The neurovascular basis of processing speed differences in humans: A model-systems approach using multiple sclerosis

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ABSTRACT

Behavioral studies investigating fundamental cognitive abilities provide evidence that processing speed accounts for large proportions of performance variability between individuals. Processing speed decline is a hallmark feature of the cognitive disorder observed in healthy aging and in demyelinating diseases such as multiple sclerosis (MS), neuromyelitis optica, and Wilson’s disease. Despite the wealth of evidence suggesting a central role for processing speed in cognitive decline, the neural mechanisms of this fundamental ability remain unknown. Intact neurovascular coupling, acute localized blood flow increases following neural activity, is essential for optimal neural function. We hypothesized that efficient coupling forms the neural basis of processing speed. Because MS features neural-glial-vascular system disruption, we used it as a model to test this hypothesis. To assess the integrity of the coupling system, we measured blood-oxygen-level-dependent (BOLD) signal in healthy controls (HCs) and MS patients using a 3T MRI scanner while they viewed radial checkerboards that flickered periodically at 8 Hz. To assess processing speed and cognitive function, we administered a battery of neuropsychological tests. While MS patients exhibited reduced ΔBOLD-processing speed relationships, we assessed the physiologic components that constitute ΔBOLD signal in healthy controls (HCs) and MS patients using a 3T MRI scanner while they viewed radial checkerboards that flickered periodically at 8 Hz. To assess processing speed and cognitive function, we administered a battery of neuropsychological tests. While MS patients exhibited reduced ΔBOLD with reductions in processing speed, no such relationships were observed in HCs. To further investigate the mechanisms that underlie ΔBOLD-processing speed relationships, we assessed the physiologic components that constitute ΔBOLD signal (i.e., cerebral blood flow, ΔCBF; cerebral metabolic rate of oxygen, ΔCMRO2; neurovascular coupling ratio) in speed-preserved and -impaired MS patients. While ΔCBF and ΔCMRO2 showed no group-differences, the neurovascular coupling ratio was significantly reduced in speed-impaired MS patients compared to speed-preserved MS patients. Together, these results suggest that neurovascular uncoupling might underlie cognitive slowing in MS and might be the central pathogenic mechanism governing processing speed decline.

1. Introduction

A multitude of previous studies have observed that some individuals consistently perform better than others across a broad range of tasks. Such observations have led to theories suggesting that a limited set of fundamental abilities or processing resources govern performance across a broad range of cognitive tasks (Baddeley, 2010; Just and Carpenter, 1992; Norman and Bobrow, 1975; Spearman, 1904; Vernon, 1983). Behavioral studies investigating these fundamental abilities provide evidence that processing speed accounts for a large proportion of performance variability between individuals (Birren and Fisher, 2014; Cerella, 1994; Earles and Salthouse, 1995; Hale et al., 1995; Rypma and D’Esposito, 1999; Salthouse, 2000, 1992; Salthouse, 1996; Tucker-Drob, 2009; Tucker-Drob et al., 2019). For instance, evidence supports a central role for processing speed in childhood development (Kail, 1985, 1986) and age-related cognitive decline (Earles and Salthouse, 1995; Hale et al., 1995; Kail, 1986, 1985; Salthouse, 2000, 1992; Salthouse, 1996). This observation is corroborated by those showing that diseases featuring processing-speed defects are also known to show higher-order cognitive deficits such as working memory and verbal fluency (Ackerman et al., 2002; Blanc et al., 2008; Chiaravalloti and DeLuca, 2008; Rao, 1995; Sivakolundu et al., 2019b).

Processing speed declines are accelerated in early-stages of diseases like multiple sclerosis (MS). Neuropsychological dysfunction, manifesting mainly as cognitive slowing, is seen in up to 65% of MS patients (Genova et al., 2009; Rao, 1995). Furthermore, higher-order cognitive...
deficits often coexist in such patients (Chiavarotti and DeLuca, 2008; Sivakolundu et al., 2019b). These observations suggest the hypothesis that processing speed might be a fundamental ability governing cognitive changes in MS (DeLuca et al., 2004). Although causal modelling work has yet to be done, extant correlational studies provide support for this hypothesis (Chiavarotti et al., 2013; DeLuca et al., 2004; Lenggenfelder et al., 2006; Owens et al., 2013). For instance, one study investigated the effects of time limitation on planning performance (Owens et al., 2013). In that study, healthy controls (HC) and MS patients performed the Tower of London task in time-limited (based on the number of moves required to complete the task) and untimed conditions. The most important result was that, whereas puzzle-completion accuracy was reduced in MS patients compared to healthy controls in the timed conditions, no such differences were observed in the untimed conditions, suggesting that MS-related processing speed dysfunction might mediate impairment across other cognitive domains.

Despite the prevalence of evidence suggesting a central role for processing speed in cognitive abilities across the lifespan and diseased populations, little remains known about the physiologic mechanisms of cognitive slowing. MS represents an ideal model for studying pathologic mechanisms that underlie cognitive slowing. It is an autoimmune inflammatory disorder of the central nervous system that globally affects the neural, glial, and vascular systems (Trapp et al., 1998). Prior research on the neural basis of cognitive slowing has mainly implicated changes in executive prefrontal systems as the neural substrate for such slowing (e.g., Rypma et al., 2006). Because processing speed is known to depend on inputs from a combination of visual, prefrontal, and motor systems (Costa et al., 2017; Stickland et al., 2019; Turner et al., 2018), we argue that it is the confluence of disruptions in these processing speed-dependent systems that contribute to cognitive slowing. As MS is known to feature brain-wide insults to the neural-glial-vascular system (Lee et al., 2012; Sivakolundu et al., 2019a; Trapp et al., 1998; Trapp and Stys, 2009), it represents a model to investigate the neural processes underlying disruptions of processing speed-dependent systems and hence cognitive slowing.

