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Effects of rhythmic-cued gait training on gait-like task related brain activation in people with multiple sclerosis

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ABSTRACT

Background: Walking impairment is one of the most debilitating symptoms of multiple sclerosis (MS). A better understanding of brain mechanisms underlying successful gait training could help to improve development of targeted therapy. We therefore investigated changes in brain activation associated with improvements in walking function after rhythmic-cued gait training.

Methods: Thirty-one people with MS (pwMS; median EDSS = 2.5, range:2.0–5.0) and 17 age- and sex-matched healthy controls (HC) completed behavioural and MRI assessments at baseline and post-intervention (four weeks after baseline). All included pwMS received a four-week actual and/or imagined gait training with rhythmic-auditory cueing, while HC received no intervention. All participants performed a bipedal ankle plantar- and dorsiflexion and a corresponding motor-imagery task during fMRI. PwMS displaying a > 5 % walking distance increase in the 2-Minute Walk Test (2MWT) from baseline to post-intervention were defined as responders.

Results: Responders did not differ from non-responders in terms of demographics, clinical variables, and walking function at baseline. Responders, non-responders, and HC showed similar movement-related brain activation at baseline. At post-intervention, responders showed decreased brain activation within the premotor cortex, precuneus, and middle frontal gyrus during the movement task. Stronger decreases within these areas were associated with higher walking function improvements in all pwMS after controlling for potential confounders. No association was observed between walking function and motor imagery-related brain activation changes.

Conclusion: Improved walking function after rhythmic-cued gait training was associated with reduced brain activation in motor planning and attention areas. This suggests a more efficient recruitment of areas subserving motor function after successful training.

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1. Introduction

Multiple Sclerosis (MS) represents one of the most common causes of neurological disability in young adults [1]. Walking impairment is a key symptom of MS [2], affecting various aspects of daily life and substantially reducing the quality of life of those affected [3]. Despite constant advancement of disease-modifying treatments (DMTs) within the last decades, disability accrual in MS cannot yet be fully prevented and functional impairment cannot be restored by DMTs alone [4].

Therefore, complementary rehabilitative treatment is crucial for mitigating disability accrual [2,5]. To date, a variety of interventions have been developed and found to improve several aspects of motor function [6,7]. Seebacher et al. [8], for example, developed a physiotherapeutic intervention for people with MS (pwMS) aimed at walking function that involves gait trainings in a home-based setting. In two previous randomised-controlled trials, Seebacher et al. compared differently cued and non-cued motor imagery trainings of gait to (passive) controls [8,9]. While all investigated motor imagery trainings significantly improved walking function, rhythmic-cued training incorporating a combination of music- and verbal cueing showed the highest positive impact on walking function, fatigue, and quality of life [8,9].

While it is generally assumed that improvements in motor function are driven by neuroplasticity [10,11], the brain mechanisms behind successful rehabilitation need to be further explored [5,12]. Functional magnetic resonance imaging (fMRI) is a powerful, non-invasive tool to investigate adaptive or maladaptive functional brain plasticity [6,13]. Specifically, task-fMRI allows to investigate brain mechanisms involved in the performance of relevant tasks by inferring on brain activation based on the blood-oxygenation level dependent (BOLD) effect [13]. So far, only a few studies have investigated the effects of motor training on task-related functional brain plasticity in pwMS, mostly focusing on upper limb tasks, and those studies have yielded mixed results [12,14–16]. This can be partly attributed to the substantial variety in interventions and study designs, but also to the considerable heterogeneity in terms of fMRI-tasks, ranging from simple finger-tapping to more complex motor-cognitive tasks [5,6], for reviews, see [14,17].

To the best of our knowledge, only one previous study has investigated the effects of motor training (resistance or endurance training) on lower-limb task-based functional brain plasticity in pwMS [18]. In this study, Tavazzi et al. [18] have reported a reduction in the extent of brain activation after four weeks of resistance or endurance training. The reduction in brain activation was not related to improvements in walking function.

The present study is the imaging sub-study of a larger, multi-centre randomised parallel trial ($n = 132$ pwMS). In this trial, Seebacher et al. [19] have shown that rhythmic-cued gait trainings (including music and verbal cues), involving either motor imagery of gait, actual gait, or a combination thereof lead to equal improvements in walking function in people with MS (pwMS).

In the present study, we aimed to investigate potential changes in lower-limb task-related brain activation after four weeks of rhythmic-cued gait training in association with improvements in walking function. Therefore, we compared brain activation (changes) between responders of the training (i.e., pwMS showing improvements in walking function), non-responders, and healthy controls (HC). Based on previous literature, we expected a decrease in brain activation within motor areas after successful training.

2. Methods

2.1. Study design

This study is part of a double-blind, randomised trial conducted in three Austrian centres (Medical Universities of Innsbruck and Graz, and Clinic for Rehabilitation Muenster; Trial registration number: DRKS00023978). Within the overall study, 132 pwMS were randomly assigned to one out of three rhythmic-cued training groups: motor imagery training, gait training or a combination of both.

Trainings were performed at home (4 times/week; 30 min/training), supported by weekly phone calls. All pwMS exercised via an audio-mix, specifically created for this study using Audacity® Version 3.0.0, which they downloaded to their respective electronic devices. The audio-mix contained weekly changing 30-min training sessions, thus, all pwMS were instructed to perform each session four times. Each session entailed training-specific instructions on music-cued kinaesthetic motor imagery vs music-cued actual gait, followed by rhythmic-cued gait exercises (e.g., "raise your knees", "take long/giant strides"). The exercises and rhythmic-cues were the same across all training groups (i.e., participants were either instructed to mentally execute the exercises using kinaesthetic motor imagery or to actually execute them on a straight path). Rhythmic-cues consisted of weekly changing music excerpts with a regular beat in a 2/4 or 4/4 metre, overlapped with metronome cues to further accentuate the beat as well as exercise-specific verbal cues (e.g., "toe-off", "step-step"). Participants were allowed to choose their preferred times and days for practicing. Further details concerning outcomes as well as a template for intervention description and replication (TIDieR) are published in the study protocol [20].

