Resting State Sensorimotor Functional Connectivity in Multiple Sclerosis Inversely Correlates With Transcallosal Motor Pathway Transverse Diffusivity

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Abstract: Recent studies indicate that functional connectivity using low-frequency BOLD fluctuations (LFBFs) is reduced between the bilateral primary sensorimotor regions in multiple sclerosis. In addition, it has been shown that pathway-dependent measures of the transverse diffusivity of water in white matter correlate with related clinical measures of functional deficit in multiple sclerosis. Taken together, these methods suggest that MRI methods can be used to probe both functional connectivity and anatomic connectivity in subjects with known white matter impairment. We report the results of a study comparing anatomic connectivity of the transcallosal motor pathway, as measured with diffusion tensor imaging (DTI) and functional connectivity of the bilateral primary sensorimotor cortices (SMC), as measured with LFBFs in the resting state. High angular resolution diffusion imaging was combined with functional MRI to define the transcallosal white matter pathway connecting the bilateral primary SMC. Maps were generated from the probabilistic tracking employed and these maps were used to calculate the mean pathway diffusion measures fractional anisotropy ($\langle FA \rangle$), mean diffusivity ($\langle MD \rangle$), longitudinal diffusivity ($\langle \lambda_1 \rangle$), and transverse diffusivity ($\langle \lambda_2 \rangle$). These were compared with LFBF-based functional connectivity measures (F_c) obtained at rest in a cohort of 11 multiple sclerosis patients and ~10 age- and gender-matched control subjects. The correlation between $\langle FA \rangle$ and F_c for MS patients was $r = -0.63$, $P < 0.04$. The correlation between all subjects $\langle \lambda_2 \rangle$ and F_c was $r = 0.42$, $P < 0.05$. The correlation between all subjects $\langle \lambda_2 \rangle$ and F_c was $r = -0.50$, $P < 0.02$. None of the control subject correlations were significant, nor were $\langle FA \rangle$, $\langle \lambda_1 \rangle$, or $\langle MD \rangle$ significantly correlated with F_c for MS patients. This constitutes the first in vivo observation of a correlation between measures of anatomic connectivity and functional connectivity using spontaneous LFBFs. *Hum Brain Mapp* 29:818–827, 2008. © 2008 Wiley-Liss, Inc.

Key words: functional connectivity; resting state; multiple sclerosis; motor cortex; low-frequency fluctuations; diffusion tensor imaging; fiber tracking; fMRI

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INTRODUCTION

Functional Connectivity With LFBF

Synchronous spontaneous low-frequency BOLD fluctuations (LFBFs) have been taken to be reflective of functional connectivity in the human brain since their first observation more than a decade ago [Biswal et al., 1995]. Recently, they have seen increasing use in a variety of studies, in particular where activation-based studies may not be well-suited to the scientific question under investigation [Anand et al., 2005; Greicius et al., 2004; Hampson et al., 2006b].

Despite the growing interest in using spontaneous synchrony in LFBF, there have only been a few studies that investigate the mechanism itself. Biswal et al. [1997a,b] and Peltier and Noll [2002] demonstrated that the signal characteristics are similar to BOLD, rather than flow or other hemodynamic contrast. Cordes et al. [2001] demonstrated that the functional contrast was limited to the frequency domain 0–0.1 Hz.

There has been some work with other modalities that motivates the existence of a mechanism for neuronal signaling in spontaneous oscillations at 0.1 Hz. Leopold et al. [2003] demonstrated in monkeys that the power in local field potential fluctuations, known to correlate strongly with BOLD contrast, is modulated at a frequency near 0.1 Hz. In addition, Kenet et al. [2003] performed a voltage sensitive dye study in cat visual cortex that showed that extensive regions of cortex exhibit spatial patterns of spontaneous neuronal activation when unstimulated that correspond to patterns observed in response to direct stimulation of the eye fields. Laufs et al. [2003] performed simultaneous electroencephalography (EEG) and functional MRI (fMRI) in resting subjects. They observed that power in the alpha band of the EEG data strongly correlated (inversely) to a broad network of brain regions typically attributed to attentional and related cognitive processes. Because the alpha band of EEG activity has long been associated with the resting, or inactive, state of brain electrical activity, this study presents additional evidence to suggest that, during rest, there exist separable networks of brain regions with correlated BOLD fluctuations.

Multiple sclerosis (MS) is a common idiopathic demyelinating disorder. MS is characterized by a waxing and waning course of neurological deficits that are separated temporally and spatially within the brain [Adams et al., 1997]. Pathologically, MS shows multi-focal areas of white matter demyelination. Often, the disease begins as a perivascular inflammation which progresses to demyelination of the adjacent white matter. Although some white matter lesions may remyelinate or repair themselves, demyelination often leads to progressive focal lesions, gliosis, and associated axonal and neuronal degeneration/loss [Bjartmar and Trapp, 2001, 2003; Bjartmar et al., 2001, 2003; Trapp et al., 1998]. Importantly for the purposes of functional connectivity, MS lesions can decrease or completely block axonal

transmission. Demonstration of slowed neuronal transmission using visual, brainstem, and somatosensory evoked potentials is typical of MS [Adams et al., 1997].

It was previously demonstrated that LFBF-based functional connectivity is reduced in MS patients when compared to healthy age and gender matched control subjects [Lowe et al., 2002]. In this report, no correlation was observed between functional connectivity and clinical measures of disease status, other than diagnosis of MS. This was likely due to the fact that clinical measures do not evaluate a single functional connection and clinical measures are likely not specific enough to exhibit a high correlation with specific pathway measures of connectivity.