Much has been learned about the mechanism of cognitive slowing from previous functional magnetic resonance imaging (fMRI) studies in MS. We and others have reported MS-related changes in fMRI signal, known as the blood-oxygen-level-dependent signal (ΔBOLD), during task performance (Faro et al., 2002; Hubbard et al., 2016; Langkilde et al., 2002; Turner et al., 2018; West et al., 2019). The nature and direction of such MS-related changes in ΔBOLD signal, however, have not been consistent. While some studies report increases in task-evoked ΔBOLD in MS compared to HCs (DeLuca et al., 2008; Mainiero et al., 2004; Stafien et al., 2002; Sweet et al., 2006), other studies have reported decreases (Faro et al., 2002; Hubbard et al., 2016; Langkilde et al., 2002). Because ΔBOLD relies on a complex physiologic interplay between neural, glial, and vascular systems, solely studying task-evoked ΔBOLD has hindered meaningful understanding of the mechanisms that underlie cognitive slowing.

One way to understand the brain mechanisms of cognitive slowing is through measurement of associations between the physiologic components underlying ΔBOLD signal and performance. Neurovascular coupling results in acute localized cerebral blood flow increases (ΔCBF) following neural activity. These increases provide vital and timely delivery of oxygen and nutrients to metabolically active neurons (Girouard and Iadecola, 2006; Howarth, 2014; Iadecola, 2017; Metea and Newman, 2000). Under normal circumstances, this coupling results in a surfeit of oxygen delivery that permits optimal neural spiking frequency and hence cognitive slowing. As MS is known to occur in MS, would lead to neurovascular uncoupling in the form of ΔCBF dysregulation in response to neurometabolic activity, and, we hypothesize, reductions in neural efficiency that would result in cognitive slowing.

We and others have investigated the integrity of the neurovascular coupling system and the extent to which alterations in this system affect cognitive performance using components of the ΔBOLD hemodynamic response function (HRF) shape (e.g., time-to-peak, amplitude, full-width-half-maximum; Hubbard et al., 2016; Turner et al., 2018). The ΔBOLD HRF models temporal changes in venous blood-oxygen content in response to neural activity (i.e., neurovascular coupling). In one study, ΔBOLD HRF was modelled for an event-related cognitive task using piecewise cubic spline functions that minimized assumptions regarding HRF shape (Turner et al., 2018). Peak amplitude was reduced in MS patients relative to healthy controls. In addition, ΔBOLD HRF time-to-peak increased with increases in reaction time for MS patients. Together, these results suggest an important role for neurovascular coupling, and vasodilatory speed necessary for the timely oxygen delivery that permits optimal neural spiking frequency and hence processing speed (Abdelkarim et al., 2019). Such changes in HRF have also been reported in other populations such as healthy aging that feature processing speed dysfunction (West et al., 2019). These results are problematic for studies that assume a direct relationship between ΔBOLD signal and neural activity in MS patients. Further, they underscore the need for using approaches that minimize shape assumptions of HRFs used in deconvolution for comparisons to populations for which neurovascular coupling is compromised (Abdelkarim et al., 2019; D’Esposito et al., 2003; Rypma and D’Esposito, 2010).

In the present study, we hypothesized that neurovascular uncoupling is a central physiologic mechanism underlying cognitive slowing. To test this hypothesis, we utilized MS as a model to investigate whether neurovascular uncoupling contributed to cognitive slowing in a processing speed-dependent system, the visual cortex (Costa et al., 2017). We examined the relationships between ΔBOLD during visual stimulation and processing speed in HCs and MS patients. To further investigate the mechanisms that underlie ΔBOLD reductions observed with reduction in processing speed, we assessed the physiologic components that constitute ΔBOLD (i.e., CBF, ΔCMRO2, neurovascular coupling ratio) in speed-preserved and-impaired MS patients. In this study we investigated the overarching hypothesis that neurovascular uncoupling might be the central pathogenic mechanism governing cognitive slowing in MS.

2. Materials and methods

2.1. Research participants

The study was approved by the University of Texas Southwestern Medical Center (UTSW) Institutional Review Board. The study group was comprised of 38 MS patients evaluated in the Clinical Center for MS at UTSW and from local MS support groups. Eighteen HCs were recruited using advertisements and flyers distributed throughout the Dallas-Fort Worth Metroplex area. Inclusion criteria for all participants were (i) male or female patients between the ages of 18 and 65 and for all MS patients, (ii) a confirmed diagnosis of a relapsing-remitting disease course based on 2010 McDonald criteria (Polman et al., 2011), and (iii) an Expanded Disability Status Scale (EDSS) score less than 7.5. Patients were also required to be (iv) clinically stable on disease modifying therapy and (v) treatments for comorbid psychiatric illness (i.e., depression, generalized anxiety disorder), if present, for at least 90 days, (vi) at least 30 days past their most recent clinical exacerbation and (vii) exposure to their last glucocorticosteroid treatment. Exclusion criteria for
all participants included (i) left-handed patients, (ii) pregnant or nursing women, (iii) history of smoking or cardiopulmonary illness (due to the use of hypercapnic gas mixture in this study; 5% CO₂ and 95% room air), (iv) prior history for neuropsychiatric conditions other than MS, and (v) contraindications to MRI scanning. Informed written consent was obtained from all participants prior to study participation.

