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RESEARCH ARTICLE

Up-regulation of DMN Connectivity in Mild Cognitive Impairment *Via* Network-based Cognitive Training

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Abstract: ***Background:*** Previous work designed a network-based protocol of cognitive training. This programme exploits a mechanism of induced task-oriented co-activation of multiple regions that are part of the default mode network (DMN), to induce functional rewiring and increased functional connectivity within this network.

Objective: In this study, the programme was administered to patients with a diagnosis of mild cognitive impairment to test its effects in a clinical sample.

Method: Twenty-three patients with mild cognitive impairment (mean age: 73.74 years, standard deviation 5.13, female/male ratio 13/10) allocated to the experimental condition, underwent one month of computerised training, while fourteen patients (mean age: 73.14 years, standard deviation 6.16, female/male ratio 7/7) assigned to the control condition underwent a regime of intense social engagement. Patients were in the prodromal stage of Alzheimer's disease (AD) as confirmed by clinical follow ups for at least two years. The DMN was computed at baseline and retest, together with other, control patterns of connectivity, grey matter maps and neuropsychological profiles.

Results: A condition-by-timepoint interaction indicating increased connectivity triggered by the programme was found in left parietal DMN regions. No decreases as well as no changes in the other networks or morphology were found. Although between-condition cognitive changes did not reach statistical significance, they correlated positively with changes in DMN connectivity in the left parietal region, supporting the hypothesis that parietal changes were beneficial.

Conclusion: This programme of cognitive training up-regulates a pattern of connectivity which is pathologically down-regulated in AD. We argue that, when cognitive interventions are conceptualised as tools to induce co-activation repeatedly, they can lead to clinically relevant improvements in brain functioning, and can be of aid in support of pharmacological and other interventions in the earliest stages of AD.

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1. INTRODUCTION

When Alzheimer's disease (AD) starts inducing the earliest measurable changes in neuropsychological functioning, it goes through a phase of mild cognitive impairment (MCI). At this stage, a patient has subjective cognitive complaints and shows objective cognitive impairment, but is not demented and still retains independence in daily life activities [1]. Although MCI patients with an amnestic presentation are those who will more likely evolve to a dementia of the AD type, non amnestic patients can also progress to AD dementia [2, 3]. A number of changes are visible in the structural and functional architecture of patients who experience either MCI due to AD, or MCI due to other aetiologies. Several studies have characterised the neural changes which affect various sub-types of MCI, including patterns of volumetric decrements [4-6], alteration of white matter fibre microstructure [7], and glucose metabolism [8]. Within this picture, disruption of network haemodynamics has also been described in MCI, with the default mode network (DMN) being a heavily affected major pathway [9]. This network is normally activated when a person engages in spontaneous, self-projecting mental activities such as autobiographical retrieval, anticipation of the future, spatial navigation, mental imaging and theory of mind [10], and, as proposed by other

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authors, in support of semantic and conceptual processing [11, 12]. The modifications seen in the intrinsic functional connectivity of the DMN seem to be of diverse nature, with studies reporting decline in the connectivity of the main DMN hubs [13, 14], and parallel research documenting increases in DMN connectivity as well that are interpreted as the result of either compensatory [15], or maladaptive processes [16].

Numerous studies have demonstrated that the integrity of the DMN is associated with optimal cognitive performance [17-20]. The DMN must in fact de-activate to allow the system to set up a task-based activation, and this mechanism is progressively impaired in the continuum between healthy ageing and AD [21]. As a consequence, an intervention that could re-establish homeostasis in connectivity of the DMN might translate into cognitive benefits, and might thus represent a potential avenue of treatment in MCI and in early stage AD dementia. On this note, this disease stage would be an ideal diagnostic window for an intervention to be put forward. In fact, patients with MCI still retain capacity for major changes in brain function triggered by cognitive interventions [22, 23].

A previous publication described a programme of cognitive exercises specifically designed to regulate functional connectivity of the DMN, and, after testing its effects in a sample of healthy adults (mean age: 66 years), it found significant increases in the posterior portion of the DMN [24]. The mechanism by which network regulation was pursued was a long-lasting task-induced co-activation of multiple DMN regions. Resting state functional connectivity shown by a pattern of regions would result from habitual co-activation during goal-directed brain function [25]. Based on this, if an experimental manipulation were able to induce recurring task-based co-activation of multiple DMN regions, this would translate into increased connectivity in the DMN. This, in turn, should lead to improved cognitive function, as a more stable connectivity within the DMN would result into a more adequate de-activation of this network at the moment of engaging in an externally directed task. Evidence has shown, in fact, that a more profound de-activation of the DMN is needed when task demands increase [26].

We applied this network-based cognitive training approach in a sample of patients who received a diagnosis of MCI for the first time. We hypothesised that: 1) regional increases in DMN connectivity would be observed as a result of the cognitive training protocol; 2) these modifications would not be generalised to other pathways of connectivity; 3) based on changes observed in patients' cognitive functioning, increased DMN connectivity would be associated with compensatory, not maladaptive mechanisms.

