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Functional and effective connectivity disruptions of the dopaminergic reward circuit in multiple sclerosis patients with depression

Giada Lombardi ,[1] Elisa Leveraro ,[2] Emilio Cipriano ,[2] Tommaso Sirito,[2] Maria Cellerino,[2] Lorenza Nasone,[2] Gianluca Serafini,[2] Lucio Ghio,[3] Andrea Escelsior,[1,2] Matilde Inglese[1,2]

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[1]IRCCS Ospedale Policlinico San Martino, Genoa, Italy
[2]Università degli Studi di Genova Dipartimento di Neuroscienze Riabilitazione Oftalmologia Genetica e Scienze Materno-Infantili, Genoa, Liguria, Italy
[3]Department of Mental Health and Addictions, ASL 3 Genovese, Genoa, Liguria, Italy

Correspondence to
Dr Matilde Inglese; m.inglese@unige.it

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ABSTRACT

Background Despite the impact of depression in multiple sclerosis (MS), the neurobiological mechanisms underlying its pathogenesis are still poorly understood. Disrupted functional connectivity (FC) within the reward circuit has been observed in major depressive disorder (MDD), highlighting its essential role in the neurobiology of depression. Here, we hypothesised that an analogous dysconnectivity may underpin depression in MS.

Methods The present study aimed to investigate FC of key nodes of the reward circuit (nucleus accumbens, NAcc and ventral tegmental area, VTA) in MS patients with depression (MS-D; n=30, 22 females), characterising differences with MS patients without depression (MS-nD; n=30, 17 females) and MDD patients without MS (n=30, 23 females). Furthermore, dynamic causal modelling (DCM) was applied to resting-state functional magnetic resonance imaging (fMRI) data to characterise effective connectivity (EC), which refers to the causal influences of brain regions involved in the circuit.

Results MS-D group showed reduced FC compared with both MS-nD and MDD, suggesting that the association of depression with MS may reflect dysfunction of the reward circuit. The DCM analysis showed inhibitory self-connections, a negative modulation of VTA in MS-D>MS-nD, a negative modulation between VTA, NAcc and the right amygdala as an effect of having depression and no EC differences for MS-D>MDD.

Conclusions The present connectivity study revealed promising results for understanding the pathophysiology of depression in MS. A combined FC-EC investigation of the reward circuit may represent a potential non-invasive in vivo MRI biomarker for understanding the onset of core depressive symptoms, supporting the development of effective and personalised therapies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Depression is a prevalent and debilitating comorbidity in multiple sclerosis (MS), but its neurobiological mechanisms remain poorly understood.

WHAT THIS STUDY ADDS

⇒ This study provides novel insights into the role of the reward circuit in MS-related depression, revealing significant functional and effective connectivity disruptions in key brain regions, such as nucleus accumbens and ventral tegmental area.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings suggest that combined functional–effective connectivity analysis could serve as a novel biomarker for MS-related depression, guiding the development of targeted and personalised therapeutic interventions for depression in MS.

INTRODUCTION

Depression is one of the most common and debilitating comorbidities in multiple sclerosis (MS), affecting up to 50% of patients and resulting in a significant reduction of their quality of life.[1] Prior MRI studies have shown associations between structural alterations in MS patients and depression, including atrophy and lesion load in frontal and temporal lobes, atrophy in hippocampus, precentral–postcentral gyri and thalamus, decreased fractional anisotropy in the cingulum, uncinate fasciculus and fornix.[2–4] Along with structural alterations, several fMRI studies revealed functional connectivity (FC) disruptions in MS patients with depression (MS-D), including decreased FC of hippocampus with dorso-lateral prefrontal and orbitofrontal cortices, cerebellum, basal ganglia and thalamic area, decreased FC of the amygdala (AMY) and frontal regions, increased FC of the anterior cingulate cortex (ACC) and decreased FC of the posterior cingulate cortex within the default mode network.[5–7] Despite such numerous findings demonstrating the impactful role of depression in MS, mechanisms associated with such anomalies are still poorly understood, making the biological background underlying this clinical association unclear. Since early studies, changes in neurotransmitters signalling have been considered one of the main biomarkers of major depression disorder (MDD).[8] Accordingly, recent data showed that signalling alterations of brain-stem monoamine nuclei, including the serotonin-producing raphe nuclei, the dopamine-producing ventral tegmental area (VTA) and substantia nigra,

