Supplementary methods

Participants

Participants were recruited via announcements at Flemish MS rehabilitation centers, the Flemish MS society and Move-to-sport network. Persons were eligible for study participation if they were over 18 years of age, had clinically definite MS and were able to walk five kilometers (km) without rest or the use of assistive devices (indicative of a maximum EDSS score of 3.5[1]). Walking ability was verified during an information session where interested MS patients jointly walked five km. Patients were excluded when they reported to have run five km in the preceding six months or in the case of a relapse in the preceding three months. The study was approved by the Medical Ethics committees of the Jessa hospital and Hasselt University. Written informed consent was obtained from all participants prior to inclusion.[2]

Neuropsychological testing

The brief repeatable battery of neuropsychological tests was administered (BRB-N), consisting of the Symbol Digit Modalities Test (SDMT), Selective Reminding Test (SRT), Word List Generation (WLG) and Spatial Recall Test (SPART).[3]

(f)MRI data collection

MRI scanning was performed at UZA Antwerp in a 3 Tesla whole-body scanner (Siemens Magnetom Trio Tim, Erlangen, Germany) using a 32-channel head coil. The protocol included a 3D T1-weighted magnetization-prepared rapid gradient-echo sequence for volumetry and registration purposes (MPRAGE; TR = 2300 ms, TE = 2.9 ms, TI = 900 ms; 176 sagittal slices of 1.2 mm thickness, 253x270 mm^2 field-of-view (FOV), in-plane resolution of 1.05x1.05 mm^2 and a flip angle of 9 degrees); a 3D fluid attenuated inversion recovery sequence for white matter lesion segmentation and filling (FLAIR; TR = 5000 ms, TE = 388 ms, TI = 1800 ms, 160 slices of 1 mm thickness with a FOV of 250x250 mm^2, an in-

plane resolution of 0.49x0.49 mm^2, flip angle 120 degrees); and a functional resting-state (eyes-closed) EPI scan consisting of 140 volumes (TR = 3000 ms, TE = 30 ms; 50 slices of 2.8mm thickness and with a 3mm slice gap; in-plane resolution of 2.5x2.5 mm^2, acquisition time 7 minutes).

Structural MRI analysis

White matter lesions were automatically segmented on FLAIR images and filled on the 3DT1 images using LEAP.[4] SIENAx was used to segment grey matter and white matter volumes and for subcortical grey matter segmentation FIRST was used (https://fsl.fmrib.ox.ac.uk/fsl). All volumes were corrected for head size.

fMRI preprocessing

All preprocessing steps were conducted using the MELODIC pipeline in FSL 5.0 (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl). This consisted of discarding the first five volumes, motion correction, spatial smoothing (6 mm full-width-at-half-maximum), high-pass filtering (100s) and removal of non-brain tissue. Subsequently, resting-state scans were registered to the 3DT1 scans and non-linearly registered to Montreal Neurological Institute (MNI152) standard space. All registrations were visually inspected and checked for excessive motion.

Atlas construction and FC calculation

After preprocessing of resting state fMRI data, the Brainnetome atlas was employed to parcellate structural and functional MRI.[5] The Brainnetome atlas in standard space was then non-linearly registered to each participant's 3DT1 image and masked for grey matter. The Brainnetome atlas consisted of 210 cortical areas and was completed with 14 subcortical regions derived from FSL's FIRST, resulting in 224 regions. This atlas was then linearly registered to fMRI space and voxels with a signal intensity in the lowest quartile were removed to limit the effect of regions with known susceptibility for artifacts (e.g. orbitofrontal cortex). In order to define the DMN, the Yeo7 RS network was overlaid on the Brainnetome atlas.[6] 38 out of 224 Brainnetome atlas regions were considered to make up the

DMN (see table S1 below). For all regions, the average time series were extracted and imported into Matlab R2012a (Natick, Massachusetts) for further analysis.