2. MATERIALS AND METHODS

2.1. Participants

Forty nine participants were enrolled in this study. All individuals were inhabitants of the Venetian archipelago, and had been referred to their first neurological assessment by their general practitioner because of suspected cognitive decline of neurodegenerative nature. Initial neurological and neuropsychological examinations were carried out as part of the clinical pipeline to establish that each candidate met inclusion and exclusion criteria. The former were based on a diagnosis of MCI according to Petersen's criteria, and a clinical profile indicating prodromal AD [1, 27]. Importantly, all participants were independent in their daily life activities. The latter were instead set as follows: diagnostic entities of clinical concern, chronic or acute cerebrovascular disease as main aetiology, history of transient ischaemic attacks, presence of uncontrolled brain seizures, peptic ulcer, cardiovascular disease, sick-sinus syndrome, neuropathy with conduction difficulties, proof of abnormal levels of folates, vitamin B12 or thyroid-stimulating hormone, significant neuropsychiatric symptoms, treatment with memantine/cholinesterase inhibitors, or medication for research purposes or with toxic effects to internal organs. Moreover, participants with significant disabilities as ascertained by evaluation of activities of daily living and instrumental activities of daily living assessment at the time of clinical profiling, or with a structural magnetic resonance imaging (MRI) scan indicating a major diagnostic category of non neurodegenerative nature that could otherwise explain the presence of cognitive symptoms were not considered for recruitment. As part of the study requirements, all participants were on stable medication for the duration of the experimental procedure.

Through blocked group allocation, twenty five patients with MCI were included in the experimental condition. Two of these patients did not complete the study, resulting in a final sample of 23. The remaining were assigned to the control condition. Ten of these patients, however, did not complete the study, as they were either lost to follow up or withdrew consent for the retest procedures. There were, therefore, fourteen patients in the final control condition sample. As shown in Tables **1** and **2**, no differences in demographic, neurostructural and cognitive variables were visible at baseline (see Section 2.5 for methodological details on the computation of these neurostructural indices). Apolipoprotein E genotypes were available for 32/37 participants. No differences in the proportion of ε_4 carriers existed between the two conditions. Also, no between-group differences in grey matter or white matter were found, as computed with voxel based morphometry (see Section 2.5 for methodological details). Furthermore, the Lesion Segmentation Tool [28] was used to extract the global load (expressed in ml) of periventricular and deep white matter hyperintensities. This was in turn converted into a percentage of the global intracranial volume, as carried out in a recent study [29]. No difference was found between the two conditions.

2.2. Experimental Manipulation

Network connectivity was stimulated with an intense programme of computerised exercises. A comprehensive description of the package can be found elsewhere [24]. Briefly, participants were asked to engage in exercises in which multiple cognitive operations were requested. These included retrieval from memory, management of interference, inhibition, working memory and logical reasoning (*e.g.,* Fig. **1**). In order to complete such tasks, the system has to rely on multiple brain regions to meet the request of all cognitive aspects. Co-activation of multiple areas, persistently demanded by the tasks day after day, would stimulate

Table 1. Demographic and neurostructural characterisation of the sample.

Variable	Experimental Condition	Control Condition	*p*
Demographic Characteristics			
Age (years)	73.74 (5.13)	73.14 (6.16)	n.s.
Education (years)	8.70 (3.69)	10.50 (5.30)	n.s.
Gender (f/m)	13/10	7/7	n.s.
ApoE Genotype			
(ε_4 carriers/non carriers/missing genotype)	14/8/2	7/3/3	n.s.
Neurostructural Volumes (ml)			
Left Hippocampus	2.30 (0.39)	2.18 (0.32)	n.s.
Right Hippocampus	2.36 (0.39)	2.21 (0.46)	n.s.
Grey Matter	654.15 (66.96)	651.30 (67.07)	n.s.
White Matter	492.78 (53.96)	487.68 (57.84)	n.s.
Total Intracranial	1500.64 (155.39)	1491.90 (158.89)	n.s.
Tissue Ratios			
Grey Matter	0.44 (0.01)	0.44 (0.01)	n.s.
White Matter	0.33 (0.01)	0.33 (0.01)	n.s.

Means and standard deviations are indicated for all variables (apart from gender). *Mann-Whitney U* tests were run to compare the two groups for age and education levels. A *chi-squared* test was run to compare the two gender ratios and genotype distributions. Since neurostructural volumes and ratios distributed normally, *independent-sample t* tests were used. n.s.: not significant.

Table 2. Neuropsychological characterisation of the sample.

Test	Experimental Condition				Control Condition				*p* Baseline Differences	Median Change Score		*p* Treatment Effect
	Baseline		Retest		Baseline		Retest			Experimental Condition	Control Condition	
	Median	IR	Median	IR	Median	IR	Median	IR				
Mini Mental State Examination	27	25-29	28	25-29	27	24-28.25	27	24.25-29	n.s.	+1	0	n.s.
Raven Progressive Matrices*	26	23-30	28	22-33	28.5	24-31.25	27	20.75-33.25	n.s.	0	0	n.s.
Letter Fluency Test*	28	20-35	28	20-35	30.5	23.5-37.25	30	24.5-36.5	n.s.	0	0	n.s.
Category Fluency Test*	28	22-37	29	24-36	27	22.5-33.25	29.5	23-38.5	n.s.	-1	+3	n.s.
Digit Cancellation Test*	48	39-54	49	45-54	49	42.75-53.5	47	43.5-55.25	n.s.	+3	+0.5	n.s.
WAIS – Similarities	19	16-23	19	15-23	19.5	14.5-22.25	18	11.5-23.25	n.s.	-1	-1	n.s.
Token Test	33.5	32-35.5	33	31-35	34	30.75-36	34	30.875-35.25	n.s.	0	-1	n.s.
Rey-Osterrieth Figure – Copy*	28.5	25.5-34	31	28-34	29.75	26-33.25	27.75	21.75-33.25	n.s.	+1	-0.25	n.s.
Rey-Osterrieth Figure – Recall*	8	4-12.5	9.5	7.5-15	10.25	5.375-12.5	9	5-13.25	n.s.	+2.5	-0.25	n.s.

(Table 2) contd….

