Brain functional connectivity alterations associated with neuropsychological post-COVID syndrome

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Abstract

Neuropsychological deficits and brain damage following SARS-CoV-2 infection are not well understood. 110 patients, with either severe, moderate or mild disease in the acute phase underwent neuropsychological and olfactory tests, as well as completed psychiatric and respiratory questionnaires at 223 ± 42 days post-infection. Additionally, a subgroup of 50 patients underwent functional magnetic resonance imaging. Patients in the severe group displayed poorer verbal episodic memory performances, and moderate patients had reduced mental flexibility. Neuroimaging revealed patterns of hypo and hyper functional connectivity in severe patients, while only hyperconnectivity patterns were observed for moderate. The default mode, somatosensory, dorsal attention and cerebellar networks were implicated. Partial least squares correlations analysis confirmed specific association between memory performances and brain functional connectivity. The severity of the infection in the acute phase is a predictor of neuropsychological post-COVID syndrome. SARS-CoV-2 infection causes long-term memory and executive dysfunctions, related to largescale functional brain connectivity alterations.

Introduction

The World Health Organization recently defined the long-term consequences of SARS-CoV-2 infection as *post-COVID-19 condition*. This refers to a multisystem condition that occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months after onset of COVID-19, with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis. To date, at least 52 clinical or biological signs have been listed [1], impacting eight different systems [2]: pulmonary, cardiovascular, hematological, renal, endocrine, gastrointestinal, dermatological, and neuropsychiatric [2].

This constellation of symptoms persists well after the acute phase of the infection and includes cognitive disorders (for a review, see [3]). Observations suggest impairment of various cognitive functions up to 3 months following COVID-19, with disruption of global cognitive efficiency [4–17], memory functions [18–22], attention [5,18,22], executive functions [5,19,21,23], logical reasoning [22] and language [5,18,21]. The etiopathogenesis of these disorders remains subject to debate, but three hypotheses have already been postulated. First, the central nervous system (CNS) may be directly damaged by SARS-CoV-2 through a pathway involving the olfactory system and ACE-2 receptors present in the CNS [24]. This hypothesis is supported directly by histopathological studies showing the presence of the virus in the CNS [24]. Second, indirect damage may result from an excessive immune or inflammatory reaction. This is supported by evidence of hyperinflammation with features of cytokine storm syndrome [25], and by studies showing a link between neuropsychiatric symptoms and immune data [26]. Third, indirect damage may result from inflammation of the endothelial cells of the vascular system, with COVID-19 being viewed as a vascular disease [27]. All three hypotheses can be supported by positron emission tomography (PET) studies revealing patterns of hypometabolism in the olfactory, frontal and limbic systems [28–30]. There is also the potential impact of the post-resuscitation / intensive care unit (ICU) syndrome in patients whose symptoms were sufficiently severe to require such treatment. Cognitive deficits after ICU, associated with

mechanical ventilation, has been demonstrated in other pathologies and are increasingly recognized [31,32] (for a recent review, see Sakusic, et al. [33]). Interestingly, this review [33] found that the factors that predicted impaired cognition and structural brain damage after hospitalization in ICU were delirium and its duration. Based on these reviews, medication (sedatives and analgesics), mechanical ventilation, extracorporeal membrane oxygenation, trophic feeding, intraoperative hypotension, and hypoxia appear not to influence the likelihood of long-term cognitive impairment [33].

The impact of respiratory severity in the acute phase of COVID-19 on chronic neuropsychological symptoms has yet to be clarified/determined. Nevertheless, some studies using validated neuropsychological testing approaches to explore the consequences of SARS-CoV-2 infection have shed some light on this issue. For example, Woo, et al. [19] and Almeria, et al. [18] compared patients who benefited from oxygen therapy with those who did not. Woo, et al. [19] found no differences, whereas Almeria, et al. [18] reported significant differences on verbal memory, visual memory, working memory, processing speed, executive function, and global cognition. Reduced performances for executive functions were only observed in ICU patients. Alemanno, et al. [5] observed better cognitive scores among patients who had been under sedation and ventilated in the ICU, compared to patients who had been hospitalized without oxygen therapy. Nevertheless, the presence of methodological limitations reduces the extent to which inferences can be drawn about the potential impact of respiratory severity in the acute phase on chronic neuropsychological deficits. Moreover, only a small number of studies have simultaneously assessed chronic neuropsychological symptoms and carried out neuroimaging. In particular, to date, no functional connectivity analyses have been conducted in patients with COVID-19, especially not in relation to neuropsychological deficits.

In this context, the objective of the present study was to test whether neuropsychological deficits 6–9 months post-infection were associated to modifications in functional brain networks, considering the severity of the respiratory symptoms in the acute phase. To this end, patients without clinical history that could be associated with neuropsychological deficits prior to infection with SARS-CoV-2 underwent a comprehensive assessment that probed multiple cognitive domains, emotion recognition, psychiatric symptoms, dyspnea, and olfaction. They were divided into three groups according to the respiratory severity of the disease in the acute phase: severe (ICU hospitalization; $n = 24$), moderate (conventional hospitalization; $n = 42$), and mild (no hospitalization; $n = 44$). Of these patients, 50 agreed to undergo MRI, for which structural visual and functional connectivity analyses were performed.

In view of our objectives, we developed two hypotheses. First, we expected neuropsychological deficits and modifications of the cerebral functional connectivity to be a function of disease severity in the acute phase [34], although moderate and mild patients might also exhibit deficits [5,19]. Second, we suspected that relationships between neuropsychological scores and changes in functional brain connectivity could be observed as a function of severity.

Methods

Participants (see Table 1)

Patients were selected among all the patients from the Geneva University Hospitals (HUG) that showed evidence of a SARS-CoV-2 infection (between March 2020 and May 2021) either by positive polymerase chain reaction (PCR) from nasopharyngeal swab and/or by positive serology while being included according to the exclusion criteria (see below). Patients were divided into three groups and included to the study at 223.07 ± 41.69 days post-infection: 24 patients who had been admitted to ICU during the acute phase of the infection (severe); 42 patients who had been hospitalized but did not require mechanical ventilation (moderate); and 44 patients who had tested positive but had not been hospitalized (mild). Of these patients, 50 agreed to undergo MRI scans (severe: n = 9; moderate: n = 21; mild: n = 20).

