# Cognitive Training Modulates Brain Hypersynchrony in a Population at Risk for Alzheimer's Disease

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## Title page

# Title: Cognitive training modulates brain hypersynchrony in a population at risk for Alzheimer's disease

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**List of abbreviations:** AD = Alzheimer's disease; BNT = Boston Naming Test; CogTr = cognitive training; EOG = electrooculogram; EKG = electrocardiogram; FC = functional connectivity; FDR = False Discovery Rate; fMRI = functional magnetic resonance imaging; GDS-SF = Geriatric Depression Scale-Short Form; HC = healthy controls; HPI = head position indicator; MCI = mild cognitive impairment; MEG = magnetoencephalography; MMSE = Mini-Mental State Examination; PLV = phase-locking value; RBMT = Rivermead Behavioral Memory Test; RSF = Rey's simple figure; SCD = subjective cognitive decline; TMT-A = Trail Making Test part A; TMT-B = Trail Making Test part B; WMS-III = Wechsler Memory Scale-III-R.

## Abstract

**Background:** Recent neuroimaging studies in humans and animal models of Alzheimer's disease (AD) demonstrated brain hyper-synchrony under amyloid burden, which is being identified as a proxy for conversion to dementia. The potential of non-pharmacological interventions to reverse this neurophysiological phenomenon in the early stages of the disease is still an open question.

**Method:** Brain synchrony modulation by cognitive training (CogTr) was examined in a cohort of healthy controls (HC, n = 41, 22 trained) and individuals with subjective cognitive decline (SCD, n = 49, 24 trained). Magnetoencephalographic (MEG) activity and comprehensive neuropsychological scores were acquired before and after completion of a ten-week CogTr program aimed at improving cognitive function and daily living performance. Functional connectivity (FC) analyses were carried out using the phase-locking value. A mixed-effects ANOVA with factors stage (pre-intervention or post-intervention), CogTr (trained or non-trained), and cognitive status (HC or SCD) was used to estimate significant FC changes across MEG recordings.

**Results:** Alpha-band FC increases were observed for the whole sample (both trained and non-trained), but the effect was different in each group. For the trained group (both HC and SCD), we report a reduction in the FC increase within temporo-parietal and temporo-occipital connections. This effect was particularly manifest in trained participants with SCD, for whom the reduction in the FC increase also correlated with enhanced cognitive performance in different neuropsychological domains (memory, language, and executive function).

**Conclusions:** CogTr programs could mitigate the increase in FC observed in preclinical AD, promoting brain synchrony normalization in groups at increased risk for developing the disease.

**Keywords:** magnetoencephalography; cognitive training; functional connectivity; subjective cognitive decline; longitudinal study.

## Background

Alzheimer's disease (AD) is a growing healthcare concern as diagnosis rates are expected to soar in the near future. By 2020, worldwide AD prevalence was estimated at 50 million [1]. The ubiquitous increase of life-expectancy, advancements in diagnosis, and the absence of effective pharmacological treatments will predictably raise the rates to 152 million cases by 2050 [2]. These figures could drop in almost 9 million patients if early treatment were to succeed in delaying the onset of the disease or in slowing down its progression by 1 year [3]. At present, pharmacological research is focused on exploring disease-modifying therapies with several agents undergoing clinical trials [4]. However, by 2020 there were no new drug approvals for the treatment of AD, and in fact, no new drug has been validated since 2003 [5].

In the last decade, animal models of AD have demonstrated an increase in neural excitability [6], mainly due to the toxicity exerted by soluble amyloid-β oligomers and amyloid-β plaques to the inhibitory terminals of GABAergic neurons [7]. This phenomenon causes the disruption of the excitatory/inhibitory synaptic balance leading to a growing probability of epileptic seizures in AD patients [8], as well as to alpha/beta hyper-synchronization in neurophysiological recordings in humans [9,10]. This aberrant hyper-synchronization

introduced by amyloid pathology seems to effectively predict conversion from mild cognitive impairment (MCI) to dementia [11,12] and appears to be an early sign of the disease in vulnerable populations [13], such as individuals with subjective cognitive decline (SCD) [14] and first-degree relatives of AD patients [15].

Although the use of antiepileptic drugs could represent a potential approach to reduce neural hyper-excitability [16], different non-pharmacological options could also contribute to promoting the resilience of the basic neurophysiological activity. Indeed, with pharmacological research reaching a plateau, non-pharmacological interventions have gained momentum, particularly cognitive training (CogTr) [17]. CogTr programs are regarded as cost-effective approaches that could be introduced in the daily routines of individuals undergoing cognitive decline, to improve their mental abilities, daily-living performance, and, whenever possible, to preserve cognitive function during the early stages of the disease. Admittedly, some reviews expressed concern after finding no satisfactory evidence of improvement following CogTr, casting doubt on previously reported benefits [18,19]. However, these conclusions could be partly explained by the substantial differences found in interventional designs and validation procedures, which could lead to underestimating the therapeutic potential of CogTr. As a matter of fact, several studies have informed of the advantages of CogTr in individuals with SCD [reviewed in 20], MCI [reviewed in 21], and mild-to-moderate AD [reviewed in 22].

Despite the positive reports, there is no currently accepted CogTr protocol to decelerate the progression of the disease [23]. Furthermore, no previous study has tested the hypothesis of how a CogTr program might modulate the state of hyper-synchrony observed in preclinical and prodromal AD. Previous neuroimaging studies have explored brain functional and

structural changes following cognitive interventions both in healthy aging [e.g., 24,25] and prodromal AD, mostly MCI [26–31]. These works used functional magnetic resonance imaging (fMRI) to generally reveal complex patterns of enhanced activity, introduced by CogTr in a way that might be specific to the degree of disease severity [26]. The sample of our study included participants in an intermediary stage between healthy aging and MCI, namely SCD. This condition is an accepted indicator of prospective cognitive impairment and dementia [32], thus renders a favorable working point along the AD continuum to attempt preventive care interventions.

In the current study, magnetoencephalographic (MEG) activity and neuropsychological scores were acquired at two time-points for a sample of healthy controls (HC) and SCD participants. This sample was blindly randomized into a non-experimental group and an experimental group that underwent a ten-week period of CogTr. Brain synchronization was analyzed using a measure of functional connectivity (FC) based on the phase coupling between neural oscillations. Changes in cognitive performance and FC across time-points were compared between the experimental and non-experimental groups. We tested: (i) whether a CogTr program could modulate resting-state FC and (ii) whether its outcome would be influenced by being in a group at increased risk for developing AD (i.e., SCD). Based on previous research, we hypothesized that the cognitive improvement promoted by CogTr should be underpinned by a neutralizing effect over the electrophysiological signature of the disease, particularly concerning aberrant hyper-synchronous connections.

## Methods and materials

### Participants

The sample of this study was recruited from the Centre for Prevention of Cognitive Impairment (Madrid Salud), the Faculty of Psychology of the Complutense University of Madrid (UCM), and the Hospital Clínico San Carlos (HCSC) in Madrid, Spain, between January 2014 and December 2015. Two-hundred thirty older adults were sequentially enrolled as part of a bigger study. All participants were aged 65−80 years, right-handed [36], and native Spanish. Research was conducted following current guidelines and regulations. The study was approved by the Ethics Committee of the HCSC, and every participant signed informed consent prior to enrolling. A detailed list of the general inclusion and exclusion criteria can be found in [14].

During the initial screening session, the following domains were assessed for every candidate: (i) memory (Rey's simple figure test form B [RSF, 37], Digit Span Test (forward and backward), Texts of Verbal Memory, and the Word List of the Wechsler Memory Scale-III-R [WMS-III (Spanish version), 38]); (ii) language (Boston Naming Test [BNT, 39], Phonemic and Semantic Fluency Tests [40]); (iii) executive function (Trail Making Test parts A and B [TMT-A and TMT-B, 41]); (iv) cognitive status (Mini-Mental State Examination [MMSE, 42], 7 Minutes Test [43]); (v) subjective memory (Rivermead Behavioral Memory Test [RBMT, 44], Memory Failures in Everyday Questionnaire (Spanish adaptation) [45]); (vi) functional capacity (Functional Activities Questionnaire [FAQ, 46]); and mood (Geriatric Depression Scale-Short Form [GDS-SF, 47]). Cognitive concerns were self-reported by the candidates in an interview with an expert clinician. Ensuingly, assignment to the SCD group was agreed on by multidisciplinary consensus after discarding possible cofounders of SCD (e.g., medication and psycho-affective disorders). SCD participants were required to be $> 60$ years at the onset of SCD, having it occurred within the last 5 years [32].

144 Out of the initial 230 candidates, 154 were apt for inclusion after discarding candidates with
145 MCI (n = 43), candidates that did not attend the first MEG session (n = 18), and candidates
146 without a valid first MEG recording (n = 15). 34 participants did not attend the second MEG
147 session, and 22 participants presented deficiencies in the recorded or source-reconstructed
148 signals. Eight participants were excluded because a T1-weighted MRI scan was not available.

149 The resulting sample of this study included 41 HC and 49 SCD participants, who had been
150 randomly split into an experimental group (n = 46, 24 of which SCD) and a non-experimental
151 group (n = 44, 25 of which SCD). The experimental group (hereafter, trained) participated in
152 the CogTr program, while no cognitive stimulation or placebo activity was appointed for the
153 non-experimental group (hereafter, non-trained). To keep the non-trained group engaged,
154 participants were offered the same CogTr program once the study was completed. All groups
155 were adjusted for age (mean (standard deviation): 70.81 (3.59) (trained HC), 71.58 (5.02)
156 (trained SCD), 69.42 (4.42) (non-trained HC), 73.24 (5.84) (non-trained SCD); p = 0.0762)
157 and sex (male/female: 9/13 (trained HC), 10/14 (trained SCD), 6/13 (non-trained HC), 6/19
158 (non-trained SCD); p = 0.5244). The complete neuropsychological, MEG, and MRI sessions
159 were repeated for all participants around 6 months after the CogTr interval. A schematic
160 representation of the study timeline can be found in **Fig. 1**.

161 ## Cognitive training

162 The CogTr program applied to the trained group was designed in 1994 at the Memory
163 Training Unit of the City Council of Madrid (UMAM, Madrid, Spain). The CogTr consisted
164 of 30 sessions (28 regular sessions and two maintaining-booster sessions) of 90 minutes,
165 organized in groups of 12 to 18 people. Assistance was controlled for all participants. All

sessions took place in the morning, three times per week, and under professional instruction. The 'UMAM method' is thoroughly detailed in [48]. Altogether, it follows a multifactorial organization, targeting cognitive stimulation (language, perception, attention), learning of cognitive strategies (by association, categorization, etc.), intervention in daily-living performance (role-playing, prospective-retrospective memory training, automatic actions rehearsal), and analysis of the meta-memory to inspect the causes and variables of one's own cognitive failures. The program also includes several activities related to the pursuit of a healthy life (eating properly, engaging in physical exercise, participating in social and leisure activities, etc.), and encourages homework hobbies such as crossword puzzles and the use of new technologies. A schematic representation of the CogTr timeline can be found in **Fig. 1**.

## Magnetic resonance imaging

Three-dimensional T1-weighted anatomical MRI scans were acquired for each participant with a 1.5 T MRI scanner (GE Healthcare, Chicago, Illinois) using a high-resolution antenna and a homogenization PURE filter (fast spoiled gradient echo sequence, with parameters: repetition time/echo time/inversion time = 11.2/4.2/450 ms, flip angle 12º, slice thickness = 1 mm, 256×256 matrix, and field of view = 256 mm).

