A novel eye-movement impairment in multiple sclerosis indicating wide-spread cortical damage

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Abstract

In multiple sclerosis remyelination trials have yet to deliver success alike to what has been achieved for relapse rates with disease course modifying treatment trials. The challenge is to have a clinical, functional outcome measure. Currently there are none which are validated, other than visual evoked potentials in optic neuritis. Like vision, quick eye movements (saccades) are heavily dependent on myelination. We hypothesised that it is possible to extrapolate from demyelination of the medial longitudinal fasciculus in the brainstem to quantitative assessment of cortical networks governing saccadic eye movements in multiple sclerosis. We have developed and validated a double-step saccadic test, which consists of a pair of eye movements towards two stimuli presented in quick succession (DEMoNS protocol). In this single-centre, cross-sectional cohort study we interrogated the structural and functional relationships of double-step saccades in multiple sclerosis. Data were collected for double-step saccades, cognitive function (extended Rao's Brief Repeatable Battery), disability (EDSS) and visual functioning in daily life (NEI-VFQ-25). MRI was used to quantify grey matter atrophy and multiple sclerosis lesion load. Multivariable linear regression models were used for analysis of the relationships between double-step saccades and clinical and MRI metrics. We included 209 individuals with multiple sclerosis (mean age 54.3 ±10.5 years, 58% female, 63% relapsing remitting multiple sclerosis) and 60 healthy control subjects (mean age 52.1 ±9.2 years, 53% female). The proportion of correct double-step saccades was significantly reduced in multiple sclerosis (mean 0.29 ±0.22) compared to controls (0.45 ±0.22, $P < 0.001$). Consistent with this, there was significantly larger double-step dysmetric saccadic error in multiple sclerosis (mean vertical error -1.18 ±1.20 degrees) compared to controls (-0.54 ±0.86 degrees, $P < 0.001$). Impaired double-step saccadic metrics were consistently associated with more severe global and local grey matter atrophy (correct responses – cortical grey matter: $\beta = 0.42$, $P < 0.001$) , lesion load (vertical error: β = -

0.28, $P < 0.001$), progressive phenotypes, more severe physical and cognitive impairment (correct responses - information processing: $\beta = 0.46$, $P < 0.001$) and visual functioning. In conclusion, double-step saccades represent a robust metric which revealed a novel eye movement impairment in individuals with multiple sclerosis. Double-step saccades outperformed other saccadic task in their statistical relationship with clinical, cognitive and visual functioning, as well as global and local grey matter atrophy. Double-step saccades should be evaluated longitudinally and tested as a potential novel outcome measure for remyelination trials in multiple sclerosis.

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Abbreviations: BRB-N = Brief Repeatable Battery of Neuropsychological tests; EDSS = Expanded Disability Status Scale; GM = grey matter; INO = internuclear ophthalmoplegia; MD = mediodorsal thalamus; NEI-VFQ-25 = National Eye Institute Visual Function Questionnaire; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis

Introduction

The pathological hallmark of multiple sclerosis is demyelination. The diagnosis relies on different brain areas being affected at different time points; so-called dissemination in space and time.[1] The success of rapid approval of disease modifying treatments by regulatory authorities was grounded in the elegant use of primary and secondary outcome measures.[2] The next challenges for the treatment of individuals with multiple sclerosis will be focused on remyelination and neuroprotection. To date no treatment trial on remyelination was successful regarding the primary clinical outcome measure.[3-6] This is a major problem, because for regulatory authorities it remains mandatory to prove efficacy for a primary, clinical, outcome measure. It has been proposed that emergence of the visual system has been a catalyst for speed of information processing in the nervous system. First by increase of axonal diameter.[7] Next by myelination of the nerves enabling quick eye movements.[8] About two thirds of the human cortex are involved in either afferent or efferent vision (Fig. 1). In this context it is relevant remembering that the pathology of demyelination in multiple sclerosis appears to be more extensive in the cortical grey matter (GM) compared to the white matter.[9] This observation is relevant for the design of functional tests of de- and remyelination in multiple sclerosis beyond to

what is already done for the optic nerve.[3] Furthermore, evidence for endogenous remyelination was shown elegantly in a post-mortem study which concluded that future clinical trials should target cortical remyelination.[9]

How can the functional change caused by de- and remyelination be measured clinically? One approach relies on the development of composite scales reflecting the complex composite pathology of the disease. Unfortunately composite scales and Z-scores are not yet accepted by regulatory authorities as a primary outcome measure. One system in which test results remain on a categorical level (e.g. diplopia yes/no, nystagmus yes/no) is eye movements. Clinically, impairments of eye movements are frequent in multiple sclerosis, affecting about 36-84% of individuals.[10-12] This makes the assessment of eye movements in multiple sclerosis a promising target for a primary clinical outcome measure.[13, 14] Recent methodological advances enable the rapid, non-invasive and precise way of detecting and quantifying eye movement deficits, by using high-frequency infrared oculography.[13-15] The classical eye movement deficit found in multiple sclerosis is an internuclear ophthalmoplegia (INO).[16-18] More recently, fixation instability and saccadic latency delay have been described.[19-21] These eye movement deficits relate to overall disability and are more prevalent in patients with advanced demyelination as present during the progressive disease course.[20, 22] An INO is an attractive target for remyelination trials because of successful response to pharmacological improvement of nerve conduction.[23, 24] A limitation of the approach is that the model only tests one single white matter tract, the MLF, of the brainstem. Therefore trial findings cannot be extrapolated to grey matter de- and remyelination of the entire brain.[9]

Accepting this limitation of the INO model, we now expanded from our earlier studies to test a potentially clinically relevant eye movement paradigm which requires interaction within (sub)cortical networks (Fig. 1).[14, 25-27] The INO results is tested through ‘single-step’ pro-saccades, in which subjects have to make a single saccade (quick eye movement) in response to a jumping target. This is a simplified approach of assessing the efferent visual system. In contrast, the here tested double-step saccades depend on more complex interplay of different cortical and subcortical network. Yet double-step saccades have not been investigated in demyelinating diseases. The double-step saccade represents a pair of saccades towards two stimuli that are briefly presented in quick succession.[25] Because the second target has already disappeared before the first saccade has started, no visual feedback can be used for the correct execution of the

second saccade and cortical input is required. The corrected execution of this second saccade requires knowledge about the metrics (especially the landing position) of the first saccade. The mechanism the visual system probably uses for this purpose is called corollary discharge, which provides an internal copy of an impending motor command from the cortex.[26, 27] This mechanism is used to update visual space when we look around and achieve a stable percept of the world.