Test	Experimental Condition				Control Condition				*p* Baseline Differences	Median Change Score		*p* Treatment Effect
	Baseline		Retest		Baseline		Retest			Experimental Condition	Control Condition	
	Median	IR	Median	IR	Median	IR	Median	IR				
Stroop Test - Time Interference*	34.5	21-59.5	29.5	18.25-64	38.5	20.5-47	30.25	20.25-49.625	n.s.	+2.5	+0.5	n.s.
Stroop Test - Error Interference*	1	0-6	1	0-4.25	1	0.75-7.25	1.75	0-7	n.s.	0	0	n.s.
Digit Span – Forward	6	5-6	6	5-6	5	5-6	5	5-6	n.s.	0	0	n.s.
Digit Span – Backwards	4	3-4	4	3-4	3	3-4	4	3-4.25	n.s.	0	+1	n.s.
Corsi Block Tapping Test	4	4-5	4	4-5	4	3.75-4	4	4-4	n.s.	0	0	n.s.
Prose Memory Test – Immediate	7	4-8	8	5-10	7	1.75-8.75	6	3.75-9	n.s.	+1	0.5	n.s.
Prose Memory Test – Delayed	7	3-11	9	6-10	6.5	2-10.5	6	1.75-13.25	n.s.	+1	0	n.s.
Paired Associates Test	8.5	6-10.5	9	6.5-11.5	10.5	6.75-13	10.25	5.875-14	n.s.	+0.5	0	n.s.
Confrontation Naming Test	18	17-20	19	19-20	18.5	17.75-20	20	19-20	n.s.	0	+1	n.s.

Mann-Whitney U tests were run to test for between-condition baseline differences in cognitive levels, and changes exerted by the design procedure. The battery of neuropsychological tests included measures of various cognitive domains, with specific focus on the aspects most profoundly affected by AD. The dataset contained four missing entries: Token Test: Control-Condition Baseline (1 datapoint), Stroop Test –Time Interference: Experimental Condition Retest (1 datapoint), Stroop Test –Error Interference: Experimental Condition Retest (1 datapoint), and Paired Associates: Control-Condition Baseline (1 datapoint). "n.s.": "not significant"; "IR": "Inter-quartile Range". Medians and interquartile ranges are reported due to the skewness normally seen in the distribution of cognitive scores. *indicates variance > 15 and range of score > 17.

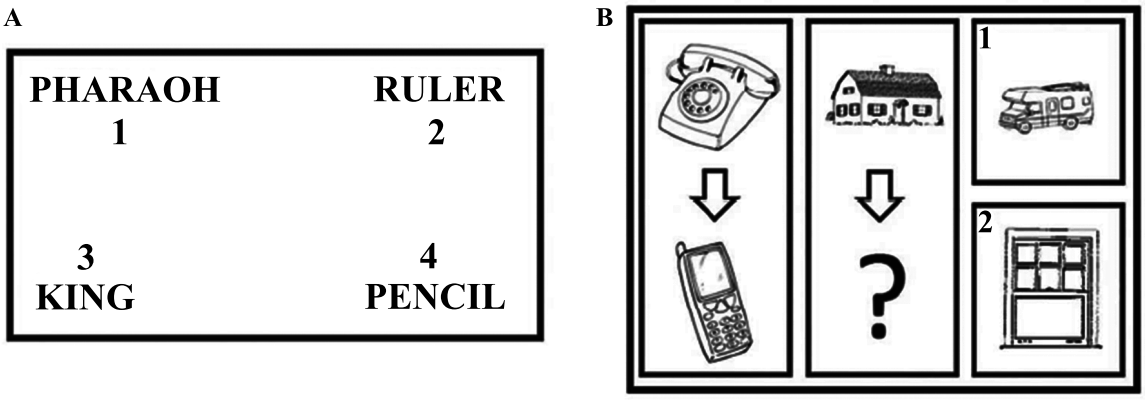

Fig. (1). Examples of tasks included in the protocol of cognitive stimulation. The task on the left (**A**) is a possible trial in English based on a mechanism of semantic interference. Four words are presented on screen and the odd one has to be selected out. The word "RULER" has two meanings. It either refers to a measuring rod, or to a person/entity who has the power. The only way to establish the alien word is to understand which of the two meanings allows the creation of a cluster of three words semantically consistent with one another. In this case the three words would be "KING", "PHARAOH" and "RULER", and, as a consequence, "PENCIL" would have to be selected as the response. This task entails mechanisms of semantic memory search (particularly visible for words with a lower frequency of use, such as "MURDER" referring to a flock of crows rather than a crime, or "MANDARIN" intended as the language spoken in China rather than the fruit), working memory to manipulate information online, and shift between the two meanings. The task on the right (**B**) is based on a series of cognitive steps. To begin with, a semantic relationship has to be extrapolated from the sequence of images on the left hand-side ("TELEPHONE" and "MOBILE PHONE"). This relationship has then to be transposed to the sequence located in the middle, where one of the two figures is missing and substituted with a question mark. Both of the two images on the right hand-side ("CAMPING VAN" and "WINDOW") are semantically related to the element included in the middle sequence ("HOUSE"), but only one would allow the resulting sequence to embody the same semantic relationship as the sequence on the left hand-side. In this case the answer is "CAMPING VAN", as it is a "portable version" of a "HOUSE", as a "MOBILE PHONE" is a portable version of a "PHONE". Engaging in this task would involve semantic processing, logical reasoning and abstract reasoning.

functional rewiring among the co-activated regions. Via voxel-based correlation methods, it had been demonstrated that the variability in performance levels on these tasks was associated with the main hubs of the posterior DMN [24]. Comparable analyses carried out on this sample confirmed this pattern. The strongest correlates were found in the medial prefrontal cortex, in the lateral temporal cortex and in the mediotemporal lobe, three computational foci of the DMN (see Supplementary Material for a detailed description of procedure and results).