The imaging sub-study was performed at the Medical University of Graz, conducting additional MRI examinations at baseline (BL) and after four weeks of training (post-intervention, PI). Only data concerning participants included in the imaging sub-study are presented here.

Since the aim of this imaging sub-study was to investigate potential neuronal mechanisms underlying improvements in walking function after rhythmic-cued gait training, we decided to split the cohort into responders and non-responders. As threshold for the definition of responders, we chose an increase in walking distance within the 2MWT of more than 5 %. This threshold was chosen to be above the expected measurement error of the 2MWT (coefficient of variation between 2 time points: 4–6 % [21]; minimally important improvement from patient/therapist perspective: 6.8–9.6 m (∼4–6 %) [22]).

We decided to perform the division between responders and non-responders irrespective of the training groups for two reasons: Firstly, based on the results of the main study, we expected similar improvements in walking function across all training groups, with no difference between the three trainings regarding changes in walking function [19]. Secondly, we expected that, even though actual and imagined gait training might lead to slightly different changes in brain function, the neuronal mechanisms that are associated with improvements in walking function after rhythmic-cued gait training should be the same. This is also supported by previous literature indicating motor imagery and actual motor training lead to similar changes in functional neuroplasticity [23].

Nevertheless, we performed sensitivity analyses comparing training groups instead of responders and non-responders for walking function as well as outcomes from task-related fMRI to rule out the possibility of an overlapping effect of training (see section *Sensitivity Analyses for Training Groups* in the Supplementary Material).

2.2. Participants

Thirty-six pwMS were included in the imaging sub-study. The enrolment period lasted from 9th February 2021, to 15th November 2022. In- and exclusion criteria for the study are described in detail elsewhere [19,20]. In brief, the main inclusion criteria were: a) definite MS [24], b) mild to moderate disability (Expanded Disability Status Scale (EDSS) [25] scores 2.0 to 5.0), and c) age $\geq$ 18 years. Main exclusion criteria were a) relapses or adjustments in DMT or physiotherapy within 3 months before inclusion, b) concomitant diseases, and c) MRI-contradictions.

Additionally, 17 age- and sex-matched healthy controls (HC) without history of neurologic or psychiatric disorders underwent MRI-scanning twice within four-week-intervals to provide reference values for MRI-analyses. The final cohort consisted of 31 pwMS and 17 HC. For a detailed study-flow, see Fig. 1.

The study was approved by the Ethics Committees of the Medical Universities of Innsbruck and Graz (22.12.2020; references 1347/2020 and 33–056 ex 20/21) and was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent prior to inclusion in the study.

2.3. Clinical and walking assessments

Clinical and demographic data were assessed at BL and included age, sex, handedness, education, disability (EDSS), disease duration, phenotype, and DMT (3 categories: no DMT, moderate-efficacy DMT, high-efficacy DMT [26]).

Two measures of walking function were assessed at both timepoints: the 2-Minute Walk Test (2MWT) [27] and the Timed 25-Foot Walk Test (T25FW) [28]. The 2MWT was performed in accordance with published guidelines [29,30]. In the T25FW, participants were asked to walk the distance of 25 ft as quickly as possible, but safely, and were allowed to use an assistive device if required. The average speed (in m/s) of two trials was used for scoring.

2.4. MRI-acquisition

MRI-data was acquired on a 3 Tesla scanner (Siemens PRISMA, Siemens Healthcare Erlangen) using a 20-channel head coil. The MRI protocol included a high-resolution structural three-dimensional (3D) T1-weighted MPRAGE sequence with 1 mm isotropic resolution (repetition time (TR) = 1900 ms, echo time (TE) = 2.7 ms). A T2-weighted 3D fluid-attenuated inversion recovery (FLAIR) sequence with 1 mm isotropic resolution was acquired to assess hyperintense T2-lesion volume in the patient cohort (TI = 1800 ms, TR = 5000 ms; TE = 393 ms, flip angle = 120°, matrix = 256x256x176). Task-based fMRI scans were acquired using a 2D echo-planar imaging (EPI) sequence with T2*-weighted BOLD contrast (voxel size = 2 mm isotropic; TR = 2500 ms; TE = 30 ms; 198 volumes, flip angle = 80°, matrix = 94x94x80, acquisition time = 8.31 min). Additionally, diffusion-weighted and resting-state imaging were performed, which are not part of this study. The scan-sessions took about 40 min including all administered sequences, leading to approximately 55 min of absolute time in the scanner including participant positioning and familiarisation with the treadmill used during task-fMRI.

2.5. Task-fMRI: procedure

The block-fMRI paradigm included a lower-limb movement condition, comprising alternating dorsi- and plantarflexion of both feet on a treadmill [31] and a corresponding motor-imagery condition.

During the motor-imagery condition, participants were asked to perform kinaesthetic motor imagery, i.e., to try to *feel* themselves performing the bipedal ankle movement without actual execution, rather than *seeing* themselves performing it [32]. Participants were instructed to perform the ankle movement and motor-imagery at the pace of instrumental music-cues, starting with the right foot. During a control condition, the same music-cues were presented and participants were asked to concentrate on the music beat only. As music-cues, four instrumental music-excerpts with a pace of 110 beats/min were selected based on the same criteria used in the interventions [33], and additionally accentuated with metronome cues. Each condition (i.e., *movement*, *motor-imagery*, and *control: listen-only*) was presented for 22.5 s (9 volumes) and followed by 15 s of total rest (6 volumes). Each condition was repeated four times in a pseudo-randomised order, assuring that no condition or music-cue occurred twice in a row. Each participant completed the paradigm in the same order.