impact brain functional configuration and activity.[9] Among these nuclei, VTA represents a core node for the mesolimbic-cortical dopaminergic system, commonly known as the *reward circuit*, which has been identified as a relevant study target to understand the onset of core symptoms of MDD, such as loss of motivation and pleasure (ie, anhedonia).[10 11] The reward circuit consists primarily of dopaminergic projections from the VTA to the nucleus accumbens (NAcc) in the ventral striatum, the prefrontal cortex (PFC), the hippocampus and the AMY.[12 13] In turn, the NAcc, receiving dense glutaminergic projections from the same areas, has been historically recognised for its crucial role in the modulation of reward-seeking and motivation-related behaviours.[14] Notably, evidence from neuroimaging studies showed disrupted FC of NAcc and VTA with the PFC and limbic regions in MDD patients, highlighting that a dysfunction of the reward circuit might play an essential role in the pathogenesis of depression.[15–17] Based on these findings, we hypothesised that an analogous neurotransmitter signalling change and connectivity disruption within the reward circuit might underpin depressive symptomatology in MS. Therefore, the present study aimed to investigate the FC of key nodes of the reward circuit in MS-D, as compared with MS patients without depression (MS-nD) and patients with major depressive disorder (MDD) without MS. Specifically, the study had two main objectives: (1) to compare MS-D and MS-nD, assessing how depression affects FC in MS patients and (2) to compare MS-D and MDD, assessing whether MS-related depression presents distinct neurobiological features relative to MDD in the absence of MS. After identifying potential functional disconnections in the two comparisons MS-D versus MS-nD and MS-D versus MDD, we aimed to further explore *effective connectivity* (EC), that is, direct causal influences, among brain regions of the circuit by applying dynamic causal modelling (DCM) to resting-state fMRI (rs-fMRI) data.[18]

Table 1 Summary of demographic and clinical data

	MS-nD	MS-D	MDD
Sample size	30	30	30
Age	42.85 (±8.21)	44.93 (±9.72)	46.26 (±11.55)
Sex (females/males)	17/13	22/8	23/7
DD	8.94 (±9.12)	7.88 (±6.55)	/
HADS-D	3.37 (±1.96)	12.73 (±1.60)	10.27 (±4.64)
EDSS, median (range)	1.75 (0–6)	2.5 (0–6)	/
MFIS	20.57 (±18.93)	51.97 (±14.41)	45.00 (±21.09)
BICAMS-z	0.23 (± 1.02)	0.11 (± 1.03)	0.34 (± 0.95)
MS therapy	**MS-nD**	**MS-D**	
Treated patients, n (%)	28 (93.33%)	27 (90%)	
Ocrelizumab, n (%)	11 (36.67%)	12 (40%)	
Natalizumab, n (%)	6 (20%)	6 (20%)	
Ofatumumab, n (%)	2 (6.67%)	2 (6.67%)	
Cladribine, n (%)	1 (3.33%)	3 (10%)	
Fingolimod, n (%)	2 (6.67%)	0	
Other, n (%)	6 (20%)	4 (13.33%)	
Untreated patients, n (%)	2 (6.67%)	3 (10%)	
Therapy for depression		**MS-D**	**MDD**
Treated patients, n (%)		7 (23.33%)	30 (100%)
Antidepressants, n (%)		6 (20%)	29 (96.67%)
Mood stabilisers, n (%)		4 (13.33%)	11 (36.67%)
Antipsychotics, n (%)		1 (3.33%)	6 (20%)
Benzodiazepines, n (%)		2 (6.67%)	10 (33.33%)

Unless otherwise specified, all values are expressed as mean (±SD). The table also contains details on MS therapy and depression therapy. Note that patients treated for depression could assume different combinations of antidepressants, mood stabilisers, antipsychotics and benzodiazepines.
BICAMS-z, Brief International Cognitive Assessment mean z-score. DD, disease duration; EDSS, Expanded Disability Status Scale; HADS-D, Hospital Anxiety and Depression Scale, subscale Depression; MDD, major depressive disorder; MFIS, Modified Fatigue Impact Scale; MS, multiple sclerosis; MS-D, multiple sclerosis with depression; MS-nD, multiple sclerosis without depression.