Diffusion Tensor Imaging and Pathway Dependent White Matter Measures

Focal macroscopic lesions alone likely do not account for all of the disability seen in MS. A significant portion of disability seen in MS may reflect ongoing/progressive degeneration of axonal pathways [Bjartmar and Trapp, 2001, 2003; Bjartmar et al., 2001, 2003; Trapp et al., 1998].

Diffusion pathway changes in MS were initially demonstrated using diffusion weighted imaging (DWI). [Castriota Scanderbeg et al., 2000; De Stefano et al., 1999; Nusbaum et al., 2000]. Werring et al. showed increases in apparent diffusion coefficient measures of normal appearing white matter (NAWM) within homologous white matter contralateral to a focal acute multiple sclerosis lesion [Werring et al., 2000a]. Multiple authors have shown that DTI can accurately detect MS lesions. Typical MS lesions demonstrate a reduction in fractional anisotropy (FA) and an increase in mean diffusivity (MD). [Bammer et al., 2000; Ciccarelli et al., 2001; Filippi et al., 2001; Guo et al., 2002; Werring et al., 1999] Several studies have also demonstrated that DTI can detect disease burden within NAWM. [Bammer et al., 2000; Ciccarelli et al., 2001; Coombs et al., 2004; Filippi et al., 2001; Ge et al., 2004; Guo et al., 2001, 2002; Rocca et al., 2003; Rovaris et al., 2002; Tievsky et al., 1999; Werring et al., 1999].

There is evidence to suggest that DTI measures are specific to the details of the axonal damage present in diseased tissue. Song et al. has demonstrated a strong correlation between transverse diffusivity and demyelination using a variety of animal models of axonal injury and demyelination [Song et al., 2002, 2003, 2005]. Further, the same studies have consistently demonstrated that longitudinal diffusivity is strongly correlated with axonal damage demonstrated by amyloid precursor protein (APP) measurements [Song et al., 2002, 2003, 2005]. In addition, Trip et al. [2006] have recently shown strong correlations between transverse diffusivity and changes in visual evoked potentials in MS subjects with optic neuritis suggesting that DTI is sensitive to underlying pathologic changes leading to delayed conduction. Combined with the large body of imaging results, we feel that this data provides good evidence that DTI, and, in particular, trans-

verse diffusivity, is an excellent surrogate marker for disease burden and axonal injury in MS.

We have shown significant increases in transverse diffusivity in MS along the transcallosal pathway connecting the bilateral supplementary motor areas (SMA) using a fiber tracking pathway based approach [Lowe et al., 2006]. In addition, we observed a correlation between the multiple sclerosis functional composite (MSFC) and transverse diffusivity of water. The component test of the MSFC most related to the SMA pathway studied with our method (Nine-hole Peg Test) showed significant correlation with transverse diffusivity, but not with longitudinal diffusivity. These results suggest that quantification of pathway disconnection secondary to axonal loss or degeneration is possible. Although this study was the first to use functionally derived fiber tracts, it also demonstrates the difficulty in using deterministic tracking methods in MS [Lowe et al., 2006]. We were unable to reliably track in the presence of lesions and so the tracking was limited to regions of NAWM. In addition, we were unable to directly measure the interhemispheric pathway between the primary motor cortices due to the presence of crossing fibers in the superior longitudinal fasciculus. In this study, we employ an improved tracking methodology based on high-angular resolution diffusion imaging (HARDI)-based fiber orientation distribution function (FODF) estimation [Sakaie and Lowe, 2007]. This FODF estimation procedure permits the use of probabilistic tracking methods that allow tracking to progress across regions of fiber inhomogeneity that are not appropriate for the single-tensor model.

The purpose of the present study is to evaluate the functional connectivity and diffusion characteristics of functionally derived pathways in a cohort of MS patients and compare these measures directly to infer the dependence of functional connectivity measures using LFBF on measures of anatomic connectivity. We demonstrate that mean pathway transverse diffusivity, which we take to be a measure of MS-related white matter disease, inversely correlates with the measure of functional connectivity between the bilateral sensorimotor regions in MS patients. An identical analysis of a cohort of approximately age and gender matched control subjects reveals no significant correlation, although the correlation in functional connectivity and transverse diffusivity was most significant when all subjects were included in the correlation.

MATERIALS AND METHODS

The following data were acquired in a cohort of MS patients, and approximately age- and gender-matched healthy control subjects. All subjects were scanned using a 12 channel receive-only head array on a Siemens Trio 3T scanner (Siemens Medical Solutions, Erlangen). All subjects were fitted for a bite bar to restrict head motion during scanning.

Scan 1, whole brain T1:T1-weighted inversion recovery turbo-flash (MPRAGE). One-hundred and twenty axial slices, thickness 1.2 mm, field-of-view (FOV) 256×256 mm, TI/TE/TR/flip angle (FA) 900/1.71/1900 ms/ 8° , matrix 256×128 , receiver bandwidth (BW) 62 kHz.

Scan 2, SPACE 3D T2. One-hundred and forty-four sagittal slices, thickness 1.2 mm, FOV 256×224 mm², matrix 256×224 , 6/8 partial Fourier acquisition, TE/TR 528/3200 ms, flip angle mode, Grappa factor = 2, 24 reference lines, BW 434 Hz/pixel.

Scan 3, SPACE 3D FLAIR. One-hundred and forty-four sagittal slices, thickness 1.2 mm, FOV 256×224 mm², matrix 256×224 , 6/8 partial Fourier acquisition, TI/TE/TR 2000/395/6500 ms, Grappa factor = 2, 24 reference lines, BW 698 Hz/pixel.

Scan 4, whole brain fieldmap. Axial Gradient Recalled echo, 32 axial slices, 4 mm thick, FOV 256×256 mm², matrix 64×64 , TE1/TE2/TR/Flip 4.89/7.35/388 ms/60, BW 260 Hz/pixel.