2.2. MRI data acquisition

MRI scans were performed on a 3T MRI scanner (Philips Medical Systems, Best, The Netherlands) equipped with a 32-channel phased array head coil at the UTSW Advanced Imaging Research Center. Participants first underwent a hypercapnia calibration experiment wherein they focused their attention on a central fixation cross for the scan duration. Second, they underwent functional MRI (fMRI) scans while they performed visual tasks (see section 2.3). During the hypercapnia calibration experiment and functional MRI scans, a dual-echo pseudo-continuous arterial spin labelling (pCASL) pulse sequence was implemented. Following these scans, high resolution T₂-weighted fluid attenuated inversion recovery (T₂ FLAIR) and T₁-weighted magnetization-prepared rapid acquisition gradient-echo (MPRAGE) images were acquired.

2.2.1. Scan parameters

MPRAGE images were acquired with the following parameters: repetition time (TR) = 8.1 ms, echo time (TE) = 3.7 ms, shot interval = 2100 ms, inversion time (TI) = 1100 ms, resolution = 1 mm³ isotropic, flip angle = 12°, field of view (FOV) = 256 mm × 204 mm × 160 mm, matrix size = 256 × 204 × 160, sagittal slice orientation.

T₂-FLAIR images were acquired with the following scan parameters: TR = 4800 ms TE = 344 ms, TI = 1600 ms, FOV = 250 mm × 250 mm × 179 mm, matrix size = 228 × 227 × 163, resolution = 1.1 mm³ isotropic, sagittal slice orientation.

Dual-echo fMRI included both pseudo-continuous arterial spin labelling (Echo 1) and BOLD images (Echo 2). Data were acquired in an axial plane with the following parameters: TR = 4000 ms, post-label delay = 1450 ms, labeling duration 1400 ms, each labeling RF-flip angle = 18°, labeling gap = 93 mm, flip angle = 90°, slice gap = 0 mm, FOV = 220 mm × 220 mm × 132 mm, matrix size = 64 × 64, slices = 22, Echo-1 TE = 13 ms, Echo-2 TE = 30 ms.

Phase contrast MRI was used to obtain baseline CBF during normocapnic and hypercapnic conditions (see section 2.2.2). The data were acquired in a transverse slice located parallel to the anterior commissure-posterior commissure (AC-PC) line and going through the superior sagittal sinus (20 mm above the sinus congruence for each participant). PC was acquired with the following scan parameters: single slice, flip angle = 15°, TR = 20 ms, TE = 6.9 ms, voxel size = 0.45 × 0.45 × 5 mm, maximum velocity encoding (VENC) = 80 cm/s, 4 signal averages, scan duration = 0.5 min.

2.2.2. Hypercapnia calibration experiment

In the hypercapnia calibration experiment, participants underwent a 10-min scan using a dual-echo pCASL pulse sequence for calibration (to calculate M, as described previously by Lu et al., 2014; see section 2.5). Participants were given a mouth-piece and a nose clip to ensure that they were only able to breathe by mouth. The mouth-piece delivered either room air or hypercapnic gas-mixture (5% CO₂ and 95% room air). The first 4 min consisted of room air (normocapnia portion), and the latter 6 min consisted of hypercapnic gas-mixture (hypercapnia portion). Normocapnia and hypercapnia portions of the experiment were controlled manually using a valve switch. Phase contrast scans were run immediately prior and immediately after the dual-echo pCASL scan to obtain global baseline CBF in the sagittal sinus during normocapnic and hypercapnic conditions (Aslan et al., 2010; Hutchinson et al., 2013a, b). End-tidal CO₂, breathing rate, heart rate, and arterial O₂ saturation from participants were monitored by capnograph and pulse oximeter throughout to ensure patient safety.

2.2.3. Visual task

Participants performed a block-designed visual task while undergoing fMRI scanning. Participants were presented with 8 Hz radial flickering checkerboard containing a central fixation cross during task blocks, and only a central fixation cross during rest blocks. The central fixation cross periodically changed in luminance. Participants were instructed to focus on the central fixation cross and press bilateral thumb buttons when they detected a luminance change in the fixation cross. The reaction time (RT) to the luminance change was recorded. Stimuli were presented across 2 runs with 12.32 s interleaved (6 task and 6 rest) blocks per run. Each run lasted 6 min, 24 s, and the entire visual task lasted just under 13 min. The dual-echo fMRI sequence was again used for image acquisition.

2.3. Neuropsychological tests

All participants underwent a comprehensive neuropsychological evaluation that involved tests performed inside (i.e., the visual task) and outside the scanner. Participants’ simple RT was obtained from the inside-scanner visual task. RT to the luminance change was averaged across all trials to obtain subjects’ RTs. Outside the scanner, participants completed a comprehensive test battery that was used to assess the following cognitive domains: (1) processing speed, measured with Digit-Symbol-Substitution Test (DSST) and Symbol-Digit-Modality Test (SDMT), (2) working memory, measured with backwards digit-span, (3) visuospatial memory, measured with 10/36 spatial-recall test, (4) verbal memory, measured with Selective-Reminding Test, (5) verbal fluency, measured with Controlled-Oral-Word-Association Test. The scores from each of the processing speed tests were z-standardized using the equation shown below:

\[ Z_i = \frac{\mu - x_i}{\sigma} \]  

Eq. 1

Where \( Z \) is the z-score for every individual \( i \), \( \mu \) is the mean across the sample, \( x_i \) is the raw processing speed test score (i.e., SDMT, DSST), and \( \sigma \) is the standard deviation across the sample. The z-scores for each of the two processing speed tests were averaged to obtain a processing speed composite for every participant. Thus, higher z-scores reflected slower individuals, and lower z-scores reflected faster individuals.