MATERIALS AND METHODS

Participants and clinical assessment

90 subjects were prospectively enrolled in the study (table 1): 60 patients with MS (30 with depression and 30 without depression) and 30 patients with MDD. Inclusion criteria for all subjects were age between 18 and 60, the ability to provide written informed consent and absence of contraindications for MRI acquisition. Inclusion criteria for MS patients were as follows: diagnosis of clinically definite MS, according to the revised McDonald's criteria,[19] Expanded Disability Status Scale (EDSS) score no greater than 6.0 at screening, diagnosis of MS made at least 2 years prior to the study (to reduce the risk of reactive depression), stable treatment for at least 1 year prior to study enrolment, no steroid administration or relapses during at least the preceding 3 months. Exclusion criteria for MS patients were treatment with Interferon beta, diagnosis of MDD prior to diagnosis of MS, family history of psychiatric disorders and depression related to traumatic events. For MDD patients, the inclusion criterion consisted of an MDD diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders criteria (DSM-5), while the exclusion criteria included a diagnosis of other psychiatric disorders, major systemic or organ comorbidities and a history of drug and/or alcohol addiction.[20] All subjects underwent MRI acquisition and neuropsychological evaluation, which included (1) the Brief International Cognitive Assessment (BICAMS), consisting of the Symbol Digit Modalities Test, the California Verbal Learning Test and the Brief Visuospatial Memory Test; (2) the Hospital Anxiety and Depression Scale (HADS) and (3) the Modified Fatigue Impact Scale (MFIS). A BICAMS composite z-score was derived by averaging the three individuals' z-scores.[21] For MS patients, disability was quantified by using the EDSS. MS patients were classified into two groups based on the depression subscale of the HADS questionnaire (HADS-D) using a cut-off of 11 points: MS-nD (score at HADS-D <11) and MS-D (score at HADS-D >11).[22] Groups were matched for age, sex and level of education. For MDD and MS-D patients, the dosages of antidepressants, mood stabilisers, antipsychotics and benzodiazepines (assumed with different combinations) were converted into chlorpromazine, lithium, imipramine and diazepam equivalents, respectively.[23] Additionally, demographic, clinical and structural MRI features of each group were preliminarily compared with a group of 30 healthy controls (HC) to assess baseline differences between healthy subjects and patients. Written informed consent was obtained from all subjects before the beginning of the study procedures, according to the Declaration of Helsinki. The protocol was approved by the local ethical standards committee (CER Liguria: 492/2021 DB id 11744).

MRI acquisition protocol

All images were acquired using a 3T MRI scanner (Prisma, Siemens Medical Systems, Erlangen, Germany) with a 64-channel head coil. The MRI protocol included (a) three-dimensional (3D) magnetisation-prepared rapid gradient-echo T1-weighted sequence (TR=2300 ms, TE=2.96 ms, TI=919 ms, resolution $1\times1\times1\,mm^3$); (b) three-dimensional (3D) sagittal T2-fluid-attenuated inversion recovery (TR=5000 ms, TE=393 ms, resolution $0.4\times0.4\times1\,mm^3$); (c) T2* echo-planar imaging (TR=720 ms, TE=33 ms, flip angle=52°, resolution $2.3\times2.3\times2.3\,mm^3$,

time points=600) for rs-fMRI (during the acquisition of this sequence, subjects were asked to rest with their eyes closed without falling asleep).

Structural MRI

Preprocessing

For MS patients, T1w and T2w were used as input to a neural network for lesions segmentation. The scalp was removed by using hd-bet to give the model the betted images as input.[24] Then, T1-hypointense lesion masks were used to fill T1 images by using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL 5.0).[25] After lesion filling, T1 images were registered to the Montreal Neurological Institute (MNI) space by using affine and non-linear transformations in Advanced Normalization Tools (ANTs).[26] For all subjects, the computational anatomy toolbox (CAT12) implemented in the Statistical Parametric Mapping (SPM) software was used for brain tissue segmentation of T1 images[27 28] (online supplemental table 2).

Functional MRI

Preprocessing

All fMRI data were preprocessed with the following pipeline: (1) realignment and unwarping for motion and susceptibility distortion correction, (2) slice timing for the correction of temporal misalignment between different slices, (3) outlier detection based on the artifact detection tool to identify potential outlier scans from the global blood-oxygen-level-dependent (BOLD) signal and the amount of subject motion in the scanner, (4) normalisation into standard MNI152 space and (5) functional smoothing by using spatial convolution with a Gaussian kernel of 6 mm full width half maximum. After the functional data had been preprocessed, denoising was applied to all images in order to remove unwanted motion, physiological and other artifactual effects from the BOLD signal. This procedure starts with the regression of confounding effects characterised by white matter and CSF timeseries (five noise components each), subject-motion regressors (three translation and three rotation parameters) plus their first derivatives (for a total of twelve noise components), a variable number of outlier scans (scrubbing, below fifty factors identified in the outlier detection preprocessing step), scan-to-scan global BOLD changes and head-motion measures (QC_timeseries, estimated during the realignment and unwarp preprocessing step) and linear trends within each session (effect of rest). Subsequently, data were filtered within the standard frequency band of 0.01–0.08 Hz, which is thought to reflect mainly neuronal fluctuations and to be less affected by physiological variables.