## Magnetoencephalography

### Data acquisition and pre-processing

MEG data were acquired at the Laboratory of Cognitive and Computational Neuroscience (UCM-UPM), in the Centre for Biomedical Technology (CTB) (Madrid, Spain) using a 306 channel (102 magnetometers, 204 planar gradiometers) Vectorview MEG system (Elekta

AB, Stockholm, Sweden) placed within a magnetically shielded room (VacuumSchmelze GmbH, Hanau, Germany). Upon their arrival, participants were informed of the MEG procedure before being conducted to the MEG room, where they were asked to sit comfortably and still. We acquired a total of four minutes of resting-state electrophysiological activity while the participants rested awake with their eyes closed. Before the MEG recording, we digitized the head shape of the participants using a 3D Fastrak digitizer (Polhemus, Colchester, Vermont). Specifically, we recorded the position of three fiducial points (nasion and right and left preauricular) and an outline of approximately 400 scalp points. In addition, we placed four head position indicator (HPI) coils in the participant's scalp (two in the mastoids and two in the forehead). The position of the HPI coils was also digitized and used to continuously monitor head movement during the recording. To capture (i) eye blinks and ocular movements and (ii) cardiac activity, we used two electrooculogram (EOG) electrodes (set above and below the left eye), and two electrocardiogram (EKG) electrodes (set diagonally across the chest) in bipolar montages. MEG data were acquired using a sampling rate of 1000 Hz and an online anti-alias band-pass filter between 0.1 and 330 Hz. Recordings were processed offline using a tempo-spatial filtering algorithm [tSSS, 49] (Maxfilter software v 2.2, correlation limit = 0.9, correlation window = 10 s) to eliminate magnetic noise originating outside the head. The same algorithm was used to compensate for head movement during the recording.

Ocular, muscular, and jump artifacts were identified using a double approach consisting of (i) the automatic artifact-detection algorithm of the Fieldtrip toolbox [50] for Matlab (R2017b, Mathworks, Inc.) and (ii) the visual confirmation of a MEG expert. An independent component-based algorithm was used to remove the effects of both EOG and EKG signals

from the data, together with external noises. Data were segmented into 4-s segments of artifact-free activity (epochs). The number of clean epochs did not differ across groups nor conditions. Due to the high redundancy found in MEG data after spatial filtering, only magnetometers' signals were used in the subsequent analyses [51].

**Source reconstruction**

Clean epochs were band-pass filtered in the classical alpha band (8 to 12 Hz) using a $1800^{th}$ order FIR filter designed using a Hamming window. To avoid edge effects, the epochs were padded with 2 seconds (2000 samples) of real data on each end, which were removed upon filtering. The source model consisted of 1220 sources placed in a homogeneous grid of 1 cm in a Montreal Neurological Institute (MNI) template that was converted to subject space by an affine transformation. MEG sources were anatomically parcellated by assigning each source to one of the 74 regions of interest defined in the Automated Anatomical Labeling atlas [AAL, 52]. The lead field was calculated with a single-shell head model [53] with a unique boundary defined by the inner skull (the combination of white matter, gray matter, and cerebrospinal fluid) generated from the individual T1-weighted MRI using Fieldtrip. Source reconstruction was performed for each subject using a Linearly Constrained Minimum Variance (LCMV) beamformer [54]. Beamformer filters were obtained using the computed lead field, the epoch-averaged covariance matrix, and a 1% regularization factor.

**Connectivity analyses**

FC was estimated under the hypothesis of phase synchronization using the phase-locking value [PLV, 55], which demonstrates high reliability across MEG recordings [56]. This measure is based upon the assumption that the degree of non-uniformity of phase differences

between two time-series should be a good estimator of their coupling. To reduce the dimensionality of the FC matrices (1220 by 1220, sources by sources), PLV values were averaged following the AAL parcellation to obtain a single PLV value between each pair of areas *A* and *B* defined therein (74 by 74, areas by areas):

$$PLV_{A,B} = \frac{1}{N_A N_B} \sum_{N_A} \sum_{N_B} \left| \frac{1}{T} \sum_{t} e^{-j\left(\varphi_{A_k}(t) - \varphi_{B_l}(t)\right)} \right|$$

where $\varphi_{A_k}(t)$ and $\varphi_{B_l}(t)$ are the instantaneous phases of signal $A_k$ and signal $B_l$ at instant $t$, $T$ is the number of temporal points per epoch, $j$ is the imaginary unit, $N_A$ is the number of sources in area *A*, and $A_k$ is the *k*th source inside this area.

## Statistical analyses

We performed a mixed-effects ANOVA with two between-subjects factors, (i) cognitive status (i.e., HC or SCD) and (ii) CogTr (i.e., trained or non-trained), and one within-subject factor (i) stage (i.e., pre-stage or post-stage) indicating the time point of the MEG recording. We tested the main effects of all three factors and every second- and third-order interaction (stage × CogTr, stage × cognitive status, CogTr × cognitive status, stage × CogTr × cognitive status). In total, the ANOVA model was applied over 2701 FC values (i.e., the symmetrical PLV values between the 74 AAL cortical areas). To attain a manageable amount of information, we performed a first analysis focused only on main effects. The outcome was corrected using a False Discovery Rate [FDR, 57] ($q = 0.05$) to address the multiple comparisons problem. Then, the second and third-order interactions were inspected only for those FC values (links) surviving multiple comparisons correction in any of the main effects. The same ANOVA model was applied to the neuropsychological scores recorded during the

pre- and post-stage. In this case, we limited the study to the interaction effect between the factors stage and CogTr (stage × CogTr) in order to determine whether the CogTr influenced the cognitive performance of the trained group at follow-up.

Finally, to explore whether the FC changes observed across MEG recordings in the trained group (HC and SCD) were associated with an improvement in cognitive performance, we searched for a relationship between the relative change in both FC and neuropsychological scores using Pearson's linear correlation coefficients. Representative neuropsychological tests were selected from the following cognitive domains: (i) memory, Digit Span Test (forward); (ii) language, BNT; and (iii) executive function, TMT-A and TMT-B (completion times). The significance level was established at 0.05 and p-values were corrected using FDR ($q = 0.2$). All the statistical analyses were performed using Matlab (R2017b, Mathworks, Inc.).

# Results

## Connectivity analyses

The mixed-effects ANOVA applied to the FC values revealed a significant main effect of stage (FDR corrected) on the whole-sample. FC in the alpha band was increased in the post-stage in 18 links spanning mainly temporo-parietal (6), fronto-parietal (4), and temporo-occipital (4) connections (lIFGor – lCu: p = 0.0002; lIFGor – rMOccL: p < 0.0001; lSTG – rCu: p = 0.0001; lSTG – rSOccL: p = 0.0001; lHeschl – rCu: p = 0.0001; lHeschl – rSOccL: p < 0.0001; rCu – rIFGor: p < 0.0001; rPrecu – rIFGt: p = 0.0002; rPrecu – rIFGor: p = 0.0001; rPrecu – rMTG: p = 0.0001; rPrecu – rITG: p < 0.0001; rPrecu – rFusiG: p < 0.0001; rPrecu – rHip: p = 0.0002; rSPG – rIFGor: p = 0.0003; rSPG – rITG: p < 0.0001; rSPG –

rFusiG: p = 0.0001; rITG – rMTG: p < 0.0001) (see **Fig. 2**). The main effects of cognitive status and CogTr were non-significant or did not survive multiple comparisons correction. The interaction effect between stage and CogTr (stage × CogTr) was significant (p < 0.05) in nine out of the aforementioned 18 links, involving mainly temporo-parietal and temporo-occipital connections (lSTG – rCu: p = 0.0218; lSTG – rSOccL: p = 0.0408; lHeschl – rCu: p = 0.0427; lHeschl – rSOccL: p = 0.0461; rPrecu – rMTG: p = 0.0053; rPrecu – rITG: p = 0.0028; rPrecu – rFusiG: p = 0.0021; rPrecu – rHip: p = 0.0001; rITG – rMTG: p = 0.0404) (see **Fig. 3**). This interaction showed that the main effect of stage was less pronounced in the trained group than in the non-trained group. In the trained group we observed a reduction in FC differences between the pre-stage and the post-stage in comparison to the non-trained group (see **Fig. 5** and **Additional file 1**).

Additionally, a triple interaction between stage, CogTr, and cognitive status (stage × CogTr × cognitive status) was significant (p < 0.05) in six out of the 18 links (including five of the nine links from the interaction between stage and CogTr). These connections involved mainly associations between the right precuneus and nodes of the right temporal lobe (middle and inferior temporal gyrus, fusiform gyrus) and the right hippocampus (lIFGor – lCu: p = 0.0383; rPrecu – rMTG: p = 0.0101; rPrecu – rITG: p = 0.0110; rPrecu – rFusiG: p = 0.0236; rPrecu – rHip: p = 0.0083; rITG – rMTG: p = 0.0328) (see **Fig. 4**). This interaction showed that the main effect of stage followed a differentiated trend in the trained SCD group. In this group, we observed that FC in the post-stage appeared to be lower than FC in the pre-stage, which was not observed in trained HC (see **Fig. 5** and **Additional file 1**).

The results described herein are summarized in **Table 1**.

## Cognitive outcomes

The mixed-effects ANOVA applied to the neuropsychological scores revealed that the interaction effect between stage and CogTr (stage × CogTr) was non-significant for all the cognitive tests included in the analysis (Digit Span Test (forward): $F = 1.282$, $p = 0.261$; Digit Span Test (backward): $F = 2.382$, $p = 0.127$; RSF (copy score): $F = 0.249$, $p = 0.619$; RSF (copy time): $F = 2.110$, $p = 0.151$; RSF (memory score): $F = 0.510$, $p = 0.477$; RSF (memory time): $F = 0.459$, $p = 0.500$; BNT: $F = 0.294$, $p = 0.589$; Phonemic Fluency Test: $F = 1.070$, $p = 0.304$; Semantic Fluency Test: $F = 0.808$, $p = 0.371$; TMT-A (hits): $F = 1.596$, $p = 0.210$; TMT-A (completion time): $F = 0.010$, $p = 0.919$; TMT-B (hits): $F = 0.138$, $p = 0.711$; TMT-B (completion time): $F = 2.940$, $p = 0.090$; MMSE: $F = 0.002$, $p = 0.960$; 7 Minutes Test: $F = 0.111$, $p = 0.740$; RBMT: $F = 0.952$, $p = 0.332$). As such, we did not observe any significant cognitive changes across sessions based on the neuropsychological scores of our sample.

## Correlation analyses

The correlation analysis between FC and neuropsychological scores was carried out for the trained group (HC and SCD). The aim of this analysis was to explore whether the FC changes observed in the trained group (i.e., maintained/reduced FC in the post-stage) were significantly associated with an improvement in cognitive performance, particularly in the case of trained SCD participants for whom the effect of CogTr was more pronounced. Relative changes in FC were calculated for the significant links obtained from the analysis of the triple interaction between stage, CogTr, and cognitive status, specifically for those involving the right precuneus (rPrecu – rMTG, rPrecu – rITG, rPrecu – rFusiG, and rPrecu –

rHip) (see **Table 1**). We observed significant correlations in the trained SCD group between the relative change in FC within some of the aforementioned connections and the relative change in performance for the cognitive measures included in the analysis. Specifically, FC changes in the trained SCD group were significantly associated with improved scores in the following neuropsychological tests: (i) Digit Span Test (forward) (rPrecu – rITG ($\rho$ = -0.4472; p = 0.0324)). (ii) BNT (rPrecu – rHip ($\rho$ = -0.4914; p = 0.0237)); rPrecu – rITG ($\rho$ = -0.4487; p = 0.0413)). (iii) TMT-A (rPrecu – rITG ($\rho$ = 0.4785; p = 0.0180)). (4) TMT-B (rPrecu – rFusiG ($\rho$ = 0.5024; p = 0.0124)). No significant associations were found for the trained HC group. The results described herein are summarized in **Table 2**.