Participants practiced the paradigm on a monitor in a separate room prior to entering the scanner and got familiarised with the treadmill once inside the scanner. Throughout the entire paradigm, participants were instructed to look at a fixation cross in the middle of the screen, not to move their heads and to relax their entire body except for their feet during the movement condition. To decrease stimulus-correlated

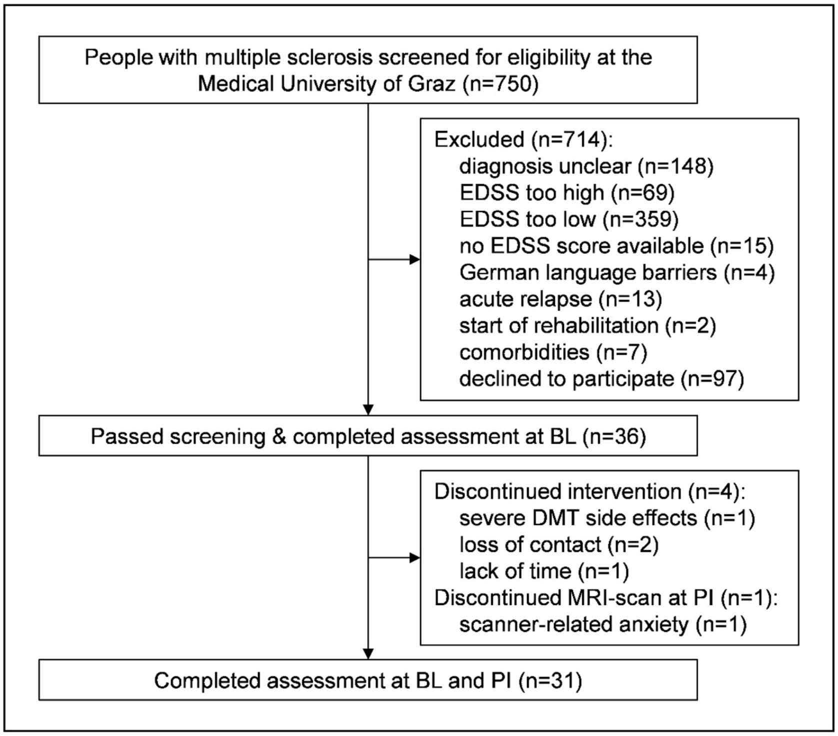

Fig. 1. Illustration of the study flow concerning people with multiple sclerosis screened at the Graz-centre who were included in or excluded from the imaging substudy. BL = baseline; DMT = disease modifying treatment; PI = post-intervention; EDSS = Expanded Disability Status Scale.

motion, participants' heads were fixed with foam-cushions and their knees flexed to approximately 135° using a soft roll and cushion beneath their knees [34]. Vision was corrected with prism lenses if necessary. During the paradigm, participants were observed to ensure the correct execution of the movements.

2.6. *Data analysis*

2.6.1. *Conventional MRI analysis*

In order to assess MS-related lesion volume (ml), hyperintense white matter lesions were segmented on FLAIR-images with the Lesion Segmentation Toolbox (SPM 12), using the automated lesion prediction algorithm (LPA) [35]. Binary lesion masks were created using fslmaths (FSL; v6.04 binarisation threshold = 0.25) and individual lesion volumes were extracted using fslstats (FSL, v6.04) [36].

After lesion-filling was performed using the FSL lesion-filling toolbox [37], normalised brain volumes were estimated on high-resolution T1-weighted images using SIENAX [36,38] (FSL, v6.04). Brain volumes were normalised for head size using the V-scaling factor derived by SIENAX. The processing pipeline for brain volume estimation was used in a previous study by our group [39] and is available online (https://github.com/neuroimaging-mug/ms-volest). Raw images and outputs from the segmentation pipeline were reviewed to ensure correctness of lesion detection.

2.6.2. *Task-fMRI preprocessing*

Task-fMRI data were pre-processed using FEAT (FMRIB's Expert Analysis Tool, v 6.00, part of FSL v6.0.4 [40]) as follows: motion correction (MCFLIRT), brain extraction (BET), spatial smoothing using a Gaussian kernel full width half maximum of 5 mm [41], high pass temporal filtering using a cut-off of 100 s (0.01 Hz), linear registration to the individual T1 image (BBR) and nonlinear registration (warp resolution 10 mm) to standard MNI152 space with 2 mm isotropic resolution. All data were visually inspected before and after pre-processing.

First-level task-fMRI analyses were performed separately for each participant and session (BL, PI). Six realignment parameters were included as covariates in first-level analyses. To further minimise the influence of head motion on signal estimation, we computed mean Framewise Displacement (FD) for each volume (command: *fsl_motion_outliers*, part of FSL) [42]. We included high motion time points (FD > 0.9 mm [43]) in the first-level analysis using confound matrices, censoring the influence of those time points from the model [44]. All participants had <20 % of high motion time points and mean FD did not differ between groups at any timepoint (see Supplementary Table 1).

2.6.3. *Statistical analysis*

Statistical analyses were performed using SPSS, v. 27.0. (Armonk, NY: IBM Corp). The level of significance was set at 0.05.

We first compared responders to non-responders (and, where relevant, to HC) in terms of demographics, clinical data, conventional MRI, and distribution of training groups. When assumptions for analyses of variance (ANOVAs) were met, group comparisons for continuous variables were performed using one-way ANOVAs (i.e., age, disease-duration, normalised brain-volume (NBV)).

For ordinal and skewed variables (i.e., education, EDSS, T2-lesion volume), groups were compared using Mann Whitney-U or Kruskal-Wallis tests. For nominal variables, Fisher's exact tests were used.

For walking test outcomes (2MWT, T25FW), we conducted mixed design ANOVAs with group (responders, non-responders, and HC) as between-subjects and time (BL, PI) as within-subjects factor.

Where appropriate, *p*-values for multiple independent tests were corrected with Benjamini-Hochberg procedure. *P*-values for post-hoc comparisons (ANOVAs) were always adjusted using the Bonferroni procedure.

2.6.4. *Task-fMRI analysis*

Second-level analyses for group comparisons were performed at BL using FLAME1 mixed-effects analyses (FMRIB's Local Analysis of Mixed Effects) [45], separately for the contrasts *movement > control* and *motor-imagery > control*. Maps were corrected for multiple comparisons using family-wise error correction (Gaussian random field theory) with a cluster-forming threshold of $z > 3.1$ and a cluster-wise significance of $p < 0.05$ [46].