FC analysis

FC analysis of rs-fMRI (rs-fMRI) data was performed with the CONN toolbox based on the SPM software implemented in MATLAB R2023b.[29] For the first-level analysis (single-subject level), FC was evaluated with a hypothesis-driven approach of ROI-to-ROI analysis. Two regions of interest (ROIs), corresponding to the two main nodes of the reward circuit, were imported in the CONN analysis setup: a mask of the VTA from the Harvard AAN-Atlas[30] and a bilateral mask of the NAcc. Their timeseries were extracted and correlated with the BOLD activity across brain regions defined in the FSL Harvard-Oxford atlas. Particularly, FC was evaluated by calculating the Pearson correlation coefficient between pairs of ROIs' BOLD timeseries, which was then transformed to a z-value with Fisher r-to-z transformation in order to improve normality (figure 1).

Dynamic causal modelling

Dynamic causal modelling (DCM) is the predominant analysis framework for the investigation of effective connectivity (EC), which refers to the causal influence among brain regions or more simply how one brain region can affect the activity of another.[18] The first step of the DCM analysis entailed the identification of ROIs to include in the model and the extraction of their timeseries (figure 2a). For the present study, specific ROIs resulting significant in the previous FC analysis and known to have an important role in the functioning of the reward circuit were selected. Particularly, for both contrasts of interest (MS-D vs MS-nD, MS-D vs MDD), the NAcc, VTA, a limbic region (insula (INS) and amygdala (AMY)) and a prefrontal region (inferior frontal gyrus (IFG) and middle frontal gyrus (MFG)) were used as ROIs to build a full model for each subject, in which the A-matrix modelled all plausible connections between nodes and inhibitory self-connections for each region (figure 2b).[31] Then, the estimated connectivity parameters were used to run a Parametric Empirical Bayes (PEB) analysis at the group level to select the model with highest probability (figure 2c).[32] (For details see online supplemental material)

Statistical analysis

Statistical analyses were performed in the Jamovi software.[33] Demographic, clinical and structural MRI characteristics of each

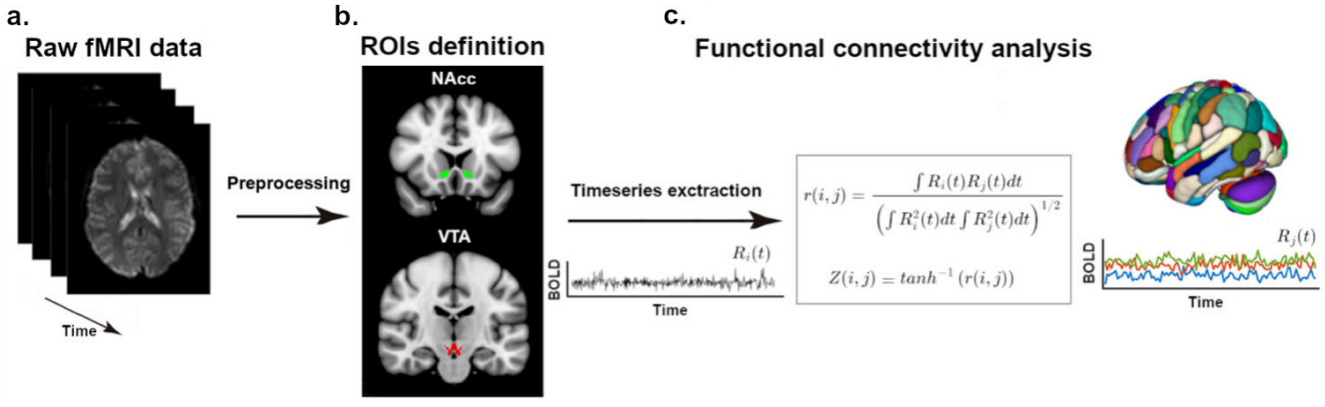

Figure 1 Functional MRI analysis pipeline. (a) Preprocessing of raw fMRI data, (b) identification of ROIs (NAcc, nucleus accumbens, and VTA, ventral tegmental area), (c) FC analysis consisting of Pearson correlation coefficient (r) calculation between BOLD timeseries of ROIs (Ri(t)) and BOLD timeseries of each brain region defined in the FSL Harvard-Oxford atlas (Rj(t)). FSL, FMRIB software library; ROIs, regions of interest.