## Discussion

The purpose of the current study was to examine whether a CogTr program could modulate brain hyper-synchrony, and whether its outcome would be influenced by being in a group at increased risk for developing AD. Our results reveal whole-sample FC increases across the two MEG recordings. The most relevant finding of the present work is that the increase in FC (hyper-synchronization) was significantly mitigated in trained participants, particularly in trained participants with SCD. For the latter, we also found a relationship between the change in FC and improved cognitive performance in different neuropsychological domains (memory, language, and executive function).

Our analysis of FC revealed whole-sample differences between the pre-stage and the post-stage in the alpha band. Specifically, this comparison exposed increased FC in the post-stage independently of whether participants had undergone CogTr or not, and regardless of their cognitive status (HC or SCD). As this study followed a longitudinal intra-individual design,

this outcome suggests an association between increments in FC and the time-gap between the MEG recordings, and thus, hints to a role of natural aging underneath the change in FC across stages. Concerning this, recent fMRI longitudinal research has contributed to unveil the non-straightforward relationship between natural aging and FC trajectories, from early adulthood up to old age, by acknowledging non-linear non-monotonic effects that were not discernible in previous cross-sectional studies [58,59]. Concretely, Staffaroni and colleagues revealed a trend towards whole-brain FC increases between ages 50-70, that peaks around 70 years and then declines in senescence [58]. On the other hand, reduced FC in aging is a widespread finding in cross-sectional fMRI studies, particularly regarding FC decrements within high-level processing networks such as the DMN [60,61]. Indeed, the DMN has received major attention in age-related FC monitoring as a consequence of the reported link between DMN FC and early-stage AD [62,63]. In our study, FC increases across stages involved regions typically ascribed to the DMN, and some of them concerned intra-network connections, which might appear conflicting with the aforementioned work, but that remains in accordance with longitudinal explorations that account for non-linearities [58]. Outside the DMN, an fMRI study by Ferreira and colleagues reported ubiquitous age-related increments of FC across inter-network connections (out of the significant connections found, 99% presented a positive correlation between age and FC) [64]. Age-related FC increases might be explained by different age-related changes, including vascular and metabolic alterations such as inflammation, dysfunction of the neurovascular unit, amyloid-β accumulation, and prion-like spread of neurotoxic proteins [58].

The study of FC has also been prominent in pathological aging, notably regarding FC changes that occur across the preclinical and prodromal stages of AD. One distinctive hallmark of AD

364 is the accumulation of soluble amyloid-β oligomers and aggregated amyloid-β plaques [65]
365 that emerge in the early stages of the disease and spread following a specific pattern of
366 regional progression [66]. The presence of amyloid-β plaques in the surroundings of
367 pyramidal neurons has been associated with the loss of GABAergic perisomatic synapses,
368 increasing the activity of pyramidal neurons under amyloid burden [7]. In view of this, in the
369 case of amyloid pathology, hyper-activity across populations of pyramidal neurons is
370 expected to increase the likelihood of synchronization between brain areas, establishing
371 aberrant connections that can be captured by the analysis of FC [11]. In our study, we found
372 increased FC in the post-stage in 18 significant links spanning mainly temporo-parietal,
373 fronto-parietal, and temporo-occipital connections. Out of those 18 links, one-third involved
374 connections with the rPrecu. This finding was particularly interesting since the precuneus
375 demonstrates a distinct vulnerability to the early deposition of amyloid-β [67]. Accordingly,
376 abnormal FC with the rPrecu might represent the expression of local excitatory responses to
377 amyloid pathology. In this regard, previous studies have established a relationship between
378 amyloid-β deposition and hyper-synchronization within nodes of the posterior DMN,
379 including the rPrecu, in asymptomatic early stages of the disease [13,68]. It must be pointed
380 out that even though we have no evidence for amyloid burden within our sample, the
381 incidence of amyloid positivity by PET has been previously addressed in cohorts of elderly
382 adults [69–71]. Specifically, Snitz and colleagues informed that 57% of their recruited SCD
383 participants turned to be amyloid positive, compared to 31% of the HC [69]. Perrotin *et al*
384 also reported an association between amyloid-β deposition in the rPrecu and reduced self-
385 confidence in memory abilities (a factor likely to be indicative of prospective SCD) [71]. In
386 view of this, our results should be interpreted considering that FC increases are influenced at
387 the same time by age-related changes (e.g., vascular and metabolic) and amyloid pathology,

388 particularly in the case of SCD participants for whom amyloid burden is expected to prevail.
389 Finally, increased FC among SCD subjects might be understood in the context of the "X
390 model" of AD conversion [11]. According to this neurophysiological model, FC rises
391 monotonically across the preclinical stages of the disease up to a turning point where it starts
392 to decrease, possibly indicating network failure eventually leading to dementia.

393 Interestingly, the aforementioned changes in FC between the pre- and the post-stages
394 followed different trends in two cases. Firstly, the statistical model revealed a significant
395 interaction between the variables *stage* and *CogTr*. This result demonstrated that FC changed
396 differently across stages in the case of participants that had undergone CogTr. In the trained
397 group, FC differences between the pre-stage and the post-stage were diminished or even
398 equilibrated when compared to the non-trained sample. Such was the case for half of the
399 original connections, mainly involving temporo-parietal and temporo-occipital links.
400 Secondly, we found a significant triple interaction between the variables *stage*, *CogTr*, and
401 *cognitive status*. This result demonstrated that, within the trained group, FC changed
402 differently across stages depending on the cognitive status. Specifically, trained SCD
403 participants exhibited decreased FC in the post-stage in one-third of the original connections,
404 a behavior that was not observed in trained HC. Importantly, two-thirds of the connections
405 involved in the triple interaction linked the rPrecu with nodes of the temporal lobe. Given the
406 association between this region and early amyloid deposition, these findings suggest that the
407 CogTr contributed to counterbalance hyper-synchronous FC patterns derived from amyloid
408 pathology. Certainly, the effect of CogTr was more pronounced in SCD participants in
409 comparison to HC. This outcome may be explained by a selective influence of the CogTr
410 over amyloid-related hyper-synchronization, which might account for enhanced results

411 within those participants most vulnerable to developing dementia. This selectiveness is in
412 consonance with previous evidence of selective FC modulation in patients at risk for AD and
413 in preclinical AD following a computerized CogTr [26]. To clarify whether the changes in
414 FC introduced by CogTr in the trained groups were indicative of improved cognitive
415 performance, we carried out a correlation analysis for a selection of neuropsychological tests.
416 This analysis revealed that the reduction in post-stage FC observed in trained SCD
417 participants was significantly associated with improved cognition in a variety of domains
418 such as memory, language, and executive function. In effect, these domains had been
419 regularly stimulated during the UMAM CogTr, albeit no significant evidence of
420 improvement could be extracted from the neuropsychological evaluations conducted at the
421 pre- and post-stages. Regarding this, it is important to note that SCD individuals exhibit a
422 self-perceived cognitive decline that virtually goes unnoticed in standardized cognitive
423 testing [32]. This singularity might challenge the detection of cognitive improvement
424 following CogTr, as evidenced by the generalized small effect sizes found after non-
425 pharmacological interventions in SCD samples [20]. In this regard, electrophysiological
426 evidence seems to precede detectability by standard cognitive tests, a finding that highlights
427 the interest of pursuing neuroimaging studies to assess the efficiency of cognitive
428 interventions, and to promote the standardization of study designs and protocols.

429 One limitation of our study is the absence of a more extended longitudinal follow-up. Follow-
430 up studies are encouraged to monitor electrophysiological and cognitive changes that might
431 become discernible at longer intervals adjusting to neuroplasticity time-scales [20].
432 Additionally, measures of amyloid-β deposition within trained samples could shed light on

433 the precise relationship between amyloid pathology and FC changes following CogTr.
434 Unfortunately, such information was not available for this cohort.

## 435 Conclusions

436 Our study offers novel evidence suggesting that CogTr contributes to mitigate brain hyper-
437 synchrony in individuals at increased risk for developing AD. If timely introduced, CogTr
438 could promote brain synchrony normalization, which might act against the progression of the
439 disease and derive benefits in cognitive performance, particularly in vulnerable populations.
440 Importantly, the electrophysiological outcome of CogTr seems to precede detectability by
441 standard neuropsychological tests, warranting the use of neuroimaging techniques to assess
442 the validity of cognitive interventions. Altogether, CogTr programs should represent a
443 valuable strategy for preventive care, providing aging individuals with the tools to boost and
444 preserve their cognitive well-being, particularly in the absence of effective pharmacological
445 treatments.

## 446 Declarations

### 447 Ethics approval and consent to participate

448 Research was conducted following current guidelines and regulations. The study was
449 approved by the Ethics Committee of the HCSC. Every participant signed informed consent.

### 450 Consent for publication

451 Not applicable.

### 452 Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.


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## Author's contributions

FM, RL-H, and AMD outlined the research project. MLD-L coordinated the neuropsychological assessment. PM and MM-P developed the cognitive training protocol and coordinated its implementation. DL-S coordinated and carried out the data collection. IS-M processed the magnetoencephalographic recordings. IS-M and RB performed the study analyses. IS-M drafted the original manuscript. RB, DL-S, PM, and FM revised the original manuscript and contributed to the final version. All authors approved the final version of the manuscript.


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## References

1. Alzheimer's Disease International. Alzheimer's Disease International: World Alzheimer Report 2015 - The global impact of dementia: An analysis of prevalence, incidence, cost and trends. London Alzheimer's Dis. Int. 2015.

2. Alzheimer's Disease International. Alzheimer's Disease International: World Alzheimer Report 2019 - Attitudes to dementia. London Alzheimer's Dis. Int. 2019.

3. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. Alzheimers Dement [Internet]. 2007;3(3):186–91. Available from: https://doi.org/10.1016/j.jalz.2007.04.381

4. Cummings J, Lee G, Ritter A, Sabbagh M, Zhong K. Alzheimer's disease drug development pipeline: 2020. Alzheimers Dement [Internet]. 2020;6(1):e12050. Available from: https://doi.org/10.1002/trc2.12050

5. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimers Res Ther [Internet]. 2014;6(4):37. Available from: https://doi.org/10.1186/alzrt269

6. Busche MA, Konnerth A. Impairments of neural circuit function in Alzheimer's disease. Philos Trans R Soc L B Biol Sci [Internet]. 2016;371(1700):20150429. Available from: http://doi.org/10.1098/rstb.2015.0429

493 7. Garcia-Marin V, Blazquez-Llorca L, Rodriguez JR, Boluda S, Muntane G, Ferrer I, et al.
494 Diminished perisomatic GABAergic terminals on cortical neurons adjacent to amyloid
495 plaques. Front Neuroanat [Internet]. 2009;Nov 20:3–28. Available from:
496 https://doi.org/10.3389/neuro.05.028.2009

497 8. Larner AJ. Epileptic seizures in AD patients. Neuromolecular Med [Internet].
498 2010;12(1):71–7. Available from: https://doi.org/10.1007/s12017-009-8076-z

499 9. Maestú F, Peña JM, Garcés P, González S, Bajo R, Bagic A, et al. A multicenter study of
500 the early detection of synaptic dysfunction in Mild Cognitive Impairment using
501 Magnetoencephalography-derived functional connectivity. Neuroimage Clin [Internet].
502 2015;9:103–9. Available from: https://doi.org/10.1016/j.nicl.2015.07.011

503 10. Bajo R, Maestú F, Nevado A, Sancho M, Gutiérrez R, Campo P, et al. Functional
504 connectivity in mild cognitive impairment during a memory task: implications for the
505 disconnection hypothesis. J Alzheimers Dis [Internet]. 2010;22(1):183–93. Available from:
506 https://doi.org/10.3233/JAD-2010-100177

507 11. Pusil S, López ME, Cuesta P, Bruña R, Pereda E, Maestú F. Hypersynchronization in
508 mild cognitive impairment: the “X” model. Brain [Internet]. 2019;142(12):3936–50.
509 Available from: https://doi.org/10.1093/brain/awz320

510 12. López ME, Bruña R, Aurtenetxe S, Pineda-Pardo JÁ, Marcos A, Arrazola J, et al.
511 Alpha-band hypersynchronization in progressive mild cognitive impairment: a
512 magnetoencephalography study. J Neurosci [Internet]. 2014;34(44):14551–9. Available
513 from: https://doi.org/10.1523/JNEUROSCI.0964-14.2014

514 13. Nakamura A, Cuesta P, Kato T, Arahata Y, Iwata K, Yamagishi M, et al. Early
515 functional network alterations in asymptomatic elders at risk for Alzheimer's disease. Sci
516 Rep [Internet]. 2017;7(1):6517. Available from: https://doi.org/10.1038/s41598-017-06876-
517 8

518 14. López-Sanz D, Bruña R, Garcés P, Martín-Buro MC, Walter S, Delgado ML, et al.
519 Functional Connectivity Disruption in Subjective Cognitive Decline and Mild Cognitive
520 Impairment: A Common Pattern of Alterations. Front Aging Neurosci [Internet]. 2017;Apr
521 21(9):109. Available from: https://doi.org/10.3389/fnagi.2017.00109

522 15. Ramírez-Toraño F, Bruña R, de Frutos-Lucas J, Rodríguez-Rojo IC, Marcos de Pedro
523 S, Delgado-Losada ML, et al. Functional Connectivity Hypersynchronization in Relatives
524 of Alzheimer's Disease Patients: An Early E/I Balance Dysfunction? Cereb Cortex
525 [Internet]. 2021;31(2):1201–10. Available from: https://doi.org/10.1093/cercor/bhaa286

526 16. Vossel KA, Tartaglia MC, Nygaard HB, Zeman AZ, Miller BL. Epileptic activity in
527 Alzheimer's disease: causes and clinical relevance. Lancet Neurol [Internet].
528 2017;16(4):311–22. Available from: https://doi.org/10.1016/S1474-4422(17)30044-3

529 17. Miotto EC, Batista AX, Simon SS, Hampstead BM. Neurophysiologic and Cognitive
530 Changes Arising from Cognitive Training Interventions in Persons with Mild Cognitive
531 Impairment: A Systematic Review. Neural Plast [Internet]. 2018;Dec 2:7301530. Available
532 from: https://doi.org/10.1155/2018/7301530

533 18. Butler M, McCreedy E, Nelson VA, Desai P, Ratner E, Fink HA, et al. Does Cognitive
534 Training Prevent Cognitive Decline?: A Systematic Review. Ann Intern Med [Internet].

535 2018;168(1):63–8. Available from: https://doi.org/10.7326/M17-1531

536 19. Clare L, Woods RT, Moniz Cook ED, Orell M, Spector A. Cognitive rehabilitation and
537 cognitive training for early-stage Alzheimer's disease and vascular dementia. Cochrane
538 Database Syst Rev [Internet]. 2003;(4):CD003260. Available from:
539 https://doi.org/10.1002/14651858.CD003260.pub2

540 20. Smart CM, Karr JE, Areshenkoff CN, Rabin LA, Hudon C, Gates N, et al. Non-
541 Pharmacologic Interventions for Older Adults with Subjective Cognitive Decline:
542 Systematic Review, Meta-Analysis, and Preliminary Recommendations. Neuropsychol Rev
543 [Internet]. 2017;27(3):245–57. Available from: https://doi.org/10.1007/s11065-017-9342-8

544 21. Belleville S. Cognitive training for persons with mild cognitive impairment. Int
545 Psychogeriatr [Internet]. 2008;20(1):57–66. Available from:
546 https://doi.org/10.1017/S104161020700631X

547 22. Kallio EL, Öhman H, Kautiainen H, Hietanen M, Pitkälä K. Cognitive Training
548 Interventions for Patients with Alzheimer's Disease: A Systematic Review. J Alzheimers
549 Dis [Internet]. 2017;56(4):1349–72. Available from: https://doi.org/10.3233/JAD-160810

550 23. Buschert V, Bokde ALW, Hampel H. Cognitive intervention in Alzheimer disease. Nat
551 Rev Neurol [Internet]. 2010;6(9):508–17. Available from:
552 https://doi.org/10.1038/nrneurol.2010.113

553 24. Chapman SB, Aslan S, Spence JS, Hart JJ, Bartz EK, Didehbani N, et al. Neural
554 mechanisms of brain plasticity with complex cognitive training in healthy seniors. Cereb
555 Cortex [Internet]. 2015;25(2):396–405. Available from:

https://doi.org/10.1093/cercor/bht234

25. Mozolic JL, Hayasaka S, Laurienti PJ. A cognitive training intervention increases resting cerebral blood flow in healthy older adults. Front Hum Neurosci [Internet]. 2010;Mar 12(4):16. Available from: https://doi.org/10.3389/neuro.09.016.2010

26. Barban F, Mancini M, Cercignani M, Adriano F, Perri R, Annicchiarico R, et al. A Pilot Study on Brain Plasticity of Functional Connectivity Modulated by Cognitive Training in Mild Alzheimer's Disease and Mild Cognitive Impairment. Brain Sci [Internet]. 2017;7(5):50. Available from: https://doi.org/10.3390/brainsci7050050

27. Belleville S, Clément F, Mellah S, Gilbert B, Fontaine F, Gauthier S. Training-related brain plasticity in subjects at risk of developing Alzheimer's disease. Brain [Internet]. 2011;134(Pt 6):1623–34. Available from: https://doi.org/10.1093/brain/awr037

28. Hampstead BM, Stringer AY, Stilla RF, Deshpande G, Hu X, Moore AB, et al. Activation and effective connectivity changes following explicit-memory training for face-name pairs in patients with mild cognitive impairment: a pilot study. Neurorehabil Neural Repair [Internet]. 2011;25(3):210–22. Available from: https://doi.org/10.1177/1545968310382424

29. Hampstead BM, Stringer AY, Stilla RF, Giddens M, Sathian K. Mnemonic strategy training partially restores hippocampal activity in patients with mild cognitive impairment. Hippocampus [Internet]. 2012;22(8):1652–8. Available from: https://doi.org/10.1002/hipo.22006

30. Rosen AC, Sugiura L, Kramer JH, Whitfield-Gabrieli S, Gabrieli JD. Cognitive training

changes hippocampal function in mild cognitive impairment: a pilot study. J Alzheimers Dis [Internet]. 2011;26 Suppl 3:349–57. Available from: https://doi.org/10.3233/JAD-2011-0009

31. Li BY, He NY, Qiao Y, Xu HM, Lu YZ, Cui PJ, et al. Computerized cognitive training for Chinese mild cognitive impairment patients: A neuropsychological and fMRI study. Neuroimage Clin [Internet]. 2019;22:101691. Available from: https://doi.org/10.1016/j.nicl.2019.101691

32. Jessen F, Amariglio RE, van Boxtel M, Breteler M, Ceccaldi M, Chételat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimers Dement [Internet]. 2014;10(6):844–52. Available from: https://doi.org/10.1016/j.jalz.2014.01.001

33. Douw L, Baayen H, Bosma I, Klein M, Vandertop P, Heimans J, et al. Treatment-related changes in functional connectivity in brain tumor patients: A magnetoencephalography study. Exp Neurol. 2008;212:285–90.

34. Nugent AC, Robinson SE, Coppola R, Zarate CA. Preliminary differences in resting state MEG functional connectivity pre- and post-ketamine in major depressive disorder. Psychiatry Res - Neuroimaging. Elsevier Ireland Ltd; 2016;254:56–66.

35. Westlake KP, Hinkley LB, Bucci M, Guggisberg AG, Findlay AM, Henry RG, et al. Resting State Alpha-Band Functional Connectivity and Recovery After Stroke. Exp Neurol. 2012;

36. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory.

598 Neuropsychologia [Internet]. 1971;9(1):97–113. Available from:
599 https://doi.org/10.1016/0028-3932(71)90067-4

600 37. Luzzi S, Pesallaccia M, Fabi K, Muti M, Viticchi G, Provinciali L, et al. Non-verbal
601 memory measured by Rey-Osterrieth Complex Figure B: normative data. Neurol Sci
602 [Internet]. 2011;32(6):1081–9. Available from: https://doi.org/10.1007/s10072-011-0641-1

603 38. Wechsler D. Escala de memoria de Wechsler-III: manual técnico. TEA Ediciones;
604 2004.

605 39. Kaplan E, Goodglas H, Weintraub S. The Boston Naming Test. 2nd ed. Lippincott
606 Williams & Wilkins; 2001.

607 40. Benton A, Hamsher S, Sivan A. Multilingual aphasia examination: manual of
608 instructions. [Iowa City] : Dept. of Neurology and Psychology, University of Iowa; 1983.

609 41. Reitan RM. Validity of the Trail Making Test as an indicator of Organic Brain Damage.
610 Percept Mot Skills [Internet]. 1958;8(7):271–6. Available from:
611 https://doi.org/10.2466/pms.1958.8.3.271

612 42. Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. A practical method for
613 grading the cognitive state of patients for the clinician. J Psychiatr Res [Internet].
614 1975;12(3):189–98. Available from: https://doi.org/10.1016/0022-3956(75)90026-6

615 43. Solomon PR, Hirschoff A, Kelly B, Relin M, Brush M, DeVeaux RD, et al. A 7 minute
616 neurocognitive screening battery highly sensitive to Alzheimer’s disease. Arch Neurol
617 [Internet]. 1998;55(3):349–55. Available from: https://doi.org/10.1001/archneur.55.3.349

618 44. Wilson, B., Cockburn, J. Baddeley AD. The Rivermead Behavioural Memory Test.
619 Reading, UK: Thames Valley Test Co; 1985.

620 45. Montejo Carrasco P, Montenegro Peña M, Sueiro MJ. The memory failures of everyday
621 questionnaire (MFE): internal consistency and reliability. Span J Psychol [Internet].
622 2012;15(2):768–76. Available from: https://doi.org/10.5209/rev_SJOP.2012.v15.n2.38888

623 46. Pfeffer RI, Kurosaki TT, Harrah CH, Chance JM, Filos S. Measurement of functional
624 activities in older adults in the community. J Gerontol [Internet]. 1982;37(3):323–9.
625 Available from: https://doi.org/10.1093/geronj/37.3.323

626 47. Sheikh JI, Yesavage JA. Geriatric Depression Scale (GDS): Recent evidence and
627 development of a shorter version. Clin Gerontol J Aging Ment Heal [Internet]. 1986;5(1-
628 2):165–73. Available from: https://doi.org/10.1300/J018v05n01_09

629 48. Montejo Carrasco P, Montenegro Peña M, Reinoso García AI, de Andrés Montes ME,
630 Claver Martín MD. Programa de Memoria. Método UMAM. Ediciones Díaz de Santos,
631 S.A.; 2001.