2.4. Visual acuity measurements

Visual acuity was assessed in each eye separately with a Sloan letter chart after correcting for any refractive errors. The acuity score was recorded as a fraction of viewing distance divided by the letter size. The Magnification Requirement (MAR) was calculated as the inverse of acuity score. MAR values were then converted to log scale. A value of 0 indicates normal visual acuity (20/20 vision) and a 0.1 increment in log (MAR) indicates one line of loss.

2.5. MRI data analyses

All dual-echo pCASL data (Echo 1, CBF; and Echo 2, BOLD) were preprocessed using Analysis of Functional Neuroimages (AFNI) software (Cox, 1996). Data were despiked and registered to the first functional volume of each dataset’s Echo 2 sequence using a heptic polynomial interpolation method to correct for motion. The transformation matrix from this registration was then applied to Echo 1 data. CBF was estimated from Echo 1 images (control and label) using surround subtraction (Liu and Wong, 2005). The Echo 2 data were registered to each participant’s anatomical data. The transformation matrix from this anatomical registration was then applied to Echo 1 data. Data were visually inspected for alignment errors. Echoes 1 and 2 data were then spatially-smoothed (8 mm) and high-pass filtered (0.0156Hz). Echo 1 (CBF) and Echo 2 (BOLD) time-series were input to a general linear
model (GLM) with a task-related block function, and 6 motion regressors and signal drift as covariates to generate voxel-wise baseline ($\beta_0$) and task-related ($\beta_1$) values, and $T$-statistics. A conjunction mask for $\Delta$BOLD and $\Delta$CBF images was created by intersecting their respective $T$-statistics. This intersection was accomplished by setting, for each voxel, a minimum $T$-statistic across each modality (average $t_{\text{BOLD}} = 1.6709$, $t_{\text{CBF}} = 1.6705$; Friston et al., 2005; Schäfer et al., 2012; Scharf and Demeure, 1991). The anatomical ROI was comprised of the occipital lobe obtained using FreeSurfer for each individual in native space. Anatomical images from every participant were parcellated based on the Desikan-Killiany atlas (Desikan et al., 2006) and reconstructed using the recon-all function in FreeSurfer. The occipital lobe for every individual comprised the following bilateral regions: lingual, pericalcarine, lateral occipital, and cuneus. The occipital lobe was then transformed back to the native space using the registration matrix that FreeSurfer used to transform the atlas (Desikan et al., 2006) and reconstructed using the LST toolbox from FLAIR images. BPF was calculated as the ratio of brain parenchymal to estimated-total intracranial volume from MPRAGE using FreeSurfer.

2.8. Statistical analysis

All analyses were performed in R (version 3.4.3) and SPSS (version 24.0). Linear regression was performed to assess associations between processing speed measures (RT, processing speed composite z-scores) and $\Delta$BOLD signal. Data were inspected and tests were performed to assure that assumptions of linearity, normality and independence of residuals were met. Linearity was assessed by visually inspecting the processing speed measures and $\Delta$BOLD signal scatterplots. Because of the non-gaussian distribution of RT (Ratliff, 1993), they were log-transformed. The residuals for processing speed composite z-scores were homoscedastic and normal.

One-way analysis of variance (ANOVA) models were performed to assess group-differences (i.e., HCs, speed-preserved MS, and speed-impaired MS; see Result 2) in RT and physiologic variables (i.e., $\Delta$BOLD, $\Delta$CBF, and, $\Delta$CMRO2). There were two outliers on $\Delta$CBF and $\Delta$CMRO2, as assessed by boxplots for values greater than 3 times the inter-quartile range (i.e., between 25th and 75th percentile). Thus, 17 HCs, 24 speed-preserved MS and 13 speed-impaired MS patients were included in the subsequent analyses. Within each group, the data were normally distributed as assessed by Shapiro-Wilk’s test of normality. Variance was homogenous for all variables as assessed by Levene’s test. All models were followed up with post-hoc t-tests and each of these tests was Bonferroni-corrected (based on $m = 3$) for multiple comparisons. A general linear model was performed to test differences in $\Delta$CBF-$\Delta$CMRO2 slope between groups. The general linear model takes the form of:

