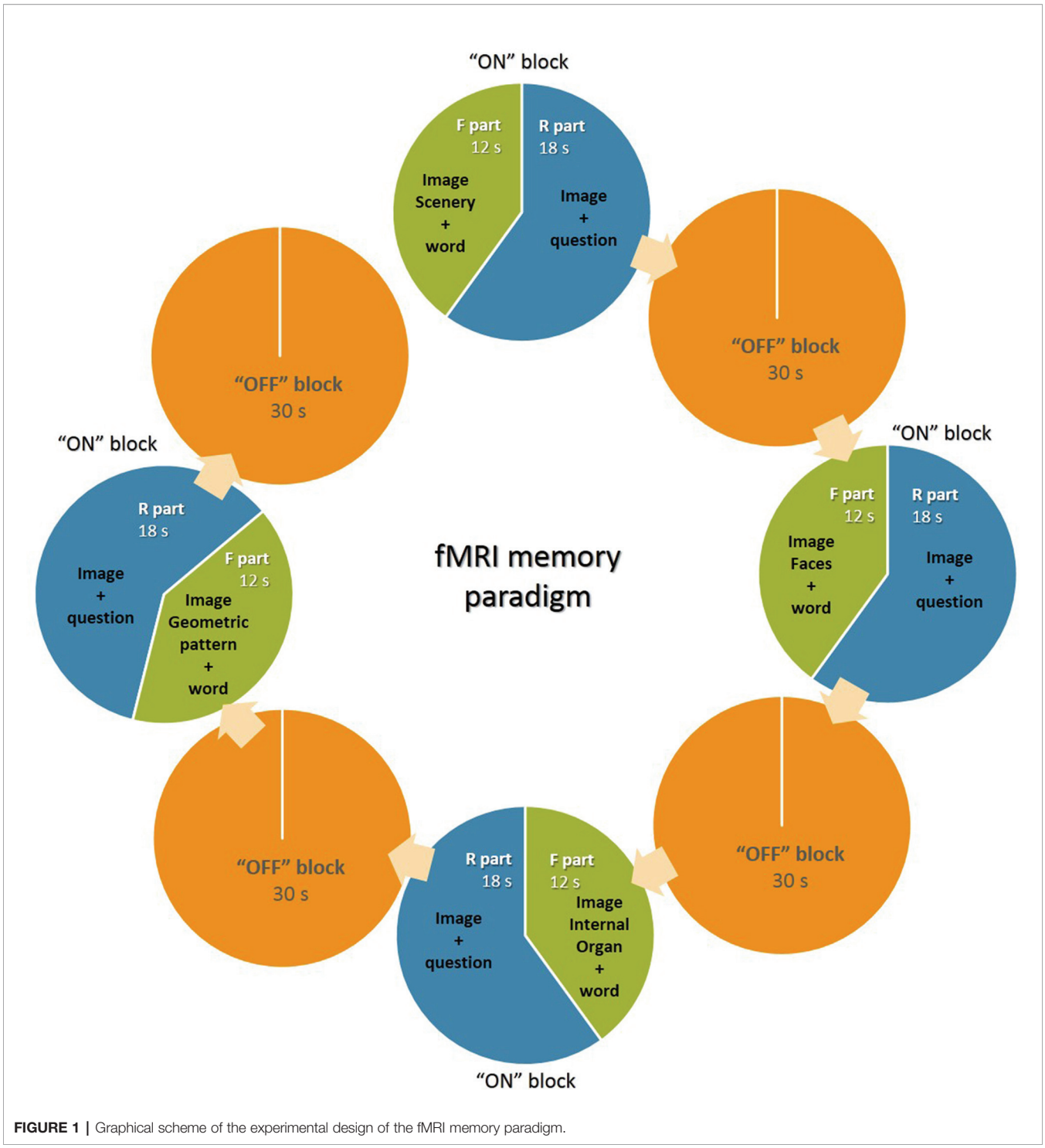

FIGURE 1 | Graphical scheme of the experimental design of the fMRI memory paradigm.

DISCUSSION

Consistent with similar studies of functional magnetic resonance imaging performance of memory tasks we determined the reliability of a memory paradigm in an experimental investigation of adult brain cortical areas that correlate with memory encoding after intensive learning in healthy individuals from both genders. Despite we did not find significant distinctions in the brain activations between males and females for the F > R and R > F contrasts, the within group results showed statistically significant differences for both the contrast maps.

The occipital residual brain activations of the F > R contrast in both genders is explicable due to the presence of visual stimuli during the fixation part. The same contrast map showed

TABLE 1 | Males peak-level in a one-sample t-test of the F > R and R > F contrast maps after intensive learning.

Anatomical localization peak activation	Cluster size (number of voxels)	pFWE-corr	MNI coordinates		
			x	y	z
F–R					
Left superior occipital gyrus	**12,583**	0.000	–12	–88	16
Right caudate nucleus	**2,348**	0.000	20	16	12
Left middle temporal gyrus	**51**	0.006	–54	–32	–2
R–F					
Left postcentral gyrus and Left supramarginal gyrus	**24**	0.022	–58	–20	30

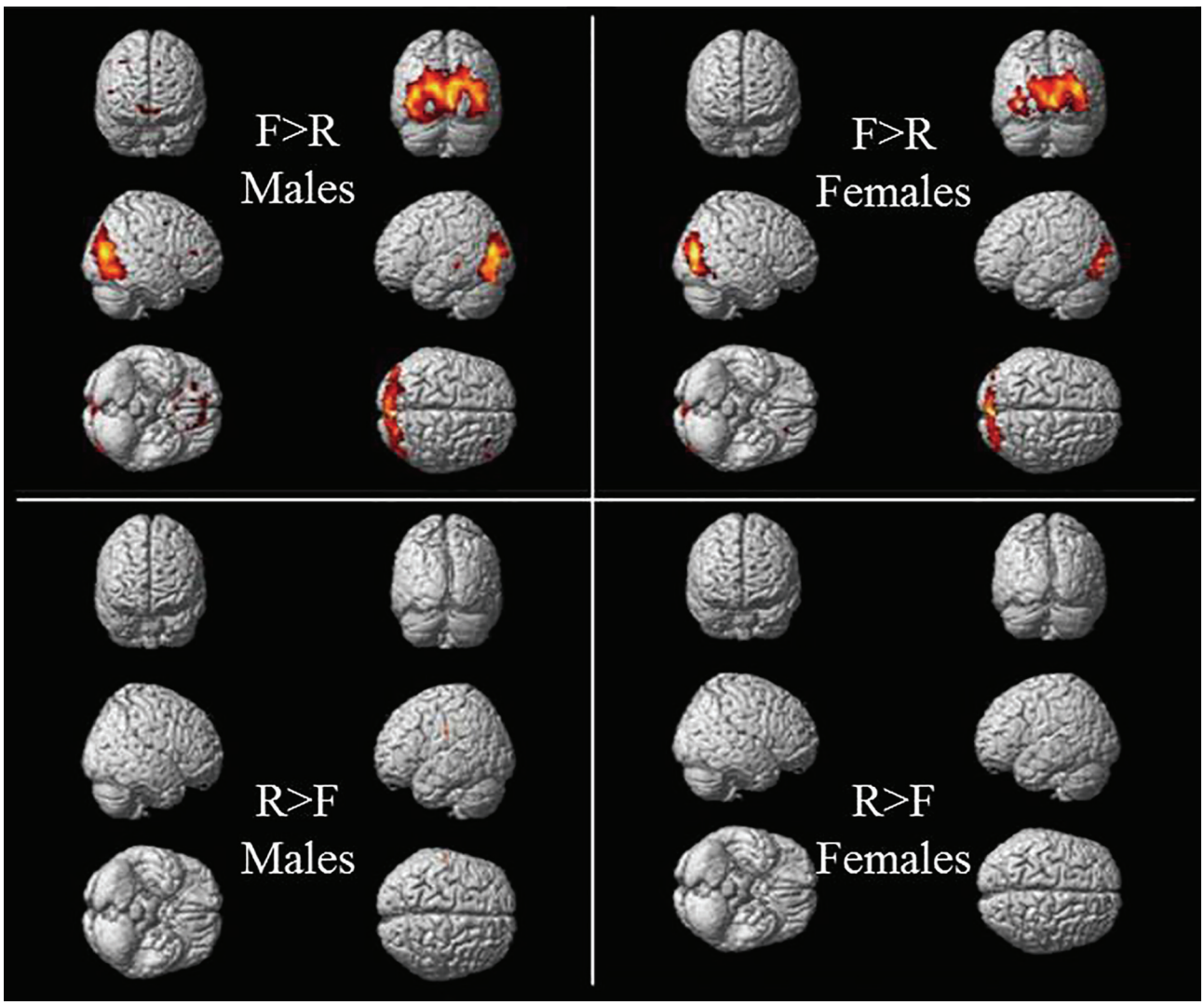

FIGURE 2 | Significant residual activations of the F > R and R > F t-contrasts in male and female subjects after intensive learning.

significant differences in the peak activation with clusters in right caudate nucleus and left middle temporal gyrus in males and right putamen and left caudate nucleus in females. Consistent with other findings (11, 12), our results indicate that memory encoding during spontaneous memory tasks including visual stimuli involve both sensory and non-sensory brain areas. Thus, occipital–temporal lobe regions are considered to participate in associative recognition and item recognition (13), and their dynamic connectivity with other cortical areas, such as basal ganglia, is essential in the memory process (14). Several studies

TABLE 2 | Females peak-level in a one-sample t-test of the F > R and R > F contrast maps after intensive learning.

Anatomical localization peak activation	Cluster size (number of voxels)	pFWE-corr	MNI coordinates		
			x	Y	z
F–R					
Right middle occipital gyrus	**6,041**	0.000	34	–80	12
Right putamen	**30**	0.008	18	12	–2
Left caudate nucleus	**22**	0.011	–16	14	0
R–F					
Right precuneus	**5**	0.031	14	–58	30
Right supramarginal gyrus	**5**	0.039	58	–24	28

highlighted the relevance of the striatal gray matter volume for memory and working memory operations (15, 16). A research report of Bauer et al., 2015 revealed "the relevance of caudate for associative memory decline in the aging brain" suggesting that the "right striatum (putamen and caudate) was correlated with recognition accuracy", whereas the "bilateral caudate was positively associated with associative learning accuracy" (17).

Furthermore, the significant peak activations of the R > F contrast resulted in clusters at the supramarginal gyrus in both males and females, although in women, due to the small number of clusters, the visualization of brain activity is almost impossible (**Figure 2**). The association brain cortex at the parietal areas of right and left hemispheres and their inferior regions in particular, including the supramarginal gyrus, mediate somatosensory integration, representation and organization of movements, motor attention and identification of motor gestures, as well as in visual word recognition (18). It is considered that left supramarginal gyrus plays a key role of the short-term memory network, preserving an abstract representation of information from serial orders, regardless of content information (19–21). As functional magnetic resonance imaging has become an important and reliable tool for investigation of brain anatomy and its function in health and disease, it becomes clear that further research of neurobiological basis of cognitive and memory domains can clarify different diagnostic prototypes and thus explain the human brain impairments in neuropsychological patients, since these are characterized by various cognitive dysfunctions (22, 23).

However, our study has several limitations. Greater sample size is probably necessary to find statistical significant differences between the genders when comparing the BOLD-activations. We found similar imaging results in the active brain regions in one of our previous studies applying the same memory paradigm and same volunteers during a period of time when the students were not subjected to intensive learning (24). Therefore, further research and statistical analysis is indispensable for evaluation of the specific differences in cortical activation areas between both conditions in order to contribute to the disclosure of the way brain works in health and disease.

CONCLUSION

The results of the present study support the recent investigations on brain plasticity and memory systems as an experimental approach in the multidisciplinary essence of translational neuroscience. Despite the limitations that should be considered in future research, the present study corroborates the findings that human brain is a dynamic structure, which reacts with complex interactions in both cortical and subcortical regions with regard to memory stimuli and tiny areas with subtle activations as supramarginal gyrus seem to play an important role in short-term memory circuits. Considering the results obtained after intensive learning and previous similar studies of brain activations at rest, we suppose that a further in-depth comparative analysis would enrich the data on how the human brain responds to stress, which in turn would be important in evaluating brain plasticity and function in disease.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Committee on Scientific Ethics, "Research Complex for Translational Neuroscience", Medical University of Plovdiv, Bulgaria. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FA-P and SS designed and performed the experimental study. FA-P and SS processed the results and wrote the manuscript. MT and AB managed the literature searches. All authors have approved the final manuscript after revision.