Because of the processing speed required this network should be vulnerable to demyelination. Demyelination will disrupt the interaction between grey and white matter essential to the control of double-step saccades. The corollary discharge mechanism is understood to be executed by an upstream pathway to the cortex, going from the superior colliculus to mediodorsal thalamus (MD) neurons, that project further to the frontal eye field.[28, 29] This predominantly GM network requires input from the posterior parietal cortex for integration of the afferent (visual) and efferent (eye movements) visual pathways (Fig. 1).[14, 30] Finally, input from the supplementary eye field is involved in coding the temporal order of sequences of saccades.[31] There is experimental evidence that inactivation of the MD leads to a systematic shift of the second saccadic landing position consistent with an impaired feedback loop to the frontal eye field.[29, 32] In humans similar deficits have been observed following a circumscribed focal ischemic stroke of the thalamus.[33, 34] Furthermore, circumscribed ischemic lesions of the focal frontal and parietal lobe resulted in impaired execution of a double-step saccadic task which concerned predominantly temporal and spatial errors, respectively.[27] Finally, transcranial magnetic stimulation of the supplementary eye field in human subjects caused a disruption of the order of the double-step saccades.[35]

Taken together, the double-step saccadic task has the potential to provide important additional insight into functioning and integration between widespread cortical and subcortical (sub)networks relying on processing speed and myelination.[7, 8] The double-step saccadic task has not yet been evaluated in multiple sclerosis. Because of the extent of demyelination in multiple sclerosis the double-step saccadic task tests should be tested as a potential outcome measure for remyelination trials in multiple sclerosis. We hypothesised that, given its reliance on cross-network integration, this task would be especially related to structural damage and cognitive functioning, on top of earlier indicated relevance for disability in demyelinating disease. Therefore, the aim of this study was first to compare double-step saccadic metrics between individuals with multiple sclerosis and healthy control subjects. Second, to test the clinical relevance of the double-step saccadic task for multiple sclerosis pathology (physically and

cognitively). Finally, to evaluate the quantitative relationship of the double-step saccade task with MRI measures, including grey matter metrics as promising imaging outcome measures for future treatment trials on remyelination.

Materials and methods

This study was approved by the Medical Ethical Committee on Human research of the Amsterdam UMC (study number 2015.227) and in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before study inclusion

Study design and participants

For this observational cross-sectional study, individuals with multiple sclerosis and healthy control subjects were included from the Amsterdam multiple sclerosis cohort, as previously described.[18, 22, 36-38]. Included patients were diagnosed with clinically definite multiple sclerosis according to the revised McDonald criteria,[39] and the disease course classified as relapsing-remitting (RRMS), secondary progressive (SPMS) and primary progressive multiple sclerosis (PPMS).[40] Study visits of included subjects took place between March 2015 and January 2018.

Clinical and cognitive assessment

All assessments were standardized in sequence and performed at the same visit, as described.[18, 22, 36] In brief, we determined disability in multiple sclerosis using the Expanded Disability Status Scale (EDSS) score[41] and cognitive function using the extended Rao's Brief Repeatable Battery of Neuropsychological tests (BRB-N)[42], including: Symbol Digit Modalities Test (information processing speed), Generation Test (verbal fluency), Selective Reminding Test (verbal memory), Word List 10/36 Spatial Recall Test (visuospatial memory), Concept Shifting Test (executive functioning), Stroop Color Word test (attention), and Memory Comparison Test (working memory). Raw test scores were corrected for effects of age, sex, and level of education based on healthy control subjects using linear regression of Z-scores. Consistent with previous work, patients who scored at least two standard deviations (i.e. $Z \leq -2$) below the average of the healthy control group on a least two cognitive domains were classified as cognitively impaired.[36, 43] High

and low contrast visual acuities were measured using Sloan letter charts.[44] Visual functioning in daily life was assessed with the National Eye Institute Visual Function Questionnaire (NEI-VFQ-25).[45, 46]

Magnetic resonance imaging

MRI data was obtained and processed as previously described on a 3-Tesla scanner (Signa HDxt; GE, Milwaukee, Wis, 8-channel coil).[47, 48] We used a high-resolution three-dimensional (3D) T1-weighted sequence and a 3D-Flair sequence. The total T2-lesion load was determined on FLAIR through automated segmentation.[49] Lesion filling was performed using Lesion Automated Processing. GM volumes were determined using FSL 5 (https://www.fmrib.ox.ac.uk/fsl). Whole brain and cortical GM volumes were calculated on the lesion-filled 3DT1 with SIENAX and then the deep GM, thalamus and cerebellum volumes were subtracted with FIRST. To assess lobar atrophy (frontal, parietal, temporal, occipital lobe), the MNI structural atlas was first individually, non-linearly registered to the lesion filled 3D T1-weighted images. Next, lobar structural atlas masks were overlaid on the GM segmentation images from SIENAX to compute lobar GM volumes (left and right volumes were summed). All volumes were normalized for head size using the SIENAX-derived V-scaling factor.

Infrared oculography

Eye movements were measured and analyzed using a validated infrared video-oculography protocol (DEMoNS).[15] Eye movements were measured binocularly with the Eyelink 1000 Plus eye tracker at 1000 Hz. The double-step sequence started with a central target shown for a random period of 1.0 – 3.5 seconds. Next, the target is shown for 67 ms at two different eccentric locations in quick succession (see Fig. 2A). Participants were instructed to look at the two target positions in the order in which they appeared. For calculation of the fixation accuracy, the target was shown once more after one second at the second position and participants were instructed to fixate it. A total of 60 double-step saccades were collected per individual (15 to each combination of target locations, which takes approximately 5 minutes). An off-line analysis was performed in Matlab (Mathworks, inc., Natick, MA). To pass quality control, a predefined level of at least 50%

of double-step saccades needed to be acceptable for a task of a participant to be included.[15] The calculation of the double-step parameters has already been described in detail.[15] In brief, Fig. 2B summarizes the classification of the two saccades. Next, we calculated latency (time between target and eye movement), amplitude or gain (size of the saccade), and direction difference (in comparison to the target direction) for the first and second saccade. Finally, we calculated the spatial error of the final eye position by determining the difference (expressed in degrees of visual angle) between the fixation before and after the reappearance of the target at the second target location. Likewise, the spatial error of the end position of the second saccade was calculated. All variables were averaged over the left and right eye for all gaze directions. Presence of INO was determined using Versional Dysconjugacy Index based thresholds.[21] All Matlab algorithms have been made available online (dx.doi.org/10.17504/protocols.io.ruad6se).

Statistical analyses

Statistical analyses were performed using Stata (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). To compare double-step saccadic parameters between individuals with multiple sclerosis and healthy control subjects, multivariable linear regression analyses were used, adjusted for sex and age. For parameters that are directly influenced by the presence of an INO (gain and peak velocity), associations were additionally adjusted for the presence of unilateral or bilateral INO. P-values below 0.05 were regarded statistically significant. Hereafter, all analyses only include double-step parameters that showed significant differences between individuals with MS and healthy controls subjects.

Next, we divided the multiple sclerosis group in two equal subgroups based on the disease duration (median split), to compare double-step parameters between the group with a (relatively) short disease duration, long disease duration and healthy control subjects, adjusted for the same confounders.

