FUNCTIONAL CONNECTIVITY IN THE BRAIN AT REST & COGNITIVE RESERVE IN THREE DIFFERENT PHENOTYPES OF MULTIPLE SCLEROSIS

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Introduction

The concept of "Cognitive Reserve" (CR) postulates the existence of functional brain mechanisms that are able to cope with cerebral damage (1). These mechanisms are believed to rely on pre-existing cognitive processes, or to enlist compensatory mechanisms. CR indicators include premorbid intelligence, formal education, lifetime experiences and current recreational activities. The CR is particularly evident in those clinical conditions which result in a progressive accumulation of cognitive disabilities (2). Cognitive functioning is frequently impaired in multiple sclerosis (MS) (3), but some patients are able to withstand considerable disease burden (e.g. white matter lesions, cerebral atrophy) without cognitive impairment. Previous studies (4) have focused on identifying potentially adaptive functional reorganization through recruitment of new brain regions that could limit expression of these deficits. Functional Magnetic Resonance Imaging (fMRI) can be used *in vivo* to detect synchronous fluctuations in blood oxygenation level-dependent (BOLD) signal across the brain at rest. This technique, which is known as resting state fMRI, is traditionally used to investigate functional connectivity (FC), which represents the synchronization of neural activity between anatomically separated brain regions (usually considering low-frequency fluctuations, ranging between 0,01 and 0,1 Hz). Synchronous low-fluctuations in BOLD signal, within different regions, across the brain at rest, have been previously detected, and defined as Resting State Networks (RSNs). Each of these RSN reflects specific cognitive, motor or sensory processes. One hypothesis suggests that the neuronal mechanisms underpinning CR results on the ability of individuals with high CR to recruit alternative brain areas or networks to preserve specific functions in the presence of structural damage. Abnormal connectivity within specific RSNs can provide useful information on the patho-physiological events underlying several neurological disorders.

Aims

Aim of this study was to investigate the association of functional connectivity (FC), measured with Resting-state (RS) fMRI, and levels of CR in three different MS phenotypes, namely Secondary Progressive (SP), Primary Progressive (PP), and Relapsing-Remitting (RR) MS. More in detail, we investigated FC within two specific networks, namely the sensory-motor network (SMN), and the default mode (DMN), as these sub-serve sensory-motor and cognitive functions, that reflect peculiar clinical features in MS.

Method

Subjects We recruited 48 patients with clinically definite MS [M/F ratio=20/28; mean (SD) age=49.26 (7.92) years, mean (SD) years of formal education=12.25 (1.93)] according to McDonalds criteria (5). 18 had a SP phenotype, 12 a PP and 18 a RR phenotype. Before MRI, all patients underwent an extensive clinical and neuropsychological examination, and fulfilled the Cognitive Reserve test. The Expanded Disability Status Scale (EDSS), the Timed 25-Foot Walk, and the 9- Hole Peg Test were administered to all patients. During MRI acquisition, subjects were instructed to keep their eyes closed, not to think of anything in particular, and not to fall asleep. All recruited patients had to be right-handed, to reduce any potential source of variability due to hemispheric dominance. See Table1 for details.

Neuropsychological assessment Two trained neuropsychologists explored the following cognitive domains: 1) Working memory and cognitive flexibility, using the Paced Auditory Serial Addition Test at 3 seconds (PASAT); 2) Information processing speed, through the administration of the Symbol Digit Modalities Test (SDMT); 3) Praxis and visuo-spatial memory abilities, assessed through the Rey Complex Figure Test–immediate (RCFC) and the 20-minutes delayed reproduction (RCFC-Recall); 4) Verbal fluency, assessed through the Word List Generation (WLG), which detects number of patients' appropriate answers, intrusions and perseveration errors. See Table1 for scoring on each measure.

Cognitive Reserve Test Scoring To evaluate the impact of CR on clinical manifestation of MS and to measure its eventual association with FC, we calculated a mean Total Score of CR and three sub-scales' mean scores measuring cognitive– (CLA), physical– (PLA), and social– (SLA) Leisure Activities (LA). Years of formal education were also recorded. See Figure2 , for scores across the three MS groups.

MRI acquisition protocol included: 1) dual-echo turbo spin echo [TSE] (TR = 6190 ms, TE=12/109 ms, matrix=256x192, FOV=230x172.5, 48 contiguous slices, slice thickness=3 mm, total scan time=4 minutes); 2) fast-FLAIR (TR = 8170 ms, TE = 96 ms); and 3) T2*-weighted EPI sensitized to BOLD contrast (TR=2080 ms, TE=30 ms, 32 axial slices parallel to AC-PC line, matrix=64x64, pixel size =3x3 mm 2, slice thickness=2.5 mm, flip angle=70°) for resting state fMRI (7-min and 20-sec period for 220 volumes).