The patients included in the experimental condition completed the entire set of 20 sessions in 20 to 35 days, typically 5 sessions a week, from Monday to Friday. Their compliance rate was on average 19.61 sessions out of 20 (standard deviation = 1.61, 451 sessions completed out of the entire set of 460 sessions). Each session was completed in 60 to 90 minutes depending on individual processing speed.

As part of the experimental design, all patients included in the control condition maintained a daily regime of intense social interactions (*e.g.,* volunteering, tour guiding, attending a club, or gardening, according to personal interests) as part of their daily-life activities of similar duration as the experimental condition. By doing so, this group of patients was exposed to an active control condition, a methodological factor of utmost importance [30]. At the same time, these patients were not requested to commit to an intense intervention based on no valid hypothesis. Compliance was then verified and confirmed for each patient during the retest clinical procedures.

2.3. Cognitive Testing

The neuropsychological examination completed prior to enrolment was also used as part of the experimental design. The battery included tests of short-term and long-term memory, lexical-semantic abilities, attention, executive functions and visuoconstructional skills (Table **2**). For the purpose of sample characterisation, baseline raw scores were compared with reference values. These were obtained from a sample of healthy participants recruited based on the same exclusion criteria, who did not meet criteria for MCI. This second sample (25 adults, 8 men) was of comparable age (mean = 71.40 years, standard deviation = 4.07, range = 65-80) and educational attainment (mean = 11.56 years, standard deviation = 5.16, range = 5-27) as the sample of 37 MCI patients (*Mann Whitney U* statistics $p > 0.05$). Presence of cognitive impairment was operationalised as a score < 1.5 standard deviations from the reference average (a > 1.5 standard-deviation threshold was instead applied to the Stroop Test). This operationalisation was preferred over the use of normative data because normative data were only available for part of the tests, and also to align sample definition to international guidelines for MCI [1]. Based on this classification, MCI patients distributed as follows: 21 amnestic multiple-domain, 1 amnestic single-domain, 8 non amnestic multiple-domain, 7 non amnestic single-domain. Frequency of domain type and domain number did not differ between the two conditions (c*hi-squared* tests $p > 0.05$).

Neuropsychological assessment was repeated for all participants at the end of the study.

2.4. MRI Acquisition

An MRI protocol inclusive of structural and functional sequences was acquired on a 1.5 T Philips Achieva system. T1-weighted, T2-weighted and FLAIR acquisitions were inspected by a senior neuroradiologist to rule out differential exclusion criteria (as per Section 2.1). T1-weighted images were also processed to investigate macro-anatomical changes induced by the experimental procedure. For this purpose, acquisitions details were as follows: sequence type: Turbo Field Echo 3D, repetition time: 7.4 ms, echo delay time: 3.4 ms, flip angle: 8°, voxel dimension 1.1 × 1.1 × 0.6 mm, field of view: 250 mm, matrix size 256 × 256 × 124.

After re-establishing the electromagnetic equilibrium via 20 s of dummy scans, resting state echo-planar functional MRI sequences were acquired in two runs of 120 volumes each. The specifics were set as follows: number of slices: 20, volume acquisition details: axial orientation, bottom-up, contiguous and gapless, repetition time: 2 s, echo delay time: 50 ms, flip angle: 90°, voxel dimensions: 3.28 × 3.28 × 6.00 mm, field of view: 230 mm.

The MRI procedures were completed at baseline and at the end of the study.

2.5. MRI Processing

The entire pipeline was completed with Statistical Parametric Mapping (SPM) 8 and toolboxes, running in Matlab R2011b (Mathworks Inc., UK).

Functional runs were pre-processed using a standard routine. Scans were initially slice-timed to homogenise the slice-to-slice temporal displacement. Then, a spatial realignment was carried out on each single run, and the output of this step was carefully examined to identify individuals with excessive in-scanner motion. A graphic representation of translational and rotational movements was visualised in SPM, and problematic movements were defined as those in excess of 3 mm or 1.5° rotation [31]. Three runs were flagged up as problematic, and were thus reduced in their initial/terminal segment to remove the affected volumes (no sequence showed excessive movements in its central section). After that, all images were normalised and registered on the SPM echo-planar template. Following normalisation, a band-pass temporal filter (0.008 to 0.1 Hz) was applied to maximise neurogenic sources of variability of the BOLD signal and minimise the impact of cardiorespiratory rhythms (which are faster than 0.1 Hz) and scanner drift (having instead a very slow rhythmicity). Finally, all scans were smoothed with a 6 mm full-width-at half-maximum Gaussian kernel.

The estimation of the DMN was carried out by means of an independent component analysis [32], implemented by the GIFT toolbox (v1.3i; mialab.mrn.org/software/gift). Briefly, this method allows the separation of independent sources of variability within the BOLD signal, and identifies latent variables (components) that show intrinsic functional connectivity, and are thus interpreted as brain networks. At the same time, this technique contributes to the separation of signal and noise-based components [33]. Thirty seven (participants) by two (timepoints) runs were included, and the

number of components to estimate was set at 20 [34]. Following agreement between two independent raters, the DMN component was identified. Three control networks were also considered: the left and right fronto-parietal networks (lFPN and rFPN), which sustain executive processing [35], and the non cognitive visual network (VN). This last network was preferred over the sensorimotor network because recent evidence suggests that mechanical properties of fine-grained motion are disrupted in AD [36]. All networks are illustrated in Fig. (**2**).

To investigate structural changes in the network territories, T1-weighted images were pre- and post-processed following voxel-based morphometry procedures [37]. Briefly, grey matter and white matter maps were separated from other tissue classes via probabilistic segmentation, using the *"new segment"* option, and the DARTEL routine was then used first to create a template using all 74 (37 baseline and 37 retest) maps, and then to register each volume to the template via flow-field deformations [38].