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MINI REVIEW
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Neural Bases of Cognitive Impairments in Post-Traumatic Stress Disorders: A Mini-Review of Functional Magnetic Resonance Imaging Findings

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Introduction: Post-Traumatic Stress Disorder (PTSD) is often associated with impairments in emotional and cognitive domains. Contrarily to the emotional sphere, neural basis underpinnings to cognitive impairments are still not well known.

Methods: We performed a bibliographic search on PUBMED of all the studies investigating the cognitive impairments in PTSD individuals. We considered only studies that applied cognitive tasks using a functional Magnetic Resonance Imaging technique. The inclusion criteria were met by nine studies.

Results: Overall, PTSD individuals reported significant impairments in the dorsolateral prefrontal cortex, anterior cingulate cortex, inferior frontal gyrus, insula, inferior temporal cortex, supplement motor area, and Default Mode Network (DMN). Moreover, abnormal activity was reported in subcortical structures (e.g. hippocampus, amygdala, thalamus) and in the cerebellum.

Limitations: Cognitive functioning was assessed using different cognitive tasks. Potential confounding factors such as age, sex, symptoms intensity, and comorbidities might have influenced the results.

Conclusion: So far, the evidence reported that PTSD is characterized by cognitive impairments in several domains, such as attention, memory and autonomic arousal, which may be due to selective dysfunctions in brain regions that are part of cortical networks, the limbic system and DMN. However, further studies are needed in order to better assess the role of cognitive impairments in PTSD and to develop more targeted therapeutic approaches.

Keywords: post-traumatic stress disorder, cognition, fMRI, selective attention, response inhibition, memory

INTRODUCTION

Post-Traumatic Stress Disorder (PTSD) is defined by the Diagnostic and Statistical Manual of Mental Disorder Fifth Edition (DSM-5) as a psychiatric disorder that can occur in people after experiencing a traumatic event, such as cataclysms, severe accidents, terroristic attacks or brutal personal assaults (1). Moreover, PTSD can occur in all people regardless of age, ethnicity, nationality, and culture, with women twice as likely as men to develop PTSD (1).

Moreover, PTSD is characterized by several symptoms, including intrusive thoughts, avoiding behaviors, negative thoughts and hyperarousal symptoms, such as irritability, sleep disorders and hypervigilance, which may, in turn, cause impairments in several cognitive domains, such as memory, attention and autonomic arousal (2). Therefore, recently, several studies on PTSD have explored the different cognitive domains using neuropsychological tests and neuroimaging techniques, including structural and functional magnetic resonance imaging (MRI) (2, 3). To the best of our knowledge, the majority of these studies focused on cognition in relation to emotion, using tasks with emotional valence stimuli (e.g. recalling traumatic event, emotion recognition tasks). Specifically, the evidence reported by neuropsychological studies showed that PTSD patients had enhanced memory performances in recalling events and items with negative emotional valence, enhanced responsivity to fear conditioning and increased attentional bias in processing threat stimuli (2). Additionally, it has also been reported that PTSD subjects were characterized by slowed goal-direction activity, impairments in recalling and learning neutral information, inability in extinction learning, as well as difficulties in remembering specific neutral and autobiographical events (2). Interestingly, in recent years, neuroimaging investigations have provided important insight on the neurobiological underpinnings of these cognitive deficits in PTSD patients. Specifically, structural and resting-state MRI studies on PTSD reported gray matter and connectivity alterations in cortical areas, such as anterior cingulate cortex (ACC), prefrontal cortex (PFC) and insula, as well as in subcortical structures, such as hippocampus and amygdala (4), all regions known to be part of a network which regulates contextual processing (5). Finally, disproportional hypervigilance and tendency to interpret neutral or safe situations as dangerous have also been considered hallmarks of PTSD (5), further supporting the presence of a dysregulation in the contextual processing network in PTSD patients, which in turn may explain impairments in modulating fear inhibition, emotion and attention regulation, as well as autonomic responses (3, 6, 7).

However, although the relation between cognition and emotion in PTSD has been well explored, to our knowledge, the study of cognitive mechanisms and their neural correlates detached from the emotional sphere is more limited. Notably, several findings showed that cognitive and emotional networks are differently affected in PTSD subjects, suggesting that cognitive and emotional domains, although strictly intertwined, need to be investigated separately (8, 9). For these reasons, in the last years, there has been an increased interest in the investigation of neuropsychological and cognitive features characterizing PTSD (2), especially for identifying the role of stress and anxiety in influencing normal cognitive processing. The evidence emerging from these studies is paramount to understanding the neural basis of PTSD, its development and its treatment, since they are critical for the social, occupational and emotional functioning (10). In this context, this review aims at summarizing all functional MRI studies (fMRI) that investigated the neural bases of cognitive impairments in PTSD using cognitive tasks.

MATERIALS AND METHODS

We carried out a bibliographic search in PubMed and Scopus using "*PTSD AND fMRI AND cognition*" and "*PTSD AND fMRI and EMOTION*" and "*PTSD AND fMRI AND cognition AND deficits*". We also used "*EMOTION*" as a keyword because in many studies cognitive domains were explored together with emotional aspects. However, for this review, we only (treatment as usual) selected the fMRI studies in which cognition was investigated outside the context of an emotional task. No time restrictions were used and we selected studies until February 2019. We excluded studies that a) employed neuroimaging techniques other than fMRI, including resting-state fMRI and real-time fMRI, b) used tasks with emotional valenced stimuli only (e.g. faces, pictures or sounds with affective contents), c) were not in humans, d) explored PTSD in relation to personality disorders, e) investigated at-risk subjects for PTSD disorder. The inclusion criteria were met by nine studies whose methods and results are summarized in **Table 1**. Specifically, for the cognitive domain explored, seven studies focused on attention and response inhibition (11, 12, 14, 16–19), one study explored memory functions (13), and one study investigated autonomic arousal (15).

RESULTS

Most of the results refer to two cognitive domains, selective attention and response inhibition, which were explored in subjects affected by PTSD through different cognitive tasks.

Specifically, the first fMRI study was performed by Thomaes et al. (18) who carried out a longitudinal study to investigate the impact of 6 months psycho-educational and cognitive behavioral stabilizing treatment in addition to the classic PTSD therapy (experimental treatment), or of classic therapy only (treatment as usual) on selective attention in PTSD patients compared to healthy controls (HC) using a Classic Stroop test. Interestingly, at baseline, the authors found that both PTSD patients and HC showed greater activations in the inferior frontal gyrus (IFG), extending to the Broca's area, dorsal ACC, supplement motor area (SMA), posterior parietal cortex, secondary visual cortex bilaterally, inferior temporal cortex and insula. Additionally, PTSD individuals also reported greater activations in the left inferior insula and dorsal ACC compared to HC. Although no

differences were observed between medicated and non-medicated patients, the authors found that patients with co-morbid major depressive disorder (MDD) displayed reduced activation in dorsal ACC compared to non-MDD patients. Moreover, at follow-up, the whole group of PTSD patients, regardless of treatment, improved the performance in the Stroop test in terms of accuracy, with subjects in the experimental therapy also showing shorter reaction times. In addition, during the task, while both groups showed a reduced activation in the premotor cortex/SMA and left inferior frontal cortex at the end of the study period, patients that followed the experimental treatment revealed decreased responses in bilateral dorsal ACC, left anterior insula and superior PFC compared to the classic therapy group.

Furthermore, in a Go/No-Go task, Jovanovic et al. (16) investigated attention and response inhibition in a sample of 41 women. Among them, 20 showed higher current PTSD symptoms (PTSD+) as opposed to the healthy remaining 21 (PTSD-). Interestingly, the behavioral responses during the Go and No-Go trials were very accurate in both groups, with no differences in the error rate. However, during the fMRI task the authors found that the PTSD+ group showed reduced activation in the ventromedial PFC compared to the PTSD- group. Additionally, attention and inhibition impairments were also explored by van Rooij et al. (19) using the stop-signal anticipation task (SSAT), a modified version of the Stop Signal Task (20), which was employed for exploring reactive and proactive inhibition in a sample of 28 PTSD male veterans, 26 male veterans without current psychiatric illnesses (combat control, CC), and 25 HC. Specifically, the authors defined reactive inhibition as the outright stopping of a response managed by motor areas, whereas proactive inhibition was defined as the anticipation of stopping, relying on the processing of contextual clues. With respect to reactive inhibition, speed inhibition was faster in HC than PTSD and combat control (CC) groups. Moreover, PTSD patients showed less reduction in activation (inhibition) in the left pre/post central gyrus compared to CC and HC groups during the SSAT. However, all groups activated neural networks usually involved in response inhibition, such as the right IFG, insula, right supramarginal gyrus, right SMA and left superior frontal gyrus, as well as deactivating the default mode network (DMN). Regarding proactive inhibition, although response time data showed that the PTSD group had reduced inhibition compared with CC and HC groups, no brain activation differences were observed between the groups during the SSAT.

Interestingly, similar results were found by Scheibel et al. (17) who investigated response inhibition and attention in 15 veterans using a modified version of the Arrows Task. During this task, the participants viewed blue or red arrows for 265 milliseconds (ms), and each was then followed by a blank screen for 200 ms and a cross hair fixation point for another approximately 2235 ms. Subjects were required to respond with a button press on the same side as the one in which the arrows were pointing when the arrows were blue, and to the opposite side when the arrows were red (17, 21). Based on the PTSD Checklist (PCL), the sample was split in 7 subjects with relatively high PTSD symptoms (HIGH PTSS; PCL≥39) and in 8 subjects with lower or no symptoms (LOW PTSS; PCL ≤ 31). The full sample analysis revealed deactivation of the posterior dorsal anterior cingulate gyrus, the right lateral frontal lobe, bilateral parietal structures and the precentral gyrus. In addition, the LOW PTSS group showed hyper-activations within both temporal lobes, the left cingulate and precentral gyri, the right thalamus and parts of the basal ganglia. Conversely, the HIGH PTSS group showed no significant activations but only extensive deactivation in cortical areas (e.g. left cingulate gyrus), subcortical structures (e.g., left amygdala, bilateral caudate), and bilateral cerebellum. Moreover, the comparison between LOW PTSS and HIGH PTSS patients highlighted a greater activation of bilateral large areas of the occipital, parietal and temporal lobes (e.g. angular gyrus, cuneus, fusiform gyrus), cerebellum, basal ganglia (e.g. left putamen), anterior and posterior cingulate gyri and the right lateral PFC within LOW PTSS individuals. Notably, there were no areas in which HIGH PTSS individuals had greater activation than LOW PTSS individuals.

Furthermore, Aupperle et al. (11) explored the neural correlates of response inhibition in a sample of 10 female PTSD patients and 12 HC with the stop signal task. The comparison between the two groups revealed greater activation in the right dorsolateral PFC, right superior temporal gyrus and the right anterior insula in the PTSD group compared to HC. Moreover, the PTSD group showed less differential activation in several DMN regions (e.g. precuneus, medial PFC). On the other hand, the HC group had more activation than the PTSD group in the left IFG, the right rostral ACC, and the lateral middle frontal gyrus than PTSD subjects. Further, HC revealed less differential activation within the anterior insula and the posterior cingulate cortex (PCC) than PTSD individuals.

Additionally, the fMRI study carried out by Clausen et al. (14) used a Multisource Interference task (MSIT) in a cohort of 39 male veterans with different levels of PTSD. Comparably to the above-mentioned findings, the authors showed that ventral ACC, rostral ACC and rostral medial PFC resulted in more activation during congruent trials, whereas incongruent trials elicited greater dorsal ACC and dorsomedial PFC activation. Moreover, the authors showed that worse PTSD symptoms were related to less rostral ACC activation. A reduced functional connectivity was found between both rostral ACC and medial PFC and lateral PFC regions. Finally, a particular form of selective attention was explored by Bluhm et al. (12) who investigated the neural bases of self-referential processing (SRP) in a sample of 20 PTSD subjects and 15 HC using an SRP task (12). The results from the within-group analyses showed that PTSD individuals had greater response in left dorsomedial PFC and in the precuneus, whereas HC had reduced activation in ventromedial PFC, dorsomedial PFC, PCC, and precuneus in the processing of self-knowledge vs. general facts contrast. Moreover, the between-group comparisons revealed that PTSD patients had reduced ventromedial PFC activation in response to self-knowledge vs. general facts compared to HC.

