Journal of Neurology, Neurosurgery & Psychiatry

rTMS affects working memory performance, brain activation and functional connectivity in multiple sclerosis patients

Journal:	*Journal of Neurology, Neurosurgery, and Psychiatry*
Manuscript ID	jnnp-2016-314224.R1
Article Type:	Research paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Hulst, Hanneke; VU University Medical Center, Department of Anatomy and Neurosciences Goldschmidt, Thomas; VU University Medical Center, Department of Anatomy and Neurosciences Nitsche, Michael; Leibniz Research Center for Working Environment and Human Factors; University Medical Hospital Bergmannsheil, Department of Neurology de Wit , Stella ; VU University Medical Center , Department of Psychiatry van den Heuvel, Odile; VU University Medical Center, Department of Psychiatry Barkhof, Frederik; VU Medical Center, MS Center, Department of Radiology and Nuclear Medicine Paulus, W; University of Gottingen, Department of Clinical Neurophysiology van der Werf, Ysbrand; VU University Medical Center, Department of Anatomy and Neurosciences Geurts, Jeroen; VU University Medical Center, Department of Anatomy and Neurosciences
Keywords:	
Specialty:	

rTMS affects working memory performance, brain activation and functional connectivity in multiple sclerosis patients

Hulst HE (PhD)[1*], Goldschmidt T (MD)[1*], Nitsche MA (MD)[2], de Wit SJ (MD)[3], van den Heuvel OA (MD, PhD)[1,3], Barkhof F (MD, PhD)[4], Paulus W (MD)[5], van der Werf YD (PhD)[1], Geurts JJG (PhD)[1]

[1] Department of Anatomy and Neurosciences, section of Clinical Neuroscience, VUmc MS center Amsterdam, Amsterdam Neuroscience, VU University Medical Center, Amsterdam, The Netherlands
[2] Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany & University Medical Hospital Bergmannsheil, dept. Neurology, Bochum, Germany
[3] Department of Psychiatry, VUmc MS center Amsterdam, Amsterdam Neuroscience, VU University Medical Center, Amsterdam, The Netherlands
[4] Department of Radiology and Nuclear Medicine, VUmc MS center Amsterdam, Amsterdam Neuroscience, VU University Medical Center, Amsterdam, The Netherlands
[5] Department of Clinical Neurophysiology, University of Göttingen, Germany,

*both authors contributed equally to this work

Correspondence to: Hanneke Hulst
VU University Medical Center
Department of Anatomy and Neurosciences
De Boelelaan 1108, 13E01 (O2 gebouw)
1081 BT Amsterdam, The Netherlands
Telephone: +31 20 4445335
Email: he.hulst@vumc.nl

Character count title: 103
Word count abstract: 249
Word count article: 3389
Number of references: 35
Number of figures: 3
Number of tables: 3

Key words: 1) Multiple Sclerosis; 2) repetitive transcranial magnetic stimulation; 3) working memory; 4) functional MRI; 5) connectivity

ABSTRACT

Objective: To investigate the effects of high-frequency repetitive transcranial magnetic stimulation (rTMS) of the right dorsolateral prefrontal cortex (DLPFC) on working memory performance, while measuring task-related brain activation and task-related brain connectivity in patients with multiple sclerosis (MS).

Methods: Seventeen MS patients and 11 healthy controls (HCs) underwent three experimental sessions (baseline, real-rTMS, sham-rTMS), all including an N-back task (3 task loads: N1, N2, N3; control condition: N0) inside the MR-scanner. Prior to imaging, real-rTMS (10Hz) was applied to the right DLPFC. The stimulation site was defined based on individually assessed N-back task activation at baseline and located using neuro-navigation. Changes in whole brain functional activation and functional connectivity with the right DLPFC were calculated.

Results: N-back task accuracy (N2 and N3) improved after real-rTMS (and not after sham-rTMS) compared to baseline (P=0.029 and P=0.015 respectively), only in patients. At baseline, MS patients, compared to HCs, showed higher task-related frontal activation (left DLPFC, N2>N0), which disappeared after real-rTMS. Task-related (N1>N0) functional connectivity between the right DLPFC and the right caudate nucleus and bilateral (para)cingulate gyrus increased in patients after real-rTMS when compared to sham stimulation.

Conclusions: In MS patients, N-back accuracy improved while frontal hyperactivation (seen at baseline relative to HCs) disappeared after real-rTMS. Together with the changes in functional connectivity after real-rTMS in patients, these findings may represent an rTMS-induced change in network efficiency in MS patients, shifting patients' brain function towards the healthy situation. This implicates a potentially relevant role for rTMS in cognitive rehabilitation in MS.

INTRODUCTION

Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system. Cognitive deficits are frequently present, affecting up to 70% of all patients[1]. These cognitive symptoms can influence the patients' life significantly, varying from minor impairments in daily living to social isolation and unemployment. Unfortunately, there are currently no treatment options available to tackle these cognitive deficits in MS.

More specifically, problems with working memory are frequent[1,3] and are more prominent in patients with a high frontal lesion load[4,5]. Functional MRI (fMRI) studies on working memory performance show frontal hyperactivation[6-9] and increased frontal inter-hemispheric connectivity[10] in cognitively non-impaired MS patients compared to healthy controls (HC). These changes likely represent functional brain reorganization, a process that is thought to underlie maintained cognitive functioning.

Using high-frequency (≥ 5Hz) repetitive transcranial magnetic stimulation (rTMS), the excitability of a particular cortical region and its connected brain regions can be enhanced[11,12]. In subjects with depression, high-frequency rTMS of the dorsolateral prefrontal cortex (DLPFC) improves working memory performance[13-15] which could not be attributed to mood improvement[13]. The advantageous effect of rTMS of the DLPFC on working memory performance makes this area particularly of interest as stimulation site for patients with MS.

The aim of the current study was to investigate the effects of a single session of high-frequency rTMS in MS patients on working memory performance, task-related brain activation (fMRI) and task-related connectivity. We expected to find improved task

performance and changes in local brain activation and connectivity of the stimulated area with other task-relevant areas in the brain after high-frequency rTMS of the right DLPFC.

METHODS

Participants and experimental design

The study protocol was approved by the ethics review board of the VU University Medical Center in Amsterdam. All subjects gave written informed consent prior to participation. All patients were diagnosed with clinically definite MS[16] and had sufficient visual acuity and upper limb motor function to perform the N-back task. Patients and HCs were matched for age, sex and education level. All participants underwent three experimental sessions (baseline, real-rTMS, sham-rTMS) in a randomized single-blind sham-controlled cross-over design (Figure 1).