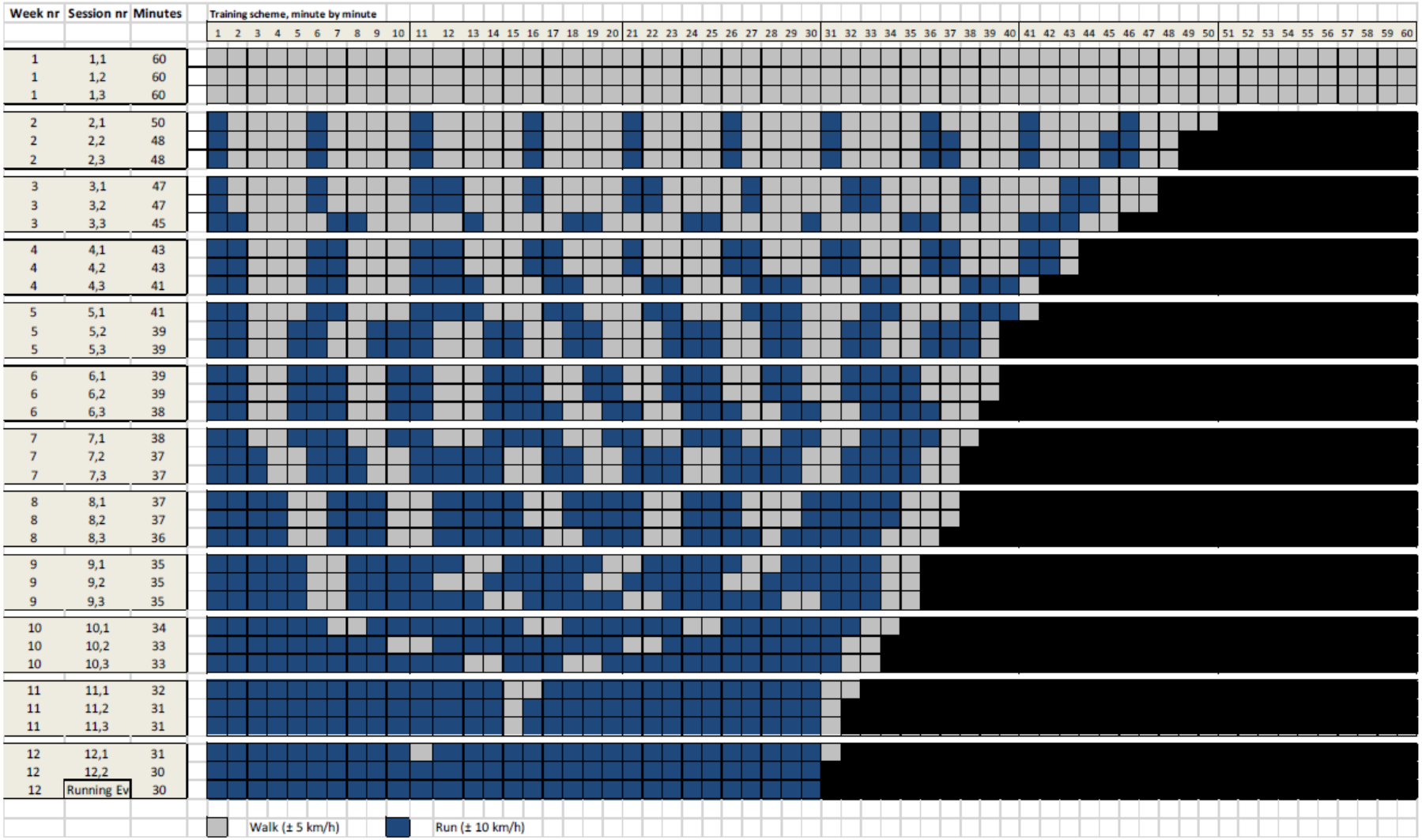
To obtain FC measures, the Pearson correlation coefficients between the left and right hippocampus and DMN were calculated separately and then averaged resulting in a single value per person representing the connectivity of the bilateral hippocampus with the DMN.

Table S1 | Brainnetome atlas regions forming the default-mode network.

