

Increased functional connectivity within memory networks following memory rehabilitation in multiple sclerosis

Victoria M. Leavitt · Glenn R. Wylie · Peter A. Girgis · John DeLuca · Nancy D. Chiaravalloti

Published online: 16 June 2012
© Springer Science+Business Media, LLC 2012

Abstract Identifying effective behavioral treatments to improve memory in persons with learning and memory impairment is a primary goal for neurorehabilitation researchers. Memory deficits are the most common cognitive symptom in multiple sclerosis (MS), and hold negative professional and personal consequences for people who are often in the prime of their lives when diagnosed. A 10-session behavioral treatment, the modified Story Memory Technique (mSMT), was studied in a randomized, placebo-controlled clinical trial. Behavioral improvements and increased fMRI activation were shown after treatment. Here, connectivity within the neural networks underlying memory function was examined with resting-state functional connectivity (RSFC) in a subset of participants from the clinical trial. We hypothesized that the treatment would result in increased integrity of connections within two primary memory networks of the brain, the hippocampal memory network, and the default network (DN). Seeds were placed in left and right hippocampus, and the posterior cingulate cortex. Increased connectivity was found

between left hippocampus and cortical regions specifically involved in memory for visual imagery, as well as among critical hubs of the DN. These results represent the first evidence for efficacy of a behavioral intervention to impact the integrity of neural networks subserving memory functions in persons with MS.

Keywords Multiple sclerosis · Memory · Neurorehabilitation · Functional connectivity · Default network

Introduction

Memory deficits are common in persons with multiple sclerosis (MS), observed in 40–65 % of the population, and have a significant impact on everyday functional activity (Chiaravalloti and DeLuca 2008). The identification of an effective treatment for memory decline represents one of the single most important goals for neurorehabilitation researchers today (DeLuca and Nocentini 2011). However, to date, few studies have explored behavioral treatments for cognitive impairment in MS, and results have been mixed (Flavia et al. 2010; Jonsson et al. 1993; Solari et al. 2004). Recent studies from our laboratory have shown that behavioral treatment for learning and memory impairment in MS resulted in improved behavioral performance (Chiaravalloti et al. 2005) and increased cerebral activation in brain regions associated with memory (Chiaravalloti et al. 2012) in samples of MS participants with documented learning and memory impairment. A 10-session behavioral intervention for improving learning and memory, the modified Story Memory Technique (mSMT) was examined in a randomized, placebo-controlled clinical trial. Treatment with the mSMT resulted in measurable improvements in new

V. M. Leavitt · G. R. Wylie · P. A. Girgis · J. DeLuca · N. D. Chiaravalloti (✉)
Neuropsychology and Neuroscience Laboratory,
Kessler Foundation Research Center,
300 Executive Drive, suite 70,
West Orange, NJ 07052, USA
e-mail: nchiaravalloti@kesslerfoundation.org

V. M. Leavitt · G. R. Wylie · J. DeLuca · N. D. Chiaravalloti
Department of Physical Medicine and Rehabilitation,
UMDNJ –New Jersey Medical School,
Newark, NJ, USA

J. DeLuca
Department of Neurology and Neurosciences,
UMDNJ –New Jersey Medical School,
Newark, NJ, USA

learning and memory on neuropsychological assessment (Chiaravalloti et al. 2005). In a follow-up study using the same intervention procedure again with persons with MS, the treatment group showed significantly increased activation using functional magnetic resonance imaging (fMRI) (Chiaravalloti et al. 2012). Specifically, relative to placebo controls, participants in the treatment group showed increased blood oxygenation-level dependent (BOLD) activation after treatment within a widespread cortical network associated with the acquisition of new information including frontal, parietal, precuneus, and parahippocampal gyrus following the 10-session behavioral intervention. The placebo control group showed no significant BOLD changes after treatment.

Functional neuroimaging provides a window into the dynamic functioning of the working brain and therefore represents an important starting point for determining the efficacy of a behavioral intervention to effect change on the neural level. In the present investigation, we examined functional connectivity within a subset of participants enrolled in the mSMT trial (Chiaravalloti et al. 2005). Functional connectivity (FC) is a method for capturing brain activity at the level of brain networks rather than at the level of a single brain area. Perhaps the most consistent finding from investigations of cognitive function using fMRI is that multiple brain regions—a network of brain regions—are involved in the performance of any particular task. While fMRI allows us to identify these regions, it does not provide information regarding the degree to which distinct neural regions communicate with one another. Functional connectivity fills this void by providing a way to investigate networks of neural activation. This is accomplished through the examination of correlated time series (derived from fMRI) across the entire brain, which allows for inferences about spatially remote yet functionally connected networks underlying cognition. As such, FC reveals the large-scale orchestration of multiple cortical regions engaged in coordinated brain activity. Moreover, when we examine FC in persons who are resting in the scanner ('resting-state' functional connectivity, or RSFC), rather than engaged in an active task, we observe cortical regions that exhibit co-occurring activation even during rest (Biswal et al. 1995). Such coordinated brain activation suggests that the areas are functionally connected.

To our knowledge, there has been only one study to examine FC subsequent to a behavioral intervention in MS (Filippi et al. 2012). Filippi et al. (2012) showed increased connectivity within the default network (DN) following a computer-based intervention targeting processing speed (PS) and executive functions in MS patients with PS and executive deficits. Here, we were interested in RSFC changes subsequent to a memory intervention within primary memory networks of the brain: the hippocampal network,

and the DN. The DN is a collection of brain regions that has been identified as more active during rest than during task performance (Buckner et al. 2008); these regions include anterior cingulate/medial prefrontal, and posterior cingulate/precuneas. Within the DN, functional connectivity is considered an important marker of memory function (Greicius et al. 2009) in both healthy and clinical populations (Buckner et al. 2008). Altered RSFC has been found in the DN of MS patients (Bonavita et al. 2011; Rocca et al. 2010; Roosendaal et al. 2010b). Importantly, abnormal DN activity/connectivity is correlated with memory deficits in MS patients (Rocca et al. 2010; Sumowski et al. 2012).