Extra safety measures were taken with regard to epileptic seizures, a possible adverse event of high frequency rTMS. In MS, a high cortical lesion load is associated with epilepsy[17], therefore patients were excluded from participation if they: (a) used medication that lowers seizure threshold; and/or (b) had ≥ 12 cortical lesions (= the mean cortical lesion load in an average MS population with comparable disease duration[18]) and/or (c) had cortical lesions in the right DLPFC as assessed on double inversion recovery (DIR). Further exclusion criteria were relapses and corticosteroid-administration 6 weeks prior to investigation.

Repetitive Transcranial Magnetic Stimulation (rTMS)

Repetitive TMS was administered with a MagPro X100 stimulator, using a figure-of-eight TMS-coil (MCF B-65; Medtronic Magoption). Resting motor threshold (RMT) of left first dorsal interosseus muscle was visually determined as described elsewhere[13].

The real-rTMS stimulation site was determined for each subject individually and was defined as the peak-voxel activation of the right DLPFC (all voxels that were significantly higher activated in all three different N-back task loads (1-back, 2-back and 3-back combined) compared to the control condition, 0-back; N123>N0 contrast). Online neuronavigation (ASA 4.6, ANT Neuro, Enschede, The Netherlands) and mechanical coil stabilization allowed precise targeting throughout the stimulation. Our rTMS protocol (10Hz, 110% RMT, 60 trains of 5sec, 25sec between trains, in total 3000 biphasic pulses in 30min) fulfilled the current international safety guidelines[19]. For sham, high-frequency rTMS (10 Hz, 60 trains of 5sec, 25sec between trains, in total 3000 biphasic pulses in 30min) was performed with a lower intensity (80% RMT) at a presumably non-effective area (2 cm posterior to the vertex)[20]. Participants were naive to rTMS and blind to stimulation condition.

Neuropsychological assessment

All subjects underwent extensive neuropsychological testing specifically aimed to investigate memory function of which working memory was assessed with the digit span and Letter-Number Sequencing, both derived from the Wechsler Adult Intelligence Scale[21]. For a detailed description of the tests used see Hulst et al., 2012[18].

MR imaging – structural MRI

MRI was performed on a 1.5T whole-body scanner (Siemens Sonata, Erlangen, Germany), using an eight-channel phased-array head coil. At all sessions a localizer, 3D-magnetization prepared rapid acquisition gradient-echo (MPRAGE, repetition time (TR)/ echo time (TE) 2700/5ms, 176 sagittal slices with 1.3mm thickness) and echo planar images (EPI) during administration of the N-back task (276 EPI, TR/TE 2570/45ms, acquisition time 12min) were performed. For white matter lesion detection turbo spin-echo proton density (PD) and T2-weighted images (TR 3130ms, TE 24/85ms, 46 axial slices with 3mm thickness) were obtained. 3D-DIR images were acquired to detect cortical lesions (TR/TE 2350/35ms, 120 sagittal slices). For each subject, the whole brain volume (gray and white matter volume separately, corrected for head size) was measured using the MPRAGE images and SIENAX [22]. White matter lesions were marked and manually outlined on the PD-weighted images using a local-threshold technique. Cortical lesions were scored on the 3D-DIR images according to consensus guidelines[23].

Visuo-spatial N-back task

In the scanner, participants performed a visuo-spatial N-back working memory task with three increasing task loads (N1, N2 and N3) and a control condition (N0)[24]. In every trial (2.8 seconds) a yellow dot randomly appeared on the screen at the left, right, bottom or top of a blue diamond. The location on the diamond corresponded to four similar locations on an MRI-compatible response box (Current Designs, Inc., Philadelphia, Pennsylvania). During the N0 condition, participants were asked to

respond immediately by pressing the corresponding button. During the N*x* conditions participants had to indicate *where* the yellow dot was one (N1), two (N2), or three (N3) trials before, while simultaneously remembering the new locations as the task continued. The task was programmed in E-Prime 1.22.0 (Psychology Software Tools, Pittsburgh, Pennsylvania) using a block design including three blocks per condition (20 trials per block; 60 trials total). The blocks were presented in order of increasing difficulty (N0, N1, N2, N3). This loop was repeated three times. The main behavioral outcome measures were the absolute number of accurate responses and the reaction time for each task load.

Functional MRI analyses

Preprocessing: All functional image analyses were performed using FSL 5.0.2 (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl). For each subject, all non-brain tissue was removed from the images (BET) and motion correction (MCFLIRT) was applied. The functional images were aligned to the subject's MPRAGE using affine registration (FLIRT) through boundary-based registration and subsequently to the MNI152 standard brain using non-linear registration (FNIRT, warp resolution: 10mm, 12 degrees of freedom).

Brain activation: For first-level FEAT analysis, high-pass filtering (230sec cut-off period) was used and spatial smoothing was performed (full-width-at-half-maximum Gaussian kernel of 5mm). We performed whole brain activation analysis and seed-based connectivity analysis using a double-gamma hemodynamic response function

in context of the general linear model using a block design. Pre-threshold masking was performed using a gray matter mask (MNI_thr25_2mm) in order to increase the statistical power by reducing the number of voxels included in the analysis.

To calculate brain activation patterns, all task loads were contrasted with the control condition (i.e. N1>N0; N2>N0; N3>N0) to find relevant areas involved in working memory performance. This approach was chosen since there is no clear *a priori* hypothesis about which degree of working memory engagement rTMS might affect the most. This allows us to be highly sensitive to small, yet meaningful, changes in response to rTMS in the different task loads.

To describe baseline differences between patients and controls, group-analysis was carried out using an unpaired t-test. Next a within-subjects (patients or controls) repeated measures design was used (paired tripled t-test) to investigate if there was an effect of real-rTMS compared to baseline and sham-rTMS. Finally, the differences in brain activation between patients and controls were investigated using a mixed effect second level analyses (FLAME). These analyses were done for the three different task loads (N1>N0, N2>N0, N3>N0).

Brain connectivity: Connectivity was calculated using a generalized psychophysiological interaction (gPPI) model to identify voxels (whole-brain) that are related to activation in a seed region in a given psychological context while controlling for other task variables[25]. For each subject individually, a seed-region was defined by computing a sphere of 6 mm around the baseline peak-voxel of task-related activation in the right DLPFC in standard space. This was the same region

that was used for neuronavigation. The time series within this seed region served as the physiological regressor. As psychological regressors we used the BOLD-response of the three contrasts of the N-back task (N1>N0, N2>N0 and N3>N0), resulting in three gPPI analyses per session. Subsequently, to perform a group analysis, all the individual seed-regions of the right DLPFC were combined in one common mask. Using this common mask, differences in connectivity between groups and between sessions were calculated.

All image analyses (activation and connectivity) were performed using a cluster correction, which allows for a correction of multiple comparisons by taking into account the activation and connectivity of associated voxels into clusters of voxels. Differences between conditions within clusters were considered significant at P≤0.05.