With regards to other cognitive domains, the last two fMRI studies included in this review explored memory and autonomic arousal in PTSD patients compared to HC.

Specifically, for the memory domain, Carrion et al. (13) investigated the role of hippocampal activity in 16 adolescents

TABLE 1 | Demographic and clinical characteristics of the fMRI studies included in the review (alphabetical order).

Study	Participants (Male/Female)	Age Mean, years (SD)	Design	MRI sequence	Clinical scale	edications	Comorbidity	Main results
Aupperle et al. (11)	10 PTSD (0/10) 12 HC (0/12)	PTSD: 34.60 (9.40) HC: 35.25 (6.44)	Stop Signal Block	fMRI (3.0T)	PCL CAPS D-KEFS	No medication	Not specified	**PTSD > HC** R Middle Frontal (dorsolateral PFC) R Superior Temporal/Insula **PTSD < HC** R Inferior Parietal L Superior medial PFC R Rostral ACC R Putamen L Caudate and Putamen R Middle Temporal R Parahippocampal gyrus L Postcentral gyrus R/L Precuneus L Paracentral Cortex L Middle Occipital R Lingual gyrus L Cerebellar Tonsil R Superior Cerebellum
Bluhm et al. (12)	20 PTSD (7/13) 15 HC (2/13)	PTSD: 39.35 (9.43) HC: 37.33 (11.52)	Self Referential Processing Block	fMRI (4.0T)	CAPS SCID CTQ	No medication	7/20 MDD 5/20 Current MDE 3/20 Dysthimia 1/20 Panic Disorder 1/20 Agoraphobia 7/20 GAD 5/20 Social Anxiety Disorder 1/20 Specific Phobia 1/20 OCD	Self-relevance vs General facts **HC > PTSD** dorsal medial PFC ventral medial PFC PCC/Precuneus **PTSD > HC** L dorsal medial PFC Precuneus
Carrion et al. (13)	16 PTSS (6/10) 11 HC (7/4)	PTSS: 13.9 (2.0) HC: 13.9 (1.9)	Verbal Declarative Memory Block	fMRI (3.0T)	CAPS-CA CBCL K-SADS WAIS	No medication	3/16 Depressive Disorder not specified 3/16 MDD 1/16 Panic Disorder 1/16 Enuresis 1/16 ADHD +C.D.+Social Phobia	Retrieval task **PTSS < HC** Bilateral Hippocampus
Clausen et al. (14)	39 PTSD (39/0)	PTSD: Not specified	Multisource Interference Block	fMRI (3.0T)	PCL-Military Version CAPS MINI	Not specified	Not specified	Congruent Blocks Rostral ACC Rostral medialPFC Ventral ACC

(Continued)

TABLE 1 | Continued

Study	Participants (Male/Female)	Age Mean, years (SD)	Design	MRI sequence	Clinical scale	edications	Comorbidity	Main results
					D-KEFS Tower Test SDMT AVLT TMT NAB Digits WTAR			<u>Incongruent Blocks</u> dorsal medial PFC dorsal ACC
Felmingham et al. (15)	11 PTSD (9/2) 11 HC (9/2)	PTSD: Not specified HC: Not specified	Auditory Oddball Event-Related	fMRI (1.5T)	CAPS SCID DASS	4/11 Antidepressants	7/11 Depression 1/11 Panic Disorder	**PTSD > HC** R rostral ACC Bilateral Hippocampus R Supramarginal gyrus R Superior Frontal Cortex R Fusiform gyrus L Thalamus R middle Frontal gyrus R Parahyppocampal gyrus R supplementary motor area L middle Cingulate **HC > PTSD** No significant clusters
Jovanovic et al. (16)	20 PTSD+ (0/20) 21 PTSD- (0/21)	PTSD+: 36.6 (3.3) PTSD-: 39.8 (2.8)	Go/No-Go Event-Related	fMRI (3T)	Modified PSS CTQ TEI STAI	No medication	Not specified	**PTSD- > PTSD+** Ventromedial PFC/rostral ACC **PTSD- < PTSD+** No significant clusters
Scheibel et al. (17)	7 PTSS + (Not specified) 8 PTSS - (Not specified)	PTSS +: 31 (5) PTSS -: 30.88 (6.36)	Arrow Task Event-Related	fMRI (3T)	P.C.L. - Civilian BSI NSI Barona IQ	PTSS+ Antidepressants: 4/7Mood Stabilizers 1/7 Pain Medication 3/7 Sleep aids 1/7 PTSS- Antidepressants: 3/8 Pain Medication 4/8 Sleep Aids 1/8	Not specified	**PTSS- > PTSS+** Bilateral Cerebellum Bilateral Basal ganglia Bilateral Cingulate gyrus Bilateral Cuneus **PTSS+ > PTSS-** No significant clusters
Thomaes et al. (18)	29 PTSD (0/33) B.L. 22 HC (0/30) B.L. 16 PTSD (0/16) A.T.	PTSD: 33.5 (11.6) HC: 35.2 (9.9) PTSD: Not specified	Classic Stroop Task Block **Treatment** TAU: Supportive care and /or pharmacotherapy EXP: Psycho-educational + Cognitive Behavioural group therapy + TAU	fMRI (1.5T)	STI CAPS BDI DES BPDSI SCID-D	19/29: SSRI 15/29: Anxiolytics	21/29: Anxiety Disorders 18/29: Depressive disorders	**BASELINE** **PTSD > HC** Bilateral Superior Frontal gyri R Parahippocampal gyrus R Fusiform gyrus L anterior Insula R dorsal ACC PPC Bilateral Premotor cortex/SMA **PTSD < HC** No significant clusters **AFTER TREATMENT**

(Continued)

TABLE 1 | Continued

Study	Participants (Male/Female)	Age Mean, years (SD)	Design	MRI sequence	Clinical scale	edications	Comorbidity	Main results
								EXP post > pre No significant clusters **EXP post < pre** Bilateral dorsal ACC R Superior frontal gyrus
L Anterior Insula Premotor Cortex/SMA **TAU post > pre:** No significant clusters **TAU post < pre** No significant clusters								
van Rooij et al. (19)	28 PTSD (28/0) 26 CC (26/0) 25 HC (25/0)	PTSD: 36.6 (10.6) CC: 37.2 (10.1) HC: 34.8 (9.5)	Stop Signal Anticipation Block	fMRI (3.0T)	CAPS SCID DSS	Anxiolitycs 1/28	15/28: Mood disorders 7/28: Anxiety disorders 2/28: Somatoform/ Pain disorders	PTSD < CC, HC (Proactive Inhibition): rostral IFG

PTSD, Post Traumatic Stress Disorder; PTSD+, Post Traumatic Stress Disorder with higher symptoms; PTSD-, Post Traumatic Stress Disorder with lower symptoms; PTSS, Post Traumatic Stress Symptoms; HC, Healthy Control; CC, Combat Control; B.L., Baseline; A.T, After Treatment; PCL, PTSD Checklist; CAPS, Clinical Administered PTSD Scale; CAPS CA, CAPS for Children/Adolescent; D-KEFS, Delian Kaplan Executive Function System; SCID, Structural Clinical Interview; CTQ, Childhood Trauma Questionnaire; CBCL, Child Behavior Checklist; K-SAD, Kidde Schedule for Affective Disorder; WAIS, Weschler Adult Intelligence Scale; MINI, Mini International Neuropsychiatric Interview; SDMT, Symbol Digit Modalities Test; AVLT, Auditory Verbal Learning Test; TMT, Trail Making Test; NAB, Neuropsychological Assessment Battery; WTAR, Weschler Test of Adult Reading; DASS, Depression, Anxiety and Stress Scale; TEI, Traumatic Events Inventory; STAI, State Trait Anxiety Inventory; BSI, Brief Symptoms Inventory; NSI, Neurobehavioral Symptom Inventory; STI, Structured Trauma Interview; BDI, Beck's Depression Inventory; DES, Dissociative Experience Scale; BPDSI, Borderline Personality Disorder Severity Index; DSS, Dissociative Symptom Scale; R, Right; L, Left; PFC, PreFrontal Cortex; ACC, Anterior Cingulate Cortex; PCC, Post Cingulate Cortex; SMA, Supplement Motor Area; IFG, Inferior Frontal Gyrus; TAU, Treatment As Usual; EXP, Experimental Treatment; SD, Standard Deviation.

with Post-Traumatic Stress Symptoms (PTSS) and 11 adolescents HC. All subjects performed a Verbal Declarative Memory Task with encoding and retrieval trials while undergoing fMRI scanning. The comparison between PTSS and HC during encoding trials showed no difference in hippocampal activation, whereas HC groups exhibited a greater activation of the right hippocampus during retrieval trials compared to PTSS. Furthermore, the authors also found a significant correlation between PTSD symptoms, specifically avoidance and numbing, and the reduction of left hippocampal activity in PTSS subjects.

Finally, Felmingham et al. (15) investigated the neural correlates associated with autonomic arousal in 11 PTSD subjects and 11 HC in response to salient stimuli in an Auditory Oddball task (AOT). The authors explored the Orienting Responses (ORs) that are critical markers of the registration of new or significant stimuli and mobilization of attention and motor responses (22). The authors used the skin conductance response (SCR), which is a powerful index of the OR since it is elicited by unexpected and potential threatening stimuli. In this regard, "oddball" stimuli (with SCR) can be considered as sudden sensory changes, as opposed to the frequent standard stimuli (without SCR). In light of this, the authors explored the response to targets both with SCR and without SCR. In relation to averaged analysis (target-background), PTSD individuals showed more enhanced activation than HC in right rostral ACC, bilateral hippocampus, right supramarginal gyrus, right superior frontal cortex, right fusiform gyrus, left thalamus, right middle frontal gyrus, right parahippocampal gyrus, right SMA, and left middle cingulate. These effects were confirmed in a subsequent analysis without individuals with depression or in treatment with antidepressants, which also revealed a major activation in right dorsal lateral frontal cortex in HC but no activity in hippocampus was found in the PTSD group. Also, the analysis between "with SCR-without SCR" targets showed that "with-SCR" targets engaged the right ventral ACC network, left supramarginal gyrus and the left inferior frontal cortex in HC. Specifically, greater activation was found in ventral ACC and in left inferior lateral frontal cortex in HC, compared to the PTSD group. In contrast, PTSD subjects showed a major engagement in dorsal ACC, right supramarginal gyrus and dorsolateral frontal regions bilaterally, displaying greater activity in bilateral dorsolateral PFC and in left supramarginal gyrus. Notably, these findings were replicated without considering depressed participants under treatment and the results showed that the PTSD group revealed greater activity in dorsal ACC than HC to "with-SCR" targets.

DISCUSSION

The majority of fMRI studies reviewed reported abnormal activations in executive functions, such as response inhibition and selective attention in PTSD patients. These functions are critical for developing a normal social and occupational life, as they have a key role in emotion regulation (10, 23). Indeed, response inhibition is the suppression of automatic responses to a situation that is no longer needed, and the appropriate adjustment of the behavioral response. Therefore, inhibition is fundamental in maintaining optimal cognitive functioning but also to suppress dysfunctional responses typical of PTSD, such as hypervigilance and intrusive thoughts (23). Similarly, impairments in selective attention contribute to developing and maintaining PTSD symptoms in several ways, such as strengthening the association between threat and stimuli during traumatic events and enhancing the detection of threatening stimuli (24).

Interestingly, from the abovementioned results emerged the hypothesis that prefrontal dysfunctions, especially in regions within the IFG, ACC, and medial/lateral PFC, might be considered key alterations characterizing PTSD patients while processing of response inhibition tasks. This is not surprising, especially because the dorsal regions of ACC and medial PFC seem to be implicated in cognitive and attention regulation (8, 25), whereas the IFG has often been associated with proactive response inhibition, a cognitive function that refers to the anticipation of stopping and relies on the processing of contextual cues (26, 27). Notably, the fMRI studies here reviewed also reported increased activation of amygdala and insula, two areas that are respectively involved in processing salient, external stimuli and in processing internal bodily states (28). Therefore, all these findings suggest that lower activation of ACC, medial and lateral PFC and IFG might reflect impairments in down-regulating amygdala and insula activation, which may ultimately lead to the cognitive deficits observed in PTSD patients (28, 29).