$$y = \beta_0 + \beta_1x + \epsilon_\alpha$$

where $\alpha = 0.38$ is the empirically-derived Grubb’s constant linking $\Delta$CBF and cerebral blood volume changes (Grubb et al., 1974; Hoge et al., 1999; Hutchison et al., 2013a; Stefanovic et al., 2004), and $\beta = 1.3$ is the empirically-derived constant related to vascular exchange and susceptibility of deoxyhemoglobin at 3T (Ances et al., 2011; Hutchison et al., 2013a, b; Leontiev and Buxton, 2007). There were no significant group-differences in M for HCs ($M = 0.1087$, $SD = 0.0725$) and MS patients ($M = 0.0835$, $SD = 0.0426$, $t(54) = 1.6301$, $p = 0.1089$). Recent research, however, suggests a change to the value of $\alpha$ as 0.23 (Chen and Pike, 2009). This change was suggested so that the value of $\alpha$ more accurately converts $\Delta$CBF to cerebral blood volume changes in the veins as $\Delta$BOLD signal is primarily dependent on venous cerebral blood volume. To ensure that the value of $\alpha$ did not have an undue effect on our results, we conducted analyses using both the values of $\alpha$ (Grubb et al., 1974) and 0.23 (Chen and Pike, 2009; see supplement). $\Delta$CMRO2 reflects the rate of cellular oxygen consumption. Dual-echo pCASL provides near-simultaneous measures of CBF and BOLD. Together, $\Delta$CBF and $\Delta$BOLD along with M allow for estimation of $\Delta$CMRO2 using the deoxyhemoglobin dilution model of $\Delta$BOLD signal change (as described by Hoge et al., 1999):
ΔBOLD-RT relationships were formally tested using a Pearson’s correlation analysis. There was a significant negative correlation between ΔBOLD and RT for MS patients, $r(35) = -0.326$ ($p = 0.0488$), but not for HCs, $r(15) = -0.040$ ($p = 0.8775$).

Second, we assessed relationships between ΔBOLD and processing speed composite $z$-scores created from performance on neuropsychological tests outside the scanner (i.e., Digit-Symbol Substitution Test, Symbol-Digit Modalities Test) in MS patients and HCs. We observed statistically significant decreases in ΔBOLD with increases in processing speed composite $z$-scores for MS patients, $r(35) = -0.3476$ ($p = 0.0350$; Fig. 1b). No such relationships between processing speed composite $z$-scores and ΔBOLD were seen for HCs, $r(15) = 0.101$ ($p = 0.7001$).

3.2. Cognitive characterization of MS patients

To specifically investigate the mechanisms that underlie cognitive slowing in MS, the MS sample was divided into speed-preserved and -impaired MS patient groups based on the RT distribution from the visual task. Speed-impaired MS patients ($n = 13$) were defined as those patients with RTs 1.5 SD higher than the HC mean RT (Sumowski et al., 2018). Speed-preserved MS patients ($n = 24$) were those that fulfilled the 2010 McDonald criteria for MS diagnosis but had intact cognition (i.e., within 1.5 SD of the HC mean RT) compared to HCs. These patients also exhibited increased disability, increased fatigue, and reduced mood compared to HCs (see Table 1).

To test for RT differences between the 3 groups (HC, speed-preserved MS, and speed-impaired MS), a one-way ANOVA was performed. Significant RT differences between groups were observed, $F(2, 51) = 54.105$ ($p < 0.0010$, partial $\eta^2 = 0.680$; Fig. 2a). Speed-impaired MS patients ($M = 3.0235$, $SD = 0.0513$) had significantly higher RT compared to speed-preserved MS patients ($M = 2.8127$, $SD = 0.0552$, $p < 0.0010$) and HCs ($M = 2.7891$, $SD = 0.0891$, $p < 0.0010$; Fig. 2a). There were no significant differences in RT between speed-preserved patients and HCs ($p = 0.8131$; Fig. 2a).

We sought to assess whether the RT group differences could be attributed to differences in visual acuity between the three groups (HC, speed-preserved MS, and speed-impaired MS). A one-way ANCOVA was performed to test for RT differences between the 3 groups after
3.3. Neurovascular uncoupling explains cognitive slowing in MS

We sought to assess the physiologic mechanisms underlying the observed ΔBOLD-processing speed relationships in MS by investigating the components that constitute BOLD signal (i.e., ΔCBF, ΔCMRO₂, neurovascular coupling ratio) in speed-preserved and speed-impaired MS patients (Hoge et al., 1999). First, we assessed group differences in visual task-evoked ΔCBF and ΔCMRO₂ between speed-preserved and -impaired MS patients using independent-samples t-tests. There were no significant differences between speed-preserved MS (M_ΔCBF = 48.9052%, SDb_ΔCBF = 28.7396% M_ΔCMRO₂ = 17.4401%, SDb_ΔCMRO₂ = 13.3196%) and speed-impaired MS patients (M_ΔCBF = 57.2700%, SDb_ΔCBF = 25.9865% M_ΔCMRO₂ = 24.8835%, SDb_ΔCMRO₂ = 15.7662%) in either ΔCBF, t(35) = −0.873, p = 0.3886 (Fig. 3a), or ΔCMRO₂, t(35) = −1.522, p = 0.1371 (Fig. 3b).

Second, we assessed relationships between these physiologic measures (ΔCBF, ΔCMRO₂) and RT in speed-preserved and -impaired MS patients using Pearson’s correlation analysis. While we observed significant reductions in ΔCBF with increases in RT for speed-impaired MS patients, r(11) = −0.6356, p = 0.0195, no such relationships were observed for speed-preserved MS patients, r(22) = −0.279, p = 0.1868. There were no significant relationships between ΔCMRO₂ and RT for speed-preserved (p = 0.3303) and -impaired MS patients (p = 0.0568).

Finally, because task-evoked ΔBOLD follows the increase in ΔCBF relative to ΔCMRO₂ (i.e., functional hyperemia due to neurovascular coupling), we hypothesized that the mechanism of ΔBOLD reductions observed in speed-impaired MS might be due to reduced neurovascular coupling. We assessed group differences in neurovascular coupling by testing for ΔCBF/ΔCMRO₂ slope differences between speed-preserved and -impaired MS patients. The ΔCBF/ΔCMRO₂ slope difference between the groups was tested using the interaction term from a general linear model. Speed-impaired MS patients had significantly higher slope compared to speed-preserved MS patients, t(23) = 2.341 (p = 0.0254), suggesting reduced neurovascular coupling in speed-impaired MS patients (see Fig. 3c and d, supplement).