Lesion volume assessment In each patient, T2-hyperintense lesions were identified by consensus of two independent observers on the short echo images of the TSE. Lesions were outlined on the same scan using a semi-automated local thresholding contouring technique (Jim 4.0, Xinapse System, Leicester, UK, http://www.xinapse.com/). FLAIR and long echo TSE scans were always used as a reference to increase the confidence in lesion identification. The total lesion volume (T2LL) was calculated for each patient. See Table1 for details about T2LL in each of the three MS groups.

RS-fMRI data analysis Functional data pre-processing was performed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm), after discarding the first 4 volumes. Images were realigned, corrected for slice-time, normalized into Montreal Neurological Institute (MNI) space, and smoothed with a 8mm3 Gaussian kernel. Finally, all images were filtered by a phase-insensitive band-pass filter (pass band 0.01-0.08 Hz) to reduce the effect of low frequency drift and high frequency physiological noise. A model-free analysis was employed by using independent component analysis (ICA) implemented in the GIFT package, in order to allow for a simultaneous separation into individual components. See Figure1, for the selected networks. Mean FC for each node of the selected network (i.e., SMN and DMN) was extracted, and correlations with clinical/neuropsychological scores and CR levels were performed, using SPSS statistical package. Group analyses of the SMN and DMN were performed by full-factorial models in SPM8, including age, years of formal education and GM fraction (normalized by intra-cranial-volume) as covariates of no interest. For each network analysis, F-contrasts masked by the main effect of groups (p cluster-level uncorr.=0.005) were performed, and the threshold for statistical significance was set at p values cluster level uncorr.<0.005. Between group changes were assessed by plotting the maxima of BOLD signal intensity in those clusters surviving the statistical threshold.

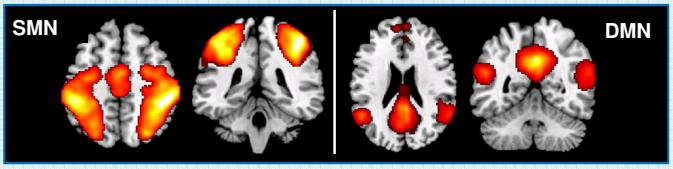

Figure1. Selected RS-fMRI networks. The sensory-motor (SMN) and the default mode network (DMN) have been studied as these sub-serve sensory-motor and cognitive functions, that reflect peculiar clinical features in MS.

Results

Table1. Demographic, clinical and neuropsychological characteristics of MS sub-groups.

Abbreviations: GM= grey matter, T2LL = T2 lesion load, EDSS= expanded disability status scale, T25-FW = Timed 25-Foot Walk, 9-HPT= 9-Hole Peg Test, PASAT-3= Paced Auditory Serial Addition Test, SDMT= Symbol Digit Modalities Test, RCFC = Copy of Complex Rey's Figure, RCFC-Recall 20 min. = Complex Rey's Figure Test–delayed, WLG= Word List Generation, SD= standard deviation. P values for One way Anova are reported, even when almost statistically significant.
****Level of significance** refers to Chi Square non-parametric test, * refers to p-value significance level for PP and SPMS patients, § refers to p-value significance level for PP and RRMS patients, ° refers to p-value significance level for SP and RRMS patients.

	SPMS N=18 (7M/11F)	PPMS N=12 (5M/7F)	RRMS N=18 (8M/10F)	p value
Age (SD) [years]	45.78(8.16)	58.33(9.05)	43.67(4.52)	<0.00*§
Years of education (SD) [years]	13.92(2.78)	10.13(2.57)	12.72(3.64)	<0.02*§
T2LL (SD) mL	14.54(10.16)	27.34(22.44)	13.76(22.78)	<0.07*§
GM fraction (SD)	0.447(0.018)	0.445(0.013)	0.450(0.014)	N.S.
Median EDSS score (range)**	6.0(3-7)	5.5(4.5-6.0)	2.0(0-6.5)	<0.001§ °
Disease duration Mean (SD) [years]	14.73(8.80)	11.30(6.06)	13.58(7.48)	N.S.
T25-FW, trial 1 Mean (SD) [minutes]	22.23(11.32)	12.08(7.05)	13.77(12.44)	0.07*
T25-FW, trial 2 Mean (SD) [minutes]	23.10(12.75)	11.76(6.39)	15.35(14.46)	0.07*
9- HPT dominant	28.39(6.87)	27.13(6.78)	21.73(3.67)	<0.019°§
9-HPT not dominant	28.63(5.13)	29.14(8.61)	22.79(2.93)	<0.03°§
PASAT-3 Mean (SD)	45.25(10.07)	57.37(14.80)	48.86(7.99)	0.01* 0.08§
SDMT Mean (SD)	46.20(14.21)	44.90(11.57)	47.44(11.77)	N.S.
RCFC Mean (SD)	33.88(2.99)	35.29(1.89)	32.92(7.31)	N.S.
RCFC -Recall Mean (SD)	17.63(8.71)	12.83(7.75)	19.31(10.30)	N.S.
WLG appropriate answers	38.00(10.84)	33.33(11.83)	35.00(8.68)	N.S.
WLG intrusions	0.20(0.44)	1.00(1.72)	0.00(0.00)	N.S.
WLG perseverations	1.80(1.64)	0.20(0.10)	0.50(0.83)	0.05*