Moreover, several studies found alterations in the modulation of the DMN (11 ,12, 14, 19), a network which remains very active when the brain is at rest and deactivates when cognitive performance is required (30, 31), which includes several brain regions, such as middle temporal lobe, PCC, medial PFC, inferior parietal lobule, and lateral temporal cortex (30–32). In recent years, interest in DMN has grown since both resting-state and functional MRI studies discovered its role in the consolidation of memory, processing of internal and external stimuli and, remarkably, in the interplay between emotional stimuli and their cognitive elaboration (31, 32). Therefore, the involvement of this network observed by the above-mentioned studies is not surprising since it suggests that PTSD individuals have difficulties in disengaging DMN during low demanding cognitive tasks, which may lead to impairments in modulating executive control during high demanding cognitive tasks. Notably, these results are also consistent with previous studies that highlighted DMN alterations in PTSD patients (33, 34). Specifically, Sripada et al. (33) found reduced functional connectivity between rostral ACC, dorsomedial PFC and hippocampus at rest. Moreover, the same areas resulted hypoactive during cognitive tasks both with and without trauma-relevant stimuli. Similar results were obtained by a study on working memory that reported abnormal reduced connectivity between the PCC, frontal, temporal and parietal regions (implicated in the switching from resting state to activation), and abnormal

enhanced connectivity between medial PFC and bilateral parahyppocampal gyrus (34). Altogether, these findings suggest that PTSD patients are characterized by an inadequate DMN functional integration during resting-state and a general DMN alteration during goal directed tasks, which may in turn cause poor performance in cognitive tasks (33, 34).

Interestingly, two studies (14, 17) also reported correlations between symptoms severity and neuropsychological performance, as well as prefrontal, parietal and temporal activity, further supporting the hypothesis that the activation of regions involved in executive control (e.g. middle frontal gyrus, inferior parietal lobule, ACC) decreased in association with greater symptom severity (9, 35). Additionally, a further negative correlation between activation and symptoms was found in subcortical structures (e.g. thalamus) and in the cerebellum, which have well-known connections with the associative cortex (36). Therefore, these findings seem to suggest that the extension of the cognitive dysfunctions observed could be connected to the intensity of the symptoms. Therefore, although these results may still be limited, they aligned with the literature, reporting significant correlation between disease severity and cognitive impairments in psychiatric diseases, such as schizophrenia and bipolar disorder (37, 38).

Finally, two studies found impairments in the autonomic arousal (15) and in the memory domain (13) in PTSD patients.

Although deficits in autonomic arousal are known to be linked to PTSD (39), fMRI studies investigating the neural correlates of this symptom are yet to be fully explored. Nonetheless, the results obtained by Felmingham et al. (15), especially the increased activation in PTSD individuals of dorsal ACC, hippocampus and supramarginal gyrus, are not surprising since these areas are known to be involved in anxiety disorders (40). Finally, it has also been reported that PTSD patients had altered activity in the hippocampus while processing a memory task (13). This finding is not surprising, especially because a robust body of literature in the last decades has consistently reported the key role played by the hippocampus in declarative memory (encoding and retrieving information), semantic and episodic memory, as well as in emotional memory [for recent reviews, see (41, 42)]. Furthermore, this result is also supported by behavioral data, suggesting that subjects affected by PTSD have poor performance in memory tasks, including both neutral and emotional stimuli, as well as severe difficulties in retrieving neutral words and autobiographical episodes (42). Finally, several neuroimaging studies also reported smaller hippocampal volumes in individuals with PTSD and abnormal hippocampal activity both during encoding and retrieval processes (4, 43, 44), ultimately suggesting that the hippocampus is a fundamental structure for cognitive and emotional processes in PTSD.

Limitations

This review should be considered in light of some limitations. First, brain activity and performance during fMRI scanning were assessed using different cognitive tasks, which may explain some heterogeneity in the results. Second, the sample considered in the reported studies was small and included patients with varying age, sex and symptoms' intensity. Third, subjects involved in the studies had comorbidities with other psychiatric diseases, such as depression and anxiety disorders, which reduced the specificity of their findings. Fourth, the majority of the fMRI studies reviewed here employed PTSD patients under various pharmacological treatments. Importantly, the use of medication should be considered carefully since it is not a confounding factor per se, though its use can influence the BOLD signal both at a neural and vascular level in a manner that is not completely clear yet (45). However, it is important to mention that the majority of PTSD patients were undergoing treatment with various medications (e.g. antidepressant, anxiolytics, mood stabilizer), thus providing a more biologically realistic estimation of the brain in PTSD. Finally, it is also important to note that the number of fMRI studies exploring cognitive domains alone in PTSD are still sparse, ultimately limiting the knowledge of neural basis of cognitive impairments associated with this disorder.

Conclusion

In conclusion, the present review suggests that PTSD seems to be characterized not only by emotional processing impairments, but also by cognitive disturbances. Specifically, PTSD patients showed abnormal activation mainly in dorsal medial PFC, IFG, ACC, hippocampus, as well as in regions that are part of the DMN, ultimately suggesting that this disorder is characterized by impairments in top-down cognitive processes involved in the modulation of external and internal stimuli response. However, future fMRI studies are warranted for an in-depth analysis of the putative biomarkers associated with cognitive symptoms often observed in this disorder. This is valid especially for resting-state MRI studies, since the investigation of functional connectivity deficits between regions and the identification of dysfunctional brain networks in PTSD patients will allow for more insights about this disease. All these strategic approaches may in turn permit the development of more targeted treatments and preventive approaches.

AUTHOR CONTRIBUTIONS

GDo and GDe wrote the manuscript. PB and CP participated in the revision and proof-reading process of the manuscript together with JS. All authors have approved the final manuscript.

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frontiers in Psychiatry

CASE REPORT
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Cognitive Impact of Cerebellar Non-invasive Stimulation in a Patient With Schizophrenia

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Cerebellum plays a role in the regulation of cognitive processes. Cerebellar alterations could explain cognitive impairments in schizophrenia. We describe the case of a 50 years old patient with schizophrenia whom underwent cerebellar transcranial direct current stimulation (tDCS). In order to study the effect of cerebellar stimulation on cognitive functions, the patient underwent a neuropsychological assessment and an eyeblink conditioning (EBC) protocol. Although the effect of brain stimulation cannot be only assessed in a single-case study, our results suggest that cerebellar stimulation may have an effect on a broad range of cognitive functions typically impaired in patients with schizophrenia, including verbal episodic, short term, and working memory. In addition to neuropsychological tests, we evaluated the cerebellar function by performing EBC before and after tDCS. Our data suggest that tDCS can improve EBC. Further clinical trials are required for better understanding of how cerebellar stimulation can modulate cognitive processes in patients with schizophrenia and healthy controls.

Keywords: schizophrenia, tDCS—transcranial direct current stimulation, cerebellum, eyeblink conditioning, cognition

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BACKGROUND

The cerebellum is involved in a broad range of cognitive functions, including working memory, emotion processing, and social cognition (1). In humans, the cerebellum represents 10% of the brain volume but contains more than 50% of its neurons (1). The posterior lobe of the cerebellum is involved in cognition and connected to associative regions such as the prefrontal cortex, whereas the anterior cerebellum is known to modulate sensory-motor cortical activity (2). As alterations of the sensory-motor parts of the cerebellum lead to motor dysmetria, abnormalities in the posterior cerebellum may have implications for cognitive dysmetria. Andreasen et al. (3) have proposed that abnormalities in the posterior cerebellum may explain some symptoms of schizophrenia.

Schizophrenia is a severe mental disorder characterized by the association of positive, negative, and cognitive symptoms. Cognitive symptoms, that often precedes the illness, have an impact on the quality of life and on the functioning of the patients (4). Deficits in working memory, attention, processing speed, visual, and verbal procedural learning have been documented in

schizophrenia (5). However, pharmacological interventions only have a very limited impact on cognitive deficits in schizophrenia. Although non-pharmacological interventions, such as cognitive remediation can improve cognitive deficits in patients, there is a clear need for new interventions to target cognitive symptoms in schizophrenia.

Neuropsychological tests are commonly used to assess cognitive functions in patients with schizophrenia. These tests require full cooperation of participants, which can be difficult in a population of patients with schizophrenia suffering from motivational deficits (6).

Eyeblink conditioning (EBC) does not rely on motivation of the subject. It is based on a simple reflex pathway and measures associative learning (7). EBC is a form of classical conditioning that consists of pairing a stimulus (conditioned stimulus—CS, auditory in our study) with an unconditioned stimulus (US, airpuff in our study) that induces an eyeblink reflex. In delay-type EBC, a tone CS precedes and co-terminates with a corneal airpuff US that elicits an unconditioned response (UR). Over repeated pairings, the CS induces a conditioned response (CR) that precedes and reduces the US. McCormick et al. first showed that electrolytic lesions of the ipsilateral cerebellum completely prevented the acquisition and retrieval of the delay EBC (8, 9). The abundant literature based on lesion, reversible inactivation, genetic manipulation, electrical stimulation, optogenetics, electrophysiology, and brain-imaging studies show that the cerebellum is necessary and sufficient for acquisition, expression, and extinction of EBC provided that the interval between CS and US stays in the range of 1 s [see review in (10, 11)]. In accordance with animal research, EBC is a relevant method to investigate cerebellar dysfunction in schizophrenia disorders (12).

Non-invasive brain stimulation techniques are commonly used in healthy adults and patients with neuropsychiatric disorders to investigate brain mechanisms or to enhance cognitive, behavioral, social, and emotion processes (13). Transcranial direct current stimulation (tDCS) is a form of neuromodulation delivering a low direct constant current over two electrodes placed on the scalp. Applied to the cerebellum, tDCS can deliver an electric field reaching the cerebellum at a strength within the range of values for modulating activity in the cerebellar neurons (14).

In healthy subjects, two studies (15, 16) reported an effect of cerebellar tDCS on EBC. Interestingly, van der Vliet et al. (16) reported an interaction between the effect of cerebellar tDCS on EBC and the BDNF Val66Met polymorphism, previously involved in cognitive deficits in schizophrenia (17).

We describe the case of a 50 years old patient with schizophrenia whom underwent posterior cerebellar transcranial direct current stimulation (tDCS). We report neuropsychological testing and monitoring of cerebellar function with EBC before and after 1 week of stimulation in the posterior cerebellum.

CASE PRESENTATION

The patient was a 50 years old man suffering from schizophrenia. During the stimulation period, the patient was stabilized under a treatment of intramuscular haloperidol (150 mg every 4 weeks) and Zopiclone (7.5 mg/j). There was no change in the patient medication during the assessment and stimulation protocol. The patient was married with two children and was discontinuously working in the construction sector. He was mostly complaining from auditory hallucinations: the patient reported that he was hearing at least once a day a male voice that was giving him orders.

Written consent was obtained from the participant prior to the study.

The patient underwent 5 days of tDCS stimulation. The post-tDCS EBC session was performed 5 days after tDCS; 7 days separated the two EBC sessions. The cerebellum was stimulated using a NeuroConn DC Stimulator (NeuroConn GmbH) with two 5 × 7 cm conductive-rubber electrodes placed over the cerebellum, 1 cm below the inion (anode) and on the right arm (cathode). Stimulation was administered during two sessions of 25 min (separated by 1 h), including 5 s of ramp-up and 5 s of ramp-down, with an intensity of 2 mA (for a total of 10 stimulation sessions). The patient was stimulated during 5 consecutive days for a total of 10 sessions. We chose this stimulation protocol based on a previous modeling study (18) and on the work of Ferruci et al. (14).

Clinical assessment included the Positive And Negative Symptom Scale (PANSS) (19) and the Auditory Hallucination Rating Scale (AHRS), before and after stimulation. Neuropsychological assessment explored key cognitive functions typically impaired in patients with schizophrenia: episodic memory, executive, and attentional functions. We selected neuropsychological tests with no test/retest effect in order to compare neuropsychological outcomes before and after stimulation (20, 21). The patient underwent a long term episodic memory test (French version of Free and cued recall−16 items, Grober & Buschke, measuring anterograde episodic verbal memory using two different verbal material) (21), two subtests of the Wechsler Adult Intelligence Scale (WAIS-IV) (digit span and spatial memory), the stroop test (Golden version) (22) and the D2 Test of attention (Brickenkamp) (20). Neuropsychological assessment was repeated 2 days before and 2 days after the stimulation protocol (**Figure 1**) by a trained neuropsychologist that was not involved in the conception of the study nor in the brain stimulation.