Table 1

| Measures [Mean (SD)] | Healthy controls | Speed-preserved MS patients | Speed-impaired MS patients |
|----------------------|------------------|-----------------------------|---------------------------|
| Age (years)          | 42.00 (11.40)    | 46.46 (9.31)                | 49.54 (11.85)             |
| Sex (%)              | 76.4%            | 59%                         | 84.6%                     |
| Dose duration (years)| 15.7662%         | 15.7662%                    | 15.7662%                  |
| Education index      | 17.13 (1.78)     | 16.17 (2.55)                | 15.22 (1.73)              |
| EDSS                 | –                | 2.88 (1.69)                 | 3.91 (2.22)               |
| Beck’s depression inventory | –       | 9.08 (6.80)                 | 8.38 (7.52)               |
| Modified fatigue impact scale | 15.50 (12.89) | 35.21 (19.83)               | 37.38 (17.65)             |
| Total lesion volume (ml) | –             | 5.78 (7.62)                 | 12.62 (12.49)             |
| BPF (%)              | 0.75 (0.07)      | 0.72 (0.07)                 | 0.68 (0.06)               |
| Binocular vision (Log Magnification Ratio) | 0.07 (0.12) | 0.08 (0.12)                 | 0.22 (0.13)               |
| 9-hole peg test      | 19.30 (2.59)     | 23.24 (8.15)                | 29.23 (9.96)              |
| Timed 25-foot walk   | 4.77 (1.06)      | 5.32 (1.28)                 | 7.82 (5.41)               |

Note: Tests for group-differences are reported without accounting for any multiple comparisons. SD = standard deviation.

* denotes significant difference between healthy controls and speed-preserved MS patients, p < 0.05.
ϕ denotes significant difference between speed-preserved MS and speed-impaired MS patients, p < 0.05.
† denotes significant difference between healthy controls and speed-impaired MS patients, p < 0.05.

Absence of a symbol denotes no statistical differences between groups, p > 0.05.

controlling for visual acuity group differences. Significant RT differences between groups were observed after controlling for visual acuity group differences, F(2, 50) = 38.218, (p < 0.001), partial η² = 0.6045; Fig. 2b).

Finally, because task-evoked ΔBOLD follows the increase in ΔCBF relative to ΔCMRO₂ (i.e., functional hyperemia due to neurovascular coupling), we hypothesized that the mechanism of ΔBOLD reductions observed in speed-impaired MS might be due to reduced neurovascular coupling. We assessed group differences in neurovascular coupling by testing for ΔCBF/ΔCMRO₂ slope differences between speed-preserved and -impaired MS patients. The ΔCBF/ΔCMRO₂ slope difference between the groups was tested using the interaction term from a general linear model. Speed-impaired MS patients had significantly higher slope compared to speed-preserved MS patients, t(23) = 2.341 (p = 0.0254), suggesting reduced neurovascular coupling in speed-impaired MS patients (see Fig. 3c and d, supplement).

4. Discussion

Processing speed changes are a hallmark of reduced cognitive ability in development, aging, and disease (DeLuca et al., 2004; Kill, 1986, 1985; Lengenfelder et al., 2006; Ryoma et al., 2006; Saltmarch, 1996; Sivakolundu et al., 2019b). In this study, we sought to test the hypothesis of a link between neurovascular coupling and processing speed. In previous work from our laboratory, and those of others, there is evidence that the neural-glial-vascular unit becomes dysfunctional in MS (Habbas et al., 2015; Hubbard et al., 2016; Jakimovski et al., 2020; Sivakolundu et al., 2019b; Turner et al., 2018). Like other conditions that feature neurovascular changes (e.g., healthy aging; Abdelkarim et al., 2019; Hutchison et al., 2013a), MS also features processing speed deficits. Thus, we utilized MS as a model to study the role of neurovascular uncoupling in processing speed dysfunction. We assessed MS-related cognitive slowing and its pathophysiologic underpinnings in a processing speed-dependent region, visual cortex. We employed a dual-echo sequence to quantify such disruptions by evaluating visual task-evoked ΔBOLD, ΔCBF, ΔCMRO₂ and neurovascular coupling ratio. Consistent with results from previous studies in our lab and others, visual-task evoked ΔBOLD was associated with processing speed measures for MS patients. As expected, those patients with processing speed deficits (i.e., speed-impaired MS patients) also exhibited higher-order cognitive deficits (i.e., working memory), implicating processing speed as a central driver of cognitive change in MS, much as has been observed in cognitive aging research (Ackerman et al., 2002; Bezdicke et al., 2016; Ebaid et al., 2017; Saltmarch, 2000, 1992; Saltmarch, 1996; Vernon, 1983; Tuck-er-Drob et al., 2009).

We further investigated the mechanism that underlies visual-task evoked ΔBOLD reductions observed in patients with processing speed deficits. We assessed the components that constitute the ΔBOLD signal, namely, visual task-evoked ΔCBF, ΔCMRO₂, and neurovascular coupling ratio (Davis et al., 1998; Hoge et al., 1999) between patients with and without processing speed deficits. While we observed no group-differences in visual task-evoked changes in either ΔCBF or ΔCMRO₂ between speed-impaired and -preserved MS patients, speed-impaired MS patients exhibited significant reductions in neurovascular coupling ratio (i.e., increases in cerebral blood flow per unit cerebral metabolic rate of oxygen; ΔCBF/ΔCMRO₂). These results implicate the glial structures (e.g., astrocytes) that mediate neurovascular coupling as a central neural mechanism underlying processing speed.