632 49. Taulu S, Hari R. Removal of magnetoencephalographic artifacts with temporal signal-
633 space separation: demonstration with single-trial auditory-evoked responses. Hum Brain
634 Mapp [Internet]. 2009;30(5):1524–34. Available from: https://doi.org/10.1002/hbm.20627

635 50. Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: Open source software for
636 advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell
637 Neurosci [Internet]. 2011;2011:156869. Available from:
638 https://doi.org/10.1155/2011/156869

639 51. Garcés P, López-Sanz D, Maestú F, Pereda E. Choice of Magnetometers and
640 Gradiometers after Signal Space Separation. Sensors (Basel) [Internet]. 2017;17(12):2926.
641 Available from: https://doi.org/10.3390/s17122926

642 52. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et
643 al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical
644 parcellation of the MNI MRI single-subject brain. Neuroimage [Internet]. 2002;15(1):273–
645 89. Available from: https://doi.org/10.1006/nimg.2001.0978

646 53. Nolte G. The magnetic lead field theorem in the quasi-static approximation and its use
647 for magnetoenchephalography forward calculation in realistic volume conductors. Phys
648 Med Biol [Internet]. 2003;48(22):3637–52. Available from: https://doi.org/10.1088/0031-
649 9155/48/22/002

650 54. Van Veen BD, van Drongelen W, Yuchtman M, Suzuki A. Localization of brain
651 electrical activity via linearly constrained minimum variance spatial filtering. IEEE Trans
652 Biomed Eng [Internet]. 1997;44(9):867–80. Available from:
653 https://doi.org/10.1109/10.623056

654 55. Lachaux JP, Rodriguez E, Martinerie J, Varela FJ. Measuring phase synchrony in brain
655 signals. Hum Brain Mapp [Internet]. 1999;8(4):194–208. Available from:
656 https://doi.org/10.1002/(SICI)1097-0193(1999)8:4%3C194::AID-HBM4%3E3.0.CO;2-C.

657 56. Garcés P, Martín-Buro MC, Maestú F. Quantifying the Test-Retest Reliability of
658 Magnetoencephalography Resting-State Functional Connectivity. Brain Connect [Internet].
659 2016;6(6):448–60. Available from: https://doi.org/10.1089/brain.2015.0416

660 57. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and
661 Powerful Approach to Multiple Testing. J R Stat Soc Ser B [Internet]. 1995;57(1):289–300.
662 Available from: https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

663 58. Staffaroni AM, Brown JA, Casaletto KB, Elahi FM, Deng J, Neuhaus J, et al. The
664 Longitudinal Trajectory of Default Mode Network Connectivity in Healthy Older Adults
665 Varies As a Function of Age and Is Associated with Changes in Episodic Memory and
666 Processing Speed. J Neurosci [Internet]. 2018;38(11):2809–17. Available from:
667 https://doi.org/10.1523/JNEUROSCI.3067-17.2018

668 59. Ng KK, Lo JC, Lim JKW, Chee MWL, Zhou J. Reduced functional segregation
669 between the default mode network and the executive control network in healthy older
670 adults: A longitudinal study. Neuroimage [Internet]. 2016;133:321–30. Available from:
671 https://doi.org/10.1016/j.neuroimage.2016.03.029

672 60. Tomasi D, Volkow ND. Aging and functional brain networks. Mol Psychiatry
673 [Internet]. 2012;17(5):549–58. Available from: https://doi.org/10.1038/mp.2011.81

674 61. Geerligs L, Renken RJ, Saliasi E, Maurits NM, Lorist MM. A Brain-Wide Study of
675 Age-Related Changes in Functional Connectivity. Cereb Cortex [Internet].
676 2015;25(7):1987–99. Available from: https://doi.org/10.1093/cercor/bhu012

677 62. Sala-Llonch R, Bartrés-Faz D, Junqué C. Reorganization of brain networks in aging: a
678 review of functional connectivity studies. Front Psychol [Internet]. 2015;6:663. Available
679 from: https://doi.org/10.3389/fpsyg.2015.00663

680 63. Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, et al. Cortical hubs

681 revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation
682 to Alzheimer's disease. J Neurosci [Internet]. 2009;29(6):1860–73. Available from:
683 https://doi.org/10.1523/JNEUROSCI.5062-08.2009

684 64. Ferreira LK, Regina ACB, Kovacevic N, Martin MDGM, Santos PP, Carneiro CDG, et
685 al. Aging Effects on Whole-Brain Functional Connectivity in Adults Free of Cognitive and
686 Psychiatric Disorders. Cereb Cortex [Internet]. 2016;26(9):3851–65. Available from:
687 https://doi.org/10.1093/cercor/bhv190

688 65. Braak H, Braak E. Demonstration of amyloid deposits and neurofibrillary changes in
689 whole brain sections. Brain Pathol [Internet]. 1991;1(3):213–6. Available from:
690 https://doi.org/10.1111/j.1750-3639.1991.tb00661.x

691 66. Grothe MJ, Barthel H, Sepulcre J, Dyrba M, Sabri O, Teipel SJ. In vivo staging of
692 regional amyloid deposition. Neurology [Internet]. 2017;89(20):2031–8. Available from:
693 https://doi.org/10.1212/WNL.0000000000004643

694 67. Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, et al.
695 Molecular, structural, and functional characterization of Alzheimer's disease: evidence for
696 a relationship between default activity, amyloid, and memory. J Neurosci [Internet].
697 2005;25(34):7709–17. Available from: 10.1523/JNEUROSCI.2177-05.2005

698 68. Jack CR, Wiste HJ, Weigand SD, Knopman DS, Lowe V, Vemuri P, et al. Amyloid-
699 first and neurodegeneration-first profiles characterize incident amyloid PET positivity.
700 Neurology [Internet]. 2013;81(20):1732–40. Available from:
701 https://doi.org/10.1212/01.wnl.0000435556.21319.e4

69. Snitz BE, Lopez OL, McDade E, Becker JT, Cohen AD, Price JC, et al. Amyloid-β Imaging in Older Adults Presenting to a Memory Clinic with Subjective Cognitive Decline: A Pilot Study. J Alzheimers Dis [Internet]. 2015;48 Suppl 1:S151–9. Available from: https://doi.org/10.3233/JAD-150113

70. Amariglio RE, Becker JA, Carmasin J, Wadsworth LP, Lorius N, Sullivan C, et al. Subjective cognitive complaints and amyloid burden in cognitively normal older individuals. Neuropsychologia [Internet]. 2012;50(12):2880–6. Available from: https://doi.org/10.1016/j.neuropsychologia.2012.08.011

71. Perrotin A, Mormino EC, Madison CM, Hayenga AO, Jagust WJ. Subjective cognition and amyloid deposition imaging: a Pittsburgh compound B positron emission tomography study in normal elderly individuals. Arch Neurol [Internet]. 2012;69(2):223–9. Available from: https://doi.org/10.1001/archneurol.2011.666

- Additional file 1.
- Word (.docx)
- Boxplots.
- Figure.

**Fig. 1 Schematic representation of the study design.** The participants assigned to the experimental group engaged in 30 CogTr sessions (28 regular sessions and two maintaining-booster sessions) of 90 minutes scheduled three times per week, while no cognitive stimulation or placebo activity was appointed for the non-experimental group. Before and after the CogTr interval, all participants underwent a MEG recording, a T1-weighted MRI scan, and a complete neuropsychological evaluation.

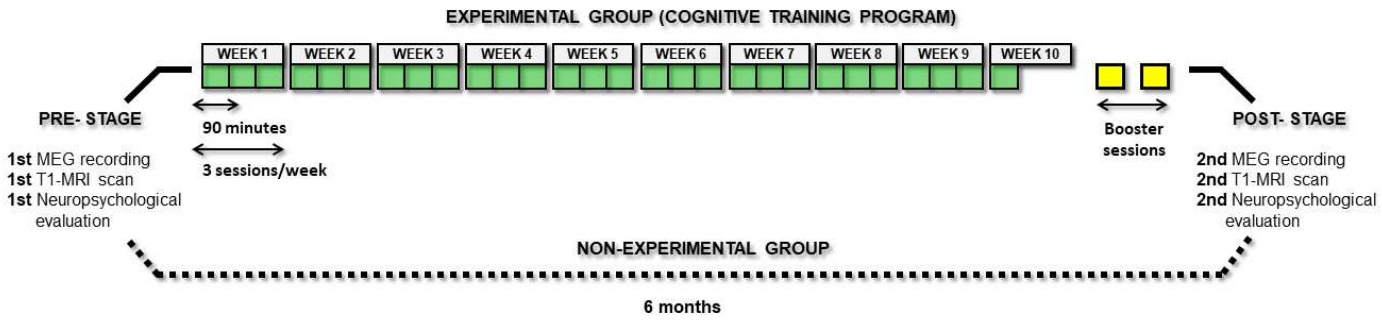


**Fig. 2 FC changes across MEG recordings.** Red lines represent significant FC increases in the post-stage (FDR corrected). Left panel: coronal (A), axial (B), and sagittal views (C and D). Right panel: Circular FC diagram. CV: Coronal view. AV: Axial view. SV: Sagittal view. RH: Right hemisphere. LH: Left hemisphere. R: Right. L: Left.

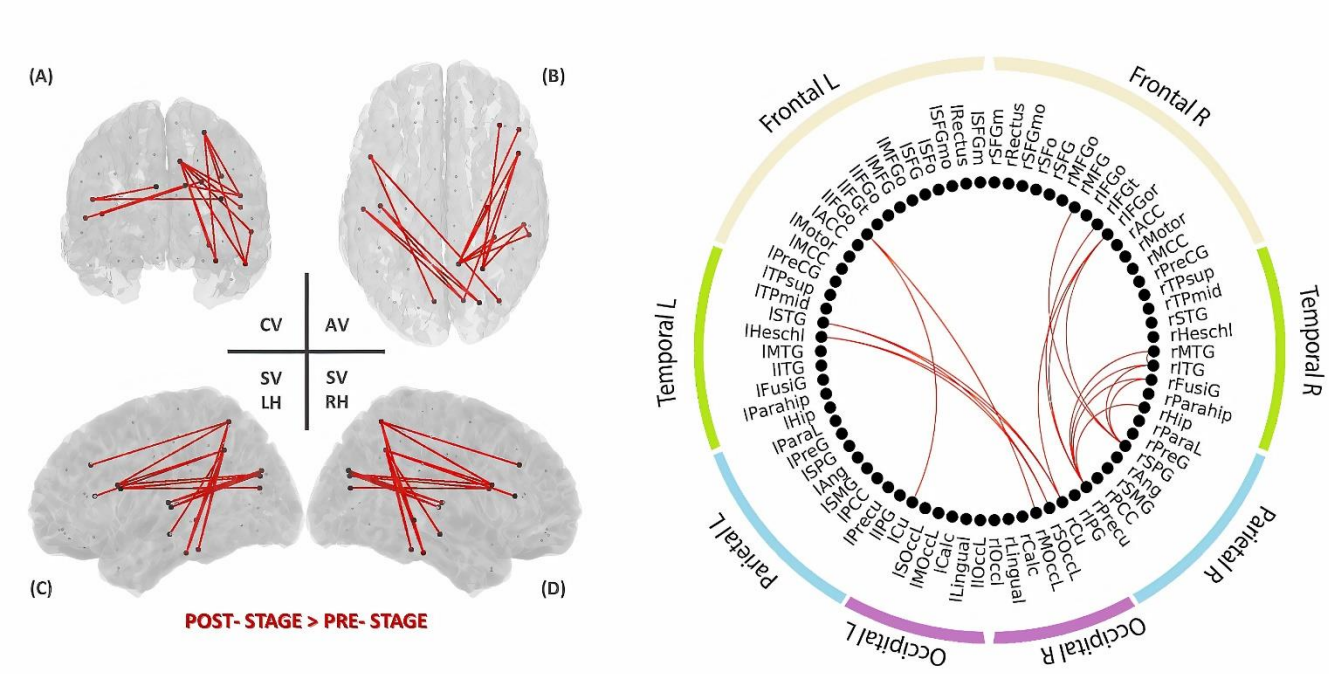


**Fig. 3 Interaction effect between stage and CogTr.** Green lines represent the connections where the interaction effect between stage (pre-stage or post-stage) and CogTr (trained or non-trained) is significant ($p < 0.05$). Left panel: coronal (A), axial (B), and sagittal views (C and D). Right panel: Circular FC diagram. CV: Coronal view. AV: Axial view. SV: Sagittal view. RH: Right hemisphere. LH: Left hemisphere. R: Right. L: Left.