The conditioning of the eyeblink reflex (EBC) was performed with a portable human eyeblink conditioning system (San Diego Instruments). The system included an infrared (IR) reflective sensor glued together with small 1.5 mm air-delivering tubing positioned just beneath the superior eyelid of the subject. The EBC device, comprising a portable airpuff and headset sound-delivering unit, controlled the timing, and intensity of both the airpuff and the sound (pure tone). It also converted the analog IR-reflection signal to numeric and sent it to a personal computer. The IR-reflection signal was collected online with the San Diego Instruments Labview software and then analyzed offline with a custom-made routine under Python (Python Software Foundation). During the overall experiment a continuous background white noise was delivered to the subject through the headset in order to provide constant ambient noise. The conditioning stimulus (CS) consisted of a 400 ms−1.2 kHz pure tone. We set the intensity of the CS such as it did not

FIGURE 1 | Stimulation protocol and clinical assessment.

trigger any startle reflex nor any detectable reaction from the subject, thereby reducing the occurrence of alpha responses. The unconditioning stimulus (US) consisted of a 50 ms airpuff whose intensity (air pressure at the tip of the tube) was set to trigger painless eyeblink in 100% of the trials. Initially, the participant was exposed to five CS alone and to five US alone stimuli to establish appropriate responses to the tone and the airpuff as well as to measure the UR prior to conditioning. We also ensured that the US did not induce any startle reaction from the participant. A conditioning trial lasted 1 s and consisted of (successively): an initial 200 ms baseline period, a 400 ms CS that co-terminated with a 50 ms US, and a final 400 ms period during which the eyeblink was recorded. An EBC block consisted of 9 successive paired presentations of CS-US and a last trial with the CS alone. An EBC session consisted of 5 successive blocks separated by an inter-trial interval randomly ranging from 2 to 12 s. The participant was passively watching a silent movie during the task.

EBC sessions: The patient underwent two EBC sessions on the same days as the clinical and neuropsychological assessments (**Figure 1**). The first one (pre-tDCS) was made two days before the first tDCS session, and the second one (post-tDCS) was made 2 days after the last tDCS session. Thus, 7 days separated the pre- and the post-tDCS EBC sessions.

The EBC signal was low-pass filtered using a 4th order Butterworth filter with a 10 Hz cutoff frequency and was offset-subtracted by deducing to the trace the averaged baseline. To estimate the percentage of CRs, we discarded trials for which a spontaneous blink occurred during the baseline. CRs were detected in a time window between 330 and 400 ms after the CS onset with a threshold of five times the standard deviation above the baseline. We visualized each trace separately afterwards to verify that the detection of CRs was correct.

Clinical and neuropsychological characteristics before and after cerebellar stimulation are reported in **Table 1**. The patient did not report any side effects, except from a slight itching in the beginning of the first session of the second day of stimulation. A careful inspection of the scalp did not evidence any cutaneous lesion. The patient did not report any headache after brain stimulation. Clinical symptoms remained stable during the stimulation protocol. Notably there was no changes in the PANSS score and the Auditory Hallucination Verbal Scale.

There was a global improvement in a large part of neuropsychological measurements (episodic memory,

TABLE 1 | Clinical and neuropsychological characteristics before and after cerebellar stimulation.

	Before stimulation	After stimulation
CLINICAL ASSESSMENT		
PANSS score:		
- Total	57	58
- Positive	22	24
- Negative	15	14
- General psychopathology	20	20
AHRS	22	23
NEUROPSYCHOLOGICAL ASSESSMENT		
Free and cued recall 16 items:		
- Recall 1		
- Free recall (pc)	5 (1–2)	7 (11–12)
- Total recall (pc)	8 (1)	11 (1–5)
- Recall 2		
- Free recall (pc)	5 (<0.1)	7 (3–4)
- Total recall (pc)	10 (<1)	13 (5)
- Recall 3		
- Free recall (pc)	7 (<1)	8 (1–2)
- Total recall (pc)	9 (<1)	12 (1)
- Delayed recall		
- Free recall (pc)	7 (<1)	11 (19–20)
- Total recall (pc)	9 (<1)	11 (1)
WAIS-IV:		
- Digit span		
- Direct order	5	6
- Indirect order	4	5
- Increasing order	6	6
- Total	15	17
- Spatial memory		
- Direct order	6	9
- Indirect order	6	6
- Total	12	15
Stroop-test golden version*:		
- Reading (pc)	74 (4–5)	82 (8–12)
- Denomination (pc)	62 (12)	61 (8–12)
- Interference (pc)	30 (5–8)	33 (12)
D2 test of attention (Brickenkamp):		
- GZ (pc)	187 (<0.1)	238 (0.5)
- % of errors (pc)	3.21 (50–75)	6.72 (25–50)
- KL (pc)	73 (4.5)	86 (8.1)

*Pc, percentiles; PANSS, Positive And Negative Symptoms Scale; AHRS, Auditory hallucinations rating scale; CGI, Global clinical impression; WAIS IV, Wechsler Adult Intelligence Scale (WAIS-IV); GZ, quantitative performance index; KL, concentration performance index; *age corrected scores.*

executive and attentional functions) before and after stimulation (**Table 1**).

We found an improvement in the long-term episodic memory, assessed with the free/cued recall 16 items (**Table 1**) test. There was an increase of performance in the delayed free recall: after the stimulation, the score of the participant was in the normal range (19–20 percentile) vs. <1 percentile before stimulation. Likewise, there was a strong improvement in the first attempt of the free recall after stimulation: the score of the participant was in the normal range [11–12 percentile) after stimulation vs. 1–2 percentile before stimulation. Two different lists of words were proposed to the participant before and after stimulation in order to avoid a test-retest effect (21).

In two tests measuring short term and working memory (digit span and spatial memory), there was an improvement in the performance of the participant in both the direct and indirect order, suggesting an effect of stimulation in both the short term and working memory.

We found an increase in the Stroop test performance. In particular, the reading performance improved from 4 to 5 percentile (pathological range) before stimulation to 8–12 percentile (normal range) after stimulation. In addition there was an improvement in the interference condition after stimulation (12 percentile) as compared to before stimulation (5–8 percentile).

Last, we measured the selective attention with the D2 test of attention (Brickenkamp). Again, there was an increase in both the quantitative performance index (GZ) and the concentration performance index (KL). In the KL index, the subject scored in the normal range after cerebellar stimulation vs. in the pathological range (<0.5 percentile) before stimulation.

Results from the EBC assessment before and after stimulation are reported in **Figures 2**, **3**. Before tDCS, the averaged block response amplitudes of the URs remained unchanged over the pre-tDCS session (**Figure 2A**), very few CRs were detected and peak latencies of averaged block signals remained constant during the overall session (**Figure 3**). Thus, 45 CS-US pairings were insufficient to induce EBC, at the end of this session only 43% of the trials displayed CRs (**Figure 3** left). This result is in agreement with previous EBC evaluation in patients with schizophrenia (22). After tDCS, the patient was rapidly conditioned and reached a final value of 83% of CRs (**Figure 3** left), as expected for a normal EBC session. Accordingly after tDCS, the averaged amplitude of the URs decreased from block to block (**Figure 2D**) while it was stable before tDCS, this progression of EBC can also be observed by monitoring the first peak latency of the responses from block to block. Before tDCS it remained stable but rapidly decreased over the blocks after tDCS (**Figure 3** right). Those features indicate the shift of the eyeblink timing toward the CS, which corresponds to a progressive change from *reflexive* toward *predictive* behavior. Thus, before tDCS the patient could not be conditioned over the EBC session while after cerebellar tDCS he displayed progressive conditioning from block to block.

DISCUSSION

We describe the case of a 50 years old patient with schizophrenia whom underwent a non-invasive cerebellar stimulation protocol. Data from clinical and psychometric evaluations including long term verbal memory, executive, and attention functions were collected before and after the stimulation, as well as data from a cerebellar-dependent eyeblink conditioning protocol.

Although we did not report changes in the positive or negative symptoms of schizophrenia before and after stimulation, there was a global improvement in psychometric measurements after stimulation. We also found an improvement in the performance in selected attention, long/short term memory, working memory, and response inhibition; cognitive domains known to be altered in patients with schizophrenia.

There was a clear improvement of EBC after stimulation. In the absence of data on healthy subjects in our conditions, we can not exclude any retest effect ("saving") in this improvement (23). This is however unlikely given that in our data: (1) no clear cut EBC could be observed at the end of the pre-tDCS session, and (2) conditioning gradually appeared during the post-tDCS EBC session, starting from an absence of predictive response (and thus showing no saving, **Figure 3**). In addition, and contrary to control subjects, patients with schizophrenia have been shown not to improve their performance during consecutive EBC sessions (24). Those lines of evidence therefore support the view that the improvement of EBC after tDCS was due to the stimulation itself and not to any retention of the first EBC session. Based on the abundant literature in both humans and animals (10, 25–27), our data indicates that the cerebellar function of the patient was basally impaired as previously described in schizophrenia (28). Our EBC assessment is consistent with several studies that reported an effect of non-invasive cerebellar stimulation on associative learning measured with EBC (15, 29). More importantly, it points out cerebellar tDCS as a powerful tool to significantly improve cerebellar function in schizophrenia.

EBC has proven to be a relevant method to investigate cerebellar dysfunction in neuropsychiatric disorders. Disentangling motivational aspects from cognitive deficits can be challenging in patients with schizophrenia. This is however important since the deficits in motivation commonly present in patients with schizophrenia can bias classic neuropsychological tests. EBC does not require active participation of the subject. In our paradigm, the patient was watching a silent movie during the experiment; in newborns, EBC can even be performed during sleep (30, 31). Thus, the outcome of EBC are unlikely to be related to motivational deficits in patients with schizophrenia.

Gupta et al. (5) found in a double-blind crossover study an effect of cerebellar tDCS on procedural learning in a population of non-clinical psychosis (NCP) population. The authors reported greater rate of motor learning in NCP population after active stimulation. We used a different stimulation protocol with the cathode electrode (return electrode) placed on the right arm, whereas Gupta el al. placed the electrode on the midline of the scalp. Although there is no consensus on the placement

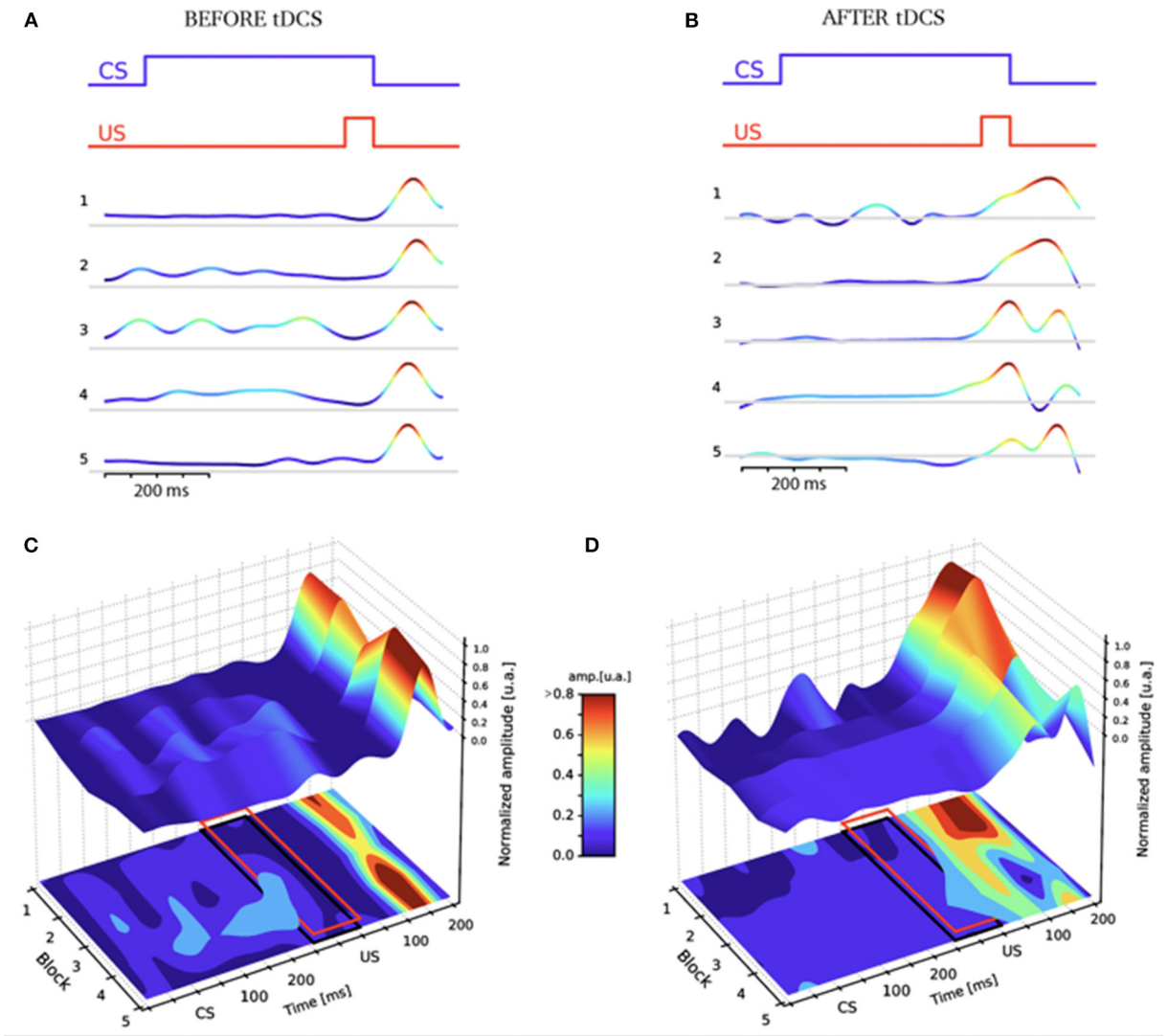

FIGURE 2 | EBC sessions before **(A,C)** and after **(B,D)** tDCS in the schizophrenic patient. The color code corresponds to the normalized response amplitude of the IR-reflected signal. The EBC protocol is depicted at the top of **(A,B)**. **(A,B)** Each block represents the average signal over the 9 CS-US trials, for experiments respectively before **(A)** and after **(B)** tDCS. **(C,D)** Plots of the IR-reflected signal for each block before **(C)** and after **(D)** tDCS. Red rectangles delimit the area where CRs were detected in **Figure 3** left. Notice that the unconditioned response (UR) peak amplitude and latency decreases block after block after tDCS **(D)**, while before the tDCS the UR peak amplitude remains constant among all the blocks, and the latency shows no trend **(C)**.