To assess changes from BL to PI, we first performed 2nd-level analyses (Fixed-Effects) separately for each participant to create individual difference-maps of activation between BL and PI. These difference-maps were fed into a 3rd-level mixed-effects model comparing groups (FLAME1, corrected for multiple comparisons using a cluster-forming threshold $z > 2.3$ and a cluster-wise significance of $p < 0.05$ [47]).

To further investigate potential linear correlations of individual activation scores extracted from relevant clusters with changes in walking function using post-hoc (sensitivity) analyses, we extracted the mean brain activation (mean z-stat values) within those clusters using FEATQUERY (part of FSL).

Firstly, post-hoc correlational analyses between mean z-stat values and absolute changes in walking function were performed across all pwMS using SPSS and visually inspected using scatter-plots to discern whether a) singular outliers or b) the threshold we set to define responders could have influenced the whole-brain results. Secondly, in sensitivity analyses, we computed partial correlations within all pwMS, controlling for either a) age and sex, b) T2-lesion volume, c) NBV, or d) baseline walking distance, to rule out the possibility that potential covariates might better explain the results obtained in whole-brain analyses. *P*-values were corrected for multiple comparisons using the Benjamini-Hochberg procedure to control for the false discovery rate.

3. Results

3.1. *Baseline characteristics*

Baseline characteristics of the entire sample are depicted in Table 1. Responders did not differ from non-responders and HC in terms of sex, age, handedness, and education (for *p*-values, see Table 1). HC had higher NBV than both patient groups, while there was no difference between responders and non-responders ($F_{(2,45)=}7.014$, $p = 0.002$; HC-Responders: $p = 0.013$; HC-Non-Responders: $p = 0.005$; Responders-Non-Responders: $p = 1.000$). There were no statistical differences between responders and non-responders regarding distribution of training groups or any of the clinical variables analysed (EDSS, disease-duration, phenotype, type of DMT, T2-lesion volume; see Table 1).

3.2. *Walking assessments*

The ANOVA conducted for the *2MWT* showed a significant effect of group ($F_{(2,43)} = 4{,}9$, $p = 0.012$, partial eta $(\eta)^2 = 0.19$), a significant effect of time ($F_{(1,\ 43)} = 22.8$, $p < 0.001$, $\eta^2 = 0.346$) and a significant interaction (group*time, $F_{(2,43)} = 14.9$, $p < 0.001$, $\eta^2 = 0.410$). Post-hoc-tests revealed a higher BL walking distance in HC than either of the patient groups (HC-responders: $M_{Difference} = 39.2$ m, 95 %CI = [7.5;70.9], $p = 0.011$; HC-non-responders: $M_{Difference} = 28.2$ m, 95 %CI = [1;55.5], $p = 0.040$), while responders and non-responders did not differ at BL ($p > 0.05$). Furthermore, post-hoc tests showed that responders significantly increased in walking distance ($p < 0.001$), while non-responders or HC did not (both $p > 0.05$, Table 2).

The ANOVA conducted for the *T25FW* showed a significant group effect ($F_{(2,43)} = 3.8$, $p = 0.029$, $\eta^2 = 0.15$), but no effects of time ($F_{(1,43)} = 0.03$, $p = 0.854$, $\eta^2 = 0.001$) or group*time ($F_{(2,43)} = 0.36$, $p = 0.700$, $\eta^2 = 0.02$). Post-hoc comparisons revealed a higher walking speed in HC compared to non-responders (HC-responders: $M_{Difference} = 0.15$ m/s, 95 %CI = [−0.20;0.50], $p = 0.888$; HC-non-responders: $M_{Difference} = 0.33$ m/s, 95 %CI = [0.03;0.63], $p = 0.026$; responders-non-responders:

Table 1

Baseline characteristics of demographic, clinical and conventional MRI variables across the study sample.

Characteristic	Responders	Non-responders	HC	*p*[a]	*p corr*[b]
Sample size, *n*	11	20	17		
Sex, female *(%)*	4 (36.4)	11 (55)	8 (41.1)	0.615	0.905
Age, years, *mean (SD)*	43 (6.4)	44.9 (9.4)	41.2 (12)	0.532	0.905
Handedness, *right (%)*	10 (90.9)	19 (95)	16 (94.1)	1.000	1.000
Education, years, *med (IQR)*	14 (5)	13.5 (5)	18 (7)	0.050	0.275
EDSS, *med (IQR)*	2.5 (1)	3.5 (1.5)	–	0.197	0.722
DD, years, *mean (SD)*	11.5 (8.7)	13.9 (9.5)	–	0.492	0.905
DMT[c], *n (%)*				0.895	1.000
No DMT	3 (27.3)	4 (20)	–		
Moderately effective DMT	4 (36.4)	9 (45)	–		
Highly effective DMT	4 (36.4)	7 (35)	–		
MS Phenotype, *n (%)*				0.215	0.676
Relapsing-remitting	8 (72.7)	16 (80)	–		
Progressive MS	3 (27.3)	4 (20)	–		
Training Group, *n (%)*				0.325	0.894
Motor Imagery	3 (27.3)	7 (35)	–		
Walking & Motor Imagery	3 (27.3)	9 (45)	–		
Walking	5 (45.5)	4 (20)	–		
T2-lesion-volume, *ml, med (IQR)*	7.6 (8.4)	7.3 (13.2)	–	1.000	1.000
NBV, cm^3, *mean (SD)*	1456.2 (50.8)	1460.2 (79.1)	1544.6 (84.8)	0.002*	0.022*

BL = baseline; DD = Disease duration; DMT = disease modifying treatment, EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; NBV = normalised brain volume T25FW = Timed 25-Foot Walk Test; 2MWT = 2-Minute Walk Test.

[a] Where data from all three groups were available, all three groups were compared, otherwise (for clinical data) only the two patient groups were compared.

[b] p-values corrected for multiple comparisons using Benjamini-Hochberg procedure (false discovery rate correction).