Hippocampal atrophy is associated with memory impairment in MS (Benedict et al. 2009; Sicotte et al. 2008), and altered functional connectivity in the hippocampus has been shown in MS patients (Roosendaal et al. 2010a). The primary hypotheses investigated in the present study were that treatment with the mSMT would result in increased RSFC within two key neural networks subserving memory functions: 1) the hippocampal network and, 2) the default network. To our knowledge, this is the first study to assess change in RSFC resulting from a behavioral memory intervention in memory-impaired persons with MS.

Methods

Participants The present study examined the data from a subset of participants who were enrolled in a larger randomized placebo-controlled clinical trial (see Chiaravalloti et al. 2012). Participants were selected for the current study based on availability of imaging data for FC analysis. This subgroup comprised 14 individuals with clinically definite MS according to the criteria of McDonald et al. (2001). Seven individuals were from the treatment group and seven individuals were from the placebo control group. The groups did not differ in age, education, estimated premorbid IQ, pre-treatment new learning ability or gender. There were no significant differences between groups in disease duration and ambulation index (see Table 1). The treatment group consisted of 6 relapsing remitting (RR) participants and 1 progressive relapsing (PR) participant; the non-treatment group was composed of 3 RR, 2 primary progressive, and 2 secondary progressive participants. Prescribed disease-modifying medications in this sample were as follows: natalizumab (2), interferon beta-1a (2), interferon beta-1b (1), glatimer acetate (4). Five participants were not currently taking disease-modifying medications.

All potential participants first signed an informed consent form approved by the Institutional Review Board of the Kessler Foundation Research Center and UMDNJ. Prior to enrollment in the study protocol, all potential participants underwent a 2-part screening: (1) an initial telephone screening

Table 1 Demographic and pre-treatment variable by group (all comparisons non-significant)

	Treatment Group M (SD) <i>n</i> =7	Control Group M (SD) <i>n</i> =7	Value
Age	49.72 (9.98)	47.71 (8.04)	<i>t</i> =-.413
Education	15.86 (3.08)	16.14 (1.86)	<i>t</i> =.210
WASI Vocabulary- scaled score (estimate of premorbid IQ)	10.86 (3.02)	9.33 (5.05)	<i>t</i> =.673
Gender	5/7 female	6/7 female	χ^2 =.424
Pretreatment learning ability (OT-SRT trials to criterion)	10.57 (2.57)	11.0 (3.06)	<i>t</i> =.284
Disease duration (in months)	186.71 (116.95)	155.167 (101.696)	<i>t</i> =-.514
Ambulation Index	8.75 (2.01)	9.33 (2.87)	<i>t</i> =.428

All comparisons are non-significant

for age, most recent exacerbation, neurological history, medications and MRI compatibility, followed by (2) an in-person screening which evaluated psychiatric and substance abuse history, visual acuity, language comprehension and new learning and memory abilities. Exclusion criteria: Persons with a history of major depressive disorder, schizophrenia, bipolar disorder I or II were excluded from the study. Persons currently taking corticosteroids and benzodiazepines were excluded. Any persons with metal in their bodies were excluded, as this would preclude eligibility for functional magnetic resonance imaging (fMRI). Inclusion criteria: All participants had to demonstrate sufficient visual acuity to see the test materials. All participants were self-reportedly right handed. Note: If a subject expressed any uncertainty regarding handedness, the Edinburgh handedness inventory (Oldfield 1971) was administered. All participants were required to demonstrate intact language comprehension, as documented by a Token Test score above 26. In order to participate in this trial, all subjects must have demonstrated objective impairment on new learning and memory abilities via neuropsychological assessment. Impairment was defined as performance at least 1.5 standard deviations below the mean of a healthy control group on the Open Trial Selective Reminding Test (OT-SRT; Chiaravalloti et al. 2009). That is, only individuals that required 8 or more trials to reach the learning criterion on the open-trial SRT were included in the study, ensuring that all subjects in the study had memory impairments. In addition to these measures, a comprehensive neuropsychological test battery was administered to each participant. The following tests were included in the full battery: Digit Span (forward and back); California Verbal Learning Test, Version 2 (CVLT-II) (Delis et al. 2000); Paced Auditory Serial Addition Tests (PASAT; 4-trial version) (Strauss et al. 2006); Symbol Digit Modality Test (A. Smith 1982); Memory Assessment Scales (Strauss et al. 2006); Prose Memory (Strauss et al. 2006); Selective Reminding Test (Strauss et al. 2006); Delis Kaplan Executive Function System (DKEFS) subtests: Trail Making, Verbal Fluency, Color-Word Interference, Tower Test (Delis et al. 2004); WAIS-III Letter-Number sequencing (Strauss et al. 2006);

Wechsler Abbreviated Scale of Intelligence (WASI) subtests: Vocabulary, Block Design, Similarities, Matrix Reasoning (Strauss et al. 2006); Wechsler Test of Adult Reading (WTAR) (Strauss et al. 2006). A condensed summary of results is shown in Table 2.