The required number of participants in each group was determined by a power analysis involving the comparison of two means. This analysis was based on the literature evaluating the short-term neuropsychological effects of COVID-19 in mild patients [19]. To achieve the desired statistical power (1 - β) of 90% and risk of Type I error (α) of 0.05, results indicated that for a one-sided hypothesis, 13 participants would be needed in each group and for a bilateral hypothesis 18. As we planned to perform nonparametric analyses, we had to increase the sample size by 15% [35], resulting in a minimum of 15 participants per group in the case of one-sided hypothesis and 21 participants per group in the case of bilateral hypothesis.

The mild and moderate groups were matched during the screening-inclusion process to the severe group for median age (mild = 57.50 years; moderate = 56.50 years; severe = 60 years), sociocultural level, and clinical variables (except for chronic renal failure) due to a limited number of available patients who were in ICU and met our exclusion criteria. Participants (n = 50) who underwent MRI were not matched during the screening-inclusion process, and all patients that agreed for the MRI study were included. Nevertheless, the groups were still comparable on sociodemographic characteristics (except gender) and severity. Participants were recruited via CoviCare program [36] following patients with post-COVID symptoms in Geneva, Switzerland (MN, OB and IG), as well as from registers from another study (LB). For each patient, we carried out a medical file review, followed by a telephone call inviting the patient to take part in the study, if all the eligibility criteria were met. Exclusion criteria were a history of neurological issues, psychiatric disorders (two of the included participants had had an episode of depression more than 10 years before their SARS-CoV-2 infection), cancer (to exclude possible chemotherapy- and radiotherapy-related cognitive impairment [37]), neurodevelopmental pathologies, pregnancy, and age above 80 years (see Fig. 1).

Table 1. Sociodemographic data and medical history

	Mild *n* = 44	Moderate *n* = 42	Severe *n* = 24	p-value”
Mean age in years (± *SD*)	56.57 (± 7.23)	56.50 (± 9.58)	62.08 (± 12.03)	.078
Mean education level [1-3] (± *SD*)	2.72 (± 0.45)	2.64 (± 0.58)	2.50 (± 0.59)	.373
Gender (% women)	34.10	35.70	20.80	.420
Handedness (% right handed)	97.70	92.90	95.80	.553
Mean days of hospitalization (± *SD*)	-	12.00 (± 12.87)	40.13 (± 32.07)	-
Diabetes in %	2.30	9.50	20.80	.083
Smoking in %	11.40	2.40	4.20	.206
History of respiratory disorders in %	11.40	11.90	25.00	.259
History of cardiovascular disorders in %	13.60	14.30	25.00	.432
History of neurological disorders in %	0	0	0	1
History of psychiatric disorders in %	2.30^{+}	2.40^{+}	4.20^{+}	.887
History of cancer in %	0	0	0	1
History of severe immunosuppression in %	0	0	0	1
History of developmental disorders in %	0	0	0	1
Chronic kidney disease in %	0	0	8.3	.026*
Sleep apnea syndrome in %	9.10	11.90	29.20	.067

Note. ns: not significant; *SD*: standard deviation; “Statistical analysis performed: Kruskal-Wallis or chi^2; $^{+}$ treated depression more than 10 years prior to COVID-19.

General procedure and ethics

A flowchart displaying the successive stages of the study according to the eligibility criteria for each experimental group is provided in Fig. 1.

After being given a full description of the study, participants provided their writtenf informed consent. The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the cantonal ethics committee of Geneva (CER-02186).

Neuropsychological assessment and other clinical outcomes

The experimental design and tests used are comparable to those used in a previous published study (Voruz et al., 2022). In addition, the detailed tests are available in SI 1.

Symptom validity and presence of noncredible symptoms

First, to validate our neuropsychological measurements, we checked the validity of patients' symptoms. Both the measurement of symptom validity (i.e., congruence) and the measurement of noncredible symptoms with the BRIEF-A showed good-to-excellent results for all participants, validating the results of the neuropsychological tests and the psychiatric symptom questionnaires.

Neuroimaging processing

Image acquisition

A total of 50 participants (mild: n = 20; moderate: n = 21; severe: n = 9) underwent MRI scans at the CIBM Center for Biomedical Imaging in Geneva, on a Siemens Magnetom PrismaFit 3 tesla scanner. Analysis revealed no significant differences between the mild, moderate and severe groups on age (mild: 55.18 ± 8.58, moderate: 54.94 ± 12.93, severe: 57.80 ± 12.49, p = .885), sociocultural level (mild: 2.76 ± 0.44, moderate: 2.78 ± 0.43, severe: 2.80 ± 0.42, p = .978) or handedness (one left-handed in the mild group), whereas a significant difference was observed for gender (p = .049), with a higher proportion of men in the severe group as compared to mild and moderate. Intergroup analysis also failed to reveal any significant differences either on the interval between infection and MRI (mild: 254.18 ± 39.52 days; moderate: 287.17 ± 45.24 days; severe: 280.80 ± 54.06 days; p = .058) and the interval between neuropsychological testing and MRI (mild: 30.47 ± 20.66 days; moderate: 39.83 ± 26.23 days; severe: 51.39 ± 25.67 days; p = .112). Data from five patients were excluded due to high movement and/or poor registration. Structural images were obtained with a T1-weighted (T1w) magnetization-prepared rapid acquisition gradient echo sequence with an isotropic voxel size of 0.9375 x 0.9375 x 0.9 mm^3 (SI 2). Resting-state functional images were acquired through a multiband accelerated echoplanar sequence with an isotropic voxel size of 2.5 x 2.5 x 2.5 mm^3, 64 slices, and repetition time of 1 s for a total of 7 min 59 s of acquisition time (480 volumes; SI 3).

Preprocessing was performed using *fMRIPrep* 20.2.3 [38], which is based on Nipype 1.6.1 [39].

Anatomical preprocessing

Each T1w volume was corrected for intensity non-uniformity using N4BiasFieldCorrection v2.1.0 [40], and skull-stripped using antsBrainExtraction.sh v2.1.0 (using the OASIS template). Spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c [41] was performed through nonlinear registration with the antsRegistration tool of ANTs v2.1.0 [42], using brain-extracted versions of both T1w volume and template. Brain tissue segmentation of cerebrospinal fluid (CSF), white matter (WM) and gray matter was performed on the brain-extracted T1w using fast [43] (FSL v5.0.9).