Behavioral statistics: Statistical analyses on demographic, clinical and behavioral variables were performed using IBM Statistical Package for the Social Sciences version 20.0. To study baseline differences between patients and HCs, unpaired t-tests were performed when variables were normally distributed; otherwise the Mann-Whitney U-test was used. As N-back accuracy measures and reaction times were not normally distributed, non-parametric statistics (Friedman's ANOVA combined with the Wilcoxon Signed Ranks Test) were performed. A *P*-value of <0.05 (two-tailed) was considered as statistically significant.

RESULTS

Nineteen MS patients and 11 HCs participated in this study. Two patients were excluded due to moderate and self-limiting adverse events (vasovagal syncopes) during determining the RMT or during real-rTMS. These incidents were most likely facilitated by an hyperextension of the neck[26]. After adjusting the neck support, no further vasovagal syncopes were observed.

Patients and controls did not significantly differ with regard to age, sex, handedness, premorbid IQ and educational level (Table 1). The parameters for rTMS (e.g. applied rTMS-intensities based on the RMT and the order of sham-rTMS and real-rTMS) did not differ between patients and controls (Table 2).

Neuropsychology

The neuropsychological test battery revealed no differences between patients and HCs in any cognitive domain. None of the patients were clinically impaired in working memory. Patients differed significantly from HCs regarding anxiety and depression measures (Table 1). Important to note is that 14/17 patients had subclinical values in the HADS-D (below cut-off 11).

Structural MRI

There were no differences between patients and HCs in normalized total brain, gray matter or white matter volume (Table 2). None of the patients had DIR-visible cortical lesions within the right DLPFC (compliant with the exclusion criteria).

Table 1. Demographical, clinical and cognitive data of healthy controls (HC) and MS patients (MS)

Demographical and clinical measures	HC (n=11)				MS (n=17)				*P*-value
Age (y)	42.3 (11.1)				43.3 (8.3)				.796
Sex (female/male) †	6/5				10/7				.826
Handedness (R/L) †	10/1				15/2				.619
Premorbid IQ	107.6 (8.3)				106.7 (11.1)				.821
Educational level†	6.0 (5.0-6.0)				6.0 (5.5-6.0)				.842
Disease type	-				13/4 (RRMS/SPMS)				-
Disease duration (y)	-				11.9y (6.8)				-
EDSS†	-				3.5 (1.5-4.5)				-
HADS-A	3.8 (1.6)				6.1 (3.4)				**.049***
HADS-D	1.8 (2.1)				5.0 (4.0)				**.023***
CIS-R	17.8 (11.5)				25.5 (11.7)				.098
Working Memory N-back accuracy	**N0†**	**N1†**	**N2†**	**N3†**	**N0†**	**N1†**	**N2†**	**N3†**	
Baseline	60 59-60	59 56-60	53 44-54	39 30-50	60 60-60	59 55.5-60	51 36-55.5	38 27-45	*ns*
Real-rTMS	60 59-60	59 56-60	54 44-58	46 34-56	60 60-60	60 56.5-60	54 50-58	44 33-51	*ns*
Sham-rTMS	60 59-60	59 58-60	58 50-60	45 42-48	60 58.5-60	58 53-59.5	53 43-57.5	44 33-47	*ns*
N-back reaction time (ms)	**N0†**	**N1†**	**N2†**	**N3†**	**N0†**	**N1†**	**N2†**	**N3†**	
Baseline	320 270-370	320 180-530	730 340-1084	520 440-900	340 320-430	260 220-610	790 510-950	660 550-920	*ns*
Real-rTMS	300 280-330	210 170-260	360 220-1030	630 450-840	360 310-460	370 250-630	570 280-960	470 450-560	*ns*
Sham-rTMS	300 270-350	200 160-610	540 200-670	470 430-560	330 290-440	260 200-400	480 290-840	550 450-770	*ns*

Demographical and clinical measures	HC (n=11)	MS (n=17)	*P*-value
Digit span			
Forward	10.3 (1.9)	9.4 (2.5)	.340
Backward†	8.0 (8.0-8.0)	7.0 (6.0-10.5)	.124
LNS	11.0 (1.3)	10.8 (2.4)	.767
Processing speed - LDST	63.8 (8.2)	56.2 (14.6)	.130
Spatial memory - LLT†	14.0 (8.0-20.0)	16.0 (6.5-39.5)	.602
Verbal memory and learning - VLGT	61.9 (5.0)	57.2 (10.6)	.185
Semantic memory			
WLG-Animals	27.6 (4.6)	24.3 (7.2)	.198
WLG-Professions	20.6 (5.4)	19.0 (5.5)	.444
WLG-M-words	13.4 (3.3)	12.4 (5.6)	.617

Data are means (standard deviation) for normally distributed variables, variables indicated with † were not normally distributed and therefore medians (interquartile range) are provided, y = years; HADS: Hospital Anxiety and Depression Scale, A: anxiety, D: depression; CIS-8: Checklist individual strength. LNS: Letter-Number Sequencing; LDST: Letter Digit Substitution Task, total number of substitutions is provided; LLT: Location Learning Task, total number of displacements is provided; VLGT: verbal learning and memory task, total number of correct items is provided; WLG: Word List Generation. *P*-value of N-back accuracy: the overall accuracy of N1, N2 and N3 was compared between HCs and MS, the baseline condition (N0) was omitted. *Significant differences were found between patients and HCs; ns = no significant differences between HCs and MS patients for the task loads separately (N0 HC versus N0 MS; N1 HC versus N1 MS; N2 HC versus N2 MS; N3 HC versus N3 MS).

Table 2. Structural MRI measures and rTMS-parameters of healthy controls (HC) and MS patients (MS)

Structural MRI measures	**HC (n=11)**		**MS (n=17)**		***P*-value**	
NBV in L [a]	1.45 (0.06)		1.44 (0.09)		.657	
NGMV in L [b]	0.76 (0.06)		0.76 (0.06)		.948	
NWMV in L [a]	0.69 (0.02)		0.68 (0.03)		.301	
T2 lesion volume in mL[†]	-		4.24 (1.48-6.89)		-	
Number cortical lesions	-		5.82 (3.40)		-	
Cortical lesions in right DLPFC	-		0 (0)		-	
rTMS parameters	Real-rTMS	Sham-rTMS	Real-rTMS	Sham-rTMS		
First session (# of subjects)	6	5	9	8	-	
RMT (%)	47.2 ±7.1	48.8 ±7.1	53.2 ±7.7	53.7 ±7.6	.061	.139
rTMS intensity[†] (%)	54 44-62	39 33-45	59 55-63.5	44 40.5-46	.161	.083
Delay rTMS / MRI (min)	5.4 ±8.7	6.5 ±3.1	6.8 ±1.6	6.0 ±1.6	-	

Data are means (standard deviation), except for [†] where because of non-normal distribution median and interquartile range are provided. NBV: normalized brain volume; NGMV: normalized gray matter volume; NWMV: normalized white matter volume; NHV: normalized hippocampal volume, L: liters, mL: milliliters. RMT (resting motor threshold) and rTMS intensity are provided in % maximal stimulator power. The latency between the end of stimulation and the beginning of the MRI measurements is provided in minutes. *P*-value: significance of difference between HC and MS group; *P*-value regarding rTMS-parameters: left value: significance of difference between real-rTMS in HCs and real-rTMS in patients; right value: significance of difference between sham-rTMS in HCs and sham-rTMS in patients.