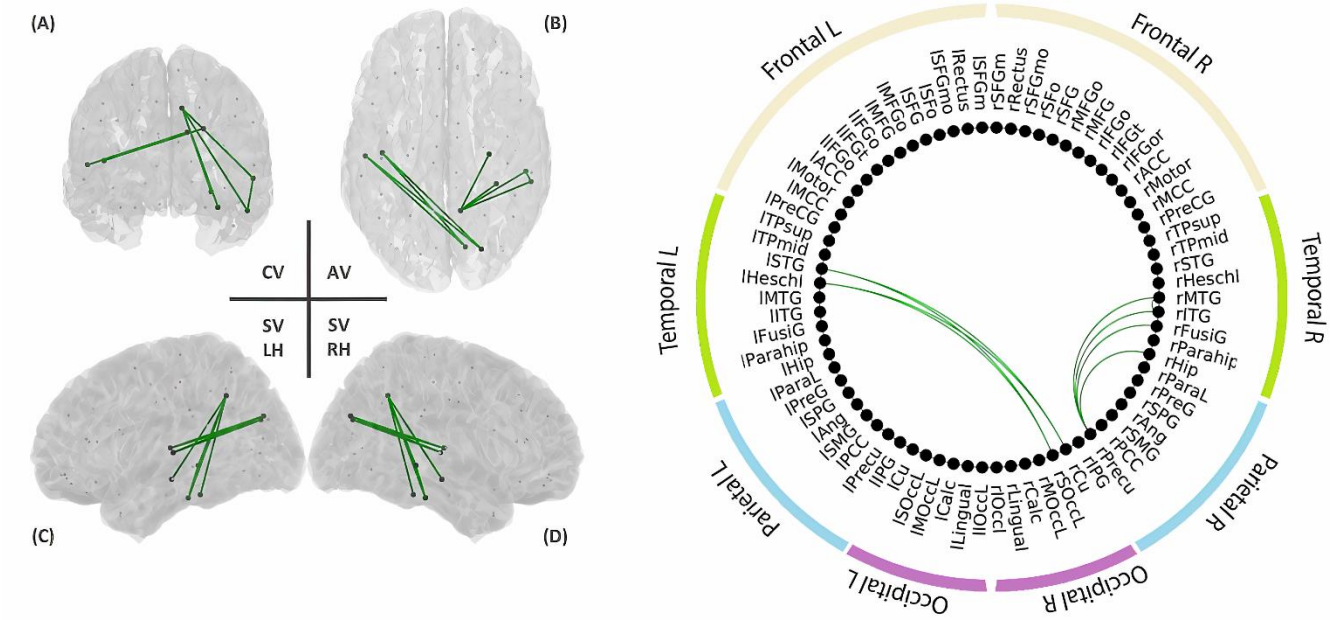


**Fig. 4 Interaction effect between stage, CogTr, and cognitive status.** Purple lines represent the connections where the interaction effect between stage (pre-stage or post-stage), CogTr (trained or non-trained), and cognitive status (HC or SCD) is significant ($p < 0.05$). Left panel: coronal (A), axial (B), and sagittal views (C and D). Right panel: Circular FC diagram. CV: Coronal view. AV: Axial view. SV: Sagittal view. RH: Right hemisphere. LH: Left hemisphere. R: Right. L: Left.

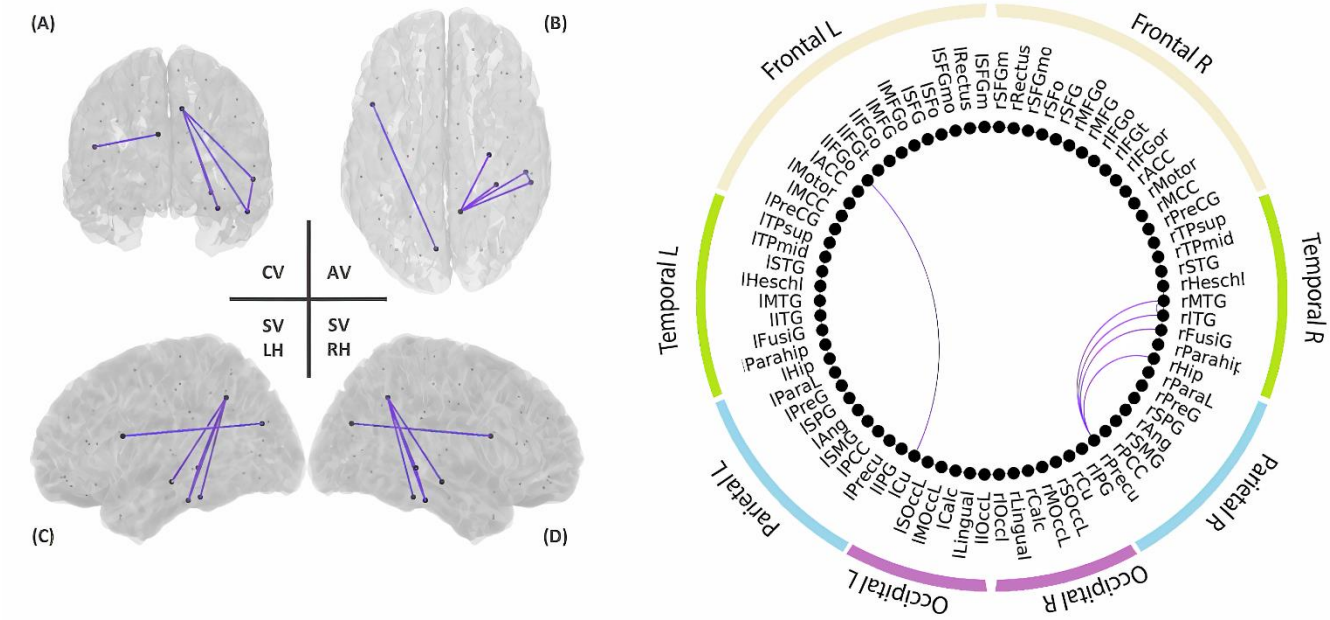


**Fig. 5 Selected boxplots of FC values in the two MEG recordings.** Boxplots of the FC values for each participant in the two MEG recordings for the connections with the rPrecu where the interaction effect between stage (pre-stage or post-stage), CogTr (trained or non-trained), and cognitive status (HC or SCD) was significant ($p < 0.05$). For each significant connection, the upper panel represents the FC values in the pre-stage and the post-stage for the whole sample. The middle panel represents the FC values in the pre-stage and the post-stage separately for non-trained participants (left) and trained participants (right). The lower panel represents the FC value in the pre-stage and the post-stage separately for the non-trained HC group (upper row, left), non-trained SCD group (lower row, left), trained HC group (upper row, right), and trained SCD group (lower row, right). The boxplots inform of each participant's FC values, the sample median, and the boundaries of the quartiles. The colored squares next to the abbreviated names of the connections emphasize the significant results for that connection: red (main effect of stage), green (double interaction between stage and CogTr), purple (triple interaction between stage, CogTr, and cognitive status).

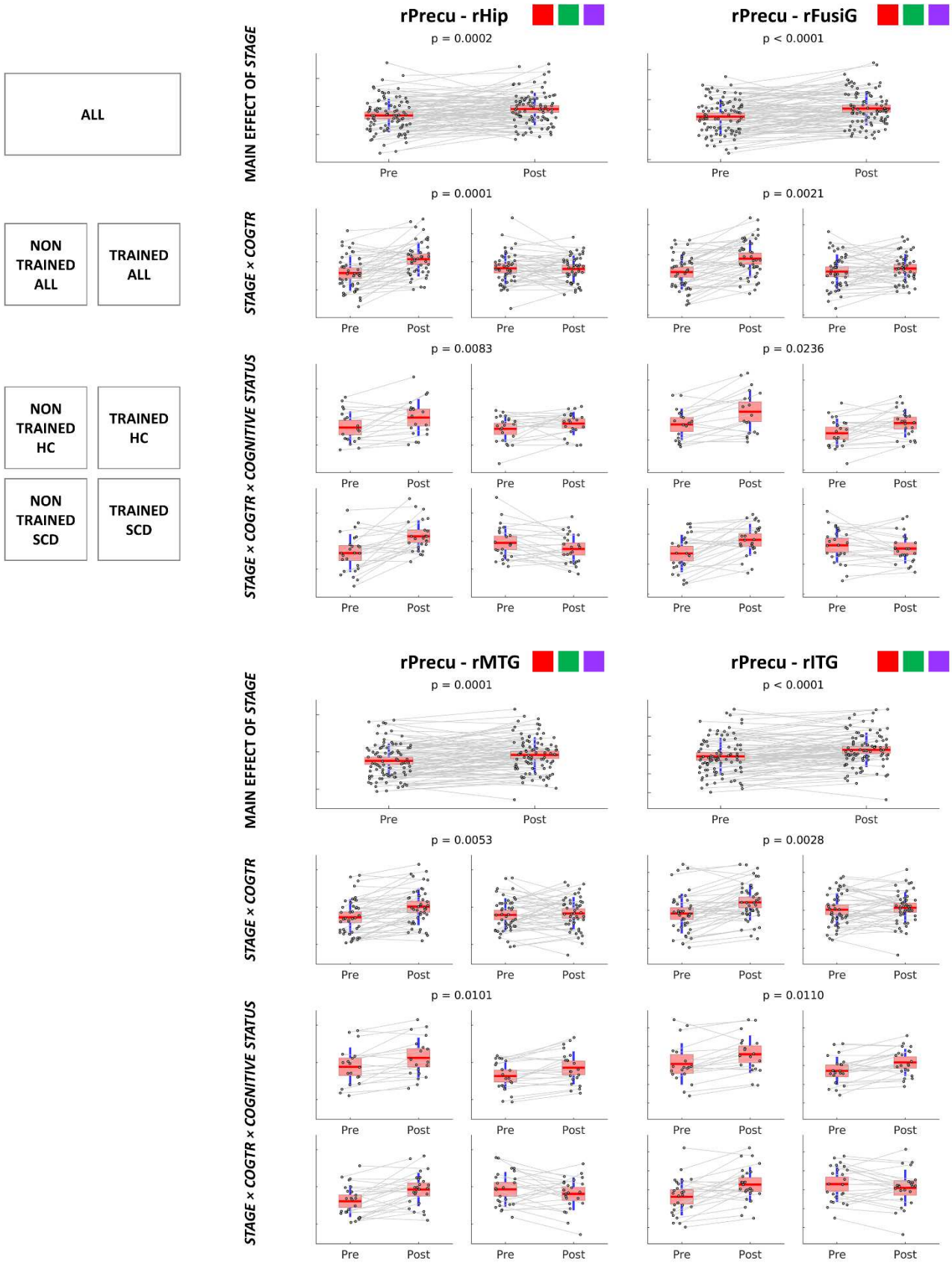

ALL
NON TRAINED ALL
TRAINED ALL
NON TRAINED HC
TRAINED HC
NON TRAINED SCD
TRAINED SCD
MAIN EFFECT OF STAGE
STAGE × COGTR
STAGE × COGTR × COGNITIVE STATUS
rPrecu - rHip
p = 0.0002
rPrecu - rFusiG
p < 0.0001
p = 0.0001
p = 0.0021
p = 0.0083
p = 0.0236
rPrecu - rMTG
p = 0.0001
rPrecu - rITG
p < 0.0001
p = 0.0053
p = 0.0028
p = 0.0101
p = 0.0110
Pre
Post