The relation between clinical/cognitive/MRI characteristics and double-step parameters in the multiple sclerosis group were investigated by multivariable linear regression analyses, again adjusted for the same confounders as the comparison between individuals with multiple sclerosis and healthy control subjects. The relation with cognitive impairment was additionally adjusted for the level of education. For parameters that showed a significant association with cognitive

impairment, the individual cognitive domain Z-scores were investigated. Likewise, for parameters that showed a significant association with NEI-VFQ-25 total score, subdomains of the questionnaire were investigated. These associations and the associations with MRI variables were corrected by the Holm-Bonferroni method for multiple comparisons.[50] Next, if multiple double-step parameters were related to the same cognitive domain, a backward linear regression analysis was performed with the cognitive domain as the dependent variable (reversed analysis). The double-step parameters were removed one by one based on the highest p-value, until a model was created with p-values below 0.05 for all parameters. This was done to investigate which (combination of) double-step parameter(s) best reflected the specific cognitive domain. Due to collinearity of the proportion of correct and acceptable responses, at most one of these two parameters remained in the final model (the one with the lowest p-value). Finally, regression analyses with MRI variables were repeated for previously investigated single-step pro-saccadic parameters[18, 20] to compare double-step associations and pro-saccadic associations (in effect size or involved regions).

Data availability

Anonymized data not published within this article will be made available through a data transfer agreement by request from any qualified investigator

Results

In total, we measured and analyzed 16.920 double-step saccades from 222 individuals with multiple sclerosis and 60 healthy control subjects. After quality control[15, 18], 15.450 double-step saccades from 209 individuals with multiple sclerosis and 60 healthy control subjects were available for further statistical analysis (see Table 1 for characteristics of the included group). Neuropsychological data were available from 169 individuals with multiple sclerosis and 54 healthy control subjects, showing cognitive impairment in 30% of all patients. MRI data were available from 165 individuals with multiple sclerosis and 54 healthy control subjects, showing a reduced whole brain volume (difference 58.5 ml, $P < 0.001$) and GM volume in patients compared to controls (difference 32.6 ml, $P < 0.001$). Patients had a mean disease duration of

21.2 (±8.4) years, a median EDSS score of 3.5 (IQR 2.5), a mean cognitive performance of Z = -0.99, with a relapsing-remitting disease course for 63% (Table 1).

Double-step saccades

The results of the double-step saccadic task are listed in Table 2. In summary, individuals with multiple sclerosis showed a significant lower proportion of correct and acceptable responses than healthy control subjects (correct responses B = -0.14, 95% CI -0.21 – -0.08, $P < 0.001$). Furthermore individuals with multiple sclerosis showed slightly more first saccades that were directed to the second target position than control subjects (B = 0.03, 95% CI 0.00 – 0.06, P = 0.044). Related to this, the direction of the first saccade deviated more from the target direction in multiple sclerosis than in controls (B = 4.1, 95% CI 1.5 – 6.6 degrees, P = 0.002). Finally, in multiple sclerosis there was a larger error of the final eye position compared to controls (absolute error B = 0.3, 95% CI 0.1 – 0.6 degrees, P = 0.006). When investigating the horizontal and vertical component of this error, individuals with multiple sclerosis showed on average a horizontal and vertical undershoot of the second target compared to healthy control subjects (Table 2). For the next steps of analyses, we included only double-step parameters that showed significant differences between individuals with multiple sclerosis and healthy control subjects (Table 2).

The median split of the multiple sclerosis group based on disease duration (cut-off 21.5 years) resulted in an 'early' multiple sclerosis group (MS-E, mean disease duration 13.9 ±3.5 years) and a 'late' group (MS-L, mean disease duration 28.0 ±5.3 years). The results of the comparison between these subgroups and the healthy control group can be found in Supplementary Table 1. In summary, the MS-L group performed significantly worse compared to healthy controls on all investigated parameters, and the MS-E group on all but one parameter. Differences between MS-L and MS-E were far less prominent and, for the vast majority, not significant.

Relationship with clinical characteristics

The disease course group differences are summarized in Fig. 3. Overall, individuals with SPMS performed worse on the double-step saccadic task than the other disease courses. They showed a

lower proportion of correct responses (B = -0.10, P = 0.004) and acceptable responses (B = -0.09, P = 0.011) and a larger absolute error of the final eye position (B = 0.32, P = 0.029) compared to individuals with RRMS. When comparing SPMS to PPMS, individuals with SPMS revealed a larger direction difference of the first saccade (B = 5.0, P = 0.046). Greater disability on the EDDS was related to worse performance on the double-step saccadic task. A higher EDSS score was associated with a lower proportion of correct responses (B = -0.03, P < 0.001) and acceptable responses (B = -0.03, P = 0.002), a larger direction difference of the first saccade (B = 1.19, P = 0.002) and the second saccade (B = 1.36, P < 0.001), a larger error absolute error of the final eye position (B = 0.01, P = 0.020), a more negative horizontal error of the final eye position (i.e. undershoot of the target, B = -0.08, P = 0.002), a more negatively vertical error of the final eye position (i.e. undershoot of the target, B = -0.10, P = 0.036).

Relationship with cognitive functioning

Cognitively impaired individuals with multiple sclerosis showed a lower proportion of correct responses (B = -0.11, P = 0.002) and acceptable responses (B = -0.11, P = 0.003), a higher proportion of first saccades directed to the second target location (B = 0.04, P = 0.046), a larger direction difference of the first saccade (B = 3.8, P = 0.016), a larger absolute error of the final eye position (B = 0.65, P < 0.001), more negatively horizontal error of the final eye position (B = -0.31, P = 0.005), more negatively vertical error of the final eye position (B = -0.86, P < 0.001) and a larger direction difference of the first saccade (B = 3.8, P = 0.017). When investigating the associations of these parameters with the Z-scores of individual cognitive domains, strong significant associations were found with all cognitive domains except for verbal fluency (Table 3). As multiple double-step parameters were related to the same cognitive domain, the association between the combination of these parameters and the cognitive domain was investigated. The final models are listed in Supplementary Table 2. For most cognitive domains the final model included only one parameter (adjusted for age, sex and education). For information processing, a combination of the proportion of correct double-step saccades (β = 0.23, P = 0.007) and the absolute error of the final eye position (β = -0.48, P < 0.001) remained significant in one model.

Relationship with visual functioning

Patients with worse double-step performance showed a significantly lower NEI-VFQ-25 score, both in the total score and in 9 of the 11 subdomain scores of the questionnaire (Supplementary Table 3). The largest effects were found for the association between the total score and absolute error of the final eye position ($B = 0.83$, $P < 0.001$) and error of the vertical eye position ($B = -1.11$, $P < 0.001$).

Relationship with MRI measures

The results of the linear regression analyses between MRI volume measures/lesion load and double-step parameters are summarized in Fig. 4A and in Supplementary Table 4. Worse double-step performance was related to a higher lesion load and more severe brain atrophy. Proportion of correct and acceptable responses and error of the final eye position (especially absolute and vertical error) were strongly associated with all MRI volume measures and lesion load. Comparing standardized regression coefficients (Fig. 4A, Supplementary Table 4), the highest effects were found for cortical GM volume (e.g association with proportion of correct double-step saccades: $\beta = 0.43$, $P < 0.001$). In Fig. 5, scatterplots show the relationship between a few double-step parameters and MRI measures. Finally, in Supplementary Table 5 and Fig. 4B-C the effect sizes of associations between pro-saccadic parameters and MRI measures are summarized. In general, lower effect sizes and less strong associations were found than for double-step parameters. After correction for multiple comparisons for the pro-saccadic associations, only the latency of pro-saccades was significantly associated to cortical GM volume ($\beta = -0.27$, $P = 0.003$) and occipital lobe volume ($\beta = 0.25$, $P = 0.003$).