Cross-sectional baseline differences *between patients and controls in N-back task accuracy, task-related brain activation and connectivity*

MS patients and HCs had similar accuracy scores and reaction times on all task load levels at baseline (Table 1). The accuracy decreased with increasing task load in patients (N0 > N1: *P*=0.033; N1 > N2: *P*<0.001; N2 > N3: *P*<0.001) and in HCs (N0 > N1: *P*=0.246; N1 > N2: *P*=0.033; N2 > N3: *P*=0.021).

Regarding task-related activation, in both groups, and in all different task load conditions a robust effect of task was found for the bilateral fronto-parietal network (Figure 2A+B). At baseline, patients compared with HCs showed higher task-related activation in the left DLPFC (N2>N0) and right temporal pole (N3>N0, Table 3, Figure 2C). No differences between the groups were detected on N1>N0. No differences in connectivity between the right DLPFC (stimulated area) and other areas in the brain were seen between patients and HCs at baseline.

Effects of stimulation - Changes in ***N-back task accuracy and reaction time*** *after rTMS*

MS patients: Improved N-back task accuracy after real-rTMS compared to baseline was observed during the 2-back (*P*=0.029) and 3-back task loads (*P*=0.015), which was not seen after sham-rTMS compared to baseline (*P*=0.312 and *P*=0.170 for 2-back and 3-back respectively). The difference in accuracy after real-rTMS compared to sham-rTMS in patients did not reach statistical significance (*P*=0.077).

Reaction times did overall not differ between the conditions, except for a significant faster reaction time after real-rTMS compared to baseline during the 3-back condition (*P*=0.016).

HCs: No change in N-back task accuracy was found between real-rTMS and baseline or between real-rTMS and sham-rTMS. On the 2-back task load, controls did improve after sham-rTMS compared to baseline (*P*=0.023).

There were no significant differences in reaction time observed for the different conditions.

*Changes in **task-related brain activation** after rTMS*

MS patients: Compared to baseline, brain activation increased in parietal and occipital regions after real-rTMS and after sham-rTMS in all three task load conditions. During the N3>N0, after sham-rTMS compared to baseline, increased activation was additionally found in the left insular region. No areas showed decreased activation after real-rTMS and after sham-rTMS compared to baseline. No differences in activation after real-rTMS compared to sham-rTMS were found (Table 3).

HCs: Compared to baseline, increased activation in multiple brain areas was seen after real-rTMS (in all three task load conditions) and after sham-rTMS (only in the N3 task load, see Table 3); Table 3 additionally provides the areas of significantly different activation after real-rTMS compared to sham-rTMS in HCs.

MS patients versus HCs: After real-rTMS, patients showed higher activation of the left inferior parietal lobule compared to HCs during N1>N0; no differences in frontal activation were detected between the two groups. After sham-rTMS, higher activation during N2>N0 in patients was detected in the left superior frontal gyrus and parietal regions compared to HCs (data not shown).

*Effects of stimulation - Changes in **connectivity of right DLPFC** after rTMS*

MS patients: No connectivity differences were detected between baseline and real-rTMS or between baseline and sham-rTMS. After real-rTMS compared to sham-rTMS, increased connectivity was detected between right DLPFC (stimulated area, seed region) and the head of the right caudate, bilateral paracingulate gyri, left anterior cingulate gyrus and frontal pole (N1>N0, Table 3, Figure 3A+B).

HCs: No connectivity differences were detected between baseline and real-rTMS or between baseline and sham-rTMS. Also no differences between real-rTMS and sham-rTMS were observed.

MS patients versus HCs: No differences in functional connectivity were detected between patients and controls.

Functional connectivity – the effect of task load

Post hoc, the results from the gPPI analysis were further investigated. We zoomed in on the significant differences in functional connectivity after real-rTMS compared with sham stimulation, i.e. within the cluster-corrected difference mask (real vs. sham

condition; contrast N1>N0, Figure 3A+B). We extracted the mean connectivity parameter estimates for N1>N0, N2>N0 and N3>N0 from each individual first level FEAT with featquery. rTMS-induced changes were defined as differences, seen after real-rTMS but not after sham-rTMS, both compared to baseline. Additionally, we contrasted the real and the sham condition.

This post-hoc analysis revealed that patients' connectivity parameter estimates of the right DLPFC augmented with increasing task load in all sessions. Furthermore, connectivity after real-rTMS was significantly higher than after sham-rTMS at all task loads (N1>N0: $P<0.001$, N2>N0: $P=0.001$, N3>N0: $P=0.003$, Figure 3D).

In healthy controls no differences in connectivity were detected and therefore this post-hoc analysis could only be performed in the patient group.

Table 3. Changes in brain activation and brain connectivity after real-rTMS and sham-rTMS

				Number of clusters	Cluster-size	Zmax	x	y	z	Location of peak voxel
Activation	**MS**	**Real > Baseline**	**N1>N0**	1	331	3.55	-10	-76	12	Intracalcarine cortex L
				2	291	3.67	24	-86	40	Lateral occipital cortex R
			N2>N0	1	1631	4.33	-12	-74	10	Intracalcarine cortex L
			N3>N0	1	211	3.87	40	-68	30	Lateral occipital cortex R
		Sham > Baseline	**N1>N0**	1	531	4.0	14	-56	12	Precuneus R
				2	342	3.71	-36	6	14	Operculum L
			N2>N0	1	244	3.67	16	-76	30	Cuneus R
				2	229	3.76	2	6	46	Paracingulate gyrus R
			N3>N0	1	555	3.70	26	-72	32	Lateral occipital cortex R
				2	259	3.83	4	4	48	Paracingulate gyrus R
				3	239	3.29	36	22	12	Operculum R
		Real > Sham	*No significant differences in N1 > N0; N2 > N0 and N3 > N0*							
Activation	**HC**	**Real > Baseline**	**N1>N0**	1	594	3.44	-6	-62	30	Precuneus L
				2	393	4.05	-24	30	-14	Frontal orbital cortex L