of the return electrode (14), we chose this location based on a modelisation study (18) to target the posterior region of the cerebellum.

Brady et al. (32) reported in a population of patients with schizophrenia, an improvement of negative symptoms after transcranial magnetic stimulation (TMS) related to dorsolateral prefrontal cortex-to-cerebellum connectivity. However, the authors did not investigate the effect of cerebellar stimulation on cognitive symptoms.

In healthy subjects, there is evidence that non-invasive cerebellar stimulation can modulate working memory, motor control, learning, and emotional processing (14). These results are in line with our case report where non-invasive cerebellar stimulation had an effect on verbal memory, executive, and attention function.

The participant did not report any significant side effects after 2 sessions of stimulation during 5 days, which is in line with previous studies showing the feasibility and good tolerance profile of cerebellar tDCS (14).

This case report supports several strengths. To the best of our knowledge, this case is the first to report the effect of tDCS on cognition (including associative learning measured with EBC) in schizophrenia. We carefully selected psychological measurements with no test/retest effects, which suggests that the cognitive improvement is related to the stimulation. In addition, there was no significant change in the positive and negative

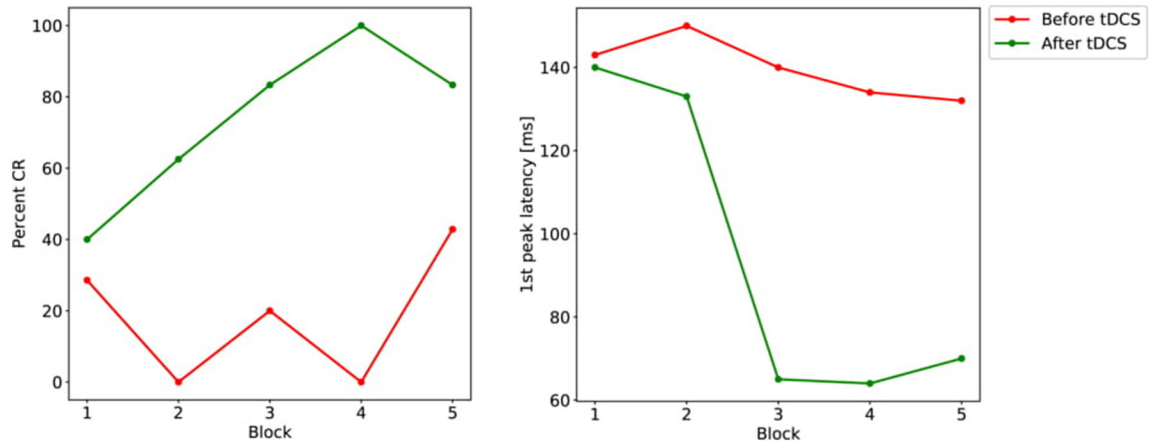

FIGURE 3 | Percentage of CRs and latency of the first peak progression from block to block before (red) and after (green) tDCS. **Left panel:** percentage of CRs from each block measured in the red rectangle indicated on **Figures 2C,D**. **Right panel:** First peak latency calculated from the data averaged in blocks in **Figures 2A,C**. Time zero for latency calculation corresponds to the onset of the US.

symptoms, suggesting again that the change in cognition are not related to a change in the symptoms of schizophrenia. Our work suggests that eyeblink conditioning can be used to assess the effect of cerebellar stimulation.

Several limits should be considered before interpreting our results. Because we only investigated the effect of stimulation in a single patient, our study remains purely qualitative. The posterior part of the cerebellum is connected to multiple regions in the associative cortex and it is difficult to target a specific domain of cognition with cerebellar brain stimulation. We were not able to measure other cognitive domains, such as social cognition that could also be modulated by cerebellar stimulation (33). However, our goal was to propose an original cognitive evaluation by combining a classic neuropsychological assessment and EBC.

In conclusion, this case report suggests that cerebellar tDCS stimulation can have an impact on cognitive impairments in patients with schizophrenia. We suggest that eyeblink conditioning, known as a relevant method to investigate cerebellar dysfunction in neuropsychiatric disorders, could be used to assess the impact of stimulation on the cerebellum in patients with schizophrenia. Further clinical trials are required to address the potential therapeutic potential of tDCS in schizophrenia.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hôpital de Ville Evrard, Unité de Recherche Clinique. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the participant for the publication of this case report.

AUTHOR CONTRIBUTIONS

CLa wrote the first draft of the manuscript. CLe setup and supervised the EBC experiments, contributed to the writing of the manuscript, and performed the EBC tests. AS-P wrote the Python routine, analyzed the EBC data, and contributed to the manuscript. FD participated in the selection of the neuropsychological tests and evaluated the patient. CF participated in the interpretation of neuropsychological assessment and to the writing of the manuscript. NB participated in the elaboration of the protocol, the clinical assessment, and the writing of the manuscript. DJ participated in the elaboration of the protocols and the writing of the manuscript.

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Dynamic Functional Connectivity Patterns in Schizophrenia and the Relationship With Hallucinations

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There is a wealth of evidence showing aberrant functional connectivity (FC) in schizophrenia but with considerable variability in findings across studies. Dynamic FC is an extension of traditional static FC, in that such analyses allow for explorations of temporal changes in connectivity. Thereby they also provide more detailed information on connectivity abnormalities in psychiatric disorders such as schizophrenia. The current study investigated dynamic FC in a sample of 80 schizophrenia patients and 80 matched healthy control subjects, replicating previous findings of aberrant dwell times in specific FC states, and further supporting a role for default mode network (DMN) dysfunction. Furthermore, relationships with hallucinations, a core symptom of schizophrenia, were explored. Two measures of hallucinations were used, one measure of current hallucination severity assessed on the day of scanning, and one trait-measure where hallucinations were assessed repeatedly over the course of 1 year. Current hallucination severity did not show a significant relationship with dynamic FC. However, the trait-measure of hallucination proneness over 1 year showed a significant relationship with dynamic FC. Patients with high hallucination proneness spent less time in connectivity states characterized by strong anti-correlation between the DMN and task-positive networks. The findings support theoretical models of hallucinations which have proposed an instability of the DMN and impaired cognitive control in patients with hallucinations. Furthermore, the results point to hallucination proneness as a potential marker for identifying distinct subgroups of schizophrenia patients.

Keywords: schizophrenia, psychosis, auditory verbal hallucinations, default mode network, neuroimaging, fMRI

INTRODUCTION

Schizophrenia (SZ) has been described as a "disconnection syndrome" (1), referring to aberrant interaction between critical areas of the brain. Neuroimaging studies have found evidence for altered resting state functional connectivity (FC) in schizophrenia patients compared to healthy control subjects, covering a range of different brain regions [see (2–5) for selected reviews]. These findings include hyper- as well as hypo-connectivity, with considerable variation in results across studies.

The inconsistencies in findings might partly stem from the fact that schizophrenia is characterized by a wide range of different symptoms which are associated with different biological underpinnings and distinct FC abnormalities. Hence, variability in sample compositions might affect the results of such studies. In particular, auditory verbal hallucinations, a key symptom of SZ, have repeatedly been linked to FC alterations. These alterations involve auditory and language regions, the default mode network (DMN), executive and cognitive control networks, and subcortical areas [see (6–8) for selected reviews].

The majority of fMRI studies that have investigated FC in schizophrenia and hallucinations, have employed methods of static connectivity, i.e. averaging FC across the scanning time. These methods work on the assumption that functional connectivity between brain regions does not change substantially across the duration of the scanning session which typically lasts 5–15 min. However, this assumption does not seem to hold true in the face of growing evidence for considerable fluctuations in fMRI FC across time (9, 10). Therefore, recent investigations of fMRI resting state FC have increasingly focused on dynamic, or time-varying, FC. These investigations aim to detect connectivity patterns that can be found across subjects and occur at different time points throughout the scanning period (11, 12). One of the main approaches for the investigation of dynamic FC is a sliding time window approach, where FC matrices are computed for consecutive portions of the scanning period. FC matrices of those windows can then be clustered based on similarities in the FC patterns in order to form a set of "connectivity states". Each connectivity state is characterized by a distinct FC pattern across a variety of brain regions and the temporal pattern of those states over time describes the functional organization of the brain [e.g., (13)]. The occurrences of FC states within an fMRI session have been linked to concurrently measured EEG signals (14, 15) and to different task conditions (16). These findings suggest that FC states reflect fluctuations in neuronal activity and cognitive states, or modes of brain functioning. Furthermore, differences in dynamic FC also reflect more stable, inter-individual differences in cognition (17).

Measures of dynamic FC also show links with mental health disorders, such as schizophrenia, and have been successful in differentiating patient groups and healthy controls (18). Importantly, dynamic FC has been found to outperform static FC measures when classifying SZ patients (19, 20). Interestingly, classification was not significantly improved when adding static FC measures to dynamic ones. This suggests that SZ-related abnormalities in static FC are also captured in dynamic FC but not vice versa. SZ patients and healthy controls have been shown to differ on a number of variables related to dynamic FC. First, patients spend more time in states with overall weak FC between networks, and less time in states with strong FC between sensory networks (18, 21–23). Second, SZ show reduced "dynamism" in FC, reflected in a reduced number of distinct FC states and reduced switching between states (24). This reduction in dynamism was also specifically related to the severity of hallucinations. On the other hand, the amount of time spent in different FC states showed no relationship with a summary measure of positive symptoms which included hallucinations (21). Together, these results could point to a unique relationship between dynamic FC and hallucinations.

The current study sought to investigate resting state dynamic FC in SZ, with a particular focus on hallucinations. Given the relative scarcity of studies on dynamic FC in SZ, the first aim was to replicate previously reported differences between SZ and HC with respect to the length of time spent in different FC states (18, 21). In addition, links between dynamic FC states and hallucinations were explored, using two different measures of hallucinations. First, effects of current hallucination severity were investigated using a measure of hallucinations on the day of scanning. However, since previous research suggests that temporary symptom severity might not show a strong relationship with dynamic FC states (21), a second, trait measure of hallucinations was included. Hallucinations were assessed repeatedly over a 1-year period and a measure of hallucination proneness was established. This second measure reflects more stable trait differences between patients. Therefore, it could indicate distinct subgroups of SZ patients, which have been suggested in previous research based on lifetime history assessments of auditory hallucinations (25). Stable differences in hallucination proneness are likely to be reflected in brain functioning and may therefore also be related to dynamic FC, which reflects a general functional organization of the brain (26). Furthermore, dynamic FC has been shown to differentiate between patient groups with different diagnoses (18, 19) and symptom-based subgroups of patients (27). Therefore, we predicted differences in connectivity state dwell times between SZ patients and healthy controls, and between patients with low versus high hallucination proneness.