Neurovascular coupling is a complex physiologic process that requires coordinated and optimal functioning of the neural-glial-vascular system (e.g., Abdelkarim et al., 2019; Howarth, 2014). It is known that, after a neuron is stimulated, action potentials propagate down the
axon via saltatory conduction. At nodes of Ranvier, potassium ions (K\(^+\)) exit the axon and are taken up by fibrous astrocytic endfeet. These astrocytes in turn utilize K\(^+\) to signal local vasculature to perfuse more local O\(_2\) (Jukkola et al., 2013). Once an action potential reaches the presynaptic terminal, neurotransmitters (e.g., glutamate; Metea and Newman, 2006), dopamine (Choi et al., 2006) are released from the presynaptic neuron. These transmitters bind to metabotropic receptors on local protoplasmic astrocytic endfeet. Postsynaptic protoplasmic astrocytes release many of the vasodilators (e.g., epoxyeicosatrienoic acid, prostaglandins) that activate local smooth muscle cells. Smooth muscle cells interact with vascular endothelia to dilate local vasculature allowing increases in local O\(_2\) via increases in CBF (see Attwell et al., 2010). These neural-glial-vascular signaling processes are vital for supplying energy resources to active neurons.

Perfusion of O\(_2\)-rich hemoglobin is an allostatic mechanism that provides for the metabolic needs of active neurons. When an action potential is generated, the neuron consumes the metabolite of O\(_2\) and glucose (i.e., adenosine triphosphate; ATP) to move ion concentrations toward their resting potentials. For example, it is estimated that postsynaptic neurons use ~50% of the ATP to pump out excess sodium and calcium ions and restore resting potentials (e.g., Attwell et al., 2010; Howarth, 2014). To supply this process, the healthy neurovascular system perfuses more O\(_2\) than necessary (by a ratio of ~2:1; Davis et al., 1998; Hoge et al., 1999; Hyder and Blumenfeld, 2004) to accommodate local neural activity (i.e., CMRO\(_2\)).

Previous work from our laboratory suggests relationships between the integrity of the neural-glial-vascular system and processing speed. We have previously demonstrated associations between processing speed and peak latency for the \(\Delta\)BOLD hemodynamic response in dorsolateral prefrontal cortex, a region of cortex known for involvement in processing speed tasks (Biswal et al., 2010; Kannurpatti et al., 2010; Motes et al., 2011; Rypma et al., 2006; Turner et al., 2018), suggesting that the time in which oxygen and glucose is delivered by cerebral blood flow to metabolically active neurons is crucial for processing speed (Abdelkarim et al., 2019). A more recent study from our lab has emphasized the importance of an effectively timed nutrient-delivery system for optimal processing speed. This study found that arterial compliance along the cerebrovascular tree (i.e., from penetrating arteries to parenchymal arterioles) was reduced in speed-impaired MS patients compared to speed-preserved MS patients (Sivakolundu et al., 2019b). This compliance is essential to sustain CBF increases for oxygen and glucose delivery upon neural stimulation. The significant reductions in visual-task evoked \(\Delta\)CBF with
increases in RT for speed-impaired but not for speed-preserved MS patients observed here are consistent with these previous results. Altered compliance along the tree could result in visual-task evoked ΔCBF reductions with increases in RT. Together, these results highlight the importance of precisely timed nutrient-delivery, mediated by neurovascular coupling, for effective cognition.

We found that neurovascular coupling was reduced in speed-impaired MS patients compared to speed-preserved MS patients. MS-related damage to the neural-glial-vascular system might cause neurovascular dysconnectivity, vascular change, and neurovascular uncoupling. MS features global insult to white-matter microstructure (e.g., astrocytes, oligodendrocytes, myelin) in both normal-appearing and lesioned tissue (Lucchinetti et al., 2000; Sivakolundu et al., 2019a; Trapp et al., 1998; Trapp and Stys, 2009). For example, MS is associated with alterations to expression of aquaporin-4 channel proteins in astrocytic end-feet, which regulate homeostasis and cellular activity (Jukkola et al., 2013). MS patients have an estimated 50% CBF reduction in normal appearing white-matter resulting from reduced astrocytic mediation of functional hyperemia (Tucker-Drob et al., 2019). A previous study from our lab found that reductions in visually-evoked ΔBOLD amplitude was associated with reduced white-matter tract integrity measured using diffusion tensor imaging (Hubbard et al., 2016).

The notion of astrocyte-mediated neurovascular uncoupling in MS receives support from a study utilizing a murine model of MS (Habbas et al., 2015). In that study, the authors found that MS mice exhibiting astrocyte dysfunction also showed reduced cognitive performance. Specifically, context-memory decline was associated with increased tumor necrosis factor α (TNFα)-mediated astrocyte signaling in the MS mice. Thus, MS-related white matter insult might compromise the ability of neurons to communicate energy needs to vasculature (i.e., neurovascular uncoupling) resulting in cognitive slowing.

The neurovascular-coupling reductions that we observed in speed-impaired MS patients suggests a central role for the neural-glial-vascular system in processing speed, a fundamental ability thought to underlie individual differences in cognition (Amato et al., 2013; DeLuca et al., 2004; Rypma and Prabhakaran, 2009; Salthouse, 2000; Salthouse,
Because these are indirect measures of the neurovascular coupling process, we cannot, however, rule out alternate hypotheses. Although visual dysfunction is known to occur in MS in the absence of ophthalmic pathologies, peripheral visual system changes could lead to reduced visual acuity and in turn, cognitive slowing (Anstey et al., 1997; Baltes and Lindenberger, 1997; Lindenberger and Baltes, 1994; Vernon, 1983).