Method The study was submitted to www.clinicaltrials.gov prior to enrolling the first participant. Upon satisfaction of the inclusion criteria, participants were randomly assigned to either the treatment group or a placebo control group. All participants completed baseline testing, consisting of a neuropsychological assessment, questionnaires to assess everyday life cognitive abilities and an fMRI scan prior to beginning treatment, as well as the same procedures with alternate forms of memory tests within one week of completing treatment. The same research assistant conducted both baseline and immediate follow-up evaluations; these assistants were blind to group membership.

Treatment protocol The treatment protocol consisted of 10 sessions of the modified Story Memory Technique (mSMT), which has been previously shown to be effective for treating new learning and memory deficits in an MS population (Chiaravalloti et al. 2005). Treatment consisted of the mSMT training twice per week for five weeks, with sessions lasting 45–60 min each. The mSMT entails two related skills: (1) imagery and (2) context. During sessions 1–4, subjects were taught to utilize imagery to facilitate the learning of verbal information. Sessions 5–8 taught participants to utilize context to facilitate learning. Sessions 9 and 10 focus on applying the mSMT to real-world settings, i.e., (1) remembering a lengthy shopping list, (2) recalling a list of errands, and (3) recalling steps in driving directions. The treatment is highly manualized; scripts are provided for the trainer.

The control group met with the trainer as often as the experimental group, but engaged in non-training oriented tasks to control for professional contact and alterations in the disease process. Tasks consisted of reading the same stories that the experimental group used and answering

Table 2 Neuropsychological test results at baseline for the sample

Domain	Test	Treatment mean \pm SD	Control mean \pm SD	t	p-value
Learning & Memory	Open Trial SRT: Trials to Criterion	10.67 \pm 2.80	11.00 \pm 3.06	-.20	0.842
	CVLT-II SDFR (total correct)	7.14 \pm 4.06	6.29 \pm 3.40	.428	0.676
	Memory Assessment Scale Prose Memory – Delayed (SS)	6.14 \pm 4.0	6.43 \pm 1.90	.033	0.867
Processing Speed	Symbol Digit Modalities Test (total correct)	38.14 \pm 18.98	46.57 \pm 7.14	-1.100	0.293
Working Memory	WAIS-IV Letter Number Sequencing (SS)	11.14 \pm 3.80	11.71 \pm 3.15	-.306	0.765
Executive Functioning	DKEFS Trail Making – Switching Trial (SS)	11.71 \pm 10.24	9.00 \pm 2.45	.682	0.508
	DKEFS Letter Fluency (SS)	9.14 \pm 2.97	9.43 \pm 4.65	-.137	0.893
	DKEFS Category Fluency (SS)	10.86 \pm 2.54	8.71 \pm 4.15	1.164	0.267
	DKEFS Tower Achievement (SS)	8.86 \pm 2.04	9.50 \pm 3.02	-.457	0.657
Pre-morbid Verbal IQ	WASI Vocabulary (SS)	13.00 \pm 5.73	10.86 \pm 3.02	.863	0.406

questions. The nonspecific task was matched to the training task for duration of contact with the examiner, and medium of presentation (computer). As such, the only difference between groups was that the key ingredients of mSMT training (imagery and context) were only provided to subjects in the experimental group.

fMRI tasks Tasks of learning and memory were completed in the scanner pre- and post-treatment. Tasks of attention were also administered. Of interest for the current study were data recorded during a visual monitoring task, the 0-Back condition of the N-Back task. During the 0-Back task, participants viewed a series of single letters and responded with a button press when a target letter (e.g. 'J') was displayed. Stimuli were presented with the E-Prime presentation software, which also recorded participants' behavioral performance (accuracy and reaction time).

Neuroimaging procedures As detailed in our prior study (Chiaravalloti et al. 2012), fMRI data were acquired on a Siemens Allegra 3T MR scanner. Images were acquired using a standard RF head coil. Contiguous slices (3.438 mm² in-plane resolution; slice thickness=4 mm) were obtained parallel to the AC-PC line. These functional images were acquired using gradient-echo echo-planar imaging (echo time=30 ms; repetition time=2000 ms; field of view=22 cm; flip angle=80°). Structural images were also acquired, using a standard T1-weighted pulse sequence: an MPRAGE (echo time=4.38 ms; repetition time=2000 ms, field of view=220 mm; flip angle=8°; slice thickness=1 mm, number of excitations=1, matrix=256 \times 256, in-plane resolution=0.859 mm²).

Resting-state functional connectivity analysis For each time series, the first five images were discarded to ensure steady state magnetization. All images were preprocessed using AFNI (Cox 1996) and FSL (Smith et al. 2004). Each time-series of images was realigned to the first remaining image of

the first series. The images were smoothed, using an 8 mm³ Gaussian smoothing kernel, and scaled to the mean intensity. The data were then deconvolved, using a boxcar function representing the time spent on the 0-back task with signal attributable to CSF, white matter, global signal, motion parameters and 3 polynomial regressors (to model signal drift) as regressors of no interest. The residuals from the deconvolution were saved and used as the resting-state data. This method for deriving resting-state data was employed by Biswal et al. (2010), and has been used in prior investigations of resting-state functional connectivity in clinical samples (e.g., Hillary et al. 2011). Resting data were then demeaned and warped into standard space. This was done by first warping the high resolution, MPRAGE image into standard space, then applying the same non-linear warping parameters to the residuals from the deconvolution.

Seeds used in connectivity analysis Neuroanatomical structures primarily implicated in memory functioning were used as seed regions for the RSFC analysis (left hippocampus, LHIPP, and right hippocampus, RHIPP). Spherical seed regions of interest (diameter=6 mm) centered at each of these regions were created. In addition, the posterior cingulate cortex (PCC), a critical hub within the DN (Fransson and Marrelec 2008; Greicius et al. 2003), was selected for placement of a third seed (diameter=6 mm). Coordinates for each of the 3 seed regions appear in Table 3.