A set of analyses was run on an additional group of structural indices to ascertain the absence of significant anatomical differences between the two groups enrolled in each condition (Table **1**). Hippocampal segmentation was carried out for baseline scans using STEPS, a pipeline that exploits multiple templates [39]. Native-space global volumes of grey matter, white matter and cerebrospinal fluid were extracted using the "*get_totals"* script for the calculation of total intracranial volumes and tissue ratios. Similarly, STEPS-based hippocampal volume was also computed.

2.6. Inferential Modelling

One-sample t test models were initially run to identify the regional contour of the four networks. To do this, all 74 maps were included in these four models. The effect of the cognitive training programme on network connectivity (first hypothesis) was tested with mixed-design full-factorial scripts modelling the condition-by-timepoint interaction (increases in connectivity seen in the experimental condition net of the increases seen in the control condition). The "inverse interaction" contrast (exclusive increases seen in the control condition) was also tested, as a methodological control. To test the possibility of a "network transfer" (second hypothesis), we modelled the effect of the interaction in the pattern of VN connectivity, for which no change was expected because of its low-order, perceptual function, as well as in the lFPN and rFPN that, albeit of cognitive relevance, were not specifically stimulated by the intervention. Each model was spatially constrained to the network 3D space of reference computed with the *one-sample t* test. The effect of the interaction was also inferred in the volumetric grey matter and white matter maps of the four networks. The third hypothesis was tested at *post hoc*, based on its reliance on the main pattern of results.

Results of all *a priori* and *post hoc* analyses were considered significant when surviving a Family-Wise-Error (FWE) corrected $p < 0.05$ at a cluster level (an uncorrected set level $p < 0.01$ threshold was chosen). All Montreal Neurological Institute (MNI) coordinates were transposed into Talairach space through a non linear transform (http://imaging.mrc-

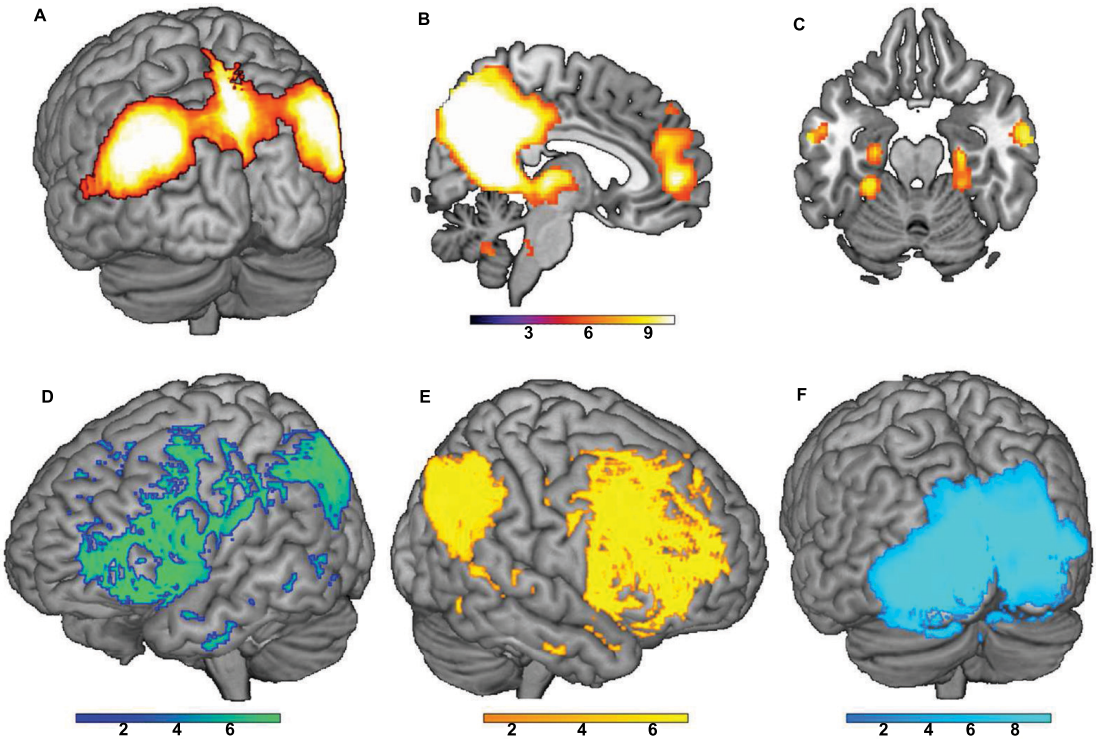

Fig. (2). The four networks investigated in this study as modelled by a *one-sample t* test. The DMN is shown in the upper half (**A-C**). The top-middle sagittal slice (**B**; MNI coordinate: x = 5) shows the inclusion of postero-medial and medial prefrontal regions, whereas the top-right axial slice (**C**; MNI coordinate: z = -17) depicts the involvement of the lateral temporal cortex and the hippocampal complex. The three control networks are shown in the lower half (**D-F**; left to right: lFPN, rFPN, VN). The colour bars indicate the *z* statistics within each component.

cbu.cam.ac.uk/downloads/MNI2tal/mni2tal-m), and were then interpreted using the Talairach Daemon Client [40].

Individual change scores were calculated for each cognitive scores, subtracting baseline from retest performance (positive scores indicated improvement; for the Stroop Test, negative scores indicated improvement). A between-group *Mann-Whitney U* test was then run to compare change scores between conditions. A Bonferroni correction was applied to control for the number of statistical comparisons (threshold $p = 0.003$).

