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Short Communication

Functional connectivity alterations in a murine model of optic neuritis

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ABSTRACT

The basis for neuronal dysfunction following inflammatory demyelination of the central nervous system (CNS) remains poorly understood. We characterized the network response to white matter injury in the anterior visual pathway using an experimental model of optic neuritis (ON), as ON is often an early manifestation of immune-mediated CNS demyelination in multiple sclerosis (MS). Optical intrinsic signal imaging was performed before and after the induction of ON in mice to measure changes in cortical network functional connectivity. We observed a greater loss of connectivity between homotopic visual cortices in ON mice compared to controls. Further, decreases in homotopic visual cortex connectivity were associated with visual acuity loss in ON mice. These results demonstrate that network connectivity changes resulting from ON can be modeled in an experimental murine system. Future studies will identify the mechanisms that cause neuronal dysfunction due to white matter injury seen in MS.

1. Introduction

Murine models such as experimental autoimmune encephalomyelitis (EAE) have been instrumental in the identification of immune mechanisms involved in the hallmark myelin and nerve damage of multiple sclerosis (MS) (Wu et al., 2011). However, it remains unclear how inflammatory demyelination of the central nervous system (CNS) produces acute as well as lasting neurologic disability. Examining neuronal changes that occur with white matter injury in EAE provides tremendous potential for uncovering the mechanisms of disability seen in MS.

Changes in visual processing are often a heralding sign of MS. The visual system is therefore an excellent candidate for assessing progression and accrual of disability in MS. Similar to MS, mice with EAE often develop optic neuritis (ON) (Bettelli et al., 2003). In fact, ON can be an isolated feature in murine models of inflammatory demyelination, with spontaneous development occurring in over 40% of mice that express the transgenic T cell receptor (TCR) specific for myelin oligodendrocyte glycoprotein (MOG) (Bettelli et al., 2003).

In humans, functional MRI (fMRI) has been used to study the presence of functional connections across brain networks, rooted in low frequency (0.008–0.09 Hz) oscillations, known as functional connectivity (FC) (Raichle, 2011). The blood oxygen level dependent (BOLD) signal typically used in fMRI is based on oscillations in local concentrations of oxy- and deoxy-hemoglobin. These changes are a proxy for neural activity and have been used to identify network structure (Raichle, 2011).

Alternatively, optical neuroimaging methods can generate hemodynamic FC mapping in animal models that do not require high-field small-animal MRI scanners and are less costly (White et al., 2011). One such technique, optical intrinsic signal (OIS) imaging, relies on changes in reflectance of incident light at various wavelengths due to absorption by hemoglobin in a manner analogous to BOLD fMRI (White et al., 2011). Transcranial FC OIS (fcOIS) imaging has already been used to reveal resting state FC networks in a murine system (White et al., 2011). Mouse fcOIS has been shown to be a sensitive assay for several neurological diseases, including ischemic stroke and Alzheimer's disease (Bauer et al., 2014; Bergonzi et al., 2015).

We recently reported connectivity changes in patients with acute ON that included loss of homotopic connectivity between the left and right primary visual cortices and a loss of anti-correlation between V1 and extra-visual regions compared to healthy subjects (Wu et al., 2015). Notably, these changes were significantly correlated with visual outcome measures, suggesting that meaningful alterations of cortical network connectivity can be detected relatively early on in the disease course. However, identifying the underpinnings of these alterations in humans is difficult. Hence, interrogating well-controlled animal model

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systems of inflammatory CNS demyelination with fcOIS could aid in the elucidation of the mechanisms underlying changes in neurologic function during MS.

The goal of this study was to determine whether connectivity changes from ON could be modeled in a murine system using fcOIS. Using a mouse model of ON, we collected behavioral measures of