Functional preprocessing

Functional data were slice-time corrected using 3dTshift from AFNI v16.2.07 [44], and motion corrected using mcflirt (FSL v5.0.9 [45]). This was followed by FLIRT (FSL) coregistration to the corresponding T1w images using boundary-based registration [46] with six degrees of freedom. Motion-correcting transformations, BOLD-to-T1w transformation and T1w-to-template (MNI) warp were concatenated and applied in a single step using antsApplyTransforms (ANTs v2.1.0), configured with Lanczos interpolation.

Physiological noise regressors were extracted with CompCor [47]. Principal components were estimated for the temporal (tCompCor) and anatomical (aCompCor) variants. A mask to exclude signal with gray matter origin was obtained by eroding the brain mask, ensuring it only contained white matter and CSF structures. Six tCompCor components were then calculated including only the top 5% variable voxels within that subcortical mask. For aCompCor, six components were calculated within the intersection of the subcortical mask and the union of CSF and WM masks calculated in T1w space, after their projection to the native space of each functional run. Framewise displacement[48] was calculated for each functional run using Nipype and volumes with a framewise displacement greater than 0.7 mm were excluded (SI 4).

Many internal operations of fMRIPrep use Nilearn [49], principally within the BOLD-processing workflow. For more details of the pipeline, see the section corresponding to workflows in the *fMRIPrep* documentation.

Behavioral statistical analyses

We compared the three groups (severe, moderate, mild) on the raw data for each neuropsychological, psychiatric, olfactory, fatigue, and dyspnea variable. Given the nonparametric distribution of the samples (as measured with Shapiro-Wilks tests), we used nonparametric Kruskal-Wallis tests. For significant ($p <$ .050) measures, Mann-Whitney *U* tests were performed for the 2 ´ 2 comparisons, with false discovery rate (FDR) corrections as function of each domain (cognition, psychiatry) and each Mann-Whitney pairwise comparison (mild vs. severe; mild vs. moderate; moderate vs. severe).

Neuroimaging statistical analysis

Structural MRI inspection. First, the neuroimaging data were visually analyzed to look for noticeable brain lesions such as microbleeds and WM damages. Groups (SI 6) were compared on the total number of microbleeds and impact on WM, with the Wahlund scale [50].

fMRI statistical analysis. The processed functional time courses were averaged into 156 regions of interest (100 cortical regions [51] that can be associated with 17 resting-state networks [52], 34 cerebellar regions [53] and 22 regions from the basal ganglia (BG) [54]), and the functional connectivity between pairs of regions was estimated by Pearson correlation. Measures of functional connectivity were converted into *z* scores with the Fisher *z* transformation and compared using two-sample *t* tests to investigate

differences between groups. The normality of functional connectivity measures was confirmed with Shapiro-Wilk tests and *p* values were FDR corrected for multiple comparisons [55].

Relationship between neuropsychological scores and brain connectivity

A partial least squares correlation (PLSC) approach was used to evaluate multivariate associations between neuroimaging and behavioural data [56]. This technique estimates latent components that consists out of linear combinations of brain functional connectivity and neuropsychological scores, respectively, to maximize their covariance across participants. The significance of the latent components was evaluated with permutation testing (1000 permutations), and the stability of the feature weights (called saliences) was assessed through bootstrapping (500 samples). Furthermore, we computed the imaging and behavioural loadings defined by the Pearson's correlation between the original neuropsychological and functional connectivity values, and their corresponding PLSC weights. Only neuropsychological and functional connectivity scores surviving the FDR correction in the intergroup comparison were considered in this analysis. The PLSC analyses were performed on 26 brain and 7 behavioural scores (including 2 covariates to account for the effects of age and sociocultural level) in the three groups (group PLS), and using the myPLS toolbox (https://github.com/danizoeller/myPLS). In post-hoc analyses, the Pearson's correlations between memory scores and the functional connectivity of specific brain-region pairs were compared across groups.

Data availability

At the end of the COVID-COG project, nonsensitive data will be made available in open access on a dedicated platform.

Results

Neuropsychological symptoms as a function of disease severity

The three groups differed significantly on i) memory encoding (RL/RI 16 - Immediate recall; $H = 17.34$, $p < .001$), ii) long-term episodic verbal memory (RL/RI 16 – Sum of 3 free recalls; $H = 9.39$, $p = .009$; Sum of 3 total recalls; $H = 6.42$, $p = .040$; Delayed free recall; $H = 11.10$, $p = .004$), iii) inhibition (Stroop Interference – Time; $H = 7.61$, $p = .022$), iv) mental flexibility (TMT B – Time; $H = 10.20$, $p = .006$; TMT B– Perseverations; $H = 13.07$, $p = .002$; TMT B-A– Time; $H = 9.96$, $p = .007$), v) logical reasoning (WAIS IV – Puzzle; $H = 6.72$, $p = .035$; WAIS IV – Matrix; $H = 6.47$, $p = .039$), and vi) emotion recognition (GERT – Emotion recognition task; $H = 8.46$, $p = .015$). None of the other effects were significant ($p > .05$ for all comparisons).

Memory encoding. Moderate patients scored significantly higher on the RL/RI 16 - Immediate recall than severe patients after FDR correction ($z = -2.43$, $p = .015$), but the other two pairwise comparisons were not

significant after FDR correction.

Long-term episodic verbal memory. Mild patients scored significantly higher on the RL/RI 16 – Sum of 3 free recalls than severe patients after FDR correction (z = -2.95, p = .003), but the other two pairwise comparisons were not significant after FDR correction. Mild patients scored significantly higher on the RL/RI 16 - Delayed free recall than severe patients after FDR correction (z = -3.26, p = .001), but the other two pairwise comparisons were not significant after FDR correction (see Fig. 1).

Mental flexibility. Mild patients performed the TMT B - Time significantly faster than moderate patients did (z = -2.70, p = .007), but the other two pairwise comparisons were not significant. Mild patients also performed the TMT B/A - Time significantly faster than moderate patients did (z = -2.62, p = .009), but the other two pairwise comparisons were not significant (see Fig. 1).

None of the other comparisons survived FDR correction (i.e., RL/RI 16 – Sum of 3 total recalls; Stroop Interference – Time; TMT B – Perseveration errors; WAIS IV – Puzzle and Matrix; GERT – Emotion recognition task).

For mean scores and standard deviations, as well as Kruskal-Wallis and Mann-Whitney U tests and p values, see SI 5.