Scan 5, whole brain isotropic 71-direction DWI. Forty-eight 2-mm thick axial slices acquired with 71 direction, $b = 1,000$ mm² s diffusion gradients, and eight $b = 0$ gradient images acquired for each slice; TE/TR = 102/7700 ms, 128×128 matrix, 256×256 mm² FOV, 5/8 partial echo, receive bandwidth = 1,628 Hz/pixel. Four volume averages were acquired per subject. For diffusion images, signals were averaged across each diffusion profile for each volume after motion correction. The motion correction was performed such that $b = 0$ volumes motion-corrected and diffusion-weighted images were co-registered to the mean, motion-corrected $b = 0$ images using FLIRT from FSL [Smith et al., 2004].

Scan 6, fMRI activation study. One-hundred and sixty volumes of 31-4 mm thick axial slices are acquired using a prospective motion-controlled, gradient recalled echo, echoplanar acquisition with TE/TR/flip = 29/2800 ms/ 80° , matrix = 128×128 , 256×256 mm² FOV, receive bandwidth = 1,954 Hz/pixel. The complex unilateral finger opposition task described below is performed during this scan.

Scan 7, whole brain LFBF fMRI study. One-hundred and thirty-two repetitions of 31-4 mm thick axial slices acquired with TE/TR = 29/2800 ms, 128×128 matrix, 256×256 mm² FOV, receive bandwidth = 1,954 Hz/pixel. The subject is instructed to rest with eyes closed and refrain from any voluntary motion.

Tasks

All tasks were performed using a pair of fiber optic data gloves (Fifth Dimension Technologies, Irvine, CA). These gloves utilize fiber optic strain gauges placed in each finger and thumb on both hands to monitor flexion of each joint. An in-house designed data acquisition system has been built to synchronize the readout of these strain gauges to the start of MRI scanning and to monitor output of the gauges with a temporal resolution of 20 ms. These

data were used to monitor task performance and to establish minimum performance criteria, described below. The following task was performed during scanning:

fMRI UFT_{complex} Unilateral (dominant hand) complex finger tapping task performed in a four-cycle “on/off” design such that each “tapping” and “rest” period is 45 s. Time: 7:28. Performed during scan 6 above.

Behavioral Data Analysis

As stated above, the purpose of monitoring task performance with fiber optic data gloves was to establish minimum performance criteria for all subjects included in the functional connectivity analysis. The following metrics were produced from the output from the datagloves:

1. *Accuracy.* The number of correct taps (in the prescribed sequence) divided by the total number of finger taps. Accuracy for each finger was computed in a similar way.
2. *Rate.* The average and variance of number of taps over a 32-s time period. This was computed for each finger and for each hand.

Statistical distributions of the accuracy, rate, asymmetry, simultaneity, and rhythm measures from the normal and MS subjects were used to establish task performance standards for both normal subjects and MS patients.

Image Post Processing

The fMRI data were postprocessed in the following manner:

1. Retrospective motion correction using 3dvolreg from AFNI [Cox, 1996].
2. Spatial filtering with Hamming filter to improve functional contrast-to-noise ratio [Lowe and Sorenson, 1997].

The functional connectivity data were postprocessed in the following manner:

1. The mean signal is calculated for each timepoint of each slice. The mean timeseries for each slice is then regressed from each slice to remove global slice effects. This is to remove global slice level effects from, for instance, respiratory-related field shifts.
2. The cardiac and respiratory signals are estimated using PESTICA [Beall and Lowe, 2007].
3. The voxel level cardiac and respiratory fluctuations are regressed out using RETROICOR as provided by AFNI [Cox, 1996; Glover et al., 2000].
4. The data are retrospectively motion corrected using 3dvolreg from AFNI [Cox, 1996].
5. Second-order motion correction is performed to regress signal fluctuations at the voxel level that cor-

relate with the calculated voxel level displacement from the motion correction parameters from 3dvolreg [Bullmore et al., 1999].

6. The data are spatially filtered with a Hamming filter to increase signal-to-noise ratio [Lowe and Sorenson, 1997].
7. The data are temporally filtered to remove all fluctuations above 0.08 Hz [Biswal et al., 1995; Lowe et al., 1998].

The DTI data were postprocessed in the following manner:

1. All image data were motion-corrected using the method described above in the acquisition description
2. All images from each of the 71 gradient directions were averaged across the four acquisitions. The eight $b = 0$ images were similarly averaged.
3. Diffusion gradient information was updated in accordance with the motion correction transformation applied to the diffusion-weighted images [Landman et al., 2007].

Image Analysis

fMRI data

The fMRI data are analyzed using a least-squares fit of a boxcar reference function, representing the 45 s off/45 s on activation paradigm, to the timeseries data of each voxel [Lowe and Russell, 1999]. The result is a whole brain Student's t map that can be thresholded to determine regions of significant involvement in the unimanual tapping task.

Functional connectivity data

The functional connectivity data are analyzed in a similar manner to that described previously in Lowe et al. [Lowe et al., 2002]. Briefly:

1. Regions of interest (ROI) are determined using the t -map calculated from the motor task data described above. The ROI for each hemisphere is defined as the region containing the highest t -value within the M1 motor regions as defined anatomically from the human motor area template (HMAT) template registered to each individual fMRI scan [Mayka et al., 2006]. ROI sizes were $\sim 1 \text{ cm}^3$ ($15 \times 15 \times 4 \text{ mm}^3$) and located to approximately follow the local anatomy for each subject (see Fig. 1 for an example ROI size and location). Right and left hemisphere ROI's are defined in the same manner.
2. A reference timeseries is produced by taking the arithmetic average for each timepoint of the most highly activated voxel, as determined from the motor task study, in left hemisphere ROI and the eight in-

plane voxels immediately surrounding it. The reference timeseries is derived from the resting state data (scan 7) for each subject.