Discussion

This study showed that double-step parameters are impaired in individuals with multiple sclerosis compared to healthy control subjects. There were robust relationships of the double-step saccades in multiple sclerosis with clinical, cognitive and visual functioning as well as GM atrophy. The correlative findings of the double-step saccade metrics with function were consistent with image

based evidence for structural deficits. There were highly significant correlations of double-step saccades with global and localised grey matter atrophy. The novel double-step saccadic task outperformed single-step saccades in the statistical relationship with function and structure. It is suggested that the double-step saccadic task, which depends on information processing speed through an extensive cortical network, is impaired because of the widespread demyelination of grey and white matter in multiple sclerosis.

Regarding structural imaging, significant associations for the double-step saccadic task were found with all investigated brain volume measures and lesion load. The highest effect was found for cortical GM volume (Fig. 4A). This suggests that widespread cortical GM pathology, of which demyelination is a large part[9], adversely affects double-step saccade performance. Compared to pro-saccades (Fig. 4B-C), double-step saccades showed much stronger associations with a range of MRI metrics. Therefore the double-step saccadic test might be a more sensitive outcome measure for detecting demyelinating pathology. Eye movements are generated and controlled by a network that spans the cortex, cerebellum and brainstem.[51] As the double-step task is a complex task, interaction between different regions of this oculomotor network is required for correct execution the task.[27-30, 32-34, 52] Of the different brain regions, occipital lobe volume showed the largest effect size in relation to double-step saccades. This suggests that the initial visual processing in the visual cortex is essential for correct execution and control of double-step saccades. However the lack of post-mortem data from our cross-sectional study limits the ability to draw conclusions about how pathology such as demyelination could cause decreased double-step saccade performance and if it is repairable by remyelination. Such studies are unlikely to happen in humans and experimental models are needed to demonstrate as a proof of principle that bespoke structural and functional connections of the oculomotor network can be modified. Neither do we yet have the longitudinal data to prove causality for grey matter de- and remyelination related interaction between structural and functional changes of the brain and double-step saccades. These data are needed from future studies to (i) expand our understanding of the networks involved in different aspects of saccadic control[53], including the role of widespread vs. local damage, and (ii) provide insight into how the responsiveness (i.e. the ability to detect changes over time) of double-step saccades is reflecting multiple sclerosis brain pathology. Responsiveness has to be evaluated by a construct approach[54] in which different hypotheses have to tested in longitudinal data (i.e. regarding direction an magnitude of

correlations of change scores, subgroup differences and changes after undergoing treatment). Taken together, there is a need to further investigate the potential of double-step saccades as a novel, reproducible and easily captured clinically relevant outcome measure reflecting grey matter pathology.

Two of the investigated double-step parameters showed the largest differences between multiple sclerosis and controls and the strongest associations within the multiple sclerosis group: performance (correct/acceptable responses) and the spatial error of the final eye position. Consistent with this observation, different cognitive domains had alternating strong associations with these two parameters (Supplementary Table 2). Moreover, they explained different parts of the variance in the regression model of information processing speed. Overall, this suggests that performance in the double-step saccadic task and the error of the final eye position can reflect different aspects of overlapping oculomotor and cognitive processing. Regarding the performance parameters, correct responses were calculated with more stringent criteria than acceptable responses. Both showed similar differences between multiple sclerosis and controls and correlations with clinical/MRI outcomes. However, as the correct responses remained significant in the backward regression models for cognitive functioning (Supplementary Table 2), we recommend to include, at the very least, this parameter for classification of double-step saccades in future studies.

Previously we described associations of single-step pro- and anti-saccades with cognitive functioning.[53] In that study, latency of pro-saccades was most strongly associated with executive functioning, information processing and working memory. Furthermore, the proportion of errors of anti-saccades was additionally strongly related to attention. In the current study, we found that double-step parameters were also related to these cognitive domains, but showed an additional strong association with visuospatial functioning. Furthermore, the association of double-step parameters (i.e. error of the final eye position) with information processing showed a larger effect than the association of single-step pro- and anti-saccadic parameters. This suggests that double-step parameters provide additional insight into the governance of oculomotor control through the widespread grey and white matter network. Future statistical modelling may consider combining parameters of the different tasks tested in one multivariable model, with the aim to simplify selection of the most relevant saccadic parameters for demyelination trials.

On review of the literature there were only very few observations on the double-step saccadic performance in disease and none in multiple sclerosis. One study[33] on 13 patients with thalamus strokes found on a group level a significant amplitude reduction of first and second saccades compared to controls. Single case analyses revealed a contraversive direction shift (away from the centre) of the second saccade in five of these patients. Similar results were found in another seven investigated patients with focal thalamic lesions[55] and 19 patients with unilateral posterior parietal lesions.[27] The reported contraversively shifted saccades could not be confirmed in the present study for multiple sclerosis. Our interpretation is that, in contrast to unilateral stroke, contraversively shifted saccades occur less frequently in multiple sclerosis because both hemispheres are affected by demyelinating pathology.

We did, however, find a significant visuospatial deficit of the final eye position in both the horizontal and vertical plane (contraversively shifted) in multiple sclerosis if compared to healthy control. This indicates a dysmetric error of the final eye position in individuals with multiple sclerosis. For reaching this final eye position, participants can make correction saccades before reaching it. Potentially, this increased error reflects the same mechanism which causes decreased spatial accuracy of memory-guided saccades, previously described in multiple sclerosis.[56] The memory-guided task involves the generation of a saccade to a previously illuminated stimulus. Spatial information of the location of the stimulus is held in working memory. It is conceivable that, similar to problems with egocentric to allocentric transformation, the network involved affects, as demonstrated by present MRI data, the lateral parietal area, hippocampus and the medial parietal regions.[57] The strong relationships between the double-step saccades and cognition may contribute to the further interrogation of the topography of visual disorientation.[58] Overall, a combination of eye movement tasks can potentially provide a cognitive oculomotor profile of different neurological diseases.[59-62]

We applied a straightforward parameter analysis and characterisation of the first and second saccades. These data were analysed statistically as separate variables. Future studies may benefit from testing a more complex approach by employing deep and machine learning. These artificial intelligence based models might not only save time, but also provide more insight into the spatial pattern of the double-step saccade as a whole. Such an artificial intelligence based approach may also permit to interrogate in more detail the grey and white matter single processing pathways which are far more complex than the relative simple model we worked on initially with brainstem