METHODS

Subjects

fMRI data were collected from 84 patients with a schizophrenia spectrum disorder (SZ) according to the ICD-10 diagnostic manual (F20–F29: Schizophrenia, schizotypal, and delusional disorders) (28). Four data sets were excluded after pre-processing, due to head movements of more than one voxel size between volumes, resulting in 80 patient data sets. Eighty healthy control subjects (HC) were individually matched with the SZ patients based on gender (60 males per group) and age (± 3 years, except for six SZ-HC pairs with a mean difference of 8.12 years). The mean age was 30.96 years (SD = 11.91) for SZ and

30.86 years (SD = 11.13) for HC. The majority of patients used antipsychotic medication, of which all used second-generation antipsychotics, with some patients in addition using first-generation antipsychotics [defined daily dose (DDD) of antipsychotics M = 1.02, SD = 0.55]. Some patients also used anti-depressants (n=8), mood stabilizers (n=2), opioids (n=1), benzodiazepines (n=13), anticholinergic (n=4), anticonvulsant (n=4), or ADHD medication (n=1). Further information on the patient sample can be found in **Table 1**. All subjects gave written informed consent to take part in the study prior to participation.

Data Acquisition

Neuroimaging Data

3T MR data were acquired at the Haukeland University Hospital in Bergen, Norway (68 patients and 80 HC), and at the Medical University of Plovdiv, Bulgaria (12 patients). In the course of the study, the MR scanner at the Bergen site was upgraded from a GE Signa HDx to GE Discovery MR750. All data acquired at the Plovdiv site were acquired on a GE Discovery MR750w, using the same MR parameters as at the Bergen site. The scanner version was included as a regressor variable of no interest in all analyses (i.e., Bergen pre-upgrade, Bergen post-upgrade, Plovdiv). The study protocol was approved by the Regional Committee for Medical Research Ethics in Western Norway (REK Vest) (REK #2016/800) and by the ethical authorities at the Medical University of Plovdiv, and conducted according to the Declaration of Helsinki.

fMRI resting state data were collected during a 5.33-min eyes-closed scan. One hundred sixty whole brain volumes were acquired, with 30 slices with a 0.5 mm gap (voxel size 1.72×1.72×3 mm) with the following parameters: repetition time (TR)/echo time (TE)/flip angle (FA)/field of view (FOV) 2000ms/30ms/90°/220mm. In addition, a structural T1-weighted image was acquired (7.42 min) using a 3D SPGR sequence with the following parameters: TR/TE/FA/FOV 7.78ms/2.94ms/14°/256mm (post-upgrade: 6.9ms/3.0ms/14°/256mm), isotropic voxel size of $1mm^3$.

Clinical Data

Hallucination severity was assessed with the P3 item of the Positive and Negative Syndrome Scale [PANSS; (29)]. The PANSS P3 item assesses hallucinations in different modalities and has a particular focus on auditory hallucinations and hearing voices (30), since these are the most common type of hallucination in psychotic patients (31, 32). Therefore, the PANSS P3 item is a measure of hallucination severity in general but the way that the interview questions are organized reflects auditory hallucinations to a greater degree than other sensory modalities. All PANSS raters were trained and certified and satisfactory inter-rater reliability was documented. For all patients, PANSS data were collected on the day of fMRI scanning. For the 12 patients from the Plovdiv site, this was the only PANSS assessment, but for the 68 patients who were scanned at the Bergen site as part of a 1-year study, additional PANSS data were acquired during up to seven additional visits to the clinic (baseline, week 1, week 3, week 6, month 3, month 6, month 9, month 12). The mean number of visits per patient was M = 5.81 (SD = 2.04), with most patients being followed for at least 6 months (mean last visit number M = 6.51, SD = 1.74). **Supplementary Table S1** provides an overview of the distributions of visits per patient. fMRI data were typically acquired at one of the first visits (visit 1, 2, or 3 in 75% of cases), but in order to account for any potential effects of the time point of scanning, the visit number was included as a regressor variable of no interest when comparing patient subgroups.

TABLE 1 | Demographic data for the whole sample of SZ patients, and for the 68 SZ patients from the Bergen site, split into non-hallucinators and hallucinators. Positive and Negative Syndrome Scale (PANSS) data are based on assessments on the day of fMRI scanning. A significant group difference between hallucinators and non-hallucinators was found for PANSS total (p =.041).

	all patients (n = 80)	non-hallucinators (n = 19)	hallucinators (n = 49)
Age	30.96 (11.91)	33.42 (11.38)	28.88 (11.15)
Gender (m/f)	59/21	13/6	36/13
PANSS P3	2.69 (1.63)	1.05 (0.23)	3.33 (1.49)
PANSS positive	16.23 (5.20)	13.53 (5.06)	16.73 (5.18)
PANSS negative	15.86 (5.00)	13.53 (5.17)	16.15 (4.63)
PANSS general	33.42 (8.99)	28.47 (8.71)	34.77 (8.85)
PANSS total	65.51 (16.39)	55.53 (16.24)	67.65 (15.09)
Medication (DDD)	1.02 (0.55)	0.96 (0.41)	1.08 (0.63)
Duration of illness	4.83 (7.71)	3.19 (5.47)	4.03 (7.15)

Data Preprocessing and Analyses

Data pre-processing was conducted with the SPM12 software package (https://www.fil.ion.ucl.ac.uk/spm/). This included realignment of functional volumes for head motion correction, coregistration of the T1 structural image to the mean functional image, normalization of functional data into MNI (Montreal Neurological Institute) space, resampling to a voxel size of 4x4x4 mm, and smoothing with a Gaussian kernel of 8 mm FWHM.

Independent Component Analysis (ICA)

Pre-processed fMRI data were further analyzed in the SPM Group ICA of fMRI Toolbox (GIFT v3.0b http://trendscenter.org/software/gift/), using the Toolbox default parameter settings. Initially, a two-step data reduction procedure was followed. First, all data sets underwent a subject-specific Principal Component Analysis (PCA) which estimated 150 components. Second, all subjects' reduced data sets were concatenated and underwent a PCA which estimated 100 components on the group level. Subsequently, group-level spatial ICA was performed on the PCA output, identifying 100 functional components equivalent to Allen et al. (13) and Damaraju et al. (21). The Infomax algorithm was used for component estimation. Subject-specific component maps and time courses were obtained from the group-level components using the GICA back reconstruction method. Components were then evaluated and excluded as artifact components if they showed a peak in white matter or cerebrospinal fluid, or if the majority of the power in the Fourier frequency spectrum of the component's time course was above 0.1 Hz (33, 34). Using component spatial maps from previous research (13) as templates, 45 components were finally identified as functional components belonging to one of eight functional networks: subcortical (four ICs: putamen, caudate, 2x thalamus),

auditory (two ICs), somatomotor (eight ICs), visual (nine ICs), insula (one IC), fronto-parietal task-positive networks (eight ICs), Default Mode Network (eight ICs), and language (five ICs).

Dynamic Functional Connectivity Analysis

Dynamic functional connectivity analyses were conducted with the GIFT Dynamic FNC Toolbox (v1.0a) equivalent to previous research (13, 21). In a first step, subjects' component time courses were detrended and despiked. A fifth-order Butterworth low-pass filter with a high frequency cutoff of 0.15 Hz was applied. Six realignment parameters obtained during head motion correction were regressed out from the time courses. For the dynamic FC analysis, overlapping time windows of 40 s (20 TRs) were taken from the scanning time in steps of 2 s (1 TR) and convolved with a Gaussian of sigma = 3 TRs in order to de-weigh volumes at the beginning and end of the windows. For each window, FC was estimated in the form of a regularized inverse covariance matrix using the Toolbox graphical LASSO method with an additional L1 norm constraint. Finally, covariance estimates were Fisher-Z-transformed. For each subject, variance associated with the scanner version was removed from the windowed FC. In order to identify FC states that reoccured across time and across subjects, the windowed FC matrices were subjected to the GIFT k-means clustering procedure. In a first step, a subset of windows (i.e. "exemplars") was selected for each subject, representing those FC matrices with maximal variability in FC. From those windows, the optimal number of clusters (k) was determined by the toolbox using the elbow criterion, defined as the ratio of within-cluster distances to between-cluster distances. The resulting k cluster centroids were used as templates for clustering all windows' FC matrices of all subjects.

Group Comparisons of Dynamic Functional Connectivity

The primary measures of interest were the state dwell times, indicating for each state the average length of the single time periods that subjects stayed in that FC state, before switching to another state. To test for differences between SZ and HC, a MANOVA was conducted with each state's dwell time as dependent variables, and SZ versus HC as an independent variable. To investigate the relationship between dwell times and hallucinations, two different measures were employed. First, the continuous P3 score on the day of fMRI scanning was used as a measure of current hallucination severity. Second, P3 assessment over the course of 1 year was used as a trait measure of hallucination proneness. This resulted in a non-hallucinator group (n=19) whose P3 score was never higher than two in the 1-year period (or alternatively one score of three compensated for by only scores of one on all other assessments), and in a hallucinator group (n = 49) whose P3 score was three or higher in at least one assessment during the 1-year period (unless compensated by only scores of one on all other assessments). Two MANCOVAs were conducted, one for each of the two hallucination measures as an independent variable, and dwell times in the different FC states as dependent variables. Age, gender, MRI visit number, and type of medication were included as regressor variables of no interest, since hallucinators and non-hallucinators were not matched with respect to these variables.

RESULTS

Dynamic Functional Connectivity States at the Whole-Group Level

K-means clustering identified five distinct connectivity states based on the windowed FC matrices (**Figure 1**). State 1 was characterized by strong positive FC within the DMN, i.e. between the different components of the DMN, and negative FC between the DMN and Task Positive Networks (TPN), especially the insula. State 2 was characterized by strong positive FC within and between sensory networks and negative FC of those networks with the DMN and TPN. State 3 was similar to State 2 but FC of sensory networks was even stronger and the negative FC with DMN and TPN was absent. Instead, sensory networks showed negative FC with subcortical networks. State 4 was characterized by overall weak FC across networks, which was positive within networks and negative between networks. State 5 was characterized by mostly positive FC which was strong within networks, in particular the DMN and language network.

Differences in Dynamic FC Between SZ Patients and HC

Two of the five FC states showed a significant difference in dwell times between SZ and HC (**Figure 2A**). SZ had significantly increased dwell time compared to HC in State 5, $F(1,158) = 4.43$, $p = .037$, but significantly reduced dwell time in State 3, $F(1,158) = 5.01$, $p = .027$. With respect to the occurrences of the two states in each group, fewer SZ than HC visited State 3 (n = 11 vs n = 23), whereas there was no difference for State 5 (n = 37 vs n = 36). There was no significant difference between SZ and HC in the number of distinct time periods in State 3 and State 5 ($p = .299$ and $p = .578$).

Relationships Between Dynamic FC and Hallucinators

Current hallucination severity, assessed with the PANSS P3 score on the day of fMRI scanning, was not significantly related to dwell times in the different FC states (all $p > .26$). However, trait hallucination proneness over a 1-year period showed a significant relationship with state dwell times (**Figure 2B**). Compared to the non-hallucinator group, the hallucinator group had significantly reduced dwell times in State 1, $F(1,26)=12.48$, $p=.002$. There was no significant difference in the number of distinct time periods in State 1 ($p=.452$). Within the hallucinator group, the severity of hallucinations, measured as the mean P3 across 1 year, did not have a significant effect on dwell times (all $p > .508$, with $p=.521$ for state 1). In order to ensure that the results were specific to hallucinations and not driven by general symptom severity, the MANCOVA was repeated twice, once corrected for the positive PANSS score across visits and once corrected for the total PANSS score across visits, and the results remained substantially the same (changes in effect size from partial $\eta^2 = .32$ to $\eta^2 = .26$ and $\eta^2 = .29$, respectively). Furthermore, neither the positive PANSS

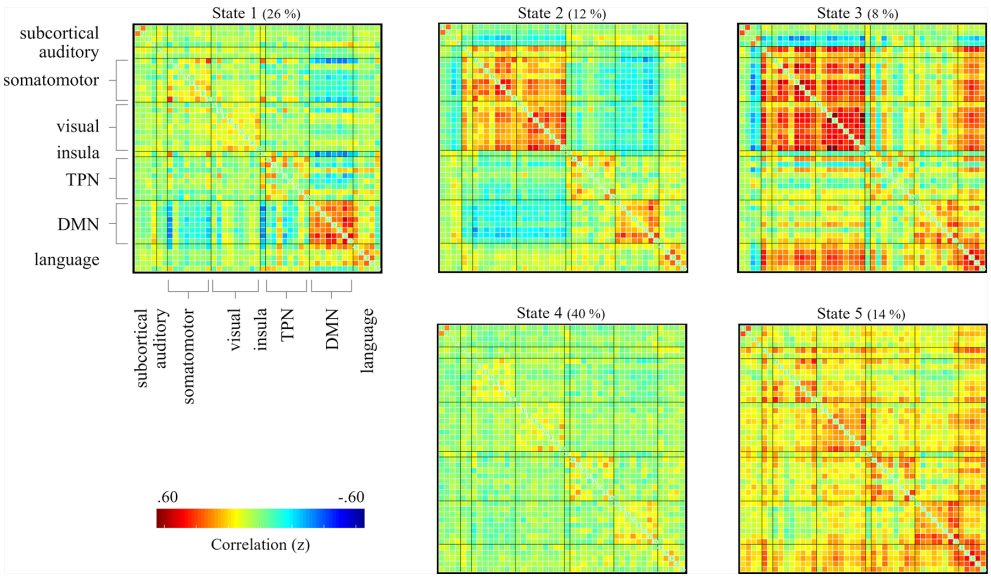

FIGURE 1 | FC matrices of the dynamic FC states identified by k-means clustering on the whole-group level (80 SZ and 80 HC). Medians of cluster centroids are displayed. In brackets are the percentages of occurrence of the five states across the scanning period. The labelling of networks that is shown for state 1, is identical for all states. FC, functional connectivity; SZ, Schizophrenia; HC, healthy control.