Correlations were calculated between the mean time-series of the voxels within each seed and each voxel in the brain, resulting in three volumes per subject (the correlations between each of our three seed regions and every voxel in the brain). To ensure that the resulting r-values were normally distributed, Fisher's r-to-z transformation was applied to the data.

The resulting z-scores were used in group-level ANOVAs for each of the three seeded regions with factors of group (Treatment vs. Control) and Time (Pre- vs. Post-treatment).

Table 3 XYZ coordinates for seed regions selected for functional connectivity analysis. LHIPP=Left hippocampus; RHIPP=Right hippocampus; PCC=Posterior cingulate cortex

	X	Y	Z
LHIPP	-30	-24	-10
RHIPP	30	-22	-10
PCC	-8	50	38

The ANOVAs allow for a comprehensive investigation of the patterns of connectivity change using a voxelwise approach for each of the three seeds. The interaction between Group and Time will be the focus of our **Results** section, as we were primarily interested in connectivity that changed across time differentially for the two groups.

All results were corrected for multiple comparisons, with corrected alpha of $p < 0.05$. However, given that this is the first study to explore change in RSFC subsequent to a behavioral memory intervention in a very small sample, we assessed the correlations in two ways. In our first analysis, we determined the cluster-level threshold of 248 contiguous voxels when the individual voxel probability was set at $p < 0.01$ (using Monte Carlo simulations). Note: The cluster threshold was calculated using original space ($4 \times 4 \times 4$ mm), yielding a cluster threshold of 31 voxels. This was converted to an appropriate threshold for our resampled data (resampled space = $2 \times 2 \times 2$ mm). In our second, exploratory analysis, we used a less stringent individual voxel probability threshold of $p < 0.05$. Here, the cluster threshold was determined to be 1000 voxels (125 voxels in original space, again, using Monte Carlo simulations). This secondary analysis at a less stringent individual voxel probability was used to permit exploration of findings that may otherwise be missed due to issues of power.

Results

Behavioral results

Due to the small sample size, nonparametric testing was utilized to examine behavioral differences between the treatment and control groups. Performance on the CVLT short delay free recall from pre- to post- intervention was examined for both the treatment and control groups. A greater proportion of treatment group participants showed greater than a 10 % improvement on CVLT short delay free recall from pre to post treatment than the control group, although this did not reach the level of statistical significance ($X^2(1) = 2.571, p = 0.109$; Table 3). Specifically, 5/7 patients in the treatment group showed improvement post treatment; disease subtypes of the responders were 4 RR, 1 PR. Significant improvements in behavioral memory performance

have been demonstrated in the treatment group, but not the placebo-control group, in larger MS samples who received the same treatment (see Chiaravalloti et al. 2005, 2012).

Resting-state functional connectivity results: group x time interaction

To assess for differences in resting-state functional connectivity between groups at baseline, t-tests were performed. The r-maps generated for each subject at baseline for each seed region used (LHIPP, RHIPP, and PCC) were compared across groups. There were no significant differences between the groups at baseline in any regions where increased connectivity was shown in the treatment group after treatment. The treatment group showed increased connectivity among several brain regions after 5 weeks of treatment with the mSMT compared to pre-treatment. The control group, in contrast, showed no increase in connectivity at post-treatment. These results are summarized in Tables 4 and 5. In what follows, we will describe all significant Group x Time interactions; for all findings, the treatment group showed increased connectivity at post-treatment. We first describe our findings for the analysis employing the more conservative threshold (individual voxel probability of $p < 0.01$). With a seed placed in the left hippocampus, the largest cluster showing increased connectivity at post-treatment was found between left hippocampus and left insula ($x = -34, y = -6, z = -10$). This cluster comprised 516 contiguous voxels. Increased connectivity was also found between left hippocampus and right parahippocampal gyrus ($x = 32, y = 8, z = -14$; 394 voxels), providing evidence for strengthened interhemispheric neural connections. The next significant cluster was found between left hippocampus and right insula ($x = 42, y = -10, z = -20$; 346 voxels), again providing evidence for increased cross-hemispheric connectivity. Increased connectivity was shown from left hippocampus to precentral gyrus ($x = 52, y = -2, z = 4$; 328 voxels). Finally, there was increased connectivity between the left hippocampus and postcentral gyrus ($x = -56, y = -18, z = 16$; 269 voxels). Using RHIPP and PCC as seed regions, there were no areas that showed a significant increase in connectivity with the more stringent ($p < 0.01$) threshold.

In order to explore these datasets more thoroughly and to protect against Type-2 error (i.e., missed effects), we relaxed

Table 4 Behavioral Changes post-intervention in the treatment group versus the control group ($X^2(1) = 2.571, p = 0.109$)

	Improvement	No Improvement
Treatment	5	2
Non-treatment	2	5

Table 5 Results for regions of increased connectivity in the Treatment group at Post-treatment as revealed by Group x Time interaction analysis (2-way mixed ANOVA), $p < 0.01$

	X	Y	Z	Cluster
LHIPP seed				
Insula	-34	-6	-10	516
Parahippocampal gyrus	32	8	-14	394
Insula	42	-10	-20	346
Precentral gyrus	52	-2	4	328
Postcentral gyrus	-56	-18	16	269