Structural MRI results as a function of disease severity

No substantial structural damage could be observed. The intergroup structural analysis failed to reveal any significant differences between groups on WM lesions using the mean score on the Wahlund scale. Concerning microbleeds, a single patient had two microbleeds, 18 patients had one microbleed, and 25 had no microbleeds. A significantly higher proportion of mild (55%) patients had at least one microbleed, compared with the moderate (18.50%) and severe (12.50%) patients (see SI 6).

fMRI connectivity results as a function of disease severity

Severe versus mild. The connectivity analysis revealed three patterns of hypoconnectivity and two patterns of hyperconnectivity in severe versus mild patients. *Hypoconnectivity*: i) weaker connectivity was observed between a subregion of the right temporoparietal network (TempPar) and the following subregions and networks: two subregions of the left and two subregions of the right somatomotor network A ($SomMot_A$) along with the right inferior parietal sulcus in the control network A ($Cont_A$); ii) weaker connectivity between the right auditory cortex in the $SomMot_B$ network and the left postcentral sulcus in the dorsal attention network B ($DorsAttn_B$); and iii) weaker connectivity between the right parahippocampal gyrus in the default mode network C (DMN_C) and the right insula in the salience ventral attentional network A ($SalVentAttn_A$). *Hyperconnectivity*: higher connectivity was found i) between the right putamen and the right central sulcus of the $SomMot_B$, and ii) within the cerebellum between the left Lobule V and bilateral Crus I.

Moderate versus mild. The connectivity analysis revealed four patterns of hyperconnectivity in moderate versus mild patients: i) higher connectivity between the right inferior parietal lobule in the default mode network A (DMN_A) and the following subregions and networks: the bilateral postcentral cortex in the default mode network B (DMN_B), the left fastigial nuclei in the cerebellum, and the right Lobule VIIIa in the cerebellum; ii) higher connectivity between the left putamen in the BG and the left auditory cortex in the right SomMotB; iii) higher connectivity between the left Crus I in the cerebellum and the following subregions: the right Lobule VIIIa in the cerebellum and the right postcentral cortex in the default mode network B (DMN_B); and iv) higher connectivity between the right dorsolateral prefrontal cortex in the control network B ($Cont_B$) and the following subregions: right superior parietal lobule in the dorsal attention network A ($DorsAttn_A$) and the right precuneus in the control network C ($Cont_C$).

Severe versus moderate. The connectivity analysis revealed three patterns of hypoconnectivity in severe versus moderate patients: i) weaker connectivity between the right frontal eye field area in the $DorsAttn_B$ network and the following subregions: the left extrastriate cortex in the visuocentral network (VisCent), the left superior extrastriate cortex in the visuoperipheral network (VisPeri) and bilateral parietal occipital cortices in the $DorsAttn_A$; ii) weaker connectivity between the right postcentral sulcus in the $DorsAttn_B$ network and the right temporo-occipital cortex in the $DorsAttn_A$ network; and iii) weaker connectivity between the right frontal medial sulcus in the $SalVentAttn_A$ network and the bilateral parietal operculum (ParOper) in the $SalVentAttn_A$ network.

Associations between neuropsychological scores and fMRI connectivity as function of disease severity (Fig. 4 and Fig. 5)

The PLSC analyses extracted one significant component ($p < 0.001$) explaining 41.96% of the covariance between functional connectivity and neuropsychological data (Fig. 4). The contributions (PLSC loadings) of the different neuropsychological scores and functional connectivity values to the multivariate correlation patterns are showed in Fig. 4 and SI 8. In mild and severe patients, better memory performances (sum of free recall and delayed free recall at 20 min) were associated with a pattern of increased connectivity including bilateral SomMotA & B, bilateral DorsAttnB, DefaultA, right TempPar and cerebellar networks. This pattern was also associated with younger age in the mild group, higher immediate recall score and socio-cultural level in the severe group as well as older age and lower socio-cultural level in the moderate group. Interestingly, and despite showing poor stability through the bootstrap process, the functional connectivity pattern was associated with poorer memory performances in the moderate patients suggesting opposite relationships than the one of the mild and severe groups. In post-hoc analyses, the Pearson's correlations between individual connections and the four behavioural scores revealed positive correlations in the mild and severe groups but not in the moderate group (see Fig. 5).

Discussion

In the present study at 6–9 months post-SARS-CoV-2 infection, behavioral results revealed reduced performance on episodic verbal memory in patients with a severe presentation of COVID-19, compared with mild and moderate ones, as well as reduced performance on mental flexibility in moderate compared to mild patients. Neuroimaging results confirmed nonstructural alterations of the brain in patients with post-COVID-19 condition[28,30] but revealed, for the first time, the presence of long-term patterns of hypo- and hyperconnectivity associated with the severity of respiratory symptoms in the acute phase. In detail, when patients with severe disease were compared with mild ones, three patterns of hypoconnectivity were revealed involving subregions of the right TempPar, left SomMotA, bilateral SomMotB, left DorsAttnB, right ContB, right DMNC, and right SalVentAttnA networks. Moreover, two patterns of hyperconnectivity were revealed involving subregions of the BG, right SomMotB network, and cerebellum. The comparison between patients with severe disease and moderate ones showed three patterns of hypoconnectivity involving subregions of the right DorsAttnB, left VisCent, left VisPer, DorsAttnA, and bilateral SalVentAttnA networks. When moderate patients were compared with mild ones, three patterns of hyperconnectivity were revealed, involving subregions of the right DMNA, bilateral DMNB, right DorsAttenA, right SomMotB, BG, and cerebellum. Finally, the multivariate PLSC approach combining behavioural and neuroimaging data revealed significant relationships between neuropsychological deficits and functional brain connectivity as a function of the disease severity. Further analysis showed similar patterns of multivariate correlation between the functional connectivity and behavioural scores in the mild and severe patients' subgroups, while opposite association could be made for the moderate group.