[c] DMTs were categorised ahead of the study [26]: Moderate-efficacy DMTs included interferon-b 1a and 1b, pegylated interferon-b 1a, glatiramer acetate, dimethyl fumarate, teriflunomide, azathioprine, and intravenous immunoglobulins. High-efficacy DMTs included alemtuzumab, cladribine, fingolimod, natalizumab, ocrelizumab, cyclophosphamide, mitoxantrone, rituximab, siponimod, ofatumumab, and ozanimod.

$M_{Difference} = 0.18$ m/s, 95 %CI = [−0.15;0.52], $p = 0.519$). Across all pwMS, there was a significant mean improvement in the 2MWT (see Table 2, $t_{(30)} = -2.3$, $p = 0.028$), but not in the T25FW ($p > 0.05$).

3.3. Task-fMRI: movement task

Whole-brain analyses revealed brain activation within primary and secondary sensorimotor areas, basal ganglia, and the cerebellum across all three groups during the movement condition (Fig. 2a, Supplementary Table 2). The F-test revealed no statistical difference in baseline brain activation between responders, non-responders, and HC.

Responders showed significant decreases in brain activation from BL to PI within precuneus and premotor cortex (Table 3), while non-responders did not show any significant decreases. HC showed decreases within the middle frontal gyrus, secondary somatosensory cortex, cingulate gyrus, and cerebellar regions (Table 3). None of the three groups showed any increases in brain activation. The F-test comparing the three groups concerning brain activation changes showed significant group differences within the middle frontal gyrus (MFG, Table 3, Fig. 3). Pairwise *t*-tests (Table 3) revealed a higher decrease in brain activation within the MFG in responders than non-responders and in HC than in responders or non-responders. Additionally, responders displayed more pronounced decreases in brain activation than non-responders within the precuneus and the premotor cortex. No other two-group comparisons revealed significant differences in activation changes.

To further investigate brain activation changes identified by whole-brain analyses, we extracted mean z-stat values from three clusters: MFG (F-test cluster), precentral gyrus and premotor cortex (mean decreases in responders; Fig. 3, left and middle). Post-hoc analyses in the entire group of pwMS revealed moderate to strong correlations between more pronounced mean brain activation decreases in the extracted clusters and improvements in the 2MWT (MFG: $r = -0.559$, 95 % CI = [−0.759;-0.247], $p = 0.003$; precuneus: $r = -0.462$, 95 % CI = [−0.698;-0.122], $p = 0.014$; premotor cortex: $r = -0.405$, 95 % CI = [−0.660;-0.053], $p = 0.024$; Fig. 3, right side). Sensitivity analyses revealed similar correlations after adjusting for age and sex, T2-lesion volume, NBV, and BL walking distance. No significant correlations were revealed for change in T25FW (all $p > 0.05$).

Table 2

Walking function outcomes at baseline and absolute change from baseline to post-intervention (post-intervention-baseline) within groups.

Characteristic	PwMS			HC
	All PwMS	Responders	Non-Resp.	$n = 17$
	$n = 31$	$n = 11$	$n = 20$	
T25FW walking speed, *m/s*				
Baseline, *M (SD)*	1.8 (0.4)	1.9 (0.5)	1.7 (0.3)	2.0 (0.3)
Change to PI, *M [95 % CI]*	0.01 [−0.1;0.1]	0.05 [−0.1;0.2]	−0.01 [−0.1; 0.1]	−0.02 [−0.1; 0.1]
2MWT, *walking distance, m*				
Baseline, *M (SD)*	163.9 (36.3)	156.9 (42.9)	167.8 (32.6)	196.1 (19.9)
Change to PI, *M [95 % CI]*	6.7[a] [0.8;12.6]	23.4[a] [16.3;30.5]	−2.5 [−7.3;2.3]	6.5 [−2.5;15.5]

CI = confidence interval; HC = healthy controls; Non-Resp. = non-responders; PI = post-intervention; PwMS = people with Multiple Sclerosis; T25FW = Timed 25 Foot Walk (results presented in metres/s); 2MWT = 2 Minute Walk Test (results presented in metres).

[a] Groups showing significant change from baseline to post-intervention.