3. The cross-correlation is calculated between the reference timeseries and the resting state study timeseries from every voxel in the brain. This corresponds to the correlation function with lag = 0.
4. The cross-correlation is converted to a Student's t [Press et al., 1986].
5. The Student's t distribution is z-score corrected by fitting a normal distribution to the full-width at half-maximum of the Student's t 's for all voxels [Lowe et al., 1998].

The result is a whole-brain map of z-scores indicating significant correlation to the reference region. Functional connectivity, F_c , is calculated as the percent of voxels in the right hemisphere region of interest over a threshold corresponding to significance $P < 0.05$ (uncorrected).

Fiber tracking

1. Using the 71 acquired diffusion profiles, the fiber orientation distribution (FOD) is calculated for each voxel [Sakaie and Lowe, 2007].
2. Using BrainVisa software (<http://www.brainvisa.info>) [Cointepas et al., 2001], a brain tissue mask is generated from the high resolution T1 weighted scan (Scan 1).
3. For each voxel, the FOD is used as the probability distribution to generate stepping directions using the three-dimensional random walk probabilistic tracking method adapted from Hagmann [Hagmann et al., 2003]. Tracks that leave the brain tissue mask are terminated.
4. Tracks are generated using the ROI in the left hemisphere SMC region selected from the fMRI data described above.
5. More than one-million tracks were propagated from the seed ROI. Tracks were terminated upon leaving the tissue mask. Tracks that pass through the right hemisphere SMC ROI selected from the fMRI data are kept as being transcallosal motor pathway tracks. It was determined that this number of iterations typically results in around 4,000 tracks passing through the right hemisphere SMC ROI, and this number was determined to be sufficient to produce a track density map with dynamic range such that stable pathway dependent measures are obtained (see below).

White matter segmentation

To properly produce white matter DTI measures, it is necessary to produce a white matter mask in the same

coordinate space as the DTI data. To do this, the following procedure was used:

1. White matter segmentation mask produce using the FSL tool FAST (www.fmrib.ox.ac.uk/fsl). A three-channel segmentation is performed using the high resolution T1 (scan 1), T2 (scan 2), and FLAIR (scan 3) volumes.
2. To permit better registration between DTI space and the anatomic images, the mean $b = 0$ image from the DTI acquisition (scan 5) is spatially unwarped using the FSL tool FUGUE and the whole brain fieldmap (scan 4).
3. The spatial transformation matrix between anatomic images and DTI is calculated using AFNI routine 3dvolreg [Cox, 1996] with the spatially unwarped mean $b = 0$ volume and the high resolution T2 volume (scan 2).
4. The white matter mask is transformed to DTI space, producing a spatially registered white matter mask $w(v)$, where v is a given voxel and $w(v) = 1$ if that voxel was found to contain white matter and $w(v) = 0$ otherwise.

DTI data

Whole brain diffusion tensor maps of FA, MD, longitudinal diffusivity (λ_1), and transverse diffusivity (λ_2) were calculated by first least-squares fitting the 71 acquired diffusion profiles to each of the six independent tensor elements, and then calculating the corresponding tensor-based values. Pathway-based DTI measures are produced by using the formula

$$\langle D \rangle = \frac{\sum_T \sum_v D(v) \cdot w(v)}{\sum_T \sum_v w(v)} \quad (1)$$

where $D(v)$ is the particular tensor-based value of interest (e.g. FA) at voxel v and $w(v)$ is the value of the white matter mask at voxel v . The inner summation is over all voxels v on track T and the outer summation is over all transcallosal motor pathway tracks, T . Note that the effect of the above equation is to weight the tensor value of a voxel by the number of times a generated track passes through it. Thus, voxels with only a few tracks passing will count very little toward the pathway tensor estimate, while tracks more central to the path will be counted very highly (see Fig. 2 for a map of track density for a typical subject). The result is $\langle FA \rangle$, $\langle MD \rangle$, $\langle \lambda_1 \rangle$, and $\langle \lambda_2 \rangle$ for every subject.

RESULTS

Eleven MS patients (seven female, age: 44.8 ± 9.5 , MSFC 0.39 ± 0.53 , EDSS: 1.9 ± 1.5). and 10 healthy controls

Figure 1.

An example corrected z-score map for a typical control subject. The reference seed is derived from the white boxed region indicated.

(9 females, age: 40.8 ± 10.25 , MSFC: 0.62 ± 0.27 , EDSS: 0.11 ± 0.33) were recruited into the imaging protocol described above. All subjects were strongly right handed (Edinburgh inventory >80 [Oldfield, 1971]) and were studied under an approved protocol from the Cleveland Clinic Institutional Review Board.

Behavioral data. Mean and standard deviation performance results described above were calculated for the healthy controls. No patients had performance measures that were more than two standard deviations away from the mean and standard deviation calculated for the normal subjects. Thus, no data were excluded on the basis of performance.

Motion characteristics. All 11 MS patients and 10 healthy controls had mean peak-to-peak displacements less than 0.4 mm, which we have determined from pilot studies to be the level of motion at which our connectivity analysis becomes affected. We attribute this to the use of a bite bar during scanning.

fMRI results. Peak-activated voxels in the bilateral M1 regions with t greater than 3.5 ($P < 0.001$, one sided, uncorrected) were detected in all subjects.

Connectivity results. Figure 1 shows typical functional connectivity maps for a single subject using the method described above. Mean connectivity was 0.17 ± 0.24 for MS patients and 0.31 ± 0.22 for controls. There was no significant difference in connectivity observed between patients and controls.