First of all, our results support previous reports of cognitive deficits in the absence of structural brain lesions in COVID-19 [57–60]. They also suggest that the severity of the initial impairment is a risk factor for the development of long-term neuropsychological consequences; this could be to a probable post-ICU/mechanical ventilation effect. The poorer performance for episodic verbal memory displayed by patients with severe disease, compared with mild and moderate patients, partially corroborate the findings of Almeria, et al. [18], who observed reduced neuropsychological performance in ICU patients. Moreover, neuroimaging results revealed patterns of hypo- and hyper-connectivity in severe patients when compared with both mild and moderate ones. Finally, the PLSC approach revealed stable associations, for severe patients, between episodic verbal memory and the right Dorsal Attentional networks, consistently with neuroimaging studies of episodic memory in healthy individuals, see [61,62], while Pearson's correlations suggested that the lower connectivity in these networks, the lower the memory performance were. Our results also question whether these cognitive effects are solely due to ICU/mechanical ventilation, and perhaps suggest a potential direct or indirect effect of a SARS-CoV-2 infection on long-term neuropsychological consequences. Although the moderate patients were not admitted in ICU and did not undergo mechanical ventilation, they still showed reduced cognitive performance, with reduced mental flexibility in comparison to the mild patients. This corroborates previous observations by Alemanno, et al. [5], who observed significantly reduced executive scores in patients who received other types of oxygen therapy than mechanical ventilation. Moreover, our neuroimaging results revealed three patterns of hyperconnectivity in moderate patients when compared

with mild ones. Finally, and as far as they were concerned, the relationships between behavioral results and brain networks revealed by the PLSC analysis were of opposite sign in the moderate patients when compared with the one of the mild and severe groups. Indeed, in the moderate group, higher measures of functional connectivity were associated with poorer scores in episodic verbal memory and older age. Interestingly, when coherent relationship between selected connection and behavioral scores were obtained for the mild and severe patients, no correlations could be observed in moderate group. In other words, these latter results pointed to a lack of association between neuropsychological score and connectivity patterns in the selected subregions for the moderate patients. From our point of view, this second pool of results, underlying a specific pattern specifically displayed by the moderate group, suggests that the neuropsychological post-COVID-19 syndrome cannot be solely attributed to a post-ICU syndrome.

An interesting hypothesis that could encompasses the results obtained with the three groups could be a potential alteration of local and global connectivity following a neurological disturbance, in this case, SARS-CoV-2 infection. Recent studies in acquired neurological (e.g., cranio-cerebral trauma), neuro-immunological (e.g., multiple sclerosis) or neurodegenerative (e.g., mild cognitive impairment or Alzheimer's disease) pathologies have highlighted patterns of both higher and lower connectivity (for a review, see[63]). Authors have suggested that hyperconnectivity is a common response following a neurological disruption, but the subsequent depletion of neural resources leads to a rapid decrease in connectivity [63]. The presence of compensatory mechanisms inducing patterns of higher connectivity in the short-term following SARS-CoV-2 infection presumably reaches a threshold of cognitive resource availability in the medium term, and eventually leads to a decrease in connectivity and the emergence of hypoconnectivity patterns. This hypothesis is consistent with our results as moderate patients showed greater connectivity than mild patients, while severe patients had lower connectivity in cortical structures, and greater connectivity in subcortical structures (putamen and cerebellum). Severe symptoms in the acute phase may induce a stronger and earlier compensatory response in the cortical networks in the short term and lead to the patterns of hypoconnectivity observed at 6–9 months post-infection, while the subcortical networks, including the BG and cerebellum, may continue to have a compensatory effect. Similarly, patients who had a moderate form in the acute phase may still be in a compensatory mode, thus explaining the increased connectivity compared with mild patients at 6–9 months post-infection. This hypothesis is emphasized by the lack of association between neuropsychological score and functional connectivity in moderate patients, suggesting that networks are engaged in different processes. Another hypothesis could be a potential alteration of local and global connectivity following a traumatic event in this case, SARS-CoV-2 infection that could enhance the effects of SARS-CoV-2. Indeed, dysconnectivity is known to be associated with PTSD and consists on hyperactivity and hyperconnectivity of the salience network which has nodes in the insula, dorsal anterior cingulate cortex, and possibly the amygdala.

Our study has several limitations. By enrolling volunteers, we may have selected the most severe cases, although a significant proportion of our sample did not report any complaints, as confirmed by the very

low mean score on the self-report QPC. This study was only performed on patients who were infected with SARS-CoV-2, and these patients had no known clinical history, posing two limitations for generalization of results. Here, we did not include a control group because the number of neuropsychological and neuroimaging data was too small for the comparison to be relevant. Moreover, with the high rates of infection, it has become more difficult to recruit subjects that have never been infected with SARS-CoV-2. Our moderate and severe groups are potentially not representative of the population of hospitalized SARS-CoV-2 patients because of their lack of comorbidities. It is important to highlight the considerable variance observed in the moderate group, as this could explain the small number of significant differences between groups. The cognitive and psychiatric, as well as functional connectivity (as described above) of the moderate group were extremely heterogeneous, suggesting that some patients had major deficits and others none, leading to nonsignificant results. The statistical comparison of behavioral data and functional connectivity revealed an imbalance between the groups and the small number of severe participants who underwent MRI may limit the generalization of this group's neuroimaging data. The acquisition of field maps was not part of the MRI protocol and correction for susceptibility distortion was not performed. Finally, the generalizability of PLS methods has been criticized and, while results stay informative about multivariate correlations within the data, the correlations from PLSC should be validated with techniques such as cross-validation.

Conclusion

Our study confirms the presence of long-term neuropsychological effects in patients who had moderate-to-severe symptoms in the acute phase of COVID-19. For the first time, nonstructural alterations of the brain (functional connectivity) were observed in patients with post-COVID-19 syndrome, who had had moderate-to-severe forms of the infection in the acute phase. These alterations were partially associated with the neuropsychological deficits we assessed. Finally, the observed neuropsychological post-COVID syndrome does not solely depend on the severity of the infection in the acute phase.