		3	214	3.86	2	36	-10	Paracingulate gyrus R
	N2>N0	1	691	4.06	2	32	-16	Frontal medial cortex R
		2	450	3.72	2	-78	36	Cuneus R
		3	216	3.04	16	-86	30	Lateral occipital cortex R
		4	188	3.81	36	-94	-6	Occipital lobe R
		5	171	3.56	-52	-26	14	Parietal operculum L
	N3>N0	1	366	3.51	16	-60	28	Precuneus R
Sham > Baseline	*No significant differences for N1 > N0 and for N2 > N0*							
	N3>N0	1	800	3.56	4	-68	40	Precuneus R
		2	203	3.70	26	16	-32	Temporal pole R
Real>Sham	**N1>N0**	*No significant differences*						
	N2>N0	1	179	4.01	-56	-28	-8	Middle temporal gyrus L
		2	251	3.60	22	-100	-8	Occipital lobe R
		3	248	3.59	-6	30	-16	Anterior cingulate cortex L
		4	191	3.48	-18	-100	-12	Occipital lobe L
	N3>N0	*No significant differences*						
Real<Sham	*No significant differences for N1 > N0 and for N2 > N0*							
	N3>N0	1	190	3.93	56	42	0	Inferior frontal gyrus R

Connectivity	**MS**	**Real > Baseline**		*No significant differences for N1 > N0; N2 > N0 and N3 > N0*						
		Sham > Baseline		*No significant differences for N1 > N0; N2 > N0 and N3 > N0*						
		Real>Sham	**N1>N0**	1	5925	3.78	9	12	6	Head of caudate nucleus R, putamen Anterior cingulate L, Paracingulate gyrus L and R, Frontal pole L
				No significant differences for N2 > N0 and for N3 > N0						
Connectivity	**HC**	**Real > Baseline**		*No significant differences for N1 > N0; N2 > N0 and N3 > N0*						
		Sham > Baseline		*No significant differences for N1 > N0; N2 > N0 and N3 > N0*						
		Real > Sham		*No significant differences for N1 > N0; N2 > N0 and N3 > N0*						

Cluster: the number of significant clusters that were detected within a certain contrast; Clustersize in number of voxels; Zmax: maximal z-value of the cluster; x, y, z: MNI-space coordinates of the Zmax. All results for activation and connectivity are cluster-corrected (P<0.05).

DISCUSSION

In this study we investigated the effects of single-session cortical excitability-enhancing rTMS on working memory performance, task-related brain activation, and task-related brain connectivity in MS patients.

Baseline differences between patients and controls

Patients that participated in this study were cognitively preserved (as measured using neuropsychological testing) and showed no differences in working memory accuracy (N-back task) compared to HCs. Additionally, no differences in functional connectivity from the right DLPFC with the rest of the brain during a working memory task were detected. However, compared to HCs, patients showed *higher task-related brain activation* at baseline in the left DLPFC (N2>N0) and the right temporal area (N3>N0) during the working memory task, suggestive of functional reorganization. The higher activation is hypothesized to 'compensate' for possible (subclinical) cognitive problems, a finding that has been reported in the previous literature[5-7].

N-back task accuracy and reaction time after rTMS

Compared to baseline, we found a slight improvement in N-back accuracy and reaction time in patients after real-rTMS, but not after sham-rTMS. This finding is promising, especially, as it is known that behavioral and neural effects of rTMS may increase more by applying multiple stimulation sessions[19]. In the current study, patients' accuracy after real-rTMS was not significantly different from the accuracy after sham-rTMS (*P*=0.077). We speculate that multiple stimulation sessions combined with an increased sample size might render this difference significant.

Changes in task-related brain activation after rTMS

Both patients and controls showed increased overall brain activation after real- and sham-rTMS compared to baseline. Interestingly, after real-rTMS (compared to baseline), the higher frontal activation (relative to HCs at baseline) disappeared in the patient group, resulting in similar brain activation patterns compared to the HCs. Concomitantly, improved accuracy and reaction time on the working memory task after real-rTMS was measured in patients only. This finding hints towards the hypothesis that any change in brain activation from the healthy control situation is unfavorable for the patients' functioning[27, 28].

Changes in task-related brain connectivity after rTMS

Increased functional connectivity during the task was detected between the right DLPFC (stimulated area), the right caudate nucleus, the bilateral paracingulate gyri, the left anterior cingulate and the frontal pole in patients with MS. We speculate that this increase in frontal connectivity after real-rTMS (compared to sham-rTMS) in MS patients may be linked to the normalization of frontal activation that becomes more identical to that of the healthy control subjects at baseline. In other words, the frontal changes seen after real-rTMS (decreased brain activation and increased functional connectivity) might reflect an improvement of frontal processing efficiency during a working memory task. Previous research in HCs showed that functional connectivity (during working memory) in the frontal gyrus correlates negatively with task-related activation and positively with working memory performance[29]. All brain regions that show increased connectivity after real-rTMS are known to be important for performing spatial working memory tasks[30-32]. Additional support comes from several studies on working memory, indicating that decreased frontal functional connectivity

(compared to controls) is detrimental for the performance in patients with neurodegenerative disease[33] or showing that differences, i.e. higher and occasionally also lower functional connectivity (compared to controls) are associated with preserved cognition in MS[10, 34].

Different response to rTMS of patients and controls

Patients and controls responded differently to real-rTMS. Consistent with a similar rTMS-study in HCs[11], the HC group did not improve in N-back accuracy after real-rTMS while patients did. Inconsistently with previous research[11] our HCs did not improve on reaction time after rTMS. This difference might best be explained by the differences in stimulation protocol (intensity, duration).

Also, in HCs no rTMS effects were seen on connectivity while there was evidence for rTMS effects in patients. We can rule out that a training effect caused these differences as the order of sham and real stimulation was counter-balanced for both the patients and HCs. Patients with MS had higher scores on depression compared to HCs, which is inherent to the disease. However, HADS-scores in 14 patients did not exceed the clinically relevant cut-off of 11, suggesting a limited influence of depressive symptoms on the outcome. For these reasons, we assume that disease-specific differences such as the patients' higher activation at baseline might account for the different effects of rTMS on activation, connectivity and N-back accuracy in patients and HCs rather than any other difference.

Future perspectives

One of the main limitations of this study is the small sample size resulting in possible underestimations of rTMS-induced changes in brain activation patterns and

connectivity measures; overestimations are less likely due to conservative cluster correction. In patients differences in task-related brain activation and changes in task-accuracy were found between baseline and real-rTMS, which ideally would also be seen between sham-rTMS and real-rTMS. However, differences between sham-rTMS and real-rTMS did not reach statistical significance in the current study. On the contrary, in patients differences in connectivity were only seen between real-rTMS and sham-rTMS and not between the two stimulation protocols and baseline.

Due to our stringent safety measures for applying rTMS, patients with extensive gray matter pathology were excluded from participation to prevent epileptic seizures. This left us with cognitively preserved MS patients. While this group is particularly of interest to study potential cognitive rehabilitation strategies to prevent future cognitive deterioration, it would be highly interesting to study the effects of rTMS in a group of patients with MS that do have overt cognitive problems and to investigate the value of rTMS in treating cognitive deficits in MS as well.