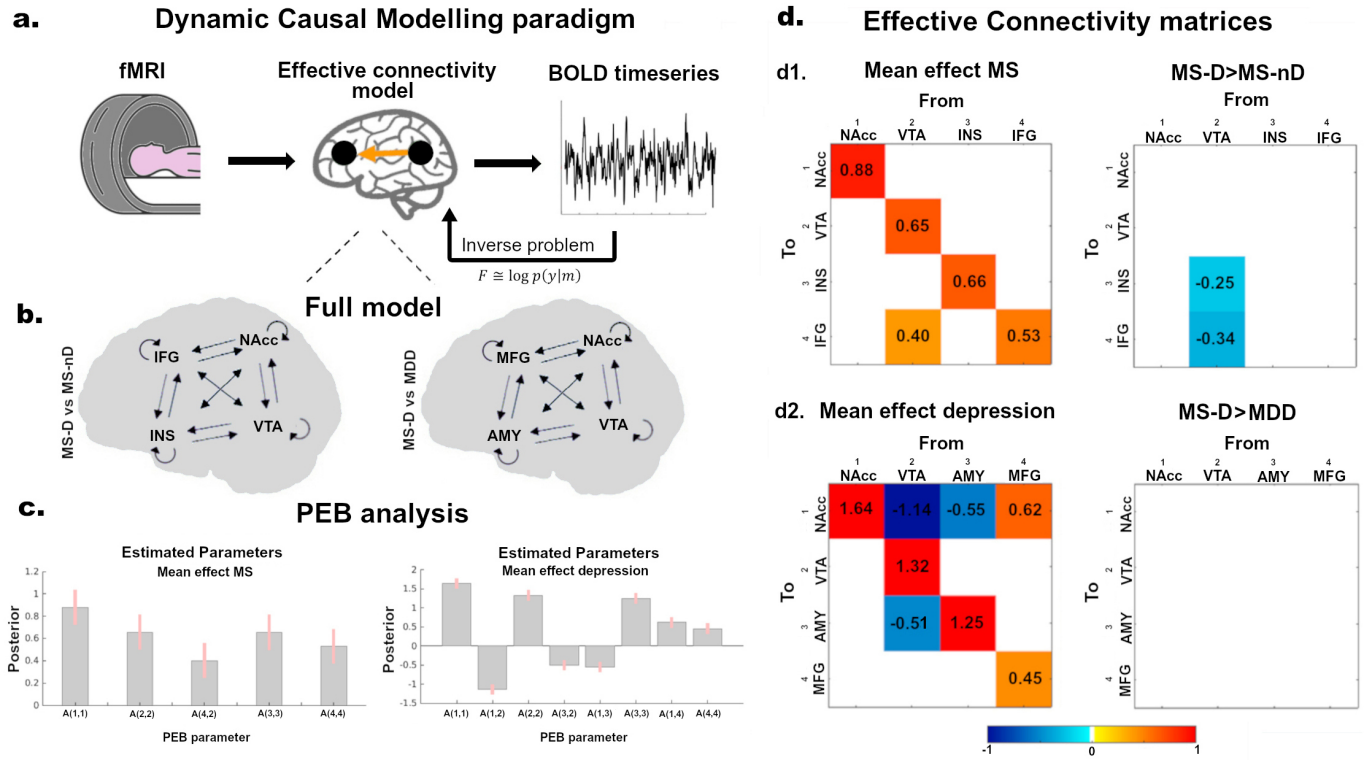

Figure 2 Dynamic causal modelling (DCM). (a) DCM paradigm consists of fMRI data acquisition and preprocessing, definition and estimation of EC models and selection of the 'best model', that is, the one which maximises the free energy F (calculated as the probability of having the observation y having the model m). (b) Definition of a 'full model' containing all plausible connections in the A-matrix. (c) By using Bayesian model comparison (BMC), the PEB analysis selects the model with the strongest evidence and calculates commonalities and differences between groups. Bar plots represent model parameters (A-matrix elements A(i,j)) surviving a thresholding at >95% of posterior probability. (d) Results of the best model can be represented in the form of connectivity matrices. For off-diagonal values (A(i,j) with i different from j), changes in EC strengths are represented in a colour scale from yellow to red if 'excitatory' (positive modulation) and from light blue to blue if 'inhibitory' (negative modulation). For values on the diagonal (A(i,j) with i=j), representing self-connections which are inhibitory by definition, the colour code is inverted. AMY, amygdala; IFG, inferior frontal gyrus; INS, insula; MDD, major depressive disorder; MFG, middle frontal gyrus; MS-D, MS patients with depression; MS-nD, MS patients without depression; NAcc, nucleus accumbens; PEB, parametric empirical Bayes; VTA, ventral tegmental area.

group were preliminarily compared with an HC group to assess baseline differences between patients and healthy subjects. Then, data of the three groups of interest were analysed using ANOVA followed by Bonferroni correction. Significant results were set at $p<0.05$. For ROI-ROI FC, two main contrasts were carried out following the two main objectives of the study: (1) MS-D versus MS-nD, to assess FC differences between MS patients with and without depression; (2) MS-D versus MDD, to assess FC differences between depressed patients with and without MS. For MS-D versus MS-nD, the ANCOVA was carried out adjusting for age, sex, EDSS and depression therapy, with statistical significance set at $p<0.05$. First, we assessed whether MFIS scores could significantly influence differences between MS subgroups by performing a preliminary Pearson correlation with each FC value. For those connections showing a significant correlation, MFIS was included as an additional covariate in the ANCOVA. Additionally, a similar correlation analysis between lesion volumes and FC metrics was performed for MS-D to exclude a possible influence of lesion load on significant FC alterations. For MS-D versus MDD, the ANCOVA was carried out, adjusting for age, sex and depression therapy, with statistical significance set at $p<0.05$.