Key: XYZ refers to the location of the voxel with the strongest connection to the seed across the group; cluster refers to the number of voxels in the cluster (voxel size $2 \times 2 \times 2$ mm). All results were statistically significant at $p < 0.01$. Cluster threshold was set at 248 contiguous voxels when the individual voxel probability was set at $p < 0.01$

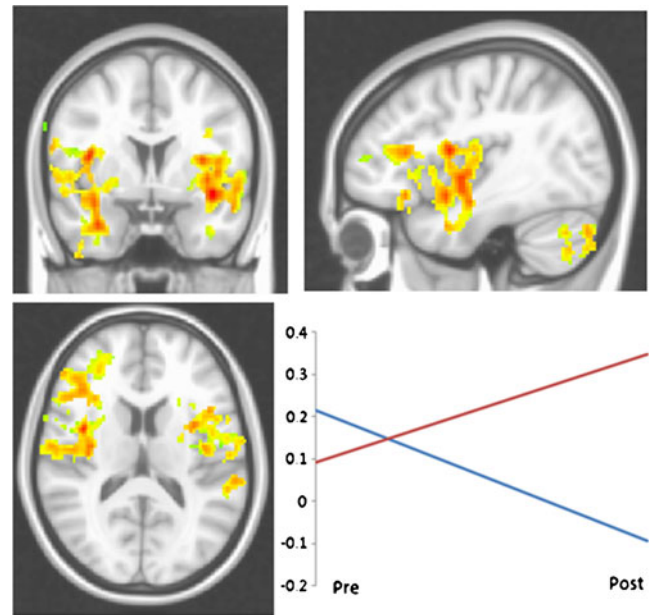
our individual voxel probability threshold to $p < 0.05$ and set the cluster threshold at 1000 voxels. This allowed identification of large regions of increased connectivity between left hippocampus and left insula ($x=40, y=2, z=14$; 4792 voxels) as well as right insula ($x=-40, y=4, z=-10$; 3970 voxels) (Table 6). Interaction plots for left and right insulae are displayed in Fig. 1.

When the right hippocampus was seeded, increased connectivity to the postcentral gyrus was shown by the treatment group ($x=-62, y=-12, z=26$; 1790 voxels). This cluster had its peak intensity (i.e., greatest r-value, or connectivity with RHIPP) in the left postcentral gyrus. Importantly however, more area within the cluster that showed increased connectivity with RHIPP was located in left precentral gyrus, left middle frontal gyrus, and left cingulate

Table 6 Results for regions of increased connectivity in the Treatment group at Post-treatment as revealed by Group x Time interaction analysis (2-way mixed ANOVA), $p < 0.05$. Cluster threshold set at 1000 contiguous voxels

	X	Y	Z	Cluster
LHIPP Seed				
Insula	40	2	14	4792
Insula	-40	4	-10	3970
Pyramis of Vermis	4	-86	-26	1515
RHIPP Seed				
Postcentral gyrus	-62	-12	26	1790
PCC Seed				
Thalamus	10	-6	-8	1260
Cerebellum ^a	38	-70	-42	894
Inferior frontal gyrus ^a	-48	28	22	734

^a Results reported did not survive correction for multiple comparisons (subthreshold)

**Fig. 1** LHIPP seed: Increased connectivity between from LHIPP to left and right insulae in the treatment group at post-treatment. Interaction plot displays increased connectivity to left insula. R-values are plotted on the ordinate; Time is plotted on the abscissa. Red line indicates Treatment subjects, blue line indicates Controls

gyrus, areas implicated in the default network (Greicius et al. 2003) (see Fig. 2).

For the seed placed in PCC, increased connectivity to the thalamus was shown after treatment ($x=10, y=-6, z=-8$; 1260 voxels) (See Fig. 3). While this cluster had its peak intensity in right thalamus, it encompassed both left and right thalami. Although the next largest cluster did not survive correction for multiple comparisons, increased connectivity was shown between PCC and right inferior semi-lunar lobule of the cerebellum ($x=38, y=-70, z=-42$; 894 voxels).

Discussion

The results of the present study reveal a behavioral treatment for learning and memory powerful enough to impact the neural networks underlying memory. These findings are extremely promising for the field of neurorehabilitation, as they represent the first evidence for increased neural connectivity resulting from a behavioral treatment targeting learning and memory impairment in MS patients. Increased functional connectivity within two primary memory networks of the brain, the hippocampal network and the default network, was shown in persons with MS after participating in a 10-session behavioral learning and memory intervention. Moreover, by showing increased functional connectivity in areas specifically related to memory for visual imagery and among critical nodes of the default network, we provide

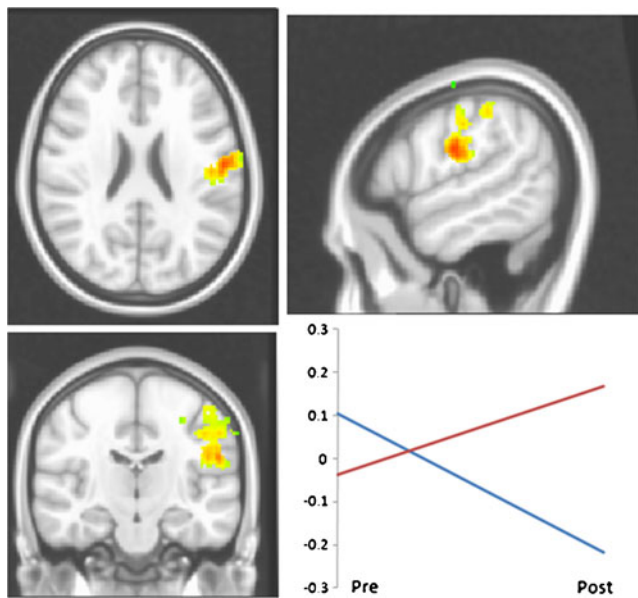


Fig. 2 RHIPP seed: Increased connectivity from RHIPP to a cluster comprising left post-central gyrus, precentral gyrus, middle frontal gyrus and cingulate gyrus. R-values are plotted on the ordinate; Time is plotted on the abscissa. Red line indicates Treatment subjects, blue line indicates Controls

the first evidence for efficacy of a behavioral intervention to impact the resting-state networks in the brain thought to be most closely associated with memory functioning.