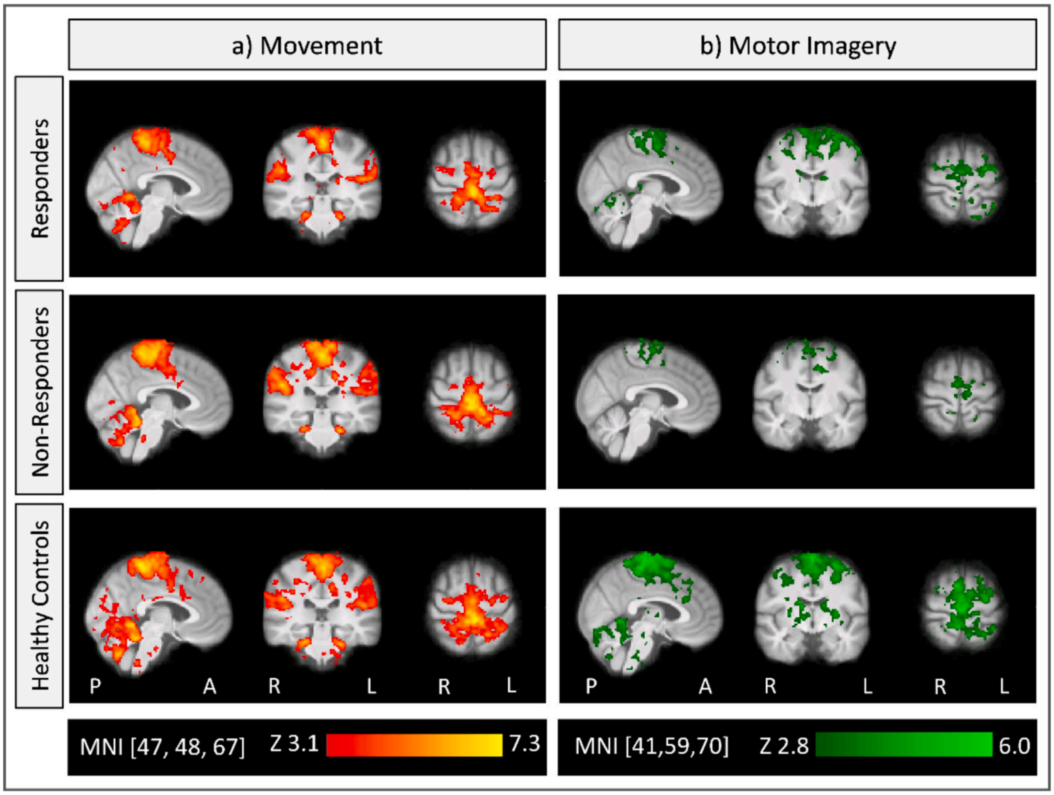

Fig. 2. Baseline brain activation during the fMRI-tasks a) Movement, b) Motor-Imagery in the three study groups (Responders, Non-Responders, Healthy Controls). Please note that for motor imagery, we adapted the visualisation threshold to $z > 2.8$ for improved visualisation. All baseline analyses were computed with a cluster-forming threshold of 3.1 and a cluster-wise significance of $p < 0.05$.

3.4. Task-fMRI: motor imagery task

The motor imagery task activated similar areas as the movement task, albeit to a lesser extent (for activation clusters, see Fig. 2b and Supplementary Table 3). At BL, the F-test revealed group differences in brain activation within the left MFG and the right Crus II (Table 4). Comparisons between groups revealed higher BL brain activation during motor imagery within HC than in all pwMS, but no difference between

Table 3
Brain activation changes from baseline to post-intervention within responders, non-responders, and healthy controls, and respective whole-brain between-group comparisons.

Anatomical region	Peak MNI-coordinates (X Y Z)	Cluster size	*z*-max
Responders (BL > PI)			
Precuneus	12–50 28	390	3.72
Premotor cortex	−6 6 56	335	3.76
Non-Responders (BL > PI)			
–	–	–	–
HC (BL > PI)			
Middle frontal gyrus L	−34 22 26	419	4.39
Cerebellum Left VIIIa, VIIIb, IX, X, VI	−12 -58 -36	738	3.94
Cerebellum Right VIIIa	38–40 -50	277	3.67
Secondary somatosensory cortex L	−48 -2 4	263	3.83
Cingulate Gyrus	0 24 24	258	3.86
Between-group comparisons: BL > PI			
F-Test			
Middle frontal gyrus L	−42 16 32	240	3.94
Responders > Non-Responders			
Precuneus	14–56 34	332	4.01
Premotor cortex	−6 6 56	260	3.72
Middle frontal gyrus L	−42 16 32	246	4.26
HC > Responders			
Middle frontal gyrus L	−36 24 24	280	3.73
HC > Non-Responders			
Middle frontal gyrus L	−38 22 28	521	4.37

BL = baseline; HC = healthy controls; L = left; PI = post-intervention; R = right; SMA = supplementary motor area; z-max = maximum z-stat values of the cluster. If available, Juelich Histological Atlas was used to label regions (premotor cortex, secondary somatosensory cortex), otherwise Harvard-Oxford Cortical Structural Atlas was used for labelling cortical and subcortical areas. The Cerebellar Atlas after normalisation with FNIRT, as incorporated in FSL, was used to label cerebellar regions.