3. RESULTS

A significant effect of the interaction was found in left superior and inferior parietal regions within the pattern of DMN connectivity (Table **3** and Fig. **3**). This interaction was further explored with *post hoc paired-sample t* tests aimed at inferring the test-retest changes in each of the two conditions. Increased DMN connectivity was found for patients assigned to the experimental condition in a midline cluster extending to precuneus and cuneus. As for the control condition, decreased connectivity was found in right and left parietal cortices (all results were FWE corrected). Although this latter result emerged from a model independent from that designed *a priori*, it overlapped to a great extent with the main cluster surviving the interaction contrast. To clarify the effect of the two conditions on the core of this region, the average *z* score was extracted from a binarised mask of the cluster. The experimental condition was associated with an average increase of 0.18 *z* scores, and the control condition was associated with an average decrease of 0.63 *z* scores (mixed-design *ANOVA*'s $F_{1, 35} = 15.08$, $p < 0.001$).

No significant findings were obtained in the analyses of VN, lFPN or rFPN connectivity, nor from the testing of the inverse interaction contrasts. Also, morphometric analyses revealed no structural modifications triggered by the training in any of the network contours. No significant change emerged from the analyses of cognitive scores.

To gain insight on whether the modification in the DMN triggered by the training were beneficial or detrimental (third hypothesis), the association between DMN *z* baseline-to-retest change scores and an index of cognitive change was investigated *a posteriori*. In order to do so, a composite measure was computed based on those tests for which baseline-to-retest changes were characterised by a sufficient degree of variability (*i.e.,* variance > 15, range of scores > 17, indicated by an asterisk in Table **2**), and could, therefore, track down baseline-to-retest change in a satisfactory way. This composite score covered the main aspects of cognition affected in neurodegeneration: long-term memory, executive functioning, visuoconstructional abilities, lexical-semantic processing and abstract reasoning. Each individual score was converted into a ratio by fractionating it by the maximum score obtained on that test at a group level either at baseline or at retest. A global individual index was then computed by averaging all the ratios. Despite a trend in the expected direction, no difference was found for this composite change ratio between the two conditions (*independent-sample* $t_{35} = 1.525$; $p = 0.136$). Since the assumption of normality was not breached for any of the two variables (*Kolmogorov-Smirnov* test of normality carried out on the distribution of standardised residuals: both p values > 0.05) linear parametric models were carried out to study the association between the parietal DMN *z* change score and the cognitive change ratio. The bivariate correlation was significant (*Pearson's* $r = 0.385$; $p = 0.019$), and so was the coefficient of partial correlation, after controlling for age, years of education, and Mini Mental State Examination score at baseline (*Pearson's* $r = 0.409$; $p = 0.016$), indicating a positive association between increase in connectivity and increase in cognitive performance. The coefficients of correlation were not significant when separate models were run in each condition.

4. DISCUSSION

This study investigated the impact of a relatively brief but intensive network-based cognitive training focussed on the DMN and based on the induced task-directed co-activation of multiple DMN regions. The construct validity of this programme had been tested in a previous study in which significant associations existed between task performance and DMN regions [24], and was confirmed in this study. Moreover, healthy seniors had shown increases in DMN connectivity, especially in parietal regions [24]. In this study patients diagnosed for the first time with MCI but highly suggestive of prodromal AD were recruited and allocated either to network-based cognitive training, or to a control condition consisting of daily activities marked by intense social engagement, but not involving computer-based training. The findings of the interaction effect indicate that the experimental condition led to up-regulation of the DMN in left parietal regions, supporting our first hypothesis. Since progression along the axis of AD neurodegeneration generates a gradual loss of functional connectivity in these pathways [13, 14], an intervention-dependent increase is a finding that deserves clinical attention.

No down-regulation and no changes in other patterns of functional connectivity were found from the other interaction contrasts. This supports the conclusion that the observed changes were specific and did not extend or transfer to other patterns of connectivity, in support of our second hypothesis.

We then broke down the significant effect of the interaction. We found that the increases in the experimental condition were milder than the decreases found in the control condition. This would be due to the influence of a sub-group of patients who showed limited or no overt response to treatment, as normally found in the study of other types of intervention, although there were no decreases as would be expected in a system affected by a progressive neurodegenerative condition. We also tried to understand whether these changes in connectivity could reflect a compensatory or maladaptive mechanism. A positive association was found between change in DMN connectivity and change in cognitive functioning, indicating a finding consistent with a beneficial compensatory mechanism. Our third hypothesis, however, was only partially supported by these findings, because the change in cognitive functioning did not differ significantly between the two conditions ($p = 0.136$), and because the correlation between change in connectivity and change in cognitive function was only found when the two conditions were combined in a single analysis. Although the cognitive improvement was just a trend in the expected direction, we

Table 3. Effect of treatment as inferred by group-by-timepoint interaction models and *post hoc* group comparisons.

Cluster Number	Cluster Extent (voxels)	Hemisphere	Brodmann Area	Peak *Z* Score at Half-Maximum	Talairach Coordinates		
					x	y	z
Up-regulation of the DMN - Effect of the Interaction							
1	113	L	7	3.33	-30	-62	44
		L	7	2.81	-32	-69	48
		L	40	2.74	-36	-54	43
Up-regulation of the DMN – Post Hoc: Increases in the Experimental Condition							
1	95	L	7	3.64	-6	-70	29
		R	7	2.80	6	-72	31
Up-regulation of the DMN – Post Hoc: Decreases in the Control Condition							
1	160	R	39	3.73	40	-58	38
		R	19	3.36	44	-70	44
2	132	L	39	3.66	-34	-60	38
		L	39	3.26	-38	-63	20
		L	7	2.90	-30	-63	53

Significant findings emerged from the inferential models testing the condition-by-timepoint interaction on the three patterns of connectivity, and *post hoc* models testing longitudinal changes in the two conditions.