DTI results. Mean motor pathway FA was 0.48 ± 0.03 for patients and 0.50 ± 0.03 for controls. Mean $\langle MD \rangle$ was $(0.779 \pm 0.071) \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ for patients and $(0.789 \pm 0.024) \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ for controls. Mean $\langle \lambda_1 \rangle$ for patients was $(1.256 \pm 0.104) \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ and $(1.302 \pm 0.029) \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ for controls. Mean $\langle \lambda_2 \rangle$ for patients was $(0.540 \pm 0.061) \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ and $(0.532 \pm 0.037) \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ for controls. None of the pathway DTI measures were significantly different between MS patients and controls.

Figure 2.

Example of transcallosal track density maps produced from the probabilistic tracking described in the text. (a) The placement of the seed and target regions derived from the fMRI activation seen in the unilateral tapping task. (b) An oblique coronal view of the trajectory of the track through the corpus callosum. (c) An axial slice at the level of the crossing fiber through the corpus callosum.

Figure 3.

Scatter plots of functional connectivity, F_c , versus (a) transcallosal motor pathway averaged transverse diffusivity, $\langle \lambda_2 \rangle$ and (b) FA for the 11 MS patients and 10 healthy controls.

Combined DTI and connectivity measure. The correlation between $\langle \lambda_2 \rangle$ and F_c for MS patients was $r = -0.63$, $P < 0.04$. None of the control correlations were significant, nor were $\langle FA \rangle$, $\langle \lambda_1 \rangle$, or $\langle MD \rangle$ significantly correlated with F_c for MS patients. The correlation between all subjects $\langle FA \rangle$ and F_c was $r = 0.42$, $P < 0.05$ and all subjects $\langle \lambda_2 \rangle$ and F_c was $r = -0.51$, $P < 0.02$. Figure 3 shows a plot of F_c as a function of $\langle \lambda_2 \rangle$ and $\langle FA \rangle$ for MS patients and controls.

It is useful to point out that there appears to be one subject data point contributing largely to the correlation of the values in Figure 3a. We could find no a priori reason to exclude this subject's DTI, fMRI, connectivity data, or tapping data, so we include these data in our report above. The effect on the correlation without this subject was to reduce the significance of the correlation ($r = -0.36$, $P < 0.11$). As discussed in the introduction, there is an a priori expectation that transverse diffusivity and functional connectivity will be inversely related. Although we have reported two-sided significances throughout this section, there is justification to report one-sided significances. In this case, the correlation of the finding without the outlier in Figure 3a is $P < 0.06$.

DISCUSSION

We report, for the first time, a measurement of the relationship between a measure of anatomic connectivity and functional connectivity in a population of patients with a known white matter impairing disease. The fact that a significant correlation was observed in a relatively small population, when imaging studies with MS populations typically require dozens of patients to observe significant effects, likely reflects the pathway dependent nature of MS.

Axonal degeneration is by its nature pathway specific. Over the recent years there has been a markedly increased interest in the role of axonal and neuronal degeneration in MS. Axonal damage has been demonstrated in acute inflammatory lesions in MS [Ferguson et al., 1997; Kornek et al., 2000; Trapp et al., 1998]. Active inflammatory lesions demonstrate the largest number of axonal transactions with the number of axonal transactions decreasing at the edge of chronic active lesions and seen to the least degree in the core of chronic active lesions [Trapp et al., 1998]. Additionally, axonal APP, a marker for axonal dysfunction and injury has been identified in active lesions as well as in the border of chronic active lesions compatible with axonal dysfunction and injury [Ferguson et al., 1997; Kornek et al., 2000]. Although axons may degenerate distal to areas of transection, myelin sheaths may persist as hollow tubes in these regions leaving the appearance of NAWM on histological staining and MRI [Bjartmar and Trapp, 2001, 2003; Bjartmar et al., 2001, 2003]. Early pathologic studies demonstrated abnormalities within NAWM [Allen and McKeown, 1979; Kornek and Lassmann, 1999]. More recently, several authors have demonstrated a reduction in axonal density in NAWM [Bjartmar et al., 2001; Evangelou et al., 2000; Ganter et al., 1999; Lovas et al., 2000; Simon et al., 2000]. Morphologic changes compatible with axonal degeneration/Wallerian degeneration have been demonstrated the distal to acute lesions using both histopathologic [Bjartmar et al., 2001] and imaging methods [De Stefano et al., 1999; Simon et al., 2000; Werring et al., 2000a]. Overall findings suggest axonal/Wallerian degeneration secondary to proximal axon transection. Thus, in the context of increasing evidence that MS is pathway specific in nature, our finding in such a small population may be due to the focus on pathway-specific anatomic and connectivity measures.

Previous studies of DTI measures of white matter damage in MS has shown that λ_2 is a stronger correlate of disease in MS than other diffusion measures such as FA and λ_1 , even suggesting that the reduction of FA in MS is a consequence of the increase in λ_2 [Henry et al., 2003; Oh et al., 2004; Lowe et al., 2006]. Our finding of stronger correlation of λ_2 with functional connectivity than FA is consistent with this picture.

Although a previous study measured a difference in connectivity between MS patients and healthy controls [Lowe et al., 2002], the fact that this study does not report a significant difference is not directly in conflict with that result. In the previous study, no significant difference in F_c was reported because the observed difference in connectivity was not observed in a single state. To discriminate between patients and controls, it was necessary to perform the connectivity measurements in both resting and continuous tapping state before a difference was observed [Lowe et al., 2002]. When plotting the F_c in each state against each other, clear discrimination was observed. We attribute this to the large intrasubject variability of the F_c measurement method. Indeed, in the current study, our intersubject variances were observed to be quite high in both patients and controls. The additional correlate of the diffusivity measure is necessary to observe a significant difference.