**Table 1 Summary of the functional connectivity results.** Asterisks indicate the significant connections for the main effect of stage, the double interaction effect between stage and CogTr, and the triple interaction effect between stage, CogTr, and cognitive status.

| Connection | Abbreviation | Main effect | Double interaction | Triple interaction |
|---|---|---|---|---|
| Left Inferior Frontal Gyrus pars opercularis – Left Cuneus | lIFGor — lCu | * | | * |
| Left Inferior Frontal Gyrus pars opercularis – Right Middle Occipital Lobe | lIFGor — rMOccL | * | | |
| Left Superior Temporal Gyrus – Right Cuneus | lSTG — rCu | * | * | |
| Left Superior Temporal Gyrus – Right Superior Occipital Lobe | lSTG — rSOccL | * | * | |
| Left Heschl Area – Right Cuneus | lHeschl — rCu | * | * | |
| Left Heschl Area – Right Superior Occipital Lobe | lHeschl — rSOccL | * | * | |
| Right Cuneus – Right Inferior Frontal Gyrus pars opercularis | rCu — rIFGor | * | | |
| Right Precuneus – Right Inferior Frontal Gyrus pars triangularis | rPrecu — rIFGt | * | | |
| Right Precuneus – Right Inferior Frontal Gyrus pars opercularis | rPrecu — rIFGor | * | | |
| Right Precuneus – Right Middle Temporal Gyrus | rPrecu — rMTG | * | * | * |
| Right Precuneus – Right Inferior Temporal Gyrus | rPrecu — rITG | * | * | * |
| Right Precuneus – Right Fusiform Gyrus | rPrecu — rFusiG | * | * | * |
| Right Precuneus – Right Hippocampus | rPrecu — rHip | * | * | * |
| Right Superior Parietal Gyrus – Right Medial Frontal Gyrus | rSPG — rMFG | * | | |
| Right Superior Parietal Gyrus – Right Inferior Frontal Gyrus pars opercularis | rSPG — rIFGor | * | | |
| Right Superior Parietal Gyrus – Right Inferior Temporal Gyrus | rSPG — rITG | * | | |
| Right Superior Parietal Gyrus – Right Fusiform Gyrus | rSPG — rFusiG | * | | |
| Right Inferior Temporal Gyrus – Right Middle Temporal Gyrus | rITG — rMTG | * | * | * |

**Table 2 Summary of the correlation analysis results.** Correlations between the relative change in both FC and neuropsychological scores in the trained group (HC and SCD). For clarity, only the connections yielding a significant relationship are included in the table. Significant values (FDR corrected) are marked in bold. *n.s*: non-significant. BNT: Boston Naming Test. TMT-A: Trail Making Test part A. TMT-B: Trail Making Test part B.

| Cognitive Test | Significant Connection | Trained HC | | Trained SCD | |
|---|---|---|---|---|---|
| | | $\rho$ | $p$ | $\rho$ | $p$ |
| BNT | rPrecu — rHip | 0.2787 | *n.s.* | -0.4914 | **0.0237** |
| | rPrecu — rITG | 0.3511 | *n.s.* | -0.4487 | **0.0413** |
| Digit Span Test (forward) | rPrecu — rITG | -0.1161 | *n.s.* | -0.4472 | **0.0324** |
| TMT-A (completion time) | rPrecu — rITG | -0.3491 | *n.s.* | 0.4785 | **0.0180** |
| TMT-B (completion time) | rPrecu — rFusiG | -0.1985 | *n.s.* | 0.5024 | **0.0124** |

# Figures

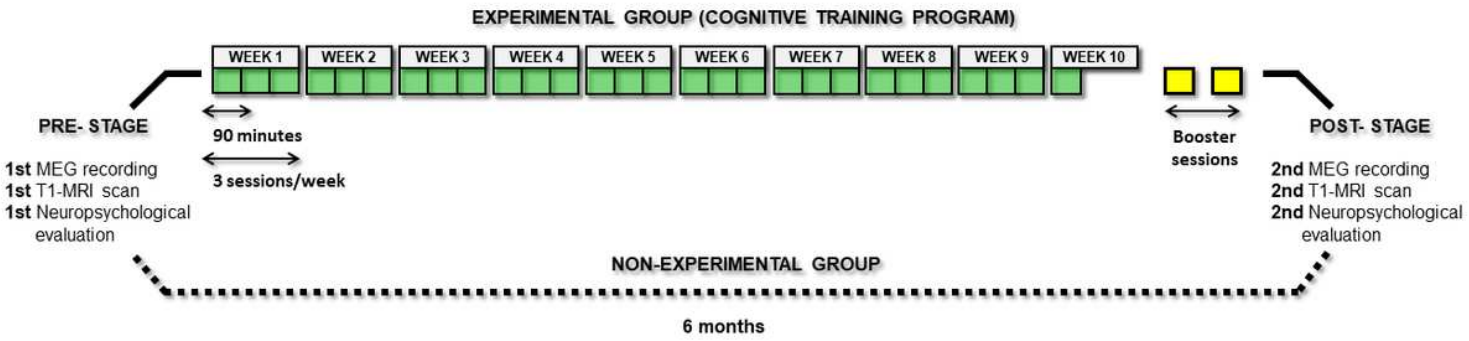



**Figure 1**

Schematic representation of the study design. The participants assigned to the experimental group engaged in 30 CogTr sessions (28 regular sessions and two maintaining-booster sessions) of 90 minutes scheduled three times per week, while no cognitive stimulation or placebo activity was appointed for the non-experimental group. Before and after the CogTr interval, all participants underwent a MEG recording, a T1-weighted MRI scan, and a complete neuropsychological evaluation.

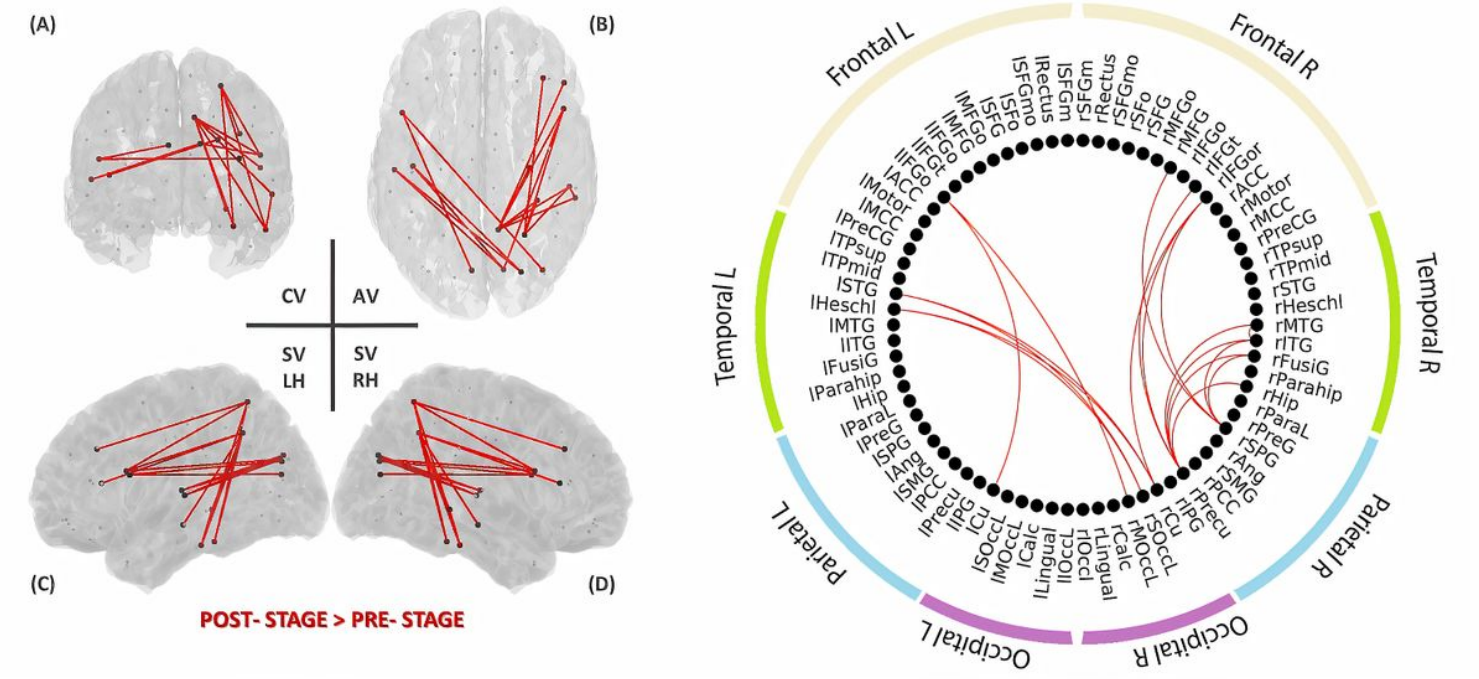
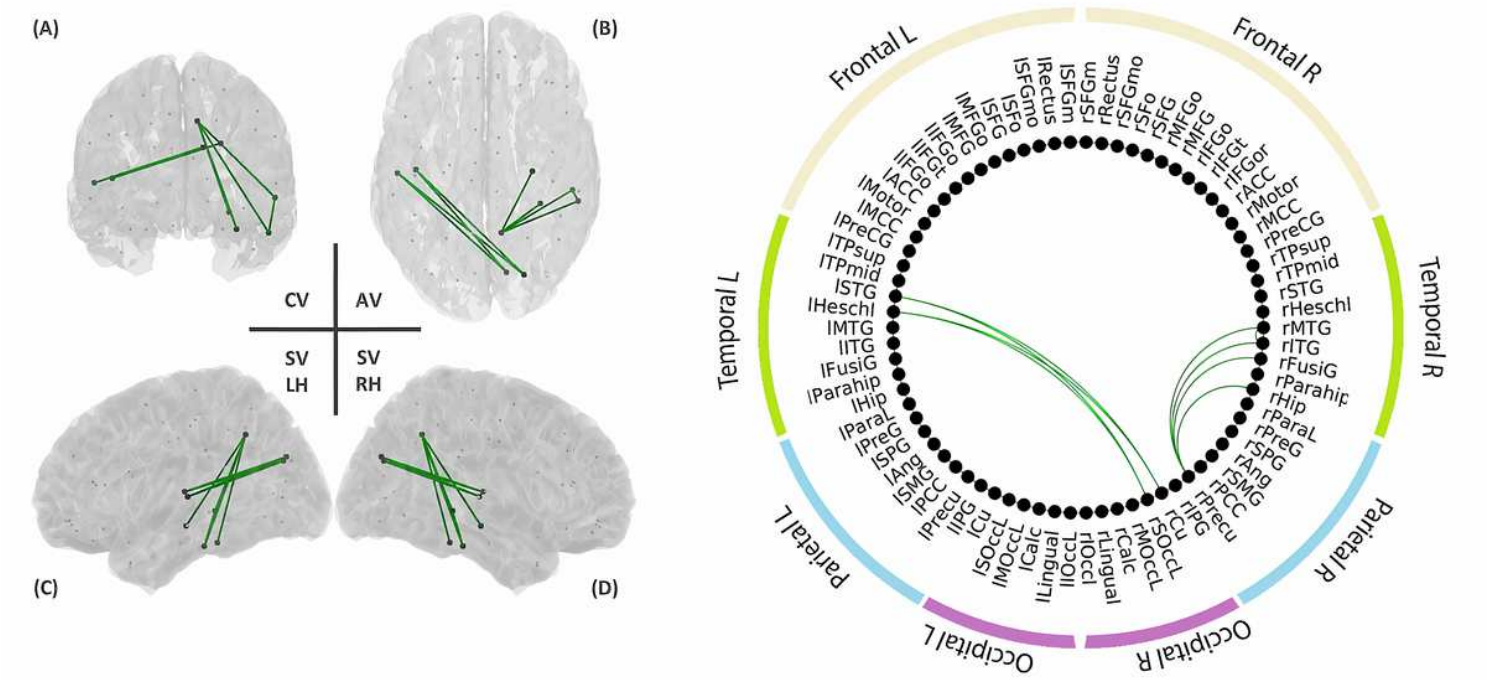