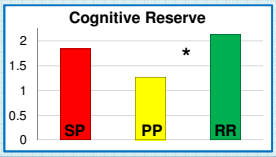

Figure2. Mean CR total scores across the 3 groups. * PP show lower CR, than RRMS, p<0.05.

Differences in clinical/neuropsychological and CR measures between MS groups are reported in Table1 and Figure2. Specific significant correlations were found within each MS group, between **CR scores and clinical/neuropsychological scores** (i.e., in the PPMS, the EDSS score positively correlated with Physical–LA and total CR score; also, a significant positive association was observed in both SP and PPMS, between RCFC and total CR score). Among the 20 components estimated by ICA, ten RSNs, already reported by others were identified (6). We examined **FC' differences across the three MS groups**, within each selected network. In the **SMN**, significant increased FC was found in SP, as compared to RRMS patients, in fronto-parieto-occipital regions. Further, PP vs SPMS/RRMS showed higher FC in sensory-motor areas, and RR vs SPMS revealed higher FC in the left Opercular cortex/Insula. When considering the **DMN**, SP showed increases in FC in frontal and occipital areas, vs RR and PPMS. Finally, PPMS revealed higher FC in frontal regions, as compared to RRMS patients. See Figure3, for FC differences in the MS groups.

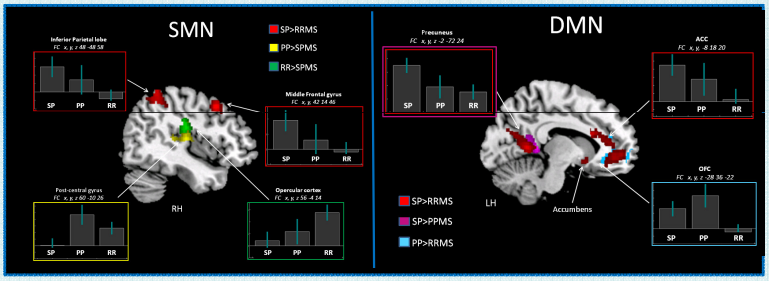

Figure3. Significant differences across the three MS phenotypes within the SMN (left panel) **and the DMN** (right panel). Plots show the BOLD signal changes in the selected blobs (p-corr.<0.005). **Abbreviations**: FC= functional connectivity; ACC= anterior cingulate cortex; OFC= orbito-frontal cortex; RH= right hemisphere; LH= left hemisphere.

Correlation analyses between FC in the SMN and DMN and CR scores have been performed (Pearson test, p<0.05). Within the **SMN**, only in the RRMS group, a significant inverse association was found between FC in almost all clusters of this network and CLA, PLA, SLA and total CR score; within the SPMS group, a significant positive association was observed between FC in the left middle Frontal Gyrus (midFG)/ACC and SLA score; and, in the PPMS group, significant inverse correlation was detected between FC in the left midFG, the insula and the CLA score. Within the **DMN**, significant inverse associations were observed in both SP and RRMS patients between total CR score and, respectively, FC in the left precuneus/PCC, and in the left ACC/Accumbens and Frontal pole/OFC.

Discussion & Conclusion

Considering CR differences across the 3 MS groups, only PP showed a significantly lower score, as compared against RRMS patients. Further, specific associations between CR and clinical/neuropsychological measures were detected within each of the 3 phenotypes. When considering FC, in line with previous literature (7), increased FC was found in SP and PP, vs RRMS patients, in fronto-parieto-occipital areas within the SMN; while, within the DMN (traditionally involved in cognition), SP, more than PP, showed increases in FC in fronto-posterior areas, when compared against RRMS. Further, lower levels of CR were associated with an iper-engagment of FC in fronto-parietal areas (in both RSNs), in the RRMS group. As well, SPMS patients showed an inverse association between levels of CR and FC in more posterior regions (i.e., precuneus/PCC), within the DMN. These findings overlap, and further explain, our RS-fMRI results, suggesting the existence of a neuronal compensation mechanism (within specific RSNs), to compensate for cognitive deficits and lower CR, in specific MS phenotypes. **Conclusions** This study suggests that the combination of CR and FC measures might help to explain, and maybe predict, MS progression, also considering potential compensation mechanisms.

References

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