RESULTS

Demographic and clinical characteristics

Groups were balanced in terms of age ($F=0.9$, $p=0.4$) and sex ($\chi^2=3.2$, $p=0.2$). Preliminary comparisons of HC with each group of patients revealed significant differences ($p<0.05$) of MFIS, BICAMS-z score and gray matter (GM) volumes. Additionally, white matter (WM) volumes were significantly different between HC and MS patients (both MS-D and MS-nD) but not between HC and MDD patients. The two MS subgroups did not differ for disease duration ($p=0.61$) and lesion volumes ($p=0.78$), while EDSS was higher in MS-D compared with MS-nD ($p=0.042$) (online supplemental table 1).

Resting-state fMRI

rs-fMRI for MS patients: MFIS significantly correlated with FC of the NAcc with the right hippocampus ($r=-0.35$, $p<0.01$), left hippocampus ($r=-0.33$, $p<0.01$) and right parahippocampal gyrus ($r=-0.33$, $p=0.01$). Once ascertained connections for which MFIS needed to be included as an additional covariate, the MS-D>MS-nD comparison revealed decreased FC of the NAcc with the left inferior frontal gyrus ($F=5.11$, $p=0.028$), right insular cortex ($F=4.29$, $p=0.043$) and brain stem ($F=5.22$, $p=0.026$) but no significant differences in FC of the VTA (figure 3a). Additionally, lesion volumes of MS-D did not correlate with significant FC alterations. The MS-D>MDD comparison revealed decreased FC of the NAcc with the MFG ($F=5.02$, $p=0.029$), right thalamus ($F=6.06$, $p=0.017$), left thalamus ($F=4.71$, $p=0.035$) and brain stem ($F=7.23$, $p=0.01$) and decreased FC of VTA with the right AMY ($F=5.73$, $p=0.02$),