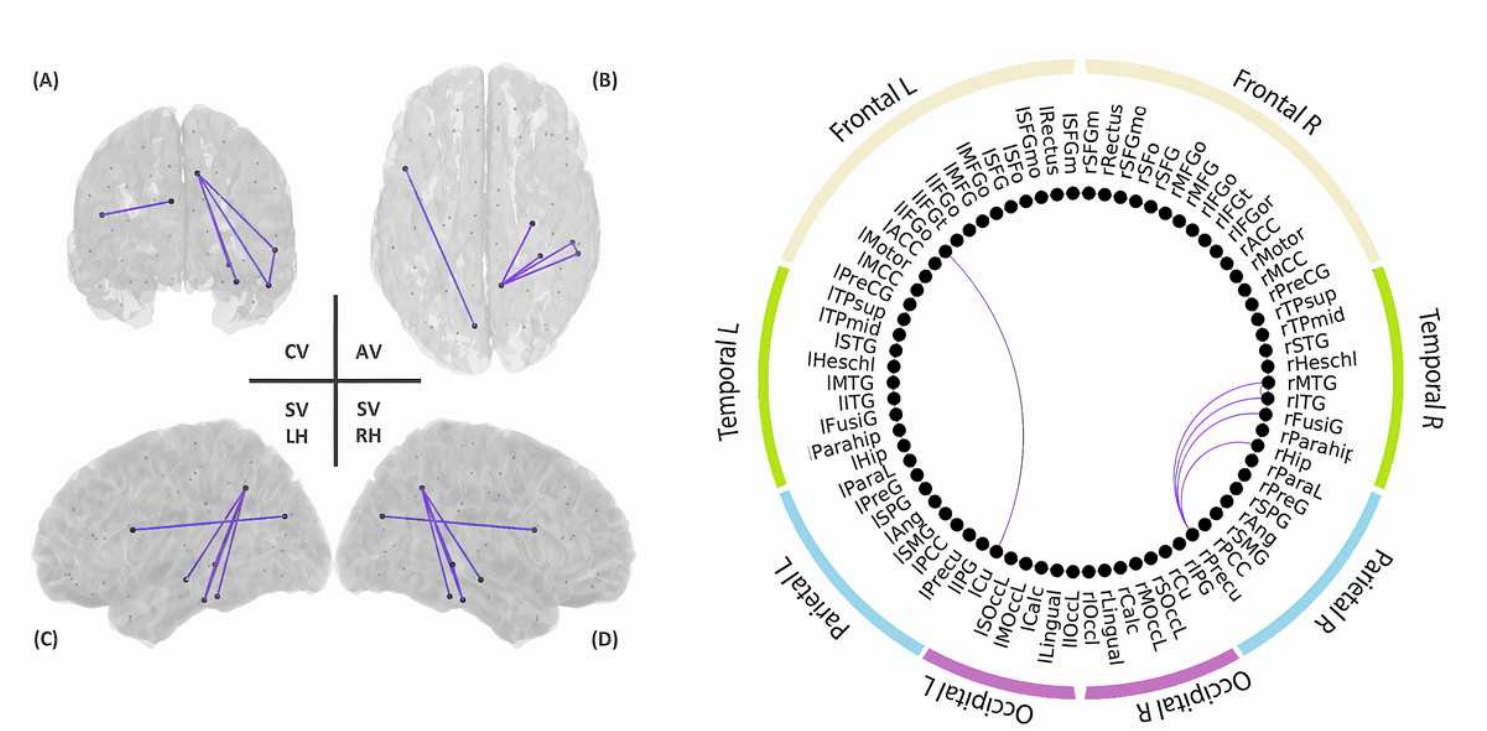

**Figure 2**

FC changes across MEG recordings. Red lines represent significant FC increases in the post-stage (FDR corrected). Left panel: coronal (A), axial (B), and sagittal views (C and D). Right panel: Circular FC diagram. CV: Coronal view. AV: Axial view. SV: Sagittal view. RH: Right hemisphere. LH: Left hemisphere. R: Right. L: Left.




## Figure 3

Interaction effect between stage and CogTr. Green lines represent the connections where the interaction effect between stage (pre-stage or post-stage) and CogTr (trained or non-trained) is significant ($p < 0.05$). Left panel: coronal (A), axial (B), and sagittal views (C and D). Right panel: Circular FC diagram. CV: Coronal view. AV: Axial view. SV: Sagittal view. RH: Right hemisphere. LH: Left hemisphere. R: Right. L: Left.



## Figure 4

Interaction effect between stage, CogTr, and cognitive status. Purple lines represent the connections where the interaction effect between stage (pre-stage or post-stage), CogTr (trained or non-trained), and cognitive status (HC or SCD) is significant ($p < 0.05$). Left panel: coronal (A), axial (B), and sagittal views (C and D). Right panel: Circular FC diagram. CV: Coronal view. AV: Axial view. SV: Sagittal view. RH: Right hemisphere. LH: Left hemisphere. R: Right. L: Left.

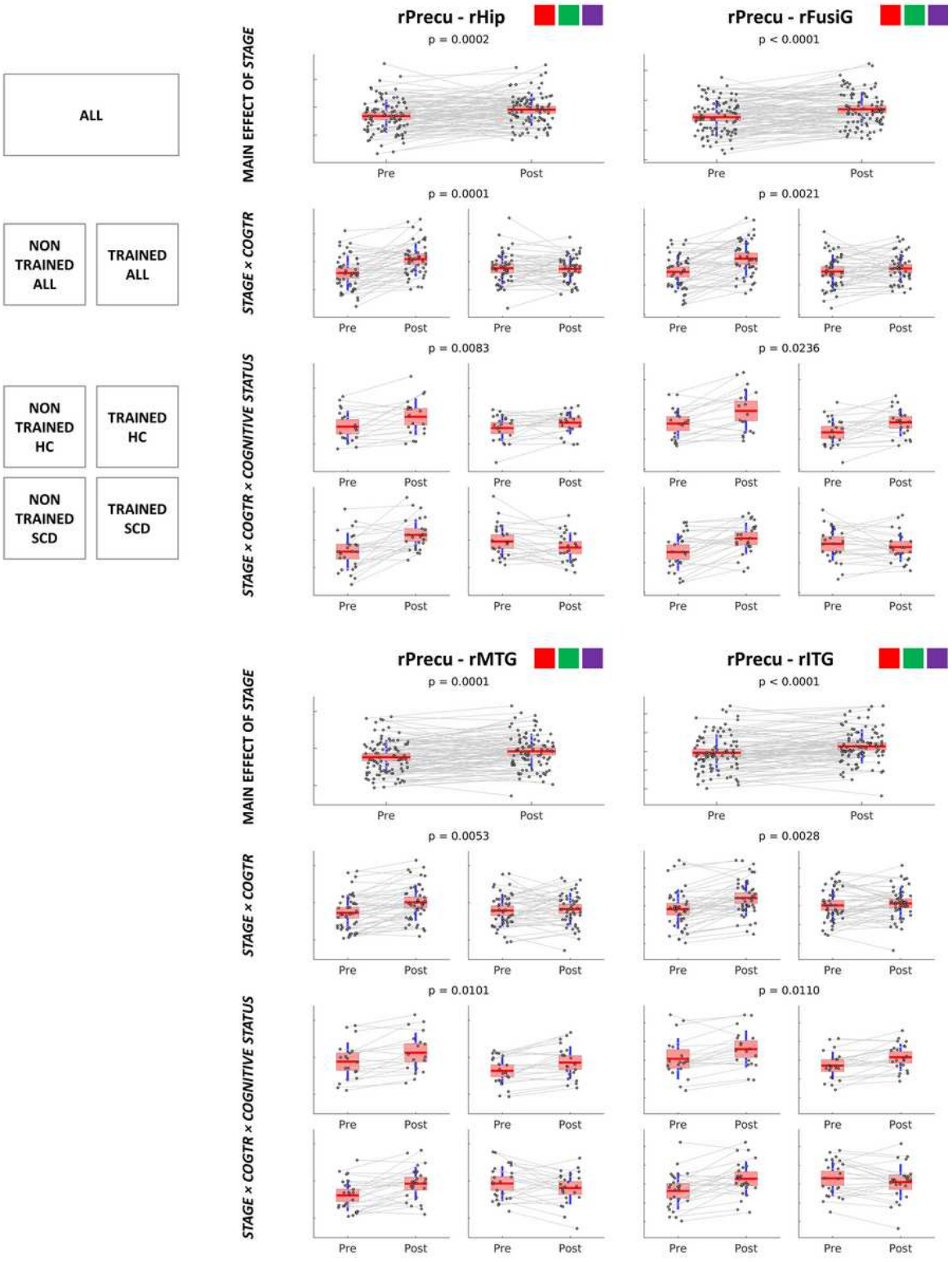


**Figure 5**

Selected boxplots of FC values in the two MEG recordings. Boxplots of the FC values for each participant in the two MEG recordings for the connections with the rPrecu where the interaction effect between stage (pre-stage or post-stage), CogTr (trained or non-trained), and cognitive status (HC or SCD) was significant ($p < 0.05$). For each significant connection, the upper panel represents the FC values in the pre-stage and the post-stage for the whole sample. The middle panel represents the FC values in the pre-stage and the post-stage separately for non-trained participants (left) and trained participants (right). The lower panel represents the FC value in the pre-stage and the post-stage separately for the non-trained HC group (upper row, left), non-trained SCD group (lower row, left), trained HC group (upper row, right), and trained SCD group (lower row, right). The boxplots inform of each participant's FC values, the sample median, and the boundaries of the quartiles. The colored squares next to the abbreviated names of the connections emphasize the significant results for that connection: red (main effect of stage), green (double interaction between stage and CogTr), purple (triple interaction between stage, CogTr, and cognitive status).

## Supplementary Files

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- Additionalfile1.docx