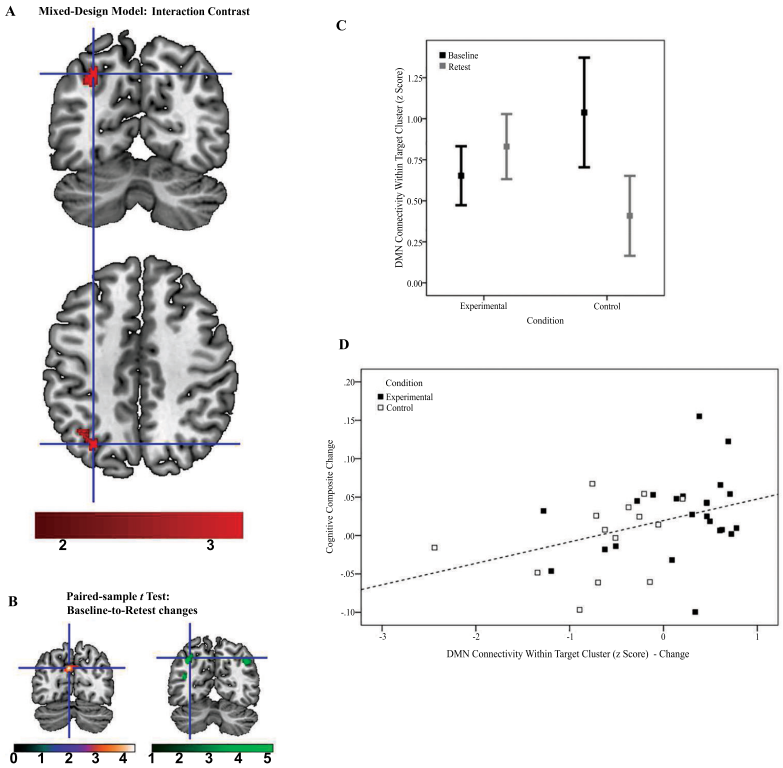

Fig. (3). Effect of the interaction contrast indicating up-regulation of DMN connectivity triggered by the experimental condition (**A**). Precuneal-cuneal increase in the experimental group and bilateral parietal decrease in the control condition (**B**) as emerged from *post hoc paired-sample t* tests of DMN connectivity. The colour bar indicates the *z* statistics. The bar graph shows the DMN *z* score averaged within the entire cluster. Means and 95% confidence intervals are illustrated (**C**). The longitudinal change was associated with changes in cognitive performance, measured as a composite score. The linear association is reported with a dotted line (**D**).

argue that this is most likely due to insufficient power and the fact that a longer exposure to these tasks would be required in MCI and early AD patients to trigger neural changes sufficiently large to generate increases measurable with clinical tests. These instruments, in fact, are not always sufficiently sensitive for capturing test-retest changes. For instance, in this sample, change scores for the verbal and visuospatial short-term memory spans ranged between -2 and +2, making these two tests highly unsuitable for capturing individual longitudinal changes. Other tests had a similarly limited variability (*e.g.,* change scores in the delayed recall of the Prose Memory Test only distributed throughout 13 integers, for a variance of just 9.99). This was the main reason why, for the creation of the composite cognitive ratio, we focussed on those tests that offer the largest possible test-retest variability and are capable of capturing a more fine-grained change. In addition, reduced novelty of assessment procedures (even when parallel forms of the tests are used) and possible practice effects are intrinsic limitations of psychometric procedures and reduce the size of any possible change effect due to treatment. A larger sample size would be required to detect significant differences if test scores were to be used as sole outcome measures.

Together with other forms of neurodegeneration, AD triggers a cascade of changes in brain structure and brain function. A number of neuroimaging techniques and post-processing/modelling procedures offer the opportunity of measuring or estimating different aspects of these progressive alterations, contributing to diagnostic accuracy, longitudinal progression monitoring, and assessment of treatment effects. The cholinergic enhancement provided by medications such as donepezil or rivastigmine, for instance, is based on a solid hypothesis, by which up-regulation of cholinergic synapses would lead to improvements in functions normally sustained by this type of neurotransmission. Similarly solid hypotheses are those behind the potentially protective/therapeutic role of other manipulations such as physical activity [41], and transcranial magnetic stimulation [42]. When it comes to cognitive interventions, on the other hand, it is more difficult to identify or extrapolate from the literature hypotheses theorising mechanisms that exploit retained neuroplasticity. Most of the studies describe protocols of exercises that are "symptomatic" in nature, which means that they target cognitive symptoms in an attempt to improve/maintain cognitive levels via repetitive stimulation of functions impaired by the disease, or by teaching the patients facilitatory strategies as computational shortcuts [43]. Studies of this type are conceived based on clinical hypotheses, in pursuit of cognitive and/or functional improvements that can be of use to the patients. On this note, it is difficult to disentangle the essence of the change triggered by multimodal interventions [44], in which cognitive exercises are flanked by other stimulation routes. Although studies based on clinical hypotheses are of utmost importance, it is also vital to understand the mechanisms by which changes are induced. To date, only a limited number of studies have tested the effects of programmes of cognitive stimulation or cognitive training with the intention of exploiting a well-specific repair mechanism. In a Japanese study carried out on a sample of patients suffering from AD, reading aloud and arithmetic training exercises were selected as part of the intervention, based on the fact that these tasks are relatively non challenging, and rely on a set of regions in which reduction of cortical metabolism is seen in AD [45]. Based on a completely different mechanism, other studies focussed on exercising abilities that are relatively preserved in MCI and AD, such as procedural memory [46], or recognition memory [47]. In this latter instance, it would be tapping regions that are spared by AD pathology and possibly mechanisms that endorse positive changes. In a further study centred on lexical-semantic skills in AD, exercises based on these abilities were designed specifically to regulate semantic memory and verbal communication, in order to foster remoulding of "other verbal-related memory networks", via a network transfer process [48]. Along these lines, we tested the efficacy of induced goal-directed co-activation to influence resting state connectivity [25], a "Hebbian" mechanism by which "regions that fire together, wire together". More approaches like these are needed to design protocols of cognitive intervention that are sustained by a strong hypothesis of neural repair. These protocols can then be implemented in clinical practice in support of other types of interventions (*e.g.,* cholinergic enhancing agents), for which the mechanisms of functioning are well known.