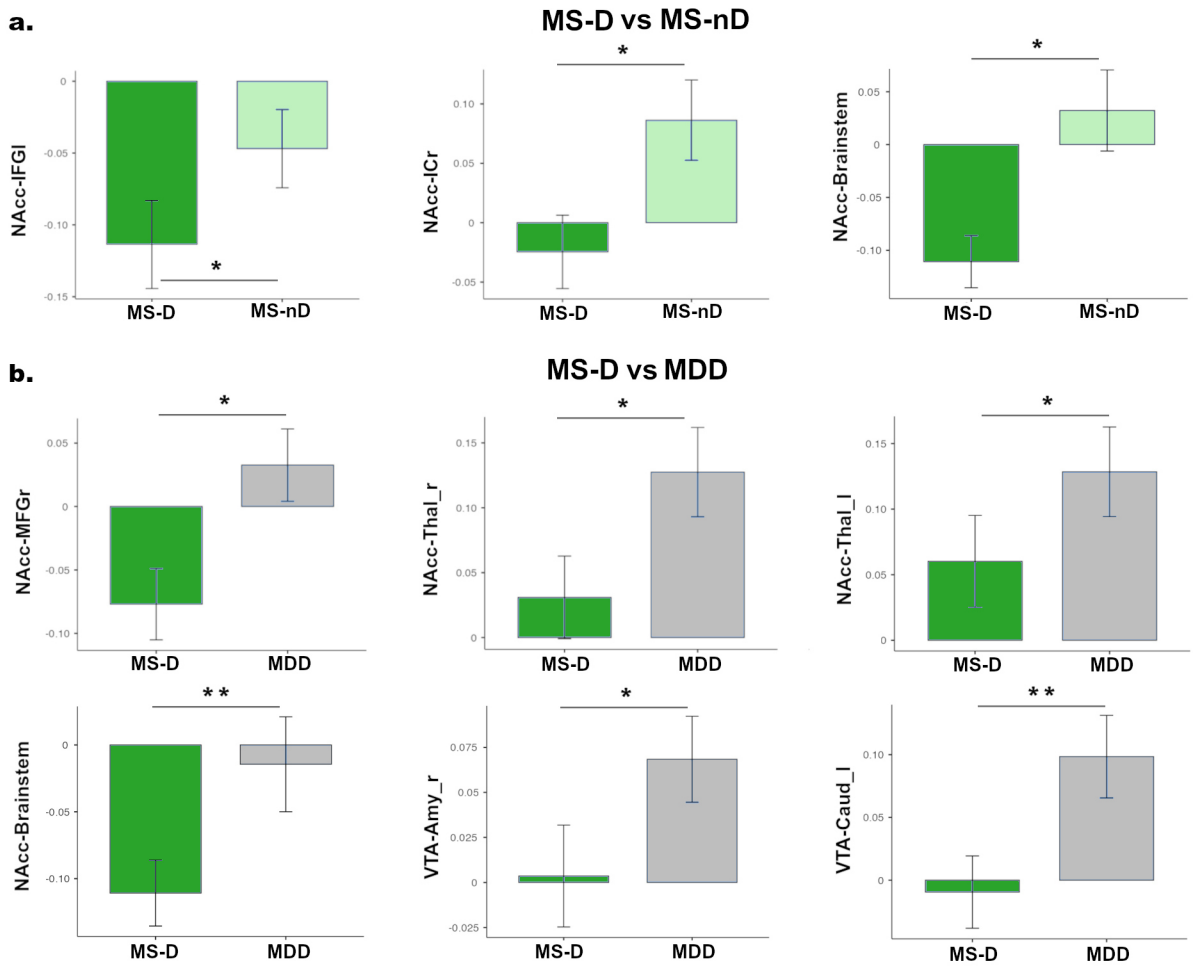

Figure 3 Results of ROI-ROI FC analysis. (a) MS-D versus MS-nD, (b) MS-D versus MDD. On the y axis, bar plots represent z-scores correlation values between ROIs. Horizontal bars indicate statistical significance (*p<0.05, **p<0.01). MDD, major depressive disorder; MS-D, MS patients with depression; MS-nD, MS patients without depression; NAcc, nucleus accumbens; ROIs, regions of interest; VTA, ventral tegmental area.

left caudate (F=9.87, p=0.003) (figure 3b) and anterior cingulate gyrus (F=3.74, p=0.056, close to significance).

Dynamic causal modelling

For the MS-D versus MS-nD contrast, the full DCM model included the following ROIs: NAcc, VTA, right INS and left inferior frontal gyrus (IFG) (figure 2b). By thresholding the Bayesian model average at >95% posterior probability (strong evidence based on the Free energy approximation), the mean effect of having MS resulted in a model with positive values of self-connections (inhibition effect) of NAcc (0.88), VTA (0.65), INS (0.66) and IFG (0.53) and a positive modulation from VTA to IFG (0.40). Additionally, the PEB analysis revealed a significant difference of effective connectivity between MS-D and MS-nD (MS-D>MS-nD), consisting of a decreased modulation from VTA to INS (−0.25) and from VTA to IFG (−0.34) in MS-D patients (figure 2). For the MS-D versus MDD contrast, the full DCM model included the following ROIs: NAcc, VTA, right AMY and right MF) (figure 2b). By using the same thresholding of >95% of posterior probability, the mean effect of having depression resulted in a model with positive values of self-connections (inhibition effect) of NAcc (1.64), VTA (1.32), AMY (1.25) and MFG (0.45); positive modulation from MFG to NAcc (0.62), but negative modulations from VTA to NAcc (−1.14), from VTA to AMY (−0.51) and from AMY to NAcc (−0.55). Instead, the PEB analysis did not reveal a significant difference in effective connectivity between MS-D and MDD (figure 2).

DISCUSSION

The present study aimed to explore neurobiological mechanisms underlying depression in MS by investigating functional and effective connectivity within the mesolimbic-cortical dopaminergic system (reward circuit), whose impairment is known to play a pivotal role in depression-related symptoms. The core strength of this work lies in providing, for the first time, insights into (1) how depressive symptomatology in MS patients could reflect dysfunction of the reward circuit and (2) shared and condition-specific functional disruptions between MS-related depression and MDD (MS-D vs MDD). Notably, the present study proposes DCM as a powerful approach for investigating causal interactions between brain regions and understanding whether and how specific clinical conditions (e.g. having depression, having MS) could impact the directionality of neural signal flow across the circuit. According to our hypothesis, MS-D presented a significant FC reduction of NAcc with the brainstem, where neurotransmitter nuclei are located, compared with both MS-nD and MDD. This result is in line with a previous work, demonstrating a functional disconnection of neurotransmitter-related nuclei in MS-D compared with MS-nD.[34] By analysing the differences between MS subgroups, MS-D showed a significant FC reduction of NAcc with INS and