Finally, this study needs to be replicated in a larger sample and should strive to enhance the rTMS effects by performing multiple stimulation sessions[35]. For clinical implications, it might be relevant to explore whether less conservative exclusion criteria are sufficiently safe, enabling inclusion of more severely impaired patients.

Conclusions

This is, to our knowledge, the first study on the effects of rTMS on cognitive performance in MS. Real-TMS resulted in decreased task-related activation in the left DLPFC, increased functional connectivity between the stimulated right DLPFC and other task-relevant areas, together with an improvement in working memory accuracy in patients with MS. With caution, we interpret these findings as an rTMS-induced

increase in processing efficiency in MS patients. This implicates a potential role for rTMS in cognitive rehabilitation in MS.

Acknowledgments: We would like to thank the Dr. Werner Jackstädt Foundation for supporting T. Goldschmidt, the Walter and Ilse Rose foundation for supporting W. Paulus and the Dutch MS Research Foundation (grant number 09-538d, 12-799) for supporting H.E. Hulst and the MS center Amsterdam. Additionally we like to thank Marie-Anne Zuidhof for her participation in this project during her internship.

REFERENCES

1. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. Lancet Neurol 2008;7:1139-1151.

2. Julian LJ, Vella L, Vollmer T, Hadjimichael O, Mohr DC. Employment in multiple sclerosis. Exiting and re-entering the work force. J Neurol 2008;255:1354-1360.

3. Benedict RH, Cookfair D, Gavett R, et al. Validity of the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). J Int Neuropsychol Soc 2006;12:549-558.

4. Foong J, Rozewicz L, Quaghebeur G, et al. Executive function in multiple sclerosis. The role of frontal lobe pathology. Brain 1997;120 (Pt 1):15-26.

5. Sperling RA, Guttmann CR, Hohol MJ, et al. Regional magnetic resonance imaging lesion burden and cognitive function in multiple sclerosis: a longitudinal study. Arch Neurol 2001;58:115-121.

6. Mainero C, Caramia F, Pozzilli C, et al. fMRI evidence of brain reorganization during attention and memory tasks in multiple sclerosis. Neuroimage 2004;21:858-867.

7. Staffen W, Mair A, Zauner H, et al. Cognitive function and fMRI in patients with multiple sclerosis: evidence for compensatory cortical activation during an attention task. Brain 2002;125:1275-1282.

8. Sweet LH, Rao SM, Primeau M, Durgerian S, Cohen RA. Functional magnetic resonance imaging response to increased verbal working memory demands among patients with multiple sclerosis. Hum Brain Mapp 2006;27:28-36.

9. Rocca MA, Valsasina P, Hulst HE, et al. Functional correlates of cognitive dysfunction in multiple sclerosis: A multicenter fMRI Study. Hum Brain Mapp 2014;35:5799-5814.

10. Cader S, Cifelli A, Abu-Omar Y, Palace J, Matthews PM. Reduced brain functional reserve and altered functional connectivity in patients with multiple sclerosis. Brain 2006;129:527-537.

11. Esslinger C, Schuler N, Sauer C, et al. Induction and quantification of prefrontal cortical network plasticity using 5 Hz rTMS and fMRI. Hum Brain Mapp 2012.

12. Rounis E, Stephan KE, Lee L, et al. Acute changes in frontoparietal activity after repetitive transcranial magnetic stimulation over the dorsolateral prefrontal cortex in a cued reaction time task. J Neurosci 2006;26:9629-9638.

13. Martis B, Alam D, Dowd SM, et al. Neurocognitive effects of repetitive transcranial magnetic stimulation in severe major depression. Clin Neurophysiol 2003;114:1125-1132.

14. O'Connor M, Brenninkmeyer C, Morgan A, et al. Relative effects of repetitive transcranial magnetic stimulation and electroconvulsive therapy on mood and memory: a neurocognitive risk-benefit analysis. Cogn Behav Neurol 2003;16:118-127.

15. Loo C, Sachdev P, Elsayed H, et al. Effects of a 2- to 4-week course of repetitive transcranial magnetic stimulation (rTMS) on neuropsychologic functioning, electroencephalogram, and auditory threshold in depressed patients. Biol Psychiatry 2001;49:615-623.

16. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 2005;58:840-846.

17. Calabrese M, De Stefano N, Atzori M, et al. Extensive cortical inflammation is associated with epilepsy in multiple sclerosis. J Neurol 2008;255:581-586.

18. Hulst HE, Schoonheim MM, Roosendaal SD, et al. Functional adaptive changes within the hippocampal memory system of patients with multiple sclerosis. Hum Brain Mapp 2011.

19. Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008-2039.

20. Loo CK, Sainsbury K, Mitchell P, Hadzi-Pavlovic D, Sachdev PS. A sham-controlled trial of left and right temporal rTMS for the treatment of auditory hallucinations. Psychol Med 2010;40:541-546.

21. Wechsler D, inventor Adult Intelligence Scale - administration and scoring manual1997.

22. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage 2002;17:479-489.

23. Geurts JJ, Roosendaal SD, Calabrese M, et al. Consensus recommendations for MS cortical lesion scoring using double inversion recovery MRI. Neurology 2011;76:418-424.

24. de Vries FE, de Wit SJ, Cath DC, et al. Compensatory Frontoparietal Activity During Working Memory: An Endophenotype of Obsessive-Compulsive Disorder. Biol Psychiatry 2013.

25. O'Reilly JX, Woolrich MW, Behrens TE, Smith SM, Johansen-Berg H. Tools of the trade: psychophysiological interactions and functional connectivity. Soc Cogn Affect Neurosci 2012;7:604-609.

26. Izzo JL, Jr., Taylor AA. The sympathetic nervous system and baroreflexes in hypertension and hypotension. Curr Hypertens Rep 1999;1:254-263.

27. Chiaravalloti ND, Genova HM, DeLuca J. Cognitive rehabilitation in multiple sclerosis: the role of plasticity. Front Neurol 2015;6:67.

28. Schoonheim MM, Meijer KA, Geurts JJ. Network collapse and cognitive impairment in multiple sclerosis. Front Neurol 2015;6:82.

29. Hampson M, Driesen NR, Skudlarski P, Gore JC, Constable RT. Brain connectivity related to working memory performance. J Neurosci 2006;26:13338-13343.

30. Courtney SM, Ungerleider LG, Keil K, Haxby JV. Object and spatial visual working memory activate separate neural systems in human cortex. Cereb Cortex 1996;6:39-49.

31. Levy R, Friedman HR, Davachi L, Goldman-Rakic PS. Differential activation of the caudate nucleus in primates performing spatial and nonspatial working memory tasks. J Neurosci 1997;17:3870-3882.