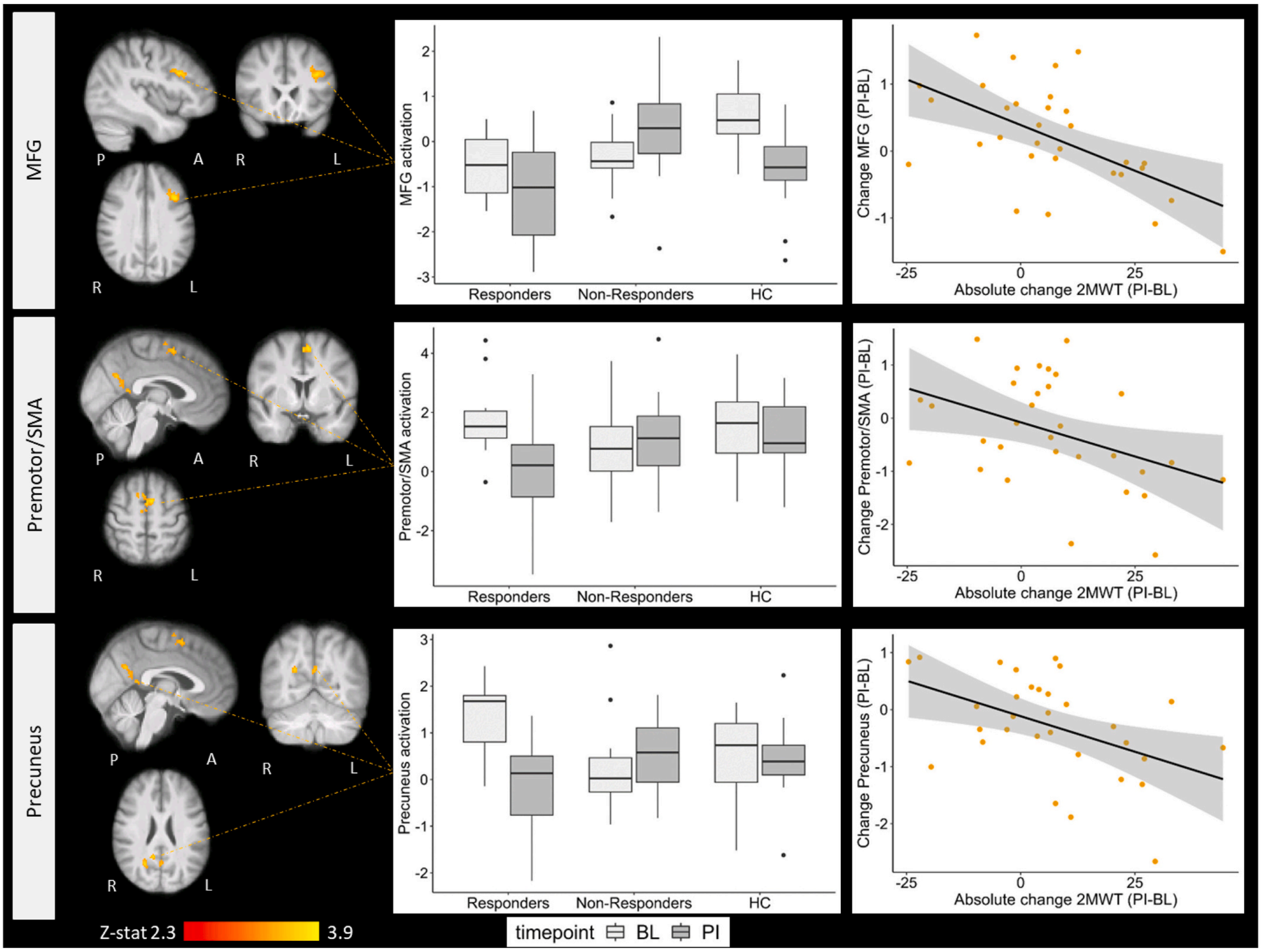

Fig. 3. Areas showing significant brain activation changes from baseline (BL) to post-intervention (PI, left) during the movement task (left), boxplots of mean brain activation within these clusters separated by study groups (responders, non-responders, HC) and timepoint (middle), and scatter-plots illustrating associations between brain activation changes and changes in walking distance within the 2-Minute Walk Test (2MWT) in people with multiple sclerosis (right).
HC = healthy controls; MFG = Medial frontal gyrus; SMA = supplementary motor area.

Table 4
Significant clusters revealed in whole-brain between-group comparisons (responders vs. non-responders vs. healthy controls) for brain activation during the motor-imagery task at baseline.

Anatomical region	Peak MNI-coordinates (X Y Z)	Cluster size	*z-max*
Between-Group comparisons: BL			
F-Test			
Middle Frontal Gyrus L	−34 24 44	188	4.72
Right Crus II	18–74 − 44	99	4.50
HC > Responders			
Middle Frontal Gyrus R	22 26 36	137	4.89
Angular Gyrus (Inferior parietal lobule L)	-44 -54 48	101	4.13
HC > Non-Responders			
Middle Frontal Gyrus L	−34 24 44	1161	5.19
Right Crus II	18–74 -44	479	4.99
Insular Cortex L	40 52 12	351	4.85
Postcentral Gyrus (Primary somatosensory Cortex R)	8–38 70	182	3.89
Precentral Gyrus (Premotor cortex R)	14–22 78		3.77
Right Crus I	32–70 -28	178	5.22
Left Crus I	−32 -58 -34	153	4.19
Middle Frontal Gyrus R	40 32 30	122	4.17

BL = baseline; HC = healthy controls; L = left; R = right; z-max = maximum z-stat values of the cluster. If available, Juelich Histological Atlas was used to label regions (premotor cortex, secondary somatosensory cortex), otherwise Harvard-Oxford Cortical Structural Atlas was used for labelling cortical and subcortical areas. The Cerebellar Atlas after normalisation with FNIRT, as incorporated in FSL, was used to label cerebellar regions.

responders and non-responders. Whole-brain analyses did not reveal any significant changes in mean brain activation within responders or non-responders. Importantly, while HC showed some mean decreases in brain activation during imagery, the F-test revealed no significant group differences regarding brain activation changes from BL to PI.

4. Discussion

In the present study, we investigated whether observed walking function changes subsequent to four weeks of rhythmic-cued gait training involving actual gait and/or motor imagery of gait are associated with changes in lower-limb task-related brain activation patterns. We observed that pwMS whose walking function improved due to rhythmic-cued gait training showed a significant reduction of movement-related brain activation in areas involved in motor function, planning and attention. Within the entire patient cohort, stronger reductions of activation within these areas were associated with higher walking test improvements from BL to PI. Meanwhile, no associations with improvements in walking function were found regarding motor imagery-related brain activation.

On closer inspection, results of our study revealed functional changes within three brain areas - premotor cortex, precuneus, and MFG. Rehabilitation-induced brain activation decreases in the premotor cortex have been previously reported in several studies [10,48], and are usually interpreted as a more efficient brain activation. The precuneus has strong connections to frontal areas and is involved in spatially guided behaviours, such as bipedal tasks and in movement planning and control [49,50]. In a recent study, changes in precuneus activity during motor-tasks were observed in progressive pwMS after multidisciplinary cognitive and motor rehabilitation [51]. Additionally, decreases in activation within the precuneus during an upper-limb visuo-motor task have been reported after upper-limb motor training [48]. The third area in which changes were observed is the MFG. Similar to our study, stronger activation decreases in the MFG have been reported to correlate with higher motor improvements after upper limb motor training [52]. The involvement of the MFG during the movement task might reflect continuous attentive effort and movement coordination [50]. Taken together, decreases in the premotor cortex, precuneus, and MFG in correlation with motor improvements seem to reflect a reduced need for planning and attention processes, which supports the hypothesis of more efficient brain activation [17].

Of note, while most previous studies did not correlate these effects with improvements in motor function [12,14,15] or did not observe a significant correlation [18], we directly associated the fMRI results with improvements in motor function in two steps. Firstly, we compared responders to the training in terms of motor function to both non-responders and age- and sex matched HC. Both responders and HC showed a pattern of decreases in mean brain activation within the three clusters, while the pattern was stable or even reversed in non-responders (Fig. 3, middle). Of note, responders showed significantly higher decreases in brain activation within all three areas compared to non-responders, which highlights a relationship between changes in walking function and in brain activation. Secondly, to ensure that effects were not merely dependent on the cut-off value defining responders, individual outliers or confounding variables, we also performed post-hoc (sensitivity) analyses across the entire sample of pwMS, which further supported the relationship obtained in whole-brain analyses. In our study, higher decreases in movement-related brain activation in areas subserving motor function, planning, and attention were associated with stronger improvements in walking function from baseline to post-intervention. Our findings therefore extend the current evidence for adaptive neural mechanisms following motor rehabilitation in pwMS [17].