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Figures

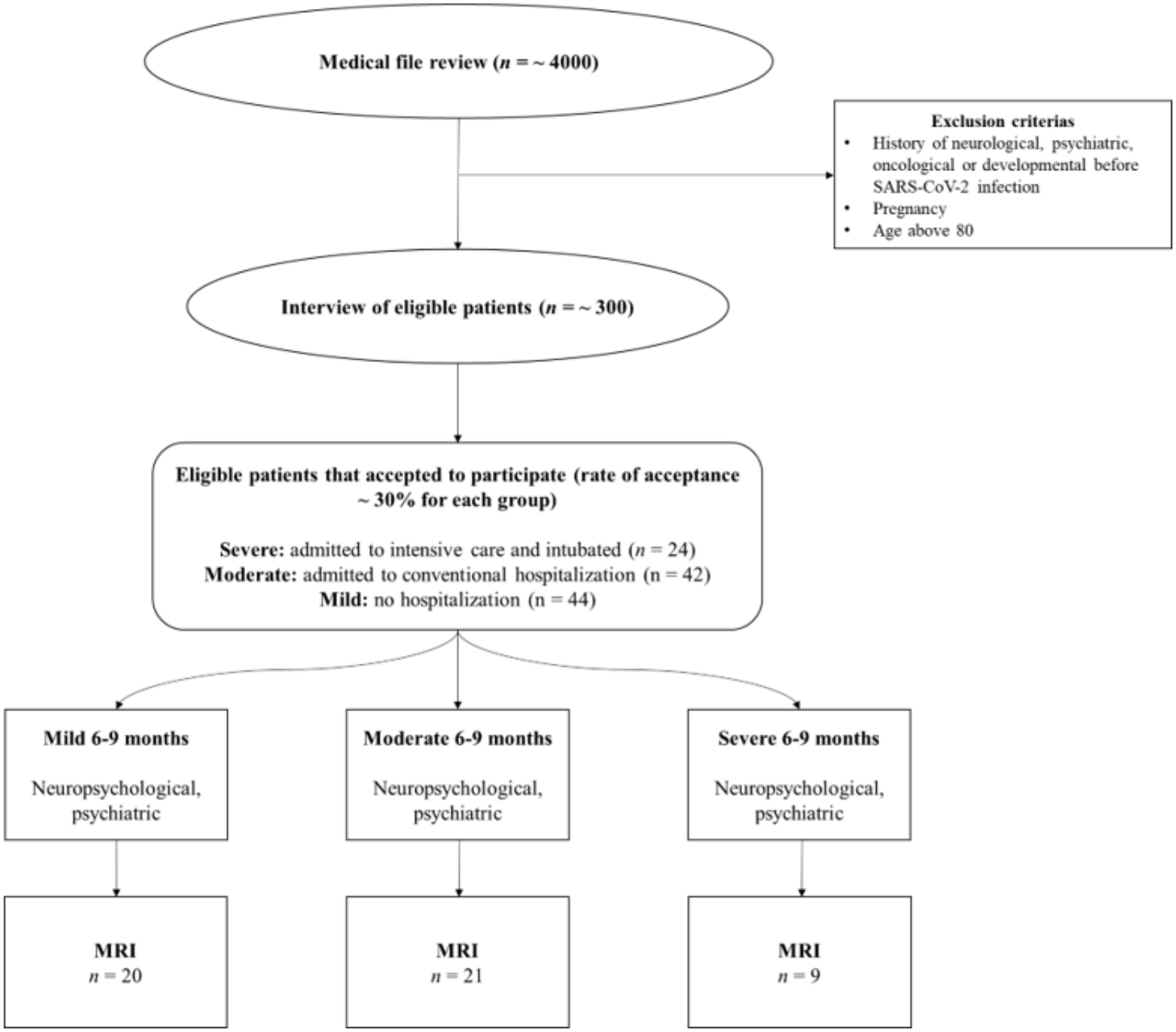

Figure 1

Flowchart of the study

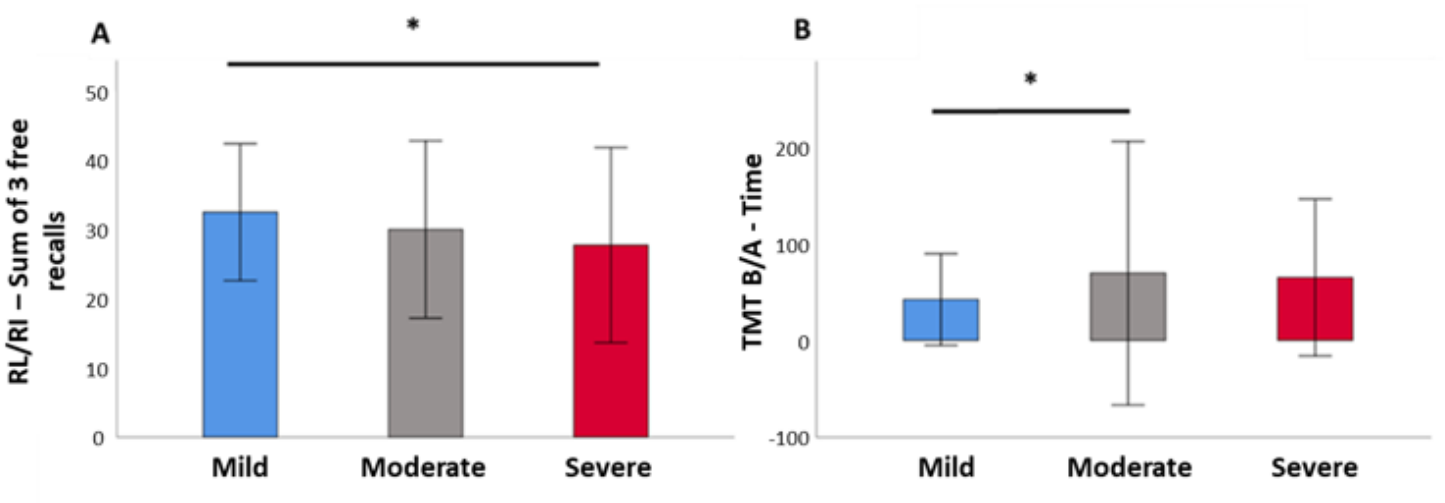

Figure 2

Intergroup comparisons for neuropsychological testing (after FDR correction). A. Severe patients performed significantly more poorly than mild patients on the RL/RI 16 – Sum of 3 free recalls. B. Moderate patients had significantly higher interference scores than mild patients on the TMT B/A - Time.