The functional changes within the DMN were accompanied by no morphometric modifications. Previous research speculated that training-related effects are the result of microstructural (rather than macrostructural) changes such as optimisation of synaptic number [49], increased myelination [50], or increased allocation of neurotransmitter and other neurobiological factors [51]. Unfortunately, it is not possible to clarify the exact biological reason behind the increase in parietal connectivity observed in this study. We speculate that a combination of mechanisms acting at synaptic level would be the most likely contributors to the up-regulation of connectivity observed in our sample. A synaptic route is supported by the pattern of findings emerged from the analysis of construct validity. In fact, the neural correlates of the exercises were centred in regions that are part of DMN (lateral temporal cortex, hippocampus, medial prefrontal cortex), but did not include any parietal cluster (see Supplementary Material section for a description of specific neural correlates broken down for each task modality). This is consistent with the idea that the DMN was up-regulated in its entirety, and highlights the role of connections between areas as major player behind the observed changes.

This study is not free from limitations. First, although it was ascertained that the sub-samples of patients allocated to the two conditions were comparable for major demographic, cognitive and neurostructural variables, it is virtually impossible to control for *all* factors that influence the mechanisms of neuroplasticity. These include concepts such as neurocognitive reserve and efficiency, for instance [52, 53], or a large number of major genetic variables in addition to genotype for the Apolipoprotein E gene [54]. We assume that any intervenient variable would have a random effect on the pattern of findings, and we acknowledge that larger samples are needed to provide further methodological protection. In any case, the two groups showed no volumetric difference in the amount of white matter damage, suggesting that at least the effect on brain tissue typically induced by vascular risk factors (a major group of intervenient variables) was compara-

ble between the two groups. This is particularly important, because evidence suggests that the DMN may be susceptible to cardiovascular risk factors, *e.g.,* reduced DMN connectivity is found in patients with mild cognitive impairment and type 2 diabetes, as compared to patients with MCI without type 2 diabetes [55]. Second, it was not possible to confirm an aetiology of AD for the MCI patients at the time of enrolment because no clinical index of disease progression was available. To minimise the risk of recruiting participants with a non neurodegenerative condition we excluded patients with major cardiovascular or psychiatric conditions. Serial clinical follow ups over several years, however, allowed confirmation of clinical diagnosis, even though no cerebrospinal fluid or amyloid positron emission tomography biomarkers could be collected in these cohorts. We acknowledge, however, that the study of DMN-based cognitive training might lead to a more precise pattern of findings if the entire sample were homogeneously showing *in vivo* evidence of AD pathology, as indicated by recent guidelines [27]. This has to be further explored in subsequent studies. This shortcoming, however, does neither influence the genuineness of the mechanism illustrated by the experimental hypothesis, nor undermine the clinical applicability of such type of cognitive intervention. In most clinical settings, in fact, clinicians very often assign a diagnosis of MCI and follow the patients up over time to shed light on the possible aetiology that triggers it.

A third point that deserves a comment and, possibly, future research attention, is the duration of the neural changes triggered by the training. Because of clinical priorities, it was not possible to include an experimental follow up for all patients. As a consequence, the durability of training-induced changes could not be determined.

An aspect of crucial importance to safeguard the solidity of interventions studies is the inclusion of an appropriate control condition [30]. Even though effort was made to include an active control condition, this was not comparable to the experimental condition in its technical delivery. It was chosen not to include a hospital-based control condition consisting of computerised exercises because it was judged not appropriate or ethically justifiable to request patients to visit the hospital on such an intense regime to complete a programme of exercises not based on a specific hypothesis. In addition, the regions that are constantly reported as the most susceptible to the placebo effect induced by control conditions, are the prefrontal cortex and the anterior cingulate cortex, and do not include the inferior parietal lobule [56]. This rules out the possibility that the observed changes were actually due to other, contingent, placebo-like aspects other than the mechanisms around which the exercises were centred.

A further consideration is linked to the choice of functional MRI to study changes in functional connectivity triggered by training. Measures of functional MRI connectivity are today one of the gold standards to test the effects of various types of intervention, *e.g.,* [57, 58]. However, brain functional changes found in certain areas might be expressed as a result of changes in multiple temporal dynamics [59]. Recent studies have documented how sophisticated parameters extracted from electroencephalographic recordings are able to separate patients with AD from controls [60, 61]. It is possible that ameliorative changes triggered by training may improve features of connectivity that are disease-specific. We argue that electrophysiological methods would allow a more specific interpretation of the spatial maps of cortical results emerged from functional MRI analysis.

CONCLUSION

In summary, we tested the beneficial effect of a network-based programme of cognitive training aimed at regulating DMN functional connectivity in a sample of patients diagnosed with MCI due to AD. An intervention-dependent up-regulation of DMN connectivity was found in left parietal regions, and this change was accompanied neither by any concomitant decreases in connectivity, nor by any modifications in other cognitive and perceptual networks. Even though improvement in cognitive functioning did not reach any statistical significance, cognitive baseline-to-retest change scores were positively associated with parietal baseline-to retest change in connectivity. This indicates that the programme of cognitive training induced changes in DMN connectivity are consistent with a compensatory mechanism.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for this study was obtained from the IRCCS Fondazione Ospedale San Camillo (Venice, Italy) institutional ethics committee (reference number CE: Protocollo11.07).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All study procedures were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

CONSENT FOR PUBLICATION

Written informed consent was obtained from all study participants.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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