pathology causing an INO.[18] Whilst the use of the double step saccadic test has advantages over the pro-saccadic test, we would caution against overlooking the relevance of an INO in a remyelination trial. Figure 1 highlights the importance the MLF and other brainstem projections have in this context. A better understanding of integration of signals from various brain regions will be required. It is not surprising that, in general, double-step saccades show lower reproducibility (intra-class correlation coefficients 0.55-0.91, Supplementary table S5 in reference 15) than pro-saccades (intra-class correlation coefficients > 0.9).[15] This is in line with the relatively high variability of performance in neuropsychological tests.[63] Related to this, healthy controls also show a relatively low percentage correct responses, with a large deviation (45% ±22% in this study, 67% ±2% in relatively young healthy subjects[15]) Investigating learning effects, adjusted instructions and practice trials of double-step saccades might optimize robustness of the test. Combined with other saccadic parameters this might lead to a prediction or machine learning model that can define cut offs for good or bad performance for different aspects of eye movement. Another limitation is that is that we included individuals with multiple sclerosis with a relatively long disease duration, likely having suffered from extensive demyelination, but this prevents us from making any assertions regarding the early disease phase. There are at least three ways in which electric network activity can be modulated: conduction block, demyelination and axonal degeneration. It will be impossible to distinguish between these three with the double step saccadic task. Axonal loss is permanent. There is no potential for recovery. Accepting that axonal loss may have an influence on the saccadic task we would not expect this to be a modifiable factor in a remyelination trial. Conduction block has been associated with paroxysmal symptoms in MS. Intermittent conduction has for example been an unwanted side effect in the lamotrigine trial, causing transient symptoms in patients.[64] Therefore conduction block variation can introduce noise into the data. One approach to test for conduction block is to provoke Uhthoff's phenomenon in MS by elevating the body temperature as elegantly demonstrated by Davis, Frohman and colleagues.[65] Because of the added time to a study protocol it may be challenging to correct for conduction block, but the possibility should be kept in mind given the rising awareness about relevance of metabolic failure in MS pathology. Finally, for the MRI associations we did not investigate sub regions of the brain, for example the mediodorsal nucleus of the thalamus. Acknowledging these limitations, it should be pointed out that the double-step saccadic task lends itself for integrated automated eye tracking recordings of more

natural scenes[66], which may in future studies help to explain the relevance for (visual) quality of life of patients .

In conclusion, this study demonstrated that the double-step saccadic task showed clinically relevant impairments in individuals with multiple sclerosis. Double-step saccade impairments were strongly related with other functional and structural tests implying demyelinating pathology in individuals with longstanding multiple sclerosis. In fact, the double-step saccades significantly outperformed single-step saccades. The double-step saccadic task should be considered as a potential outcome measure for remyelination trials.

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Competing interests

-J.A. Nij Bijvank reports no competing interests.

-S.N. Hof reports no competing interests.

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-L.J. van Rijn reports no competing interests.

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Supplementary material

Supplementary material is available at *Brain* online.

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Figure Legends

Figure 1 Areas and pathways involved in oculomotor control. (A) Schematic overview of important structures and pathways in the visual system. The afferent visual pathways (blue lines) originates in the retina, where bipolar cells connect with the retinal ganglion cells (RGCs), which project to the lateral geniculate nucleus (LGN) in the thalamus (dark blue lines). From the LGN the signal continues through the optic radiations (light blue lines) towards the primary visual cortex. Preliminary processing takes places in, amongst others, the middle temporal cortex (not shown), which is involved in the perception of motion. These regions are connected with the posterior parietal cortex (parietal eye field and precuneus), which are responsible for constructing a spatial representation of the environment and directing spatial attention. The efferent visual pathway (green lines) originates in a broad network of cerebral regions, extending from parietal and (pre)frontal areas, which connect through the thalamus to the superior colliculus (light green lines). From the posterior parietal cortex, direct connections with the superior colliculus exist, which can generate reflexive eye movements. For more volitional eye movements and broad-sale cognitive processing, information passes from the parietal to the (pre)frontal cortex. Regions in this area are thought to be involved in, amongst others, decisional and predictive processes, performance monitoring and motivation and modulation of motor commands. The frontal regions are directly connected to the superior colliculus and indirectly via the basal ganglia, the latter connections are related to evaluating the significance of an action. Through connections with the three ocular motor nuclei (oculomotor, trochlear (both not shown) and abducens nucleus), signal are sent to cranial nerves (III, IV and VI) innervating the extraocular muscles (dark green line). The thalamus is a widely connected region which is responsible for directing visual attention and relaying the various information from other sources necessary for oculomotor control. The cerebellum (not shown) is also a key component in the efferent visual pathway, with connections to areas in both the brainstem and the prefrontal cortex, and is involved in fine motor and cognitive control of eye movements. [Reproduced with permission from Coric D, Nij Bijvank JA, van Rijn LJ, Petzold A, Balk LJ. The role of optical coherence tomography and infrared oculography in assessing the visual pathway and CNS in multiple sclerosis. Neurodegener Dis Manag. 2018 Oct;8(5):323-335. doi: 10.2217/nmt-2018-0011]. **(B+C)** Important brainstem areas involved in the control of eye movements are shown in a sagittal (B) and coronal (C) cross

section of the brainstem: three oculomotor nuclei (III, IV, VI), the interstitial nucleus of Cajal (INC), the superior colliculus, the Rostral interstitial nucleus of medial longitudinal fasciculus (riMLF), the medial longitudinal fasciculus (MLF), , the mediadorsal nucleus (MD) in the thalamus. The nucleus raphe interpositus (not shown) lies close to the midline, at the level of the abducens nucleus (VI) [modified with permission from Petzold A, Paine M, Faldon M, Riordan-Eva P, Bronstein AM, Gresty MA, Plant GT. Synchronised paroxysmal ocular tilt reaction and limb dystonia. Neuroophthalmology 2009;33:217-236.].

Figure 2 The double-step saccadic task. (A) The target locations of the double-step saccadic task. One example of a sequence of target locations is shown with the solid dots and the arrows. The fixation point (F) is located in the centre of the screen and disappears after a random interval of 1.0 to 3.5 seconds. Then the target reappears at the first target location (T1) and second target location (T2) for 67 ms each. The target will reappear at T2 after 1 second. After 1.5 seconds, the target will move back to the centre of the screen. Other target locations are shown with the dashed circles. T1 is located 8 degrees of visual angle left or right from the centre, the second 8 degrees in an oblique direction up or down from the first target. **(B)** Classification of double-step saccades. The proportion of double-step in the different classification groups (against the total number of included double-step saccades) was used for further the statistical analysis.

Figure 3 Double-step parameters across disease courses. Box and whisker plots showing the distribution of double-step parameters across multiple sclerosis disease courses and compared to healthy control subjects. The horizontal line inside the box indicates the median and the asterisk the mean of the group. HC: Healthy control subjects, RR: relapsing-remitting multiple sclerosis, SP: secondary progressive multiple sclerosis, PP: primary progressive multiple sclerosis, *: p-value below 0.05, **: p-value below 0.005.

Figure 4 Effect sizes of associations between MRI volume variables and double-step and pro- saccadic parameters. Associations with p-values below 0.05 are shown (see Supplementary Table 3 and 4 for all associations). Standardized regression coefficients (symbol) and 95% CI (bars) are plotted. **(A)** Effect sizes (β) of linear regression analyses between MRI

variables and double-step parameters. **(B)** Effect sizes (standardized odds ratio) of logistic regression analyses between MRI variables and presence of internuclear ophthalmoplegia. **(C)** Effect sizes (β) of linear regression analyses between MRI variables and other pro-saccadic parameters. CGM: cortical grey matter; DGM: deep grey matter

Figure 5 Associations between MRI volumes and double-step parameters. Exemplar scatterplots showing the unadjusted association between MRI volume variables and the proportion of correct responses **(A-D)** and the absolute error of the final eye position **(E-H).** The linear fit (solid line) with 95% confidence interval (dashes lines) is shown. In the upper left corner the standardized regression coefficient and p-value of the unadjusted association is shown. For the adjusted associations, see Fig. 3A and Supplementary Table 4. FEP: final eye position.