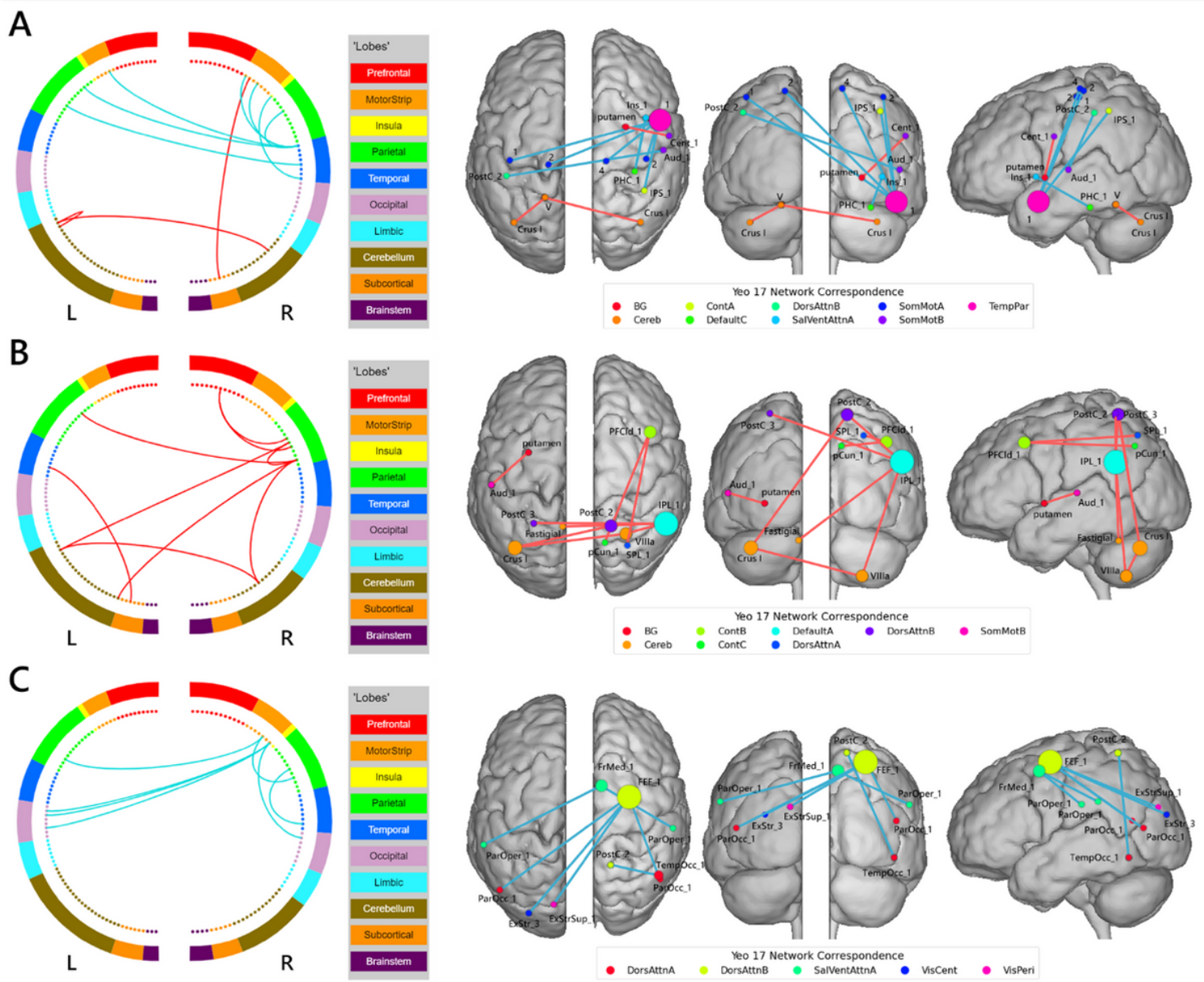

Figure 3

Patterns of significantly different functional connectivity in the intergroup comparison. Differences in functional connectivity between brain structures shown schematically (left) and in a network representation on a glass brain (right) when comparing mild versus severe (A), mild versus moderate (B) and moderate versus severe (C). Blue lines indicate a decrease in the connectivity measurement (mean decrease = -0.3), red lines indicate an increase in the connectivity measurement (mean increase = 0.3), and node size corresponds to the number of connections. Statistical significance was FDR-corrected for multiple comparisons ($p < 0.05$ FDR). **Networks:** BG: basal ganglia; Cereb: cerebellum; ContA: control A; DefaultA and DefaultC: default mode A and C; DorsAttnA and DorsAttnB: dorsal attention A and B; SalVentAttA: salience ventral attention A; SomMotA and SomMotB: somatosensory motor A and B; TemPar: temporoparietal; VisCent: visual central; VisPeri: visual peripheral. **Regions:** Aud: auditory cortex; Cent: central sulcus; ExStr: extrastriate cortex; ExStrSup: superior extrastriate cortex; FEF: frontal eye field

area; FrMed: frontal medial sulcus; INS: insula; IPL: inferior parietal lobule; IPS: inferior parietal sulcus; ParOcc: parietal occipital cortex; ParOper: parietal operculum; pCun: precuneus; PFCld: dorsolateral prefrontal cortex; PHC: parahippocampal gyrus; PostC: postcentral region; SPL: superior parietal lobule; TempOcc: temporo-occipital cortex. Figures were created with BioImage Suite (https://bioimagesuiteweb.github.io/webapp/index.html).