Unlike reports from similar studies of DTI measures in MS patients and controls, we report no significant differences in FA or transverse diffusivity between patients and controls. We attribute this to both the small population and the fact that the cohort of MS patient may have had lesser disease involvement than previous studies. In our previous study [Lowe et al., 2006], we observed a difference of $0.046 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ in transverse diffusivity between patients and controls with mean patient EDSS 2.4, as compared with $0.008 \times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$ with mean patient EDSS 1.9 for the current study. The study of Oh et al. [2004] for example, had a much large sample population with a mean EDSS of 4.1.

With that said, the main finding of this study could be attributed to the pathway specific nature of both the connectivity measure and the disease process that leads to impaired anatomic connectivity.

Although no significant correlation was seen between F_c and λ_2 in healthy controls, as stated above, the difference between the correlation in MS patients and controls was not significant, and when taking the population as whole, the correlation was more significant than with MS patients alone. Thus, the reason for the lack of correlation in healthy controls can possibly be attributed to the lack of range in white matter connectivity in the healthy population.

This study's finding of a significant correlation between anatomic and functional connectivity bridges a gap in the study of these effects. Recent studies of functional connectivity using LFBF correlations have shown a significant correlation with task performance [Hampson et al.,

2006a,b] as well as disease state [Anand et al., 2005; Greicius et al., 2004; He et al., 2007; Lowe et al., 2002]. Studies of DTI measures of anatomic connectivity have been shown to correlate with many disease states almost since the inception of the technique [Song et al., 2004; Werring et al., 1999, 2000b]. More recently, DTI measures have been shown to correlate with task performance [Beaulieu et al., 2005; Dougherty et al., 2007; Lowe et al., 2006]. Thus, in this context, it is not surprising that anatomic measures of connectivity show a strong correlation with functional connectivity measures in a population of patients with known white matter impairment.

CONCLUSION

We report an initial observation of a significant inverse correlation between DTI measures of white matter integrity along the transcallosal pathway connecting the bilateral SMC and functional connectivity as assessed with LFBF in the resting state in a population of MS patients and healthy control subjects. This observation constitutes the first direct link between an in vivo measurement of anatomic connectivity and functional connectivity in the resting state.

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REFERENCES

- Adams RD, Victor M, Ropper AH (1997): In: Adams RD, Victor M, Ropper AH, editors: Principles Of Neurology, 6th ed. New York: McGraw-Hill: pp 903–921.
- Allen IV, McKeown SR (1979): A histological, histochemical and biochemical study of the macroscopically normal white matter in multiple sclerosis. *J Neurol Sci* 41:81–91.
- Anand A, Li Y, Wang Y, Wu J, Gao S, Bukhari L, Mathews VP, Kalnin A, Lowe MJ (2005): Activity and connectivity of brain mood regulating circuit in depression: A functional magnetic resonance study. *Biol Psychiatry* 57:1079–1088.
- Bammer R, Augustin M, Strasser-Fuchs S, Seifert T, Kapeller P, Stollberger R, Ebner F, Hartung HP, Fazekas F (2000): Magnetic resonance diffusion tensor imaging for characterizing diffuse and focal white matter abnormalities in multiple sclerosis. *Magn Reson Med* 44:583–591.
- Beall EB, Lowe MJ (2007): Isolating physiologic noise sources with independently determined spatial measures. *Neuroimage* 37: 1286–1300.
- Beaulieu C, Plewes C, Paulson LA, Roy D, Snook L, Concha L, Phillips L (2005): Imaging brain connectivity in children with diverse reading ability. *Neuroimage* 25:1266–1271.
- Biswal B, Hudetz AG, Yetkin FZ, Haughton VM, Hyde JS (1997a): Hypercapnia reversibly suppresses low-frequency fluctuations in the human motor cortex during rest using echo-planar MRI. *J Cereb Blood Flow Metab* 17:301–308.