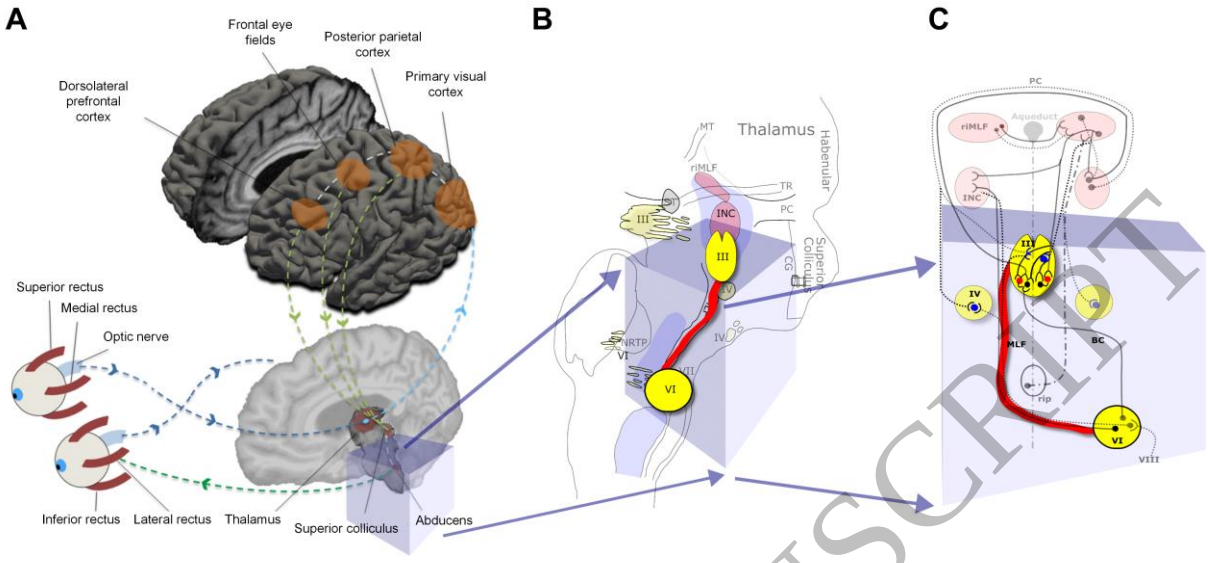

Figure 1
160x73 mm (6.8 x DPI)

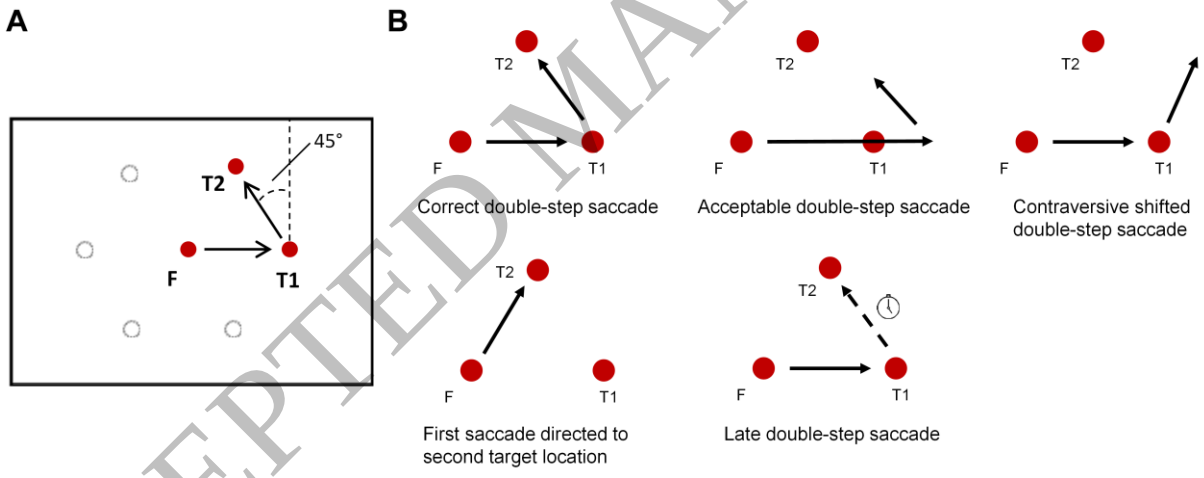

Figure 2
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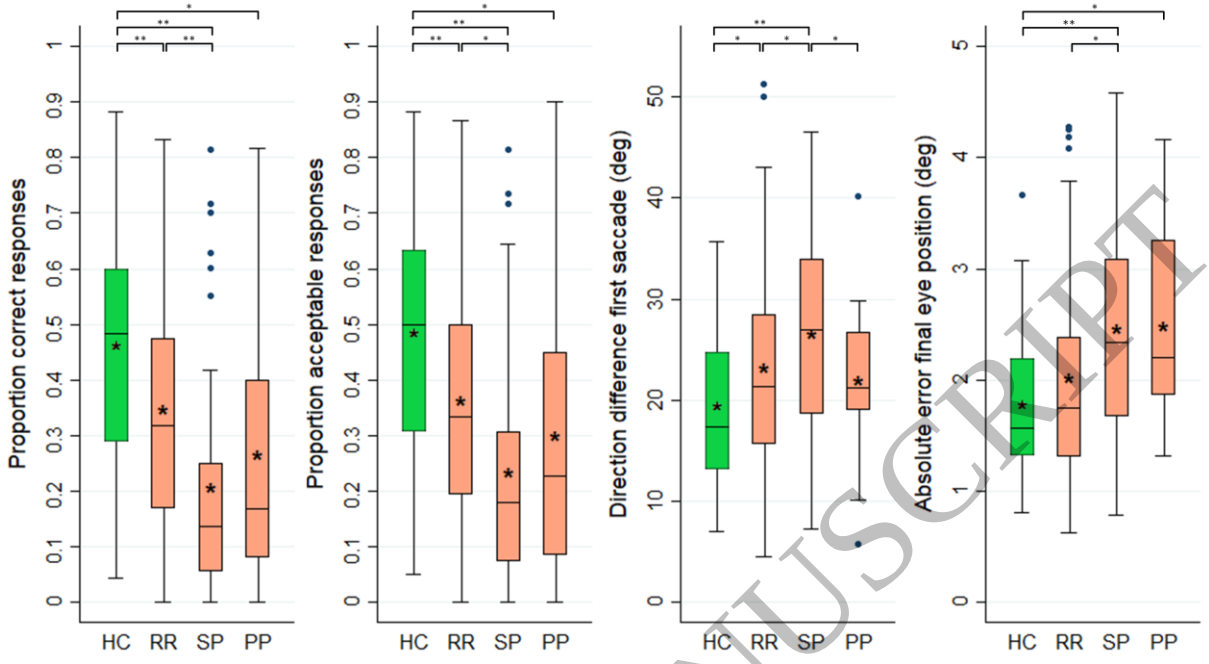