IFG. The INS is a site of multisensory integration, involved in the regulation of autonomic and physiological responses to rewarding and emotional stimuli and whose projections to the NAcc are known to critically influence motivated behaviour.[35] On the other hand, a decreased FC between IFG and NAcc is associated with a desynchronisation between the executive control network and the reward circuit, resulting in impaired regulation and processing of negative reward information.[36] Notably, it has been demonstrated that NAcc deep-brain stimulation (DBS) modulates the activity of INS and PFC (which includes IFG), contributing to the therapeutic effect of DBS for psychiatric disorders.[37] These findings highlight that a significant FC decrease of the NAcc with these two brain areas in MS-D could lead to abnormal functionality of the reward circuit. The DCM analysis of MS patients provided further data: the PEB analysis revealed positive self-connections common across MS subjects (corresponding to more inhibition) and a decreased EC (negative modulation) from the VTA to INS and IFG as a result of MS-D>MS-nD. A reduced excitatory modulation from VTA to the INS and PFC may indicate a potential dysregulation of dopaminergic signalling in MS-D, which could have implications for affective and motivational processes.[38] Notably, results of the VBM analysis showing no significant GM volumetric differences in MS-nD>MS-D support the hypothesis that depressive symptoms in MS may be attributed to a functional reorganisation of neurotransmitter activity rather than structural alterations. In addition, lesion volumes of MS-D did not correlate with significant FC alterations, excluding a direct impact of MS-related lesions on the functional impairment of the reward circuit. By comparing depressed patients (MS-D vs MDD), MS-D still presented a more significant FC disruption of NAcc with many brain regions, including the MFG and thalamus. Moreover, MS-D>MDD resulted in decreased FC of VTA with the right AMY, left caudate and, close to significance, ACC. Notably, despite FC differences between MS-D and MDD, DCM analysis of these groups did not show significant differences in causal influences between brain regions: both MS-D and MDD presented positive self-connections (corresponding to more inhibition), a very strong inhibition of the VTA on the NAcc, as well as negative modulation of the connection from the AMY to the NAcc, a pathway implicated in motivated behaviour.[39] Some potential limitations of the current study should be considered. First, the relatively small sample size of each group may have limited the statistical power of our analyses. Future studies with a larger number of patients are needed to confirm and extend these results. Second, the classification of MS patients in MS-D and MS-nD was based on scores of the HADS scale in patients with at least 2 years since MS onset to rule out reactive depression. Despite not replacing a full psychiatric evaluation, the HADS is a well-validated and widely used screening tool for depression in clinical populations.[40] For future studies, a more comprehensive diagnostic approach could provide a more detailed characterisation of depression in MS patients. Third, the DCM analysis was carried out by considering specific brain regions, resulting significant in the FC analysis and known to be involved in the reward circuit. However, future research should consider a more detailed EC model to characterise causal interactions of the circuit among a greater number of nodes. Finally, longitudinal studies would be valuable in determining whether and how EC could dynamically evolve over time and be associated with changes in FC alterations, perhaps related to antidepressants' efficacy. In conclusion, the present study sheds new light on the neurobiological mechanisms underlying depression in MS. Particularly, MS-D exhibited more significant FC disruptions within the reward circuit compared with both MS-nD and MDD, pointing out two main findings: (1) the presence of depression in MS further compromises FC relative to MS without depression, highlighting the impact that this comorbidity could play in intensifying dysfunctions already present in the MS brain; (2) MS associated with depression may exacerbate FC alterations usually seen in MDD without MS. Moreover, the study underscores the potential of applying DCM to fMRI data to better understand, besides the functional correlation of activity between brain regions, the directionality of signal flow and their causal relation. A combined FC-EC analysis approach could represent a potential non-invasive in vivo MRI biomarker for the elucidation of the pathophysiology underlying the onset of depression in MS, providing new targets for the development of more efficacious and personalised therapies.

Contributors GL contributed to data collection, performed data analysis and wrote the first draft of the manuscript; EL contributed to data collection and data analysis; EC contributed to data analysis; TS contributed to data collection; MC contributed to data interpretation; LN contributed to data collection; GS, LG and AE contributed to data collection and consulting for the development of the project; MI conceptualised the study, supervised the development of the study and contributed to data interpretation. MI is the guarantor of the study (m.inglese@unige.it). All coauthors read the manuscript and approved its final version.

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Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by CER Liguria: 492/2021 - DB id 11744. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

ORCID iDs
Giada Lombardi https://orcid.org/0009-0002-7749-307X
Elisa Leveraro https://orcid.org/0009-0005-0018-4449
Emilio Cipriano https://orcid.org/0000-0003-0304-0529

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