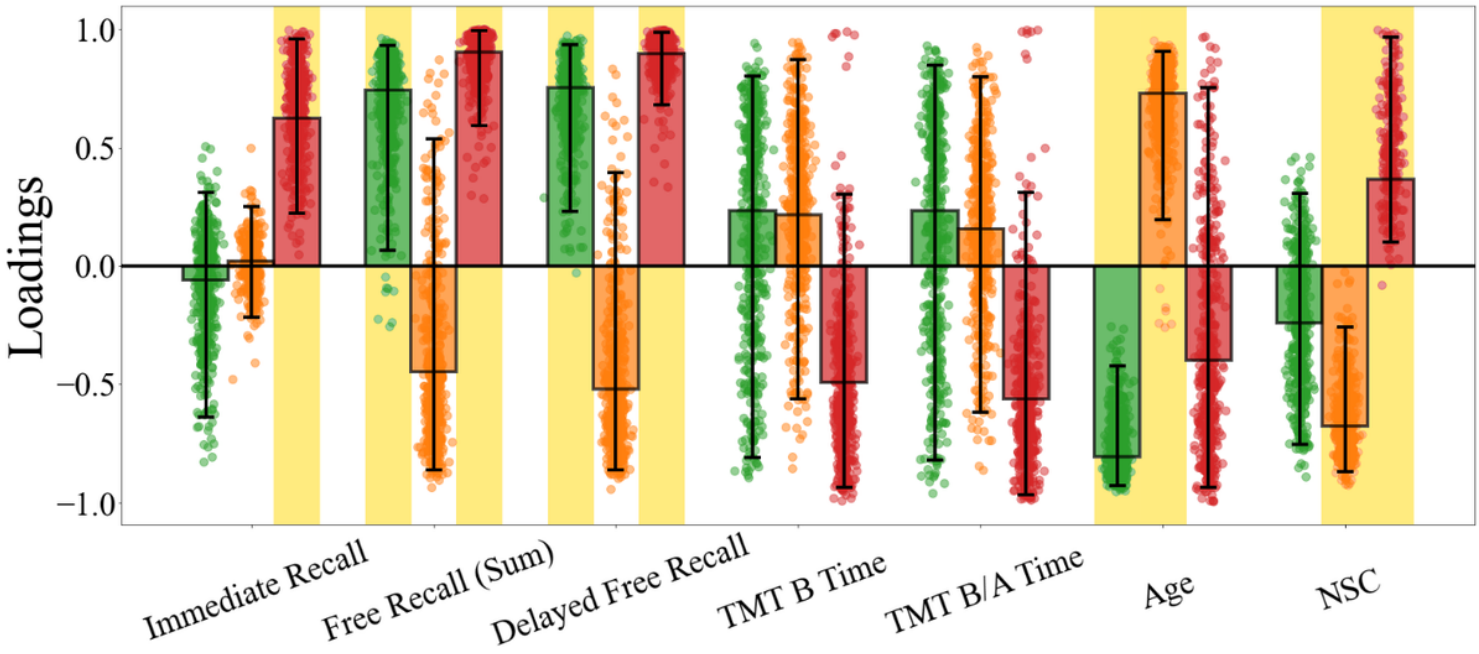

Figure 4

PLSC results, neuropsychological loadings of latent component 1 (p < 0.001, 41.96% covariance) for the mild (green), moderate (orange) and severe (red) groups. A large positive (or negative) weight indicates a larger contribution of the specific feature to the multivariate correlation pattern. Yellow highlights indicate weights significantly different from zero, dots show the bootstrap samples and error bars indicate the 95% confidence interval.

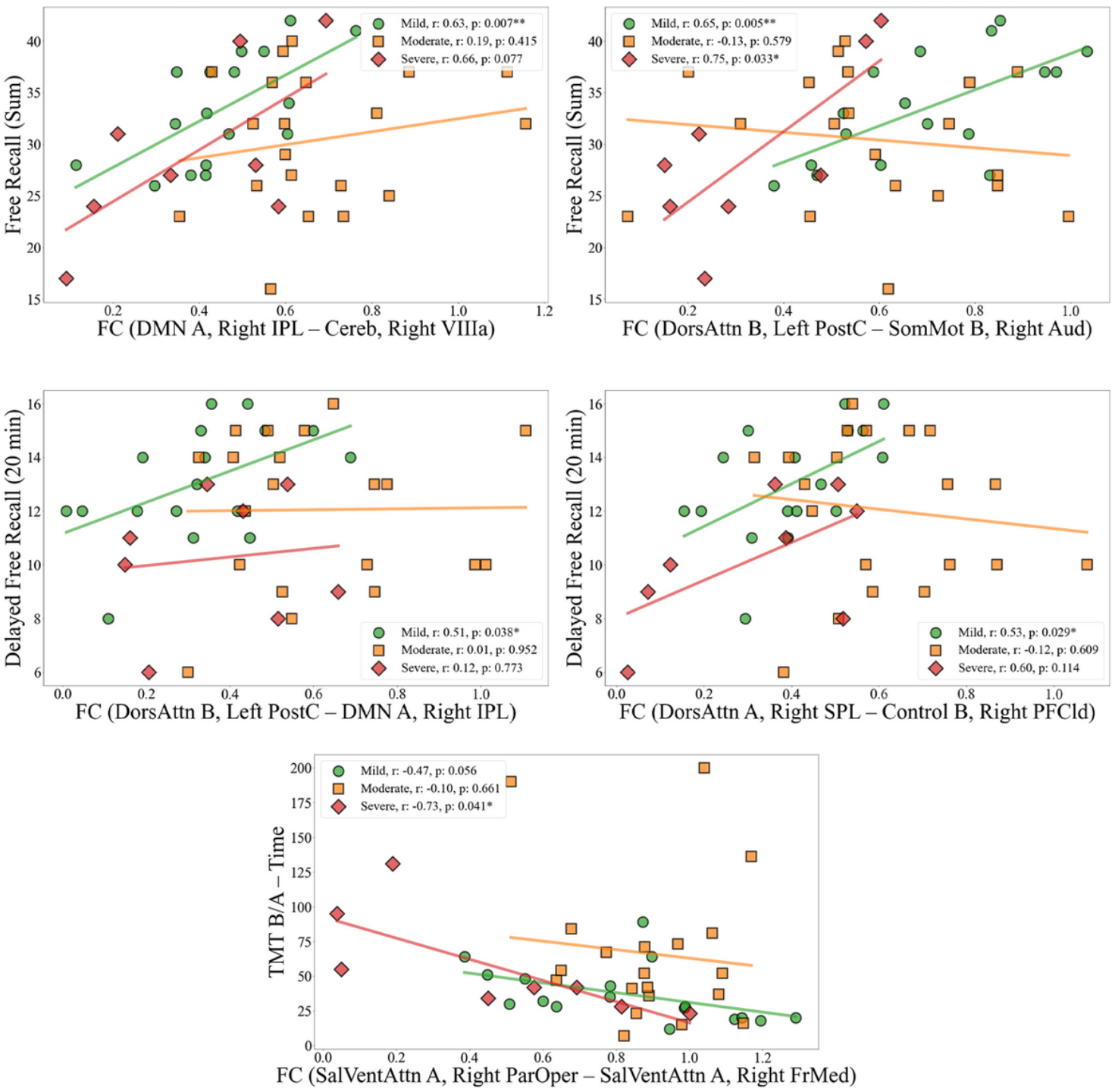

Figure 5

Correlations between functional connectivity and behavioral scores as a function of severity. Each symbol represents a subject. Lines represent the least square regression fit to the data for each group, with green, yellow and red corresponding to mild, moderate and severe groups, respectively. Pearson's *r* and p-values are shown in the legend.

* $p < 0.05$, ** $p < 0.01$

Supplementary Files

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