Previous studies investigating the effect of MS-related optic neuritis on visually-evoked \( \Delta \text{BOLD} \) signal showed that this signal is reduced in visual cortex in MS patients with a previous history of optic neuritis compared to those with no such previous history. In our study, we found speed-impaired MS patients had reduced visual acuity compared to speed-preserved MS patients. Nonetheless, the group-differences in RT remained significant, after accounting for visual acuity. In addition, demyelinating lesions along the visual tract might also lead to visual cortex dysfunction and thus, cognitive slowing. Future studies are needed to ascertain whether the processing speed deficit in MS is based on lower-level peripheral visual tract changes (i.e., ophthalmic pathologies, or lesion-mediated visual tract disconnection) or changes in higher-level processing speed dependent regions (i.e., visual cortex, motor cortex, prefrontal cortex).

Future work is also essential to disentangle neural, glial, and vascular contributions to MS-related neurovascular uncoupling. For instance, in our sample, we observed slightly (but not significantly) higher M values in the HC group compared to the MS group. M is a subject-specific constant that represents maximal \( \Delta \text{BOLD} \) elicited upon passive vasodilation using carbon dioxide. Physiologically, M can be considered as a normalization constant to partially account for the vasculature variability between individuals (Hoge et al., 1999; Hutchison et al., 2013a). We conducted a power analysis to determine the sample size required to elicit statistically significant group-differences in M. Considering alpha error probability of 0.05, we would need 89 participants in each group (a total of 178 participants) to elicit group-differences. This slight difference in M might reflect the vascular dysfunction observed in MS by our group and others (D’haeseleer et al., 2011; Sivakolundu et al., 2019; Marshall et al., 2014).

Some limitations must be weighed in any conclusions from the present study. This study is limited by the low temporal resolution of the dual-echo sequence. Such low-resolution precluded us from utilizing functions that minimize shape assumptions such as cubic splines to model the long HRFs (i.e., 32s) that are sampled every 4s. It is worth noting that, in the context of block designs, the effect of HRF shape differences would be relatively minimal compared to event-related designs. With improvement in MRI technologies, higher temporal resolution in dual-echo sequences will be possible permitting analysis methods that minimize the shape assumptions necessary to model block-design HRFs. Nonetheless, with measures from the dual-echo sequence, we found evidence for neurovascular uncoupling in MS patients and its contributions to MS-related cognitive slowing. In this study, we also used RT to assess its relationships with physiologic measures. RT was chosen because it was calculated directly from the visual task used to elicit \( \Delta \text{CBF} \) and \( \Delta \text{CMRO}_2 \) in the visual cortex. In addition, RT from the visual task provided a more direct assessment of participants’ speed within the scanner environment during which the physiologic measures were assessed. Future studies are however required to assess relationships between physiologic measures obtained from other processing speed dependent regions (e.g., pre-frontal cortex) and RT obtained from more direct in-
scanner processing speed tasks (e.g., fMRI-adapted digit symbol substitution test; Rypma et al., 2006).

In summary, we sought to investigate the neural mechanisms that underlie individual differences in processing speed. We assessed psychologic changes in a processing speed-dependent system (i.e., visual cortex). Because MS is known to feature global insults to the neural-glial-vascular system and is known to feature widely-prevalent speed deficits, we utilized MS as a model to identify its underlying neural mechanisms. We found that neurovascular coupling was reduced in speed-impaired MS patients compared to speed-preserved MS patients in the visual cortex suggesting that neurovascular coupling might be a central mechanism of processing speed. According to our model (Fig. 4), processing speed depends on the integrity of the neural-glial-vascular system that provides timely and sufficient oxygen and nutrients to meet the high neurometabolic cost of the high-frequency action potentials required for optimal cognitive function (Goldman-Rakic, 1995). Disruption of this system, such as occurs in healthy aging, dementia, and MS, would result in abnormal CBF responsiveness to neurometabolic demand upon neural activation (i.e., neurovascular uncoupling), leading to altered neural function, reduced processing speed, and reduced cognition.

Author contributions

DS and BR conceptualized and designed the study. DS, KW, MZ, and MT acquired the data. DS, KW, MZ, MT, YZ, DA, JS, HL, DO, and BR analyzed and interpreted the data. DS, MT, DA, DO, and BR drafted and critically revised the manuscript.

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Declaration of competing interest

DS, KW, MT, DA, YZ, HL, JS, MZ, BR report no disclosures related to the work presented here. DO received advisory and consulting fees from Celgene, EMD Serono, Genentech, Genzyme, and Novartis and research support from Biogen.

CRediT authorship contribution statement

Dinesh K. Sivakolundu: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. Kathryn L. West: Data curation, Formal analysis. Mark Zuppichini: Data curation, Formal analysis. Monroe P. Turner: Data curation. Dema Abdelkarim: Formal analysis, Data curation. Yuguang Zhao: Formal analysis, Data curation. Jeffrey S. Spence: Formal analysis, Data curation. Hanzhang Lu: Formal analysis, Data curation. Darin T. Okuda: Formal analysis, Data curation, Writing - original draft, Writing - review & editing. Bart Rypma: Conceptualization, Formal analysis, Data curation, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

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