- Biswal BB, Van Kylen J, Hyde JS (1997b): Simultaneous assessment of flow and BOLD signals in resting-state functional connectivity maps. *NMR Biomed* 10:165–170.
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS (1995): Functional connectivity in the motor cortex of resting human brain. *Magnetic Resonance in Medicine* 34:537–541.
- Bjartmar C, Trapp BD (2001): Axonal and neuronal degeneration in multiple sclerosis: Mechanisms and functional consequences. *Curr Opin Neurol* 14:271–278.
- Bjartmar C, Trapp BD (2003): Axonal degeneration and progressive neurologic disability in multiple sclerosis. *Neurotox Res* 5:157–164.
- Bjartmar C, Kinkel RP, Kidd G, Rudick RA, Trapp BD (2001): Axonal loss in normal-appearing white matter in a patient with acute MS. *Neurology* 57:1248–1252.
- Bjartmar C, Wujek JR, Trapp BD (2003): Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. *J Neurol Sci* 206:165–171.
- Bullmore ET, Brammer MJ, Rabe-Hesketh S, Curtis VA, Morris RG, Williams SC, Sharma T, McGuire PK (1999): Methods for diagnosis and treatment of stimulus-correlated motion in generic brain activation studies using fMRI. *Hum Brain Mapp* 7:38–48.
- Castriota Scanderbeg A, Tomaiuolo F, Sabatini U, Nocentini U, Grasso MG, Caltagirone C (2000): Demyelinating plaques in relapsing-remitting and secondary-progressive multiple sclerosis: Assessment with diffusion MR imaging. *AJNR Am J Neuroradiol* 21:862–868.
- Ciccarelli O, Werring DJ, Wheeler-Kingshott CA, Barker GJ, Parker GJ, Thompson AJ, Miller DH (2001): Investigation of MS normal-appearing brain using diffusion tensor MRI with clinical correlations. *Neurology* 56:926–933.
- Cointepas Y, Mangin JF, Garnero J, Poline JB, Benali H (2001): BrainVISA: Software platform for visualization and analysis of multi-modality brain data. *Neuroimage* 13:S98.
- Coombs BD, Best A, Brown MS, Miller DE, Corboy J, Baier M, Simon JH (2004): Multiple sclerosis pathology in the normal and abnormal appearing white matter of the corpus callosum by diffusion tensor imaging. *Mult Scler* 10:392–397.
- Cordes D, Haughton VM, Arfanakis K, Carew JD, Turski PA, Moritz CH, Quigley MA, Meyerand ME (2001): Frequencies contributing to functional connectivity in the cerebral cortex in “resting-state” data. *AJNR Am J Neuroradiol* 22:1326–1333.
- Cox RW (1996): AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 29:162–173.
- De Stefano N, Narayanan S, Matthews PM, Francis GS, Antel JP, Arnold DL (1999): In vivo evidence for axonal dysfunction remote from focal cerebral demyelination of the type seen in multiple sclerosis. *Brain* 122 (Pt 10):1933–1939.
- Dougherty RF, Ben-Shachar M, Deutsch GK, Hernandez A, Fox GR, Wandell BA (2007): Temporal-callosal pathway diffusivity predicts phonological skills in children. *Proc Natl Acad Sci USA* 104:8556–8561.
- Evangelou N, Esiri MM, Smith S, Palace J, Matthews PM (2000): Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. *Ann Neurol* 47: 391–395.
- Ferguson B, Matyszak MK, Esiri MM, Perry VH (1997): Axonal damage in acute multiple sclerosis lesions. *Brain* 120 (Pt 3):393–399.
- Filippi M, Cercignani M, Inglese M, Horsfield MA, Comi G (2001): Diffusion tensor magnetic resonance imaging in multiple sclerosis. *Neurology* 56:304–311.
- Ganter P, Prince C, Esiri MM (1999): Spinal cord axonal loss in multiple sclerosis: a post-mortem study. *Neuropathol Appl Neurobiol* 25:459–467.
- Ge Y, Law M, Johnson G, Herbert J, Babb JS, Mannon LJ, Grossman RI (2004): Preferential occult injury of corpus callosum in multiple sclerosis measured by diffusion tensor imaging. *J Magn Reson Imaging* 20:1–7.
- Glover GH, Li TQ, Ress D (2000): Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. *Magn Reson Med* 44:162–167.
- Greicius MD, Srivastava G, Reiss AL, Menon V (2004): Default-mode network activity distinguishes Alzheimer’s disease from healthy aging: evidence from functional MRI. *Proc Natl Acad Sci USA* 101:4637–4642.
- Guo AC, Jewells VL, Provenzale JM (2001): Analysis of normal-appearing white matter in multiple sclerosis: comparison of diffusion tensor MR imaging and magnetization transfer imaging. *AJNR Am J Neuroradiol* 22:1893–1900.
- Guo AC, MacFall JR, Provenzale JM (2002): Multiple sclerosis: diffusion tensor MR imaging for evaluation of normal-appearing white matter. *Radiology* 222:729–736.
- Hagmann P, Thiran JP, Jonasson L, Vandergheynst P, Clarke S, Maeder P, Meuli R (2003): DTI mapping of human brain connectivity: statistical fibre tracking and virtual dissection. *Neuroimage* 19:545–554.
- Hampson M, Driesen NR, Skudlarski P, Gore JC, Constable RT (2006a): Brain connectivity related to working memory performance. *J Neurosci* 26:13338–13343.
- Hampson M, Tokoglu F, Sun Z, Schafer RJ, Skudlarski P, Gore JC, Constable RT (2006b): Connectivity-behavior analysis reveals that functional connectivity between left BA39 and Broca’s area varies with reading ability. *Neuroimage* 31:513–519.
- He Y, Wang L, Zang Y, Tian L, Zhang X, Li K, Jiang T (2007): Regional coherence changes in the early stages of Alzheimer’s disease: A combined structural and resting-state functional MRI study. *Neuroimage* 35:488–500.
- Henry RG, Oh J, Nelson SJ, Pelletier D (2003): Directional diffusion in relapsing-remitting multiple sclerosis: a possible in vivo signature of Wallerian degeneration. *J Magn Reson Imaging* 18:420–426.
- Kenet T, Bibitchkov D, Tsodyks M, Grinvald A, Arieli A (2003): Spontaneously emerging cortical representations of visual attributes. *Nature* 425:954–956.
- Kornek B, Lassmann H (1999): Axonal pathology in multiple sclerosis. A historical note. *Brain Pathol* 9:651–656.
- Kornek B, Storch MK, Weissert R, Wallstroem E, Steffler A, Olsson T, Linington C, Schmidbauer M, Lassmann H (2000): Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. *Am J Pathol* 157:267–276.
- Landman BA, Farrell JAD, Patel NL, Mori S, Prince JL (2007): DTI Fiber Tracking: The Importance of Adjusting DTI Gradient Tables for Motion Correction. CATNAP—A Tool to Simplify and Accelerate DTI Analysis. Paper presented at the Human Brain Mapping, Chicago.
- Laufs H, Krakow K, Sterzer P, Eger E, Beyerle A, Salek-Haddadi A, Kleinschmidt A (2003): Electroencephalographic signatures of attentional and cognitive default modes in spontaneous brain activity fluctuations at rest. *Proc Natl Acad Sci USA* 100:11053–11058.
- Leopold DA, Murayama Y, Logothetis NK (2003): Very slow activity fluctuations in monkey visual cortex: implications for functional brain imaging. *Cereb Cortex* 13:422–433.