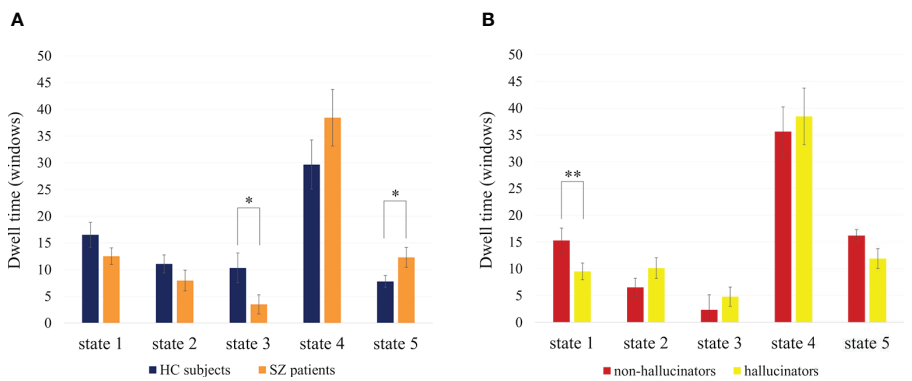

FIGURE 2 | Mean dwell times (and SEM) in the different FC states per group. **(A)** Shows the comparison of HC subjects and SZ patients. **(B)** Shows the comparison of hallucinators and non-hallucinators. Dwell times are given in number of windows (1 window = 1 TR = 2s). Significant differences in dwell times are marked with * for $p < .05$ and ** for $p < .01$. FC, functional connectivity; TR, repetition time.

subscale nor the total PANSS score were significantly related to dwell times in State 1 (p=.130 and p=.151).

DISCUSSION

This study investigated dynamic FC in SZ patients compared with HC subjects and explored relationships with hallucinations, one of the key symptoms of SZ. On the whole-group level, dynamic FC analyses revealed five FC states which reoccurred over time and across subjects and which were characterized by distinct FC patterns across a variety of functional networks. These FC states were highly similar to those found in previous research using the same methods (13, 21). Dwell times in different states showed relationships with SZ diagnosis as well as hallucinations.

Dynamic FC Differences Between SZ Patients and HC Subjects

While all FC states occurred in the SZ as well as in the HC group, the two groups differed with respect to the length of time periods spent in two out of the five states. Firstly, SZ had significantly longer dwell times than HC in states that were characterized by positive FC within and between networks and particularly strong FC within the DMN and the language network (State 5). The DMN has repeatedly been reported to show abnormal FC patterns in SZ, with the majority of reviews

concluding predominantly increased FC within the DMN (3, 35, 36). This finding has been suggested to reflect thought disturbances in relation to an increased internal focus of cognitive processing in SZ patients (37, 38). The fact that SZ patients in the current study were not more likely to reach states with high within-DMN connectivity but stayed in these states significantly longer than HC, might indicate that they become "stuck" in these states of increased internal processing focus. Furthermore, those states were also characterized by overall positive FC across all brain regions, which suggests a weak segregation between different functional networks and could be related to impairments in differentiating between internal versus external thought contents (36).

The results also showed a significant difference between SZ and HC in dwell times in State 3. That is, HC stayed longer in states with strong connectivity within and between sensory networks and strong anti-correlation with subcortical areas, as has been reported previously (18, 21, 22). This FC state has been suggested to reflect a state of low alertness or drowsiness (13, 21). Shorter dwell times for SZ compared to HC could simply reflect higher levels of arousal or anxiety in patients, which would make them less likely to be in a state of relaxation or drowsiness. Even though this explanation is speculative, it is supported by the fact that more HC than SZ patients ever reached this state at all.

Overall, the differences between SZ and HC with respect to dwell times in the different FC states largely replicate previous findings (21, 23), even though some group differences did not pass the significance threshold in the current study (e.g., increased dwell time for SZ compared to HC in states with overall weak connectivity between all networks). Given the smaller sample size in the current study, this might be related to lower statistical power or it could indicate that some state differences are more generalizable across different sample compositions than others.

Dynamic FC Differences Between Hallucinators and Non-Hallucinators

The second aim of the study was to explore the relationship between dynamic FC and hallucinations within the SZ sample. As expected (21), the time spent in different FC states was not significantly related to a measure of current hallucination severity on the day of fMRI scanning. However, assessing hallucinations as a trait variable across a 1-year period and differentiating between patients with high versus low hallucination proneness in that period, showed that those two subgroups of patients differed in dynamic FC. Specifically, hallucinators spent less time than non-hallucinators in states with strong anti-correlation between the DMN and task-positive networks (TPN).

Reduced DMN-TPN anti-correlation has previously been found in SZ patients in studies using static FC. These findings have been interpreted in the light of impaired differentiation between internal and external focus in cognitive processing (35, 36). These impairments might be particularly relevant for hallucinations, which constitute an internally generated stimulus which is attributed to an external source [cf. (39, 40)]. Abnormalities in DMN connectivity have also been linked to hallucinations directly (6). A recently proposed model of auditory hallucinations centers around aberrant DMN connectivity, alongside auditory cortex abnormalities (41). Specifically, it was suggested that a reduced anti-correlation between the DMN and the central executive network causes a collapse of states with an internal processing focus. The resulting DMN withdrawal then leads to a state of increased focus on auditory processing and consequently to the experience of hallucinations. The fact that hallucinators in the current study could not uphold DMN-TPN anti-correlation states for as long as non-hallucinators, might reflect the hypothesized collapse of internal processing states, which are maintained by DMN-TPN anti-correlation. Therefore, the findings are also in line with a previously proposed hallucination-related instability of the DMN (42) and a dysfunction of the cognitive control network (43).

Hallucinations as a Potential Marker for Subgroups of SZ

The current study suggests that hallucinations could be an indicator for identifying distinct subgroups of SZ patients. Importantly though, relationships of hallucinations with dynamic FC were only present for trait hallucination proneness but not for current hallucination severity. This indicates that the general vulnerability to experience hallucinations is reflected in functional brain organization and that this vulnerability is detectable even in periods where hallucination severity is low (as indicated by patients with high hallucination proneness but low current hallucination severity). Within the hallucination-prone subgroup, there was no effect of hallucination severity over time, which suggests that differences in dynamic FC reflect a categorical vulnerability to experience hallucinations.

Differences between hallucinators and non-hallucinators persisted when correcting for total symptom severity and positive symptoms. Furthermore, neither total symptom severity nor positive symptoms were related to state dwell times. This points to a unique relationship of hallucinations with dynamic FC patterns and further supports the potential value of hallucinations as a marker for differentiating SZ subgroups. The measure of hallucination proneness over a 1-year period might be related to assessments of lifetime history of hallucinations, which has previously been shown to discriminate between SZ subgroups in a classification study (25). In fact, classification accuracy was higher for the hallucinators versus non-hallucinators subgroups of patients than for the SZ versus HC groups. This led the authors to the conclusion that hallucination-based patient subgroups might be a more useful entity than traditional diagnosis groups. In the current study, dynamic FC was susceptible to both, SZ diagnosis as well as hallucination proneness, but with stronger relationships with for hallucination proneness. Interestingly, the FC states that showed effects of SZ diagnosis and the states that showed hallucination effects, were both characterized by strong FC within the DMN. It is therefore possible that the two effects are related, with states of DMN-TPN anti-correlation (State 1) potentially compensating for DMN hyper-connectivity (State 5). That is, SZ patients generally spend more time in states with DMN hyper-connectivity, which has been associated with misattributions of internal thought contents to external sources (36–38). However, in non-hallucinators, states of DMN-TPN anti-correlation modulate DMN functioning, which might make these misattributions less likely and hence protect against hallucinations.

Limitations and Directions for Future Research

Despite the potential of dynamic FC as a method for investigations of naturally occurring fluctuations in brain functioning, some limitations should be considered when interpreting dynamic FC findings. First, while dynamic FC corresponds to fluctuations in cognitive states (14, 16) and stable inter-individual cognitive differences (17), the interpretations of the single FC states with respect to underlying cognitive processes are still largely unclear. Even though FC patterns can be interpreted based on the knowledge about the functional networks involved, future research should investigate direct links between dynamic FC states and cognitive states, for example using task-based fMRI paradigms. With respect to hallucinations, it would be interesting to apply dynamic FC to fMRI data from symptom capture designs. This would allow an investigation of the states that are associated with time periods in which patients are known to experience hallucinations, or time periods that precede or succeed hallucinations.

Second, dynamic FC analyses require a number of choices with respect to different analysis parameters, such as window size, and the selection of functional networks. While it has been shown that some of these choices do not significantly affect the results (13, 21), the current study nonetheless used previously validated analysis parameters in order to enhance comparability between studies and offer a replication of previous findings (21). However, relationships between dynamic FC and hallucinations, which were based on a relatively small sample in the current study, should be subjected to further testing and replication.

A further critical point concerns the fact that fMRI data in the current study were collected on different scanners. While this is very common, in particular when studying clinical populations, great care should be taken when combining fMRI data from multiple sources, in order to avoid effects of sampling bias and scanner-related measurement parameters (44). In the present study, scanner-related variance was removed from the data so that all reported results are corrected for potential site- or scanner-related effects.

The current study did not assess the degree to which patients experienced hallucinations during fMRI scanning. Therefore, it is impossible to determine if and how potentially occurring hallucinations during scanning may have affected the results. This is particularly true for the analysis on current hallucination severity, which was assessed on the day of scanning and might therefore be related to hallucination frequency during scanning. Since this analysis did not show any significant effect on dynamic FC, it is difficult to speculate about a potential role of ongoing hallucinations in the current findings.

The current study used the PANSS P3 item as a measure of hallucination severity. Therefore, it is not possible to disentangle the effects of hallucinations in different sensory modalities. However, given the focus in the PANSS P3 interview situation on auditory hallucinations (30), and the relative prevalence of auditory hallucinations compared to other types of hallucinations (31, 32), it is likely that the results primarily reflect effects of auditory hallucinations. The reported hallucination-related differences were centered around FC abnormalities in the DMN and TPN and were interpreted in the context of theoretical models that were developed to explain auditory verbal hallucinations (41, 43). However, since the DMN and TPN are involved in general cognitive functioning, independent of a particular sensory modality, it is conceivable that their dysfunction does not only play a role in auditory hallucinations but in hallucinations generally. Future studies that assess different modalities of hallucinations separately, should explore to which degree they share underlying mechanisms, and to which degree modality-dependent differences exist.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Regional Committee for Medical Research Ethics in Western Norway (REK-Vest 2016/800); Medical University of Plovdiv. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SW contributed data analysis and interpretation of the data and wrote the first draft of the manuscript. EJ contributed conception and design of the study. RK, E-ML, SK, and DS contributed to the data collection. KK organized the database. KH contributed to the conception and design of the study and interpretation of the data. All authors contributed to manuscript revision.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00227/full#supplementary-material

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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