Figure 3
160x88 mm (6.8 x DPI)

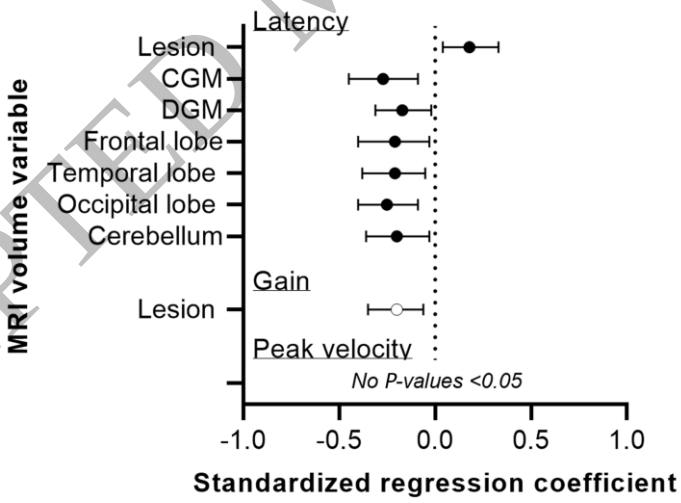

Figure 4
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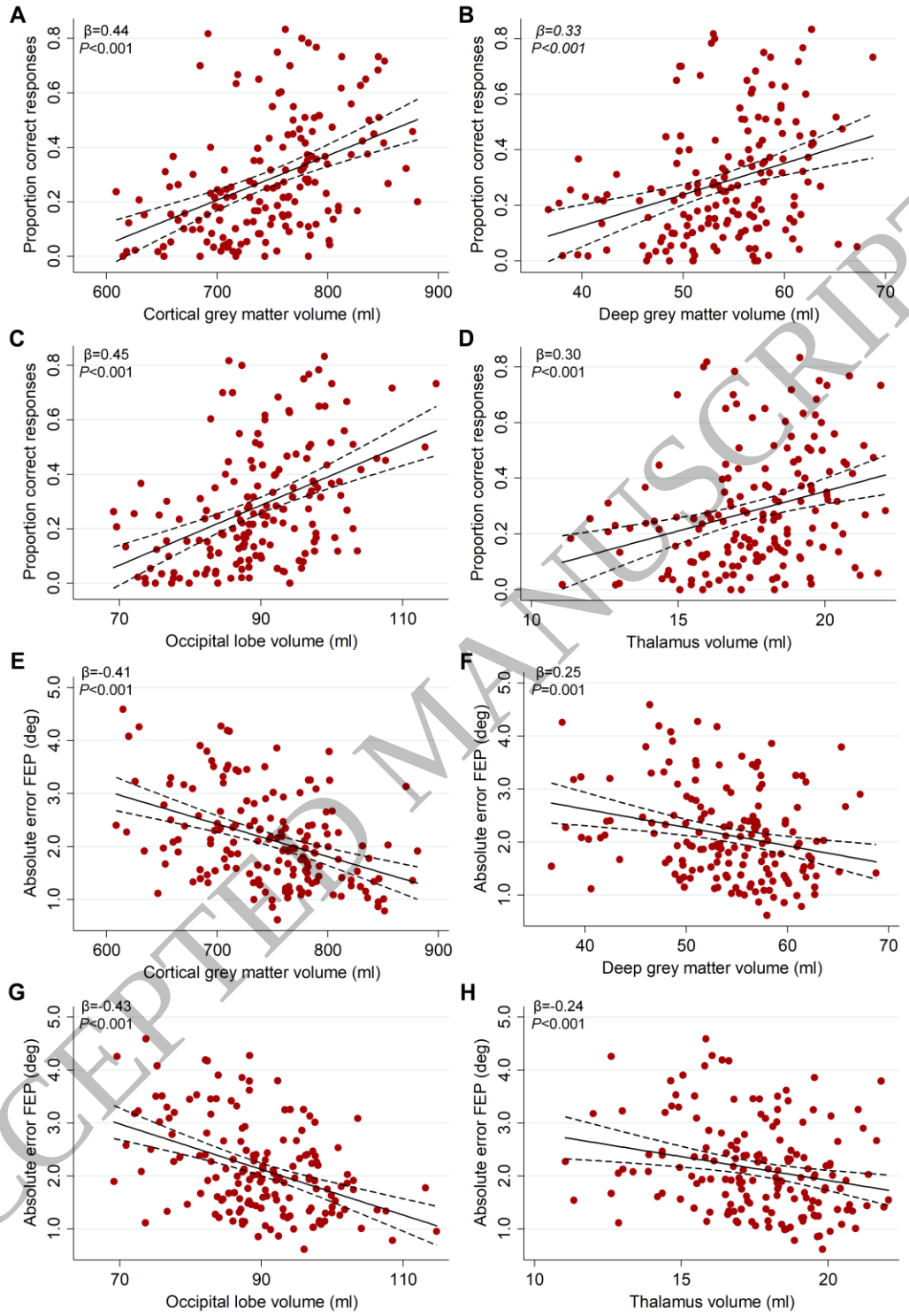

Figure 5
160x227 mm (6.8 x DPI)

Table 1 Demographic and clinical characteristics of the healthy control subjects and individuals with MS

	Individuals with MS ***n*** **= 209**	**Healthy control subjects** ***n*** **= 60**
Sex, *n*, female (%)	142 (68)	32 (53)
Age, years (SD)	54.3 (±10.5)	52.1 (±9.2)
Level of education, median (range)[a]	4 (1–7)	6 (1–7)
Disease duration, years (SD)	21.1 (±8.4)	N/A
EDSS score, median (IQR, total range)	3.5 (2.5, 0.0–8.5)	N/A
Disease course		
Relapsing-remitting, *n* (%)	130 (63)	N/A
Secondary progressive *n* (%)	54 (26)	N/A
Primary progressive *n* (%)	18 (9)	N/A
Unclassifiable, *n* (%)	3 (1)	N/A
High-contrast visual acuity, best eye[b]	56.4 (±7.2)	N/A
Low-contrast visual acuity, best eye[c]	31.2 (±12.0)	N/A
Optic neuritis history, *n* (%)[d]	97 (50)	N/A
Internuclear ophthalmoplegia, *n* (%)[e]	68 (33)	N/A
Average cognition, Z-score[f]	−1.0 (±0.9)	0.2 (±0.5)

MS = multiple sclerosis ; EDSS = expanded disability status scale; IQR = interquartile range; N/A = not applicable

[a]Education is ranked from 1 (did not finish primary education) to 7 (university degree). Education data available for 195 individuals with MS and 54 healthy control subjects.

[b]High-contrast visual acuity data available for 191 individuals with MS.

[c]Low-contrast visual acuity data available for 151 individuals with MS.

[d]Optic neuritis information available for 193 individuals with MS.

[e]Internuclear ophthalmoplegia information available for 207 individuals with MS.

[f]Neuro-psychological data available for 169 individuals with MS and 54 healthy control subjects.