- Lovas G, Szilagyi N, Majtenyi K, Palkovits M, Komoly S (2000): Axonal changes in chronic demyelinated cervical spinal cord plaques. *Brain* 123(Pt 2):308–317.
- Lowe MJ, Russell DP (1999): Treatment of Baseline Drifts in fMRI Time Series Analysis. *Journal of Computer Assisted Tomography* 23:463–473.
- Lowe MJ, Sorenson JA (1997): Spatially filtering functional magnetic resonance imaging data. *Magnetic Resonance in Medicine* 37:723–729.
- Lowe MJ, Horenstein C, Hirsch JG, Marrie RA, Stone L, Bhattacharyya PK, Gass A, Phillips MD (2006): Functional pathway-defined MRI-diffusion measures reveal increased transverse diffusivity of water in multiple sclerosis. *Neuroimage* 32:1127–1133.
- Lowe MJ, Mock BJ, Sorenson JA (1998): Functional connectivity in single and multislice echoplanar imaging using resting-state fluctuations. *Neuroimage* 7:119–132.
- Lowe MJ, Phillips MD, Lurito JT, Mattson D, Dziedzic M, Matthews VP (2002): Multiple sclerosis: Low-frequency temporal blood oxygen level-dependent fluctuations indicate reduced functional connectivity initial results. *Radiology* 224:184–192.
- Mayka MA, Corcos DM, Leurgans SE, Vaillancourt DE (2006): Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. *Neuroimage* 31:1453–1474.
- Nusbaum AO, Lu D, Tang CY, Atlas SW (2000): Quantitative diffusion measurements in focal multiple sclerosis lesions: correlations with appearance on T1-weighted MR images. *AJR Am J Roentgenol* 175:821–825.
- Oh J, Henry RG, Genain C, Nelson SJ, Pelletier D (2004): Mechanisms of normal appearing corpus callosum injury related to pericallosal T1 lesions in multiple sclerosis using directional diffusion tensor and 1H MRS imaging. *J Neurol Neurosurg Psychiatry* 75:1281–1286.
- Oldfield RC (1971): The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9:97–113.
- Peltier SJ, Noll DC (2002): T(2)(*) dependence of low frequency functional connectivity. *Neuroimage* 16:985–992.
- Press W, Flannery BP, Teukolsky SA, Vetterling WT (1986): *Numerical Recipes: The Art of Scientific Computing*. (First ed.). Cambridge: Cambridge University Press.
- Rocca MA, Iannucci G, Rovaris M, Comi G, Filippi M (2003): Occult tissue damage in patients with primary progressive multiple sclerosis is independent of T2-visible lesions—a diffusion tensor MR study. *J Neurol* 250:456–460.
- Rovaris M, Iannucci G, Falautano M, Possa F, Martinelli V, Comi G, Filippi M (2002): Cognitive dysfunction in patients with mildly disabling relapsing-remitting multiple sclerosis: An exploratory study with diffusion tensor MR imaging. *J Neurol Sci* 195:103–109.
- Sakaie KE, Lowe MJ (2007): An objective method for regularization of fiber orientation distributions derived from diffusion-weighted MRI. *Neuroimage* 34:169–176.
- Simon JH, Kinkel RP, Jacobs L, Bub L, Simonian N (2000): A Wallerian degeneration pattern in patients at risk for MS. *Neurology* 54:1155–1160.
- Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazky RK, Saunders J, Vickers J, Zhang Y, De Stefano N, Brady JM, Matthews PM (2004): Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23(Suppl 1):S208–219.
- Song SK, Kim JH, Lin SJ, Brendza RP, Holtzman DM (2004): Diffusion tensor imaging detects age-dependent white matter changes in a transgenic mouse model with amyloid deposition. *Neurobiol Dis* 15:640–647.
- Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH (2003): Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. *Neuroimage* 20:1714–1722.
- Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH (2002): Demyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage* 17:1429–1436.
- Song SK, Yoshino J, Le TQ, Lin SJ, Sun SW, Cross AH, Armstrong RC (2005): Demyelination increases radial diffusivity in corpus callosum of mouse brain. *Neuroimage* 26:132–140.
- Tievsy AL, Ptak T, Farkas J (1999): Investigation of apparent diffusion coefficient and diffusion tensor anisotropy in acute and chronic multiple sclerosis lesions. *AJNR Am J Neuroradiol* 20:1491–1499.
- Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L (1998): Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 338:278–285.
- Trip SA, Wheeler-Kingshott C, Jones SJ, Li WY, Barker GJ, Thompson AJ, Plant GT, Miller DH (2006): Optic nerve diffusion tensor imaging in optic neuritis. *Neuroimage* 30:498–505.
- Werring DJ, Brassat D, Droogan AG, Clark CA, Symms MR, Barker GJ, MacManus DG, Thompson AJ, Miller DH (2000a): The pathogenesis of lesions and normal-appearing white matter changes in multiple sclerosis: a serial diffusion MRI study. *Brain* 123(Pt 8):1667–1676.
- Werring DJ, Clark CA, Barker GJ, Thompson AJ, Miller DH (1999): Diffusion tensor imaging of lesions and normal-appearing white matter in multiple sclerosis. *Neurology* 52:1626–1632.
- Werring DJ, Toosy AT, Clark CA, Parker GJ, Barker GJ, Miller DH, Thompson AJ (2000b): Diffusion tensor imaging can detect and quantify corticospinal tract degeneration after stroke. *J Neurol Neurosurg Psychiatry* 69:269–272.