During the motor imagery task, our analyses revealed similar areas of brain activation as during actual movement, albeit to a lesser extent, which is in line with previous literature [53,54]. Interestingly, pwMS showed a smaller extent of brain activation at BL than HC in 2 brain areas. Both of these areas, the MFG and cerebellum, have been consistently reported to be involved in gait imagery [54,55]. So far, evidence on brain activation during motor imagery in pwMS is scarce [56]. A previous study, using an upper-limb motor imagery task (squeezing a foam ball) [56], reported lower brain activation in relapsing-remitting pwMS compared to patients with clinically-isolated syndrome, which was interpreted as failure to recruit compensatory mechanisms. However, as we did not observe any association between walking function and brain activation during the motor imagery task, we cannot discern whether, in our study, lower activation during the imagery task in pwMS compared to HC is (mal-)adaptive.

Of note, while improvements in walking function were associated with brain activation decreases during gait-like movement in all patients, we found no such associations with brain activation during the corresponding motor-imagery task. These results suggest that improvements in walking function do not necessarily come along with altered recruitment of brain areas during a motor imagery task [14]. Therefore, choosing a task similar to the trained function seems essential for assessment of rehabilitation induced changes.

Another integral aspect of our study was the rhythmic cueing. Previous literature has shown positive effects of rhythmic-cueing on walking function in neurological disorders [8,57,58], as well as on fatigue and quality of life – especially, when a combination of music and verbal cueing was applied [8,9]. In terms of the underlying mechanisms, it was postulated that synchronising movements with rhythmic-auditory cues (sensorimotor synchronisation) may facilitate planning and execution of cyclical movements such as gait due to the strong connection between auditory and motor systems in the brain [59,60]. Because of the reported clinical benefits, all pwMS in this study received the rhythmic cueing during training and fMRI-tasks, which in turn makes it impossible for us to investigate the individual effect of rhythmic cueing on brain activation (changes).

There are several strengths and limitations worth discussing. A major strength was that we designed the fMRI-tasks as similar as possible to the trainings by using lower-limb tasks and music- and metronome-cues, so that we could investigate effects of the trained functions more thoroughly. This advantage comes with the limitation that lower-limb tasks might lead to more head-movement within the scanner than simpler finger tapping tasks. However, we mitigated the possible effect of head motion by taking precautions during scanning, at pre-processing and during statistical analyses. We therefore are confident that head motion did not have a relevant influence on our outcomes.

Since we aimed to explore changes in brain activation that accompany successful training, we decided to apply an a-priori threshold for the definition of responders. This allowed us to directly compare responders to non-responders in terms of several possibly confounding variables. Although we chose a threshold above the measurement error [21], we are aware that it lies below clinically meaningful changes. Importantly, the mean percent increase in walking distance in responders (15.8 %; 95 % CI [10.1;21.4]) was considerably larger than the established threshold. Furthermore, to ensure that effects were independent of the chosen threshold, we computed (partial) correlations for brain activation changes and improvements in the 2MWT across the entire patient cohort.

Another point worth discussing is the presence of different training groups. While the comparison of training groups was not an aim of our study but the objective of the overall-study, encompassing a large enough sample of participants ($n = 132$) [19], pwMS in our sample were also randomised to all three training groups. Therefore, to minimize the possibility that our effects were caused by training differences, we investigated the distributions of training groups within responders and non-responders and, additionally, always checked behavioural and MRI-results for potential confounding effects by training groups.

Another point to be considered is that, while our sample is certainly among the largest of previous studies on that topic, the sample size is still rather small. Considering the large number of patients screened in order to obtain this sample size (~750 pwMS), multi-centric designs could help to confirm the obtained results while maintaining reasonable recruitment intervals.

5. Conclusions

In conclusion, our results show that pwMS whose walking function improved with rhythmic-cued gait training showed a significant reduction in movement-related brain activation within brain areas subserving motor function, planning and attention. Stronger reductions of activation in these areas were associated with higher increases in walking function from BL to PI. This pattern suggests more efficient brain activation after successful training. Furthermore, our study highlights the task-dependency of neuroplasticity, in showing the different brain reorganisation patterns between a movement and a motor-imagery task, which should be taken into consideration in future studies.

Ethics statement

The study was approved by the Ethics Committees of the Medical Universities of Innsbruck and Graz (22.12.2020; references 1347/2020 and 33-056 ex 20/21) and was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent prior to inclusion in the study.

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CRediT authorship contribution statement

Birgit Helmlinger: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Barbara Seebacher:** Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Stefan Ropele:** Methodology, Writing – review & editing. **Stefanie Hechenberger:** Investigation, Writing – review & editing. **Bettina Heschl:** Investigation, Writing – review & editing. **Gernot Reishofer:** Methodology, Writing – review & editing. **Sara Jordan:** Investigation, Writing – review & editing. **Christian Tinauer:** Methodology, Writing – review & editing. **Sebastian Wurth:** Investigation, Writing – review & editing. **Paola Valsasina:** Methodology, Supervision, Writing – review & editing. **Maria Assunta Rocca:** Supervision, Writing – review & editing. **Massimo Filippi:** Writing – review & editing. **Rainer Ehling:** Writing – review & editing. **Markus Reindl:** Writing – review & editing. **Michael Khalil:** Investigation, Writing – review & editing. **Florian Deisenhammer:** Funding acquisition, Writing – review & editing. **Christian Brenneis:** Conceptualization, Writing – review & editing. **Christian Enzinger:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Daniela Pinter:** Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2025.123426.

Data availability

Requests will be reviewed by the corresponding author and study sponsor, who will consider the feasibility and appropriateness of the request and the credentials of the requester. If the request is deemed reasonable and in line with scientific purposes, de-identified participant data will be shared by the corresponding author with colleagues who made the request. To ensure data protection and responsible usage, requesters will be required to sign a data sharing agreement before obtaining access to the requested data.

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