The treatment that was employed, the mSMT, provides participants with a strategy for encoding information

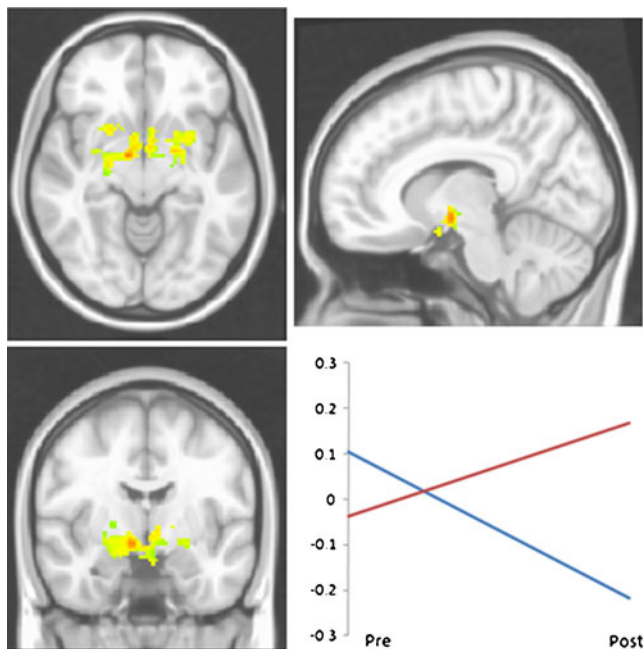


Fig. 3 PCC seed: Increased connectivity was shown by treatment group from PCC to thalamus at post-treatment. R-values are plotted on the ordinate; Time is plotted on the abscissa. Red line indicates Treatment subjects, blue line indicates Controls

through the use of visual imagery. The results of an analysis of neural connectivity in the hippocampal memory network showed increased FC between left hippocampus and left insula. These two areas are implicated in memory for visual imagery, specifically tasks involving memory for imagined scenes (Bird et al. 2010). Importantly, increased connectivity was also shown between left hippocampus and right insula, which demonstrates greater integrity of cross-hemispheric connections after treatment. The right hippocampus showed increased connectivity to left postcentral gyrus, further evidence for increased integrity of efferent corticocortical cross-hemispheric connections.

Connectivity within the default network was examined. While the hippocampus and its diverse cortical projections have traditionally been considered the primary learning and memory center of the brain, the default network has recently taken its place alongside the hippocampal network as a critical, and distinct, memory network. The key distinction between the two networks is that activity within the default network is reflective of the brain's intrinsic activity. That is, the structures comprising the default network exhibit some degree of coordinated activation at all times, including during sleep. Therefore, whereas the hippocampus is involved in effortful encoding of information, the DN appears to be involved in constant, 'off-line' consolidation of memories taking place even during active cognitive processes (see, e.g., Sumowski et al. 2012). Moreover, while active participation in memory tasks activates a subset of regions, observation of the resting-state memory network reveals co-activation of a more comprehensive set of memory-related regions (Fox and Raichle 2007). In addition, resting-state functional connectivity allows inferences about the integrity of DN function in the absence of behavioral output, thereby bypassing the need for interpreting the interplay of performance and neurophysiological output. As such, while there is some neuroanatomical overlap between the hippocampal memory network and the default network, the two networks likely subserve separable aspects of memory function. Increased connectivity was shown in the DN of participants in the treatment group at post-treatment, with no group differences pre-treatment. Specifically, increased connectivity was shown between PCC and thalamus, as well as the subthreshold finding of increased PCC-cerebellum connectivity. The thalamus and the cerebellum have been identified as important subcortical hubs of the default network (Tomasi and Volkow 2011). Our findings of increased integrity of connections within the default network both augment and complement the increased connectivity shown within the hippocampal network.

The present results augment previously reported findings of increased fMRI activation and behavioral improvements after treatment with the mSMT (Chiaravalloti et al. 2012). Chiaravalloti et al. (2012) showed increased BOLD

activation in the treatment group during performance of a memory task in regions associated with memory and visual imagery (e.g., middle frontal gyrus, precentral gyrus, and inferior frontal gyrus, left and right middle temporal gyrus, superior parietal lobule, precuneus, posterior cingulate, and supramarginal gyrus), but not in the control group. Additionally, greater activity in right MFG was correlated with improved memory performance in the treatment group. The findings of the current study accord precisely with the fMRI activation results, insofar as increased resting-state functional connectivity was seen among networks critically involved with memory in the treatment group after treatment.

Whereas task-active fMRI results provide information about how brain regions are utilized during performance of a memory task, the results of functional connectivity analysis provide evidence for neural change that is apparent even beyond the context of task performance. That is, the findings of increased RSFC more generally show that treatment impacts the integrity of the very neural networks known to underlie memory functioning in healthy individuals (Buckner et al. 2008). Additionally, studies in healthy subjects show that changes in default network RSFC are brought about as a result of sleep (Sweet et al. 2010) and aerobic exercise (Voss et al. 2010). As such, there is accumulating evidence to suggest that the integrity of DN connectivity may serve as a proxy for overall cognitive well-being. This is particularly exciting because it provides the field with a new target for treatments and interventions, a target that has already been shown to be impacted by healthy behaviors.