32. Owen AM, Evans AC, Petrides M. Evidence for a two-stage model of spatial working memory processing within the lateral frontal cortex: a positron emission tomography study. Cereb Cortex 1996;6:31-38.

33. Thiruvady DR, Georgiou-Karistianis N, Egan GF, et al. Functional connectivity of the prefrontal cortex in Huntington's disease. J Neurol Neurosurg Psychiatry 2007;78:127-133.

34. Au Duong MV, Boulanouar K, Audoin B, et al. Modulation of effective connectivity inside the working memory network in patients at the earliest stage of multiple sclerosis. Neuroimage 2005;24:533-538.

35. Baumer T, Lange R, Liepert J, et al. Repeated premotor rTMS leads to cumulative plastic changes of motor cortex excitability in humans. Neuroimage 2003;20:550-560.

FIGURE LEGENDS

Figure 1. Flow chart of the experimental design

Each participant underwent three sessions (baseline, real-rTMS and sham-rTMS). There was a minimum washout period of two weeks between session 2 and session 3**.** At baseline, structural MRI scans (T1, T2, proton-density, DIR) and functional MRI (visuo-spatial N-back task) were obtained. At baseline, the highest activation (peak-voxel) in the right DLPFC was determined during the N-back task (N123>N0). This was done for every participant individually and served as the target for neuronavigation to perform real-rTMS. The order of real and sham stimulation was randomized across participants to control for order effects.

Figure 2. BOLD activation during N-back task at N2>N0 at baseline

Besides typical brain activation patterns in MS patients and controls during the N-back task at baseline, we found frontally higher brain activations in MS patients as compared to controls. A+B: Mean activation in MS patients (A) and HCs (B) at baseline show the expected fronto-parietal activation with a right-sided preponderance. C: Higher brain activation in MS patients as compared to controls at baseline (cluster-corrected ($Z=2.3$), $P<0.05$). The left DLPFC shows higher activation in MS patients than in controls during N-back task performance. R indicates right side, images are shown using radiological convention.

Figure 3. Higher connectivity with right DLPFC in MS patients after real-rTMS compared to sham-rTMS

A+B: The head of the left caudate nucleus, bilateral paracingulate gyri, left anterior cingulate gyrus and frontal pole were more strongly connected with the stimulated area after real-rTMS compared to sham-rTMS (at N1>N0, cluster-corrected, $P<0.05$). C: stimulated area (right DLPFC, combined mask of 6 mm kernel around the baseline activation-peaks in the right DLPFC (N123>N0 contrast) of every subject). D: Parameter estimates (PE) of the area with increased connectivity from the stimulated area after real-rTMS (seen in A+B). Connectivity after real-rTMS was significantly higher than after sham-rTMS at all task loads (N1>N0: $P<0.001$, N2>N0: $P=0.001$, N3>N0: $P=0.003$, paired tripled T-test) and connectivity increases with task load: at N3>N0 PE was higher than at N2>N0 and N1>N0 ($P=0.012$ and $P=0.003$, respectively, all sessions together, paired tripled T-test). R indicates right side, images are shown using radiological convention.

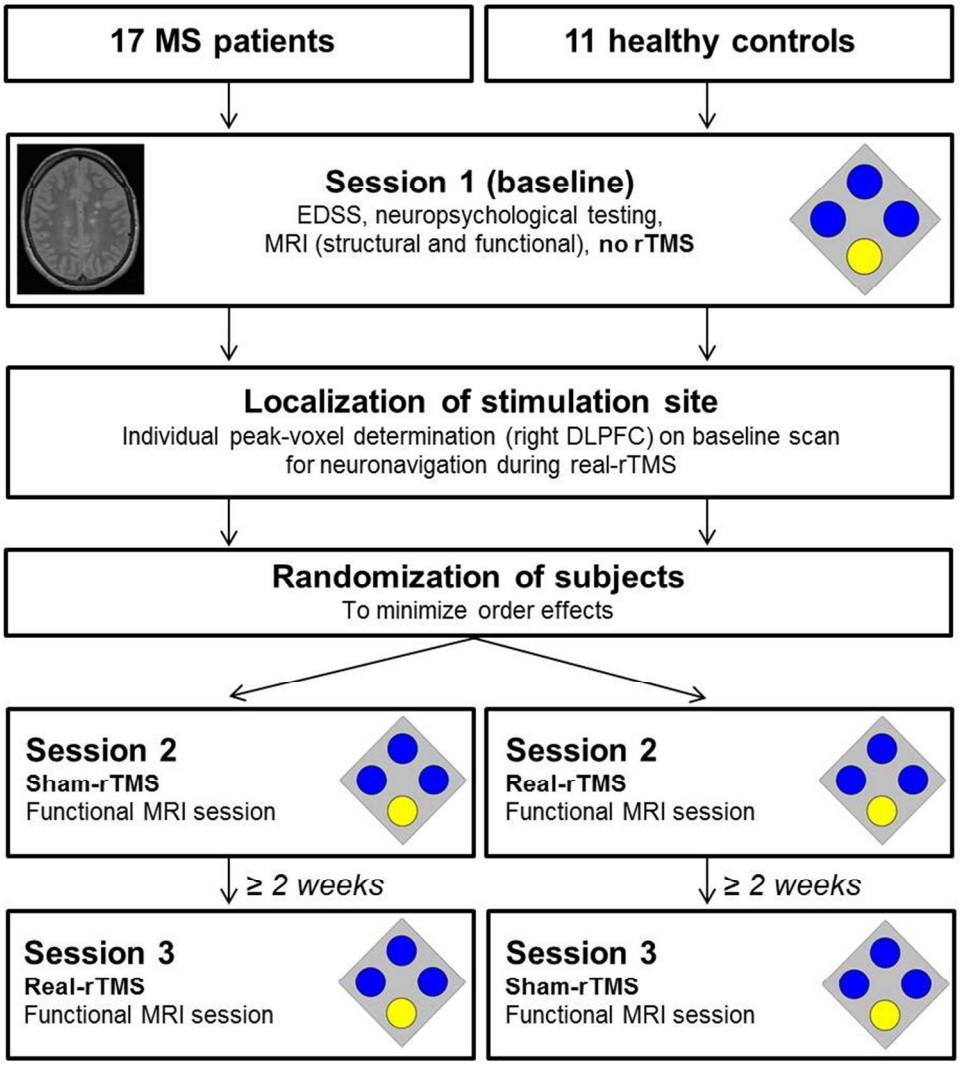

Figure 1
Figure 1
162x175mm (150 x 150 DPI)