BNA area number	BNA label	BNA coordinates	BNA area number	BNA label	BNA coordinates
3	*A8dl, dorsolateral area 8*	-18, 24, 53	**84**	*A21r, rostral area 21*	51, 6, -32
4	*A8dl, dorsolateral area 8*	22, 26, 51	**87**	*aSTS, anterior superior temporal sulcus*	-58, -20, -9
5	*A9l, lateral area 9*	-11, 49, 40	**88**	*aSTS, anterior superior temporal sulcus*	58, -16, -10
6	*A9l, lateral area 9*	13, 48, 40	**95**	*A20il, intermediate lateral area 20*	-56, -16, -28
11	*A9m,medial area 9*	-5, 36, 38	**113**	*TL, area TL (lateral PPHC, posterior parahippocampal gyrus)*	-28, -32, -18
13	*A10m, medial area 10*	-8, 56, 15	**121**	*rpSTS, rostroposterior superior temporal sulcus*	-54, -40, 4
14	*A10m, medial area 10*	8, 58, 13	**141**	*A40c, caudal area 40(PFm)*	-56, -49, 38
23	*A8vl, ventrolateral area 8*	-33, 23, 45	**143**	*A39rv, rostroventral area 39(PGa)*	-47, -65, 26
27	*A10l, lateral area10*	-26, 60, -6	**144**	*A39rv, rostroventral area 39(PGa)*	53, -54, 25
33	*A45c, caudal area 45*	-53, 23, 11	**153**	*A31, area 31 (Lc1)*	-6, -55, 34
35	*A45r, rostral area 45*	-49, 36, -3	**154**	*A31, area 31 (Lc1)*	6, -54, 35
39	*A44v, ventral area 44*	-52, 13, 6	**175**	*A23d, dorsal area 23*	-4, -39, 31
41	*A14m, medial area 14*	-7, 54, -7	**176**	*A23d, dorsal area 23*	4, -37, 32
42	*A14m, medial area 14*	6, 47, -7	**178**	*A24rv, rostroventral area 24*	5, 22, 12
43	*A12/47o, orbital area 12/47*	-36, 33, -16	**179**	*A32p, pregenual area 32*	-6, 34, 21
44	*A12/47o, orbital area 12/47*	40, 39, -14	**181**	*A23v, ventral area 23*	-8, -47, 10
51	*A12/47l, lateral area 12/47*	-41, 32, -9	**182**	*A23v, ventral area 23*	9, -44, 11
81	*A21c, caudal area 21*	-65, -30, -12	**187**	*A32sg, subgenual area 32*	-4, 39, -2
83	*A21r, rostral area 21*	-53, 2, -30	**188**	*A32sg, subgenual area 32*	5, 41, 6

BNA = Brainnetome atlas

Figure S1 | Training schedule showing the exercise type (i.e. running or walking) per minute of each training on the x-axis. The y-axis shows the training duration per week and training session.



Supplementary Results

Participants

In the original study[2], 18 participants in the intervention group and 17 in the wait list control group completed the trial. Of these persons, four (two in each group) did not have imaging data due to MR contraindications and logistical problems. For two pwMS (one in each group), imaging data could not be analyzed due to errors in the automated segmentation. The current analysis, therefore, included 15 participants in the intervention group and 14 in the wait list control group.

Neuropsychology

To establish whether the relationship between SPART delta-scores and FC of hippocampus-DMN delta-scores was specific or also present in other cognitive domains, the delta scores of the other cognitive tests were related to the FC delta-scores. The results can be found in Table S2 below and show that for no other cognitive test significant associations were observed.

Table S2 | Cognitive test scores and relationships to functional connectivity. F-statistics represent group*time interaction effects on 2x2 ANOVAs. Correlation analyses of the delta scores of each test and the delta scores of hippocampus-DMN FC were corrected for age.

	Intervention group (n=15)	Control group (n=14)	F-statistic	p-value	Correlation Δ-test to Δ-FC hip-DMN	p-value
SDMT pre	92.2 (14.5)	84.6 (12.8)				
SDMT post	93.7 (16.3)	87.3 (10.3)	.14	.711	.168	.411
PASAT pre	49.6 (5.7)	47.5 (11.5)				
PASAT post	51.9 (8.1)	48.9 (6.4)	.05	.819	.028	.894
SRT pre[a]	51.5 [43.75-54.0]	51.5 [47.5-53.0]				
SRT post[a]	50.0 [38.0-56.0]	52.5 [42.75-59.25]	.457	.505	-0.17	.415
WLG pre	30.6 (9.1)	32.1 (10.4)				
WLG post	32.1 (7.8)	31.9 (8.2)	.36	.556	-.145	.490

SDMT = symbol digits modalities test; PASAT = paced auditory serial addition test; SRT = serial reminding test; WLG = word list generation; pre = baseline test; post = follow-up test. [a] Median [IQR]

Supplementary references

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