The findings of the present study are encouraging in that they represent the first demonstration of increased neural connectivity in an MS sample subsequent to a behavioral intervention, although a limitation is the small sample size. Clearly, future research to extend these findings in a larger MS sample is warranted. Nonetheless, confidence in our findings is bolstered by an inspection of individual subject data which revealed that 7/7 treatment group participants showed expected patterns of increased connectivity subsequent to treatment.

Another limitation of the present study is the heterogeneity of patients in regard to disease subtype. Specifically, the composition of the treatment and non-treatment groups differed: treatment, 6/7 RR; non-treatment, 3/7 RR. Within the treatment group, those showing memory improvements were 4 RR, 1 PR. These findings leave us wondering whether relapsing-remitting patients are more likely to benefit from treatment. Disease subtype therefore represents a factor that should be examined in follow-up work. An additional consideration for future work will be the effects of the treatment on non-verbal memory, which was not assessed in the present study. Given that the intervention employs visual imagery as a strategy for effective encoding of verbal information, it would be interesting to test whether

treatment benefits are specific to verbal memory, or whether visual memory is impacted as well.

Finally, the results of the present study suggest that increases in RSFC within the brain's memory networks may serve as a precursor to behavioral change (i.e., improved memory) in MS patients, although this remains to be tested in future studies with larger samples and longitudinal follow-up. Roosendaal et al. (2010a) found altered hippocampal RSFC in persons with MS who had not yet elicited memory impairment, suggesting that such changes may precede cognitive decline. Although here we showed increased neural connectivity subsequent to treatment, the behavioral results did not reach the level of statistical significance. One possible explanation for this is the small sample size, a clear limitation of the present study. Another possibility is that neural change precedes behavioral change, as shown by Roosendaal et al. (2010a), albeit in the opposite direction. Finally, the generalizability of benefits of increased RSFC is an important area for future research. Quality of life, mood, and fatigue in persons with MS will likely be favorably impacted by a memory treatment shown to improve behavioral performance, increase neural activation, and increase connectivity among the neural networks subserving memory functions.

Acknowledgments This work was funded by National Institutes of Health grants (grant number R01 HD045798 and HD045798-S to N.D.C.); National Multiple Sclerosis Society (training grant-MB0003 to J.D.) and the Kessler Foundation.

References

- Benedict, R. H., Ramasamy, D., Munschauer, F., Weinstock-Guttman, B., & Zivadinov, R. (2009). Memory impairment in multiple sclerosis: correlation with deep grey matter and mesial temporal atrophy. *Journal of Neurology, Neurosurgery, and Psychiatry*, *80*(2), 201–206. doi:10.1136/jnnp.2008.148403.
- Bird, C. M., Capponi, C., King, J. A., Doeller, C. F., & Burgess, N. (2010). Establishing the boundaries: the hippocampal contribution to imagining scenes. *Journal of Neuroscience*, *30*(35), 11688–11695. doi:10.1523/JNEUROSCI.0723-10.2010.
- Biswal, B., Yetkin, F. Z., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic Resonance in Medicine*, *34*(4), 537–541.
- Biswal, B. B., Mennes, M., Zuo, X. N., Gohel, S., Kelly, C., Smith, S. M., et al. (2010). Toward discovery science of human brain function. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(10), 4734–4739. doi:10.1073/pnas.0911855107.
- Bonavita, S., Gallo, A., Sacco, R., Corte, M. D., Bisecco, A., Docimo, R., et al. (2011). Distributed changes in default-mode resting-state connectivity in multiple sclerosis. *Multiple Sclerosis*, *17*(4), 411–422. doi:10.1177/1352458510394609.
- Buckner, R. L., Andrews-Hanna, J. R., & Schacter, D. L. (2008). The brain's default network: anatomy, function, and relevance to disease. *Annals of the New York Academy of Sciences*, *1124*, 1–38. doi:10.1196/annals.1440.011.