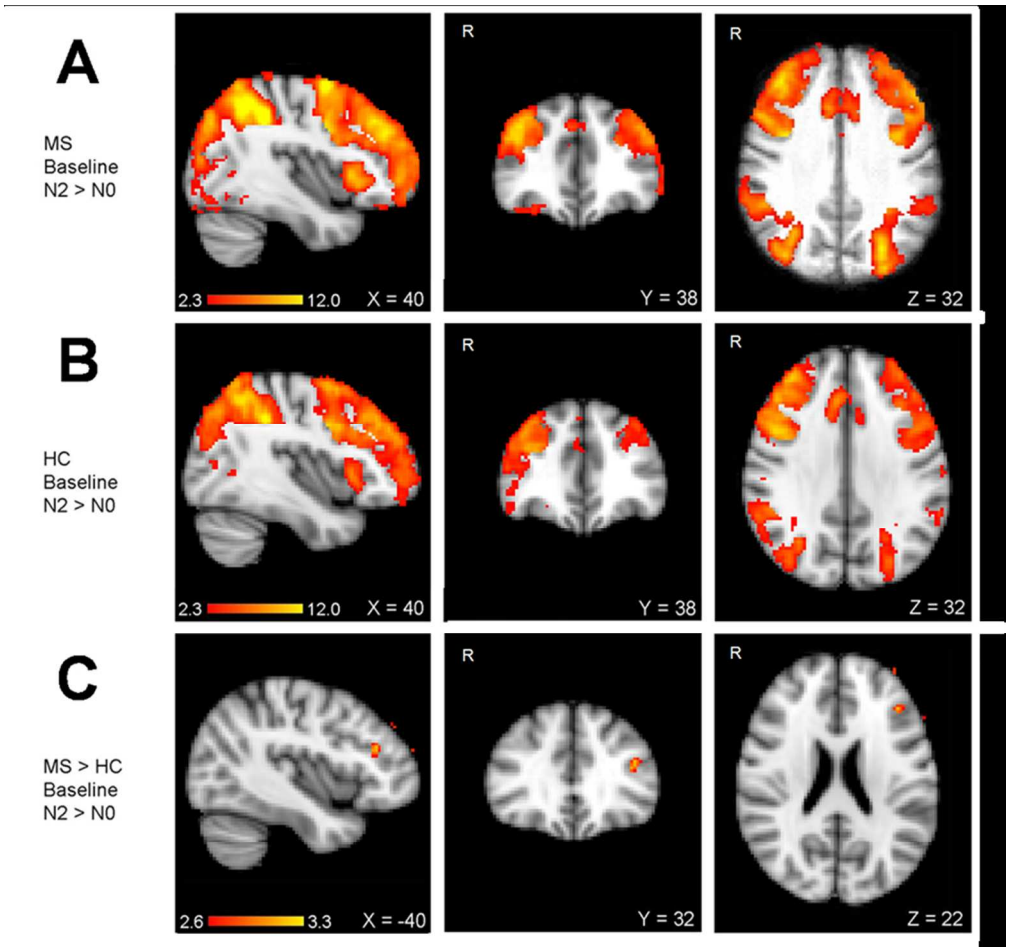

Figure 2
Figure 2
159x150mm (150 x 150 DPI)

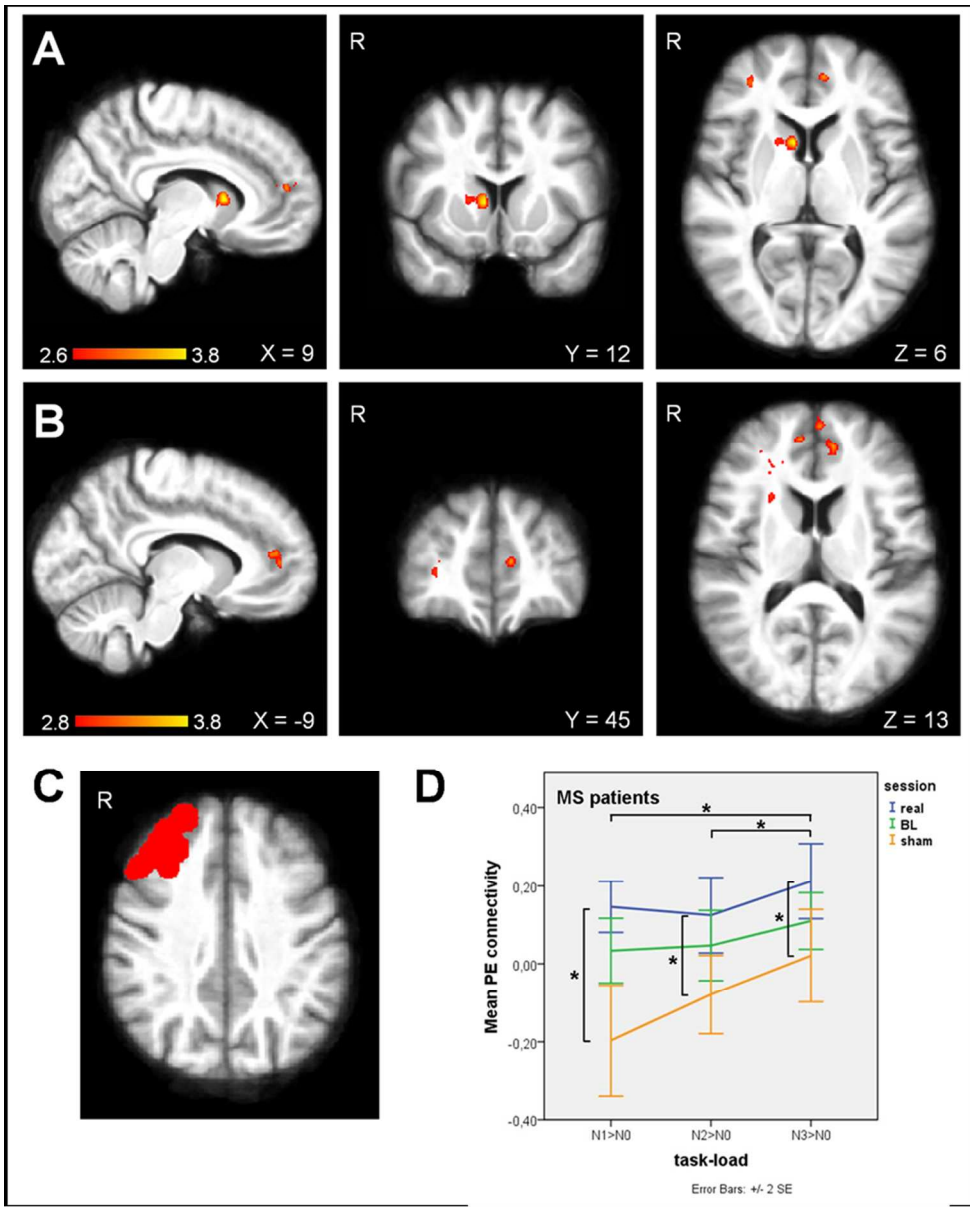

Figure 3
Figure 3
159x198mm (150 x 150 DPI)