Table 2 Comparison of double-step saccadic parameters between individuals with MS and healthy control subjects

Parameter	Individuals with MS	Healthy control subjects	Difference HC−MS[a]	
	Mean, SD	Mean, SD	B (95% CI)	*P*-value
Proportions				
Correct double-step saccades	0.29 ± 0.22	0.45 ± 0.22	−0.14 (−0.21 to −0.08)	**<0.001**
Acceptable double-step saccades	0.31 ± 0.22	0.48 ± 0.22	−0.15 (−0.21 to −0.08)	**<0.001**
Contraversive double-step saccades	0.02 ± 0.02	0.02 ± 0.03	−0.01 (−0.01 to 0.00)	0.185
S1 to second target	0.14 ± 0.12	0.10 ± 0.08	0.03 (0.00 to 0.06)	**0.044**
Late double-step saccades	0.11 ± 0.10	0.07 ± 0.11	0.03 (−0.00 to 0.06)	0.085
Latencies (ms)				
First saccade, all	314.8 ± 71.2	314.0 ± 54.8	−0.9 (−20.2 to 18.4)	0.928
Correct first saccade	299.5 ± 86.6	309.8 ± 68.8	−11.1 (−34.9 to 12.9)	0.366
First saccade to second target	329.2 ± 100.0	319.7 ± 86.6	9.9 (−18.3 to 38.1)	0.517
Correct double-step saccades	281.3 ± 82.9	304.1 ± 70.1	−21.3 (−44.7 to 1.7)	0.162
Intersaccadic interval, correct	390.4 ± 137.7	405.9 ± 125.6	−21.9 (−61.1 to 17.5)	0.275
Accuracy and velocity				
Direction difference S1 (deg)	23.7 ± 9.1	19.0 ± 7.6	4.1 (1.5 to 6.6)	**0.002**
Direction difference S2 (deg)	22.3 ± 9.0	20.4 ± 6.7	1.7 (−0.9 to 4.2)	0.177
Peak velocity S1 (deg/s)	285.5 ± 62.8	293.0 ± 57.0	−0.3 (−18.9 to 18.4)	0.978
Peak velocity S2 (deg/s)	267.0 ± 62.3	281.1 ± 58.6	−10.8 (−29.8 to 8.2)	0.263
Amplitude S1 (deg)	6.0 ± 1.1	6.3 ± 1.0	−0.2 (−0.5 to 0.1)	0.154
Gain S2	0.91 ± 0.12	0.92 ± 0.11	−0.02 (−0.06 to 0.01)	0.179
Horizontal error end position S2[a] (deg)	−0.10 ± 0.72	−0.10 ± 0.75	−0.07 (−0.28 to 0.14)	0.546
Vertical error end position S2[b] (deg)	−0.86 ± 0.78	−1.02 ± 0.83	−0.15 (−0.40 to 0.09)	0.223
Absolute error FEP (deg)	2.1 ± 0.9	1.7 ± 0.6	0.3 (0.1 to 0.6)	**0.006**
Horizontal error FEP[b] (deg)	−0.16 ± 0.64	0.19 ± 0.71	−0.34 (−0.53 to −0.15)	**<0.001**
Vertical error FEP[c] (deg)	−1.18 ± 1.20	−0.54 ± 0.86	−0.54 (−0.86 to −0.22)	**0.001**

MS = multiple sclerosis; S1 = first saccade; S2 = second saccade; ms = milliseconds; deg = degrees of visual angle; s = second; FEP = final eye position.

[a]The comparison between MS and controls is the results of linear regression analyses, adjusted for sex and age. The comparisons of peak velocity, amplitude, gain and end position of the second saccade were additionally adjusted for the presence of unilateral or bilateral internuclear ophthalmoplegia.

[b]A negative number indicates a horizontal undershoot of the target (horizontally located more to the edge of the screen compared to the fixation position after reappearance of the target), a positive number a horizontal overshoot of the target.

[c]A negative number indicates a vertical undershoot of the target (vertically located more to the center of the screen compared to the fixation position after reappearance of the target), a positive number a vertical overshoot of the target.

Table 3 Adjusted associations between cognitive domain Z-scores and double-step parameters

Double-step parameter (dependent variable)	Cognitive domain (independent variable)	Adjusted model			
		B	95% CI B	β	*P*-value
Proportion correct double-step saccades	Executive functioning	0.040	0.018 to 0.061	0.28	**<0.001**
	Verbal memory	0.044	0.593 to 0.977	0.25	**0.001**
	Information processing	0.093	0.066 to 0.120	0.46	**<0.001**
	Visuospatial memory	0.045	0.021 to 0.069	0.28	**<0.001**
	Working memory	0.036	0.013 to 0.059	0.24	**0.002**
	Attention	0.039	0.012 to 0.066	0.22	**0.006**
Proportion acceptable double-step saccades	Executive functioning	0.041	0.019 to 0.063	0.28	**<0.001**
	Verbal memory	0.041	0.028 to 0.078	0.23	**0.004**
	Information processing	0.093	0.065 to 0.121	0.45	**<0.001**
	Visuospatial memory	0.045	0.021 to 0.070	0.27	**<0.001**
	Working memory	0.039	0.015 to 0.062	0.26	**0.001**
	Attention	0.041	0.013 to 0.069	0.22	**0.004**
Direction difference first saccade	Executive functioning	−1.129	−2.120 to −0.138	−0.18	0.026
	Verbal memory	−1.267	−2.521 to −0.013	−0.17	0.048
	Information processing	−1.799	−1.642 to −0.444	−0.21	0.010
	Visuospatial memory	−1.538	−2.638 to −0.437	−0.22	**0.006**
Absolute error FEP	Executive functioning	−0.265	−0.349 to −0.180	−0.45	**<0.001**
	Information processing	−0.472	−0.575 to −0.368	−0.58	**<0.001**
	Visuospatial memory	−0.117	−0.221 to −0.013	−0.18	0.028
	Working memory	−0.189	−0.285 to −0.092	−0.31	**<0.001**
	Attention	−0.248	−0.359 − −0.137	−0.25	**<0.001**
Horizontal error FEP	Executive functioning	0.099	0.028 to 0.170	0.22	**0.007**
	Information processing	0.186	0.092 to 0.281	0.30	**<0.001**
	Visuospatial memory	0.099	0.021 to 0.178	0.20	0.013
	Working memory	0.089	0.013 to 0.165	0.19	0.021
Vertical error FEP	Executive functioning	0.342	0.230 to 0.455	0.44	**<0.001**
	Verbal memory	0.158	0.008 to 0.309	0.17	0.039
	Information processing	0.591	0.446 to 0.735	0.54	**<0.001**
	Visuospatial memory	0.180	0.043 to 0.316	0.20	0.010
	Working memory	0.268	0.141 to 0.394	0.33	**<0.001**
	Attention	0.305	0.157 to 0.452	0.30	**<0.001**

Only associations with *P*-values below 0.05 are shown. Bold P-values represent *P*-values of associations that survived multiple comparisons correction. The associations between cognitive domain Z-scores and double-step saccades was investigated with linear regression analyses, adjusted for age, sex, level of education. Unstandardized regression coefficients (B) with 95% confidence interval (CI) are shown, as well as standardized regression coefficients (β).