- Chiaravalloti, N. D., & DeLuca, J. (2008). Cognitive impairment in multiple sclerosis. *Lancet Neurology*, 7(12), 1139–1151. doi:10.1016/S1474-4422(08)70259-X.
- Chiaravalloti, N. D., DeLuca, J., Moore, N. B., & Ricker, J. H. (2005). Treating learning impairments improves memory performance in multiple sclerosis: a randomized clinical trial. *Multiple Sclerosis*, 11(1), 58–68.
- Chiaravalloti, N. D., Balzano, J., Moore, N. B., & DeLuca, J. (2009). The Open-Trial Selective Reminding Test (OT-SRT) as a tool for the assessment of learning and memory. *Clinical Neuropsychology*, 23(2), 231–254. doi:10.1080/13854040802121158.
- Chiaravalloti, N. D., Wylie, G., Leavitt, V., & DeLuca, J. (2012). Increased cerebral activation after behavioral treatment for memory deficits in MS. *Journal of Neurology*. doi:10.1007/s00415-011-6353-x.
- Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research*, 29(3), 162–173.
- Delis, D. C., Kramer, J. H., Kaplan, E., & Ober, B. A. (2000). *California Verbal Learning Test—Second Edition (CVLT-II)*. San Antonio, TX.
- Delis, D. C., Kramer, J. H., Kaplan, E., & Holdnack, J. (2004). Reliability and validity of the Delis-Kaplan Executive Function System: an update. *Journal of International Neuropsychological Society*, 10(2), 301–303. doi:10.1017/S1355617704102191.
- DeLuca, J., & Nocentini, U. (2011). Neuropsychological, medical and rehabilitative management of persons with multiple sclerosis. *Neurorehabilitation*, 29(3), 197–219. doi:10.3233/NRE-2011-0695.
- Filippi, M., Riccitelli, G., Mattioli, F., Capra, R., Stampatori, C., Pagani, E., et al. (2012). Multiple Sclerosis : Effects of Cognitive Rehabilitation on Structural and Functional MR Imaging Measures—An Explorative Study. *Radiology*, 262(3):932–940.
- Flavia, M., Stampatori, C., Zanotti, D., Parrinello, G., & Capra, R. (2010). Efficacy and specificity of intensive cognitive rehabilitation of attention and executive functions in multiple sclerosis. *Journal of Neurological Sciences*, 288(1–2), 101–105. doi:10.1016/j.jns.2009.09.024.
- Fox, M. D., & Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nature Reviews Neuroscience*, 8(9), 700–711. doi:10.1038/nrn2201.
- Fransson, P., & Marrelec, G. (2008). The precuneus/posterior cingulate cortex plays a pivotal role in the default mode network: Evidence from a partial correlation network analysis. *NeuroImage*, 42(3), 1178–1184. doi:10.1016/j.neuroimage.2008.05.059.
- Greicius, M. D., Krasnow, B., Reiss, A. L., & Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, 100(1), 253–258.
- Greicius, M. D., Supekar, K., Menon, V., & Dougherty, R. F. (2009). Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cerebral Cortex*, 19(1), 72–78. doi:10.1093/cercor/bhn059.
- Hillary, F. G., Slocumb, J., Hills, E. C., Fitzpatrick, N. M., Medaglia, J. D., Wang, J., et al. (2011). Changes in resting connectivity during recovery from severe traumatic brain injury. *International Journal of Psychophysiology*, 82(1), 115–123. doi:10.1016/j.ijpsycho.2011.03.011.
- Jonsson, A., Korfitzen, E. M., Heltberg, A., Ravnborg, M. H., & Byskov-Ottosen, E. (1993). Effects of neuropsychological treatment in patients with multiple sclerosis. *Acta Neurologica Scandinavica*, 88(6), 394–400.
- McDonald, W. I., Compston, A., Edan, G., Goodkin, D., Hartung, H. P., Lublin, F. D., et al. (2001). Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Annals of Neurology*, 50(1), 121–127.
- Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, 9(1), 97–113.
- Rocca, M. A., Valsasina, P., Absinta, M., Riccitelli, G., Rodegher, M. E., Misci, P., et al. (2010). Default-mode network dysfunction and cognitive impairment in progressive MS. *Neurology*, 74(16), 1252–1259. doi:10.1212/WNL.0b013e3181d9ed91.
- Roosendaal, S. D., Hulst, H. E., Vrenken, H., Feenstra, H. E., Castelijns, J. A., Pouwels, P. J., et al. (2010a). Structural and functional hippocampal changes in multiple sclerosis patients with intact memory function. *Radiology*, 255(2), 595–604. doi:10.1148/radiol.10091433.
- Roosendaal, S. D., Schoonheim, M. M., Hulst, H. E., Sanz-Arigita, E. J., Smith, S. M., Geurts, J. J., et al. (2010b). Resting state networks change in clinically isolated syndrome. *Brain*, 133(Pt 6), 1612–1621. doi:10.1093/brain/awq058.
- Sicotte, N. L., Kern, K. C., Giessler, B. S., Arshanapalli, A., Schultz, A., Montag, M., et al. (2008). Regional hippocampal atrophy in multiple sclerosis. *Brain*, 131(Pt 4), 1134–1141. doi:10.1093/brain/awn030.
- Smith, A. (1982). *Symbol digit modalities test manual*. Los Angeles: Western Psychological Services.
- Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E., Johansen-Berg, H., et al. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, 23(Suppl 1), S208–219. doi:10.1016/j.neuroimage.2004.07.051.
- Solari, A., Motta, A., Mendozzi, L., Pucci, E., Forni, M., Mancardi, G., et al. (2004). Computer-aided retraining of memory and attention in people with multiple sclerosis: a randomized, double-blind controlled trial. *Journal of Neurological Sciences*, 222(1–2), 99–104. doi:10.1016/j.jns.2004.04.027.
- Strauss, E., Sherman, E. M. S., & Spreen, O. (2006). *A compendium of neuropsychological tests: Administration, norms, and commentary*. Oxford: Oxford University Press.
- Sumowski, J. F., Wylie, G., Leavitt, V. M., Chiaravalloti, N., & DeLuca, J. (2012). Default network activity is a sensitive and specific biomarker of memory in MS. *Multiple Sclerosis*. doi:10.1177/1352458512448267.
- Sweet, L. H., Jerskey, B. A., & Aloia, M. S. (2010). Default network response to a working memory challenge after withdrawal of continuous positive airway pressure treatment for obstructive sleep apnea. *Brain Imaging and Behavior*, 4(2), 155–163. doi:10.1007/s11682-010-9095-y.
- Tomasi, D., & Volkow, N. D. (2011). Functional connectivity hubs in the human brain. *NeuroImage*, 57(3), 908–917. doi:10.1016/j.neuroimage.2011.05.024.
- Voss, M. W., Prakash, R. S., Erickson, K. I., Basak, C., Chaddock, L., Kim, J. S., et al. (2010). Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. *Frontiers in Aging Neuroscience*, 2. doi:10.3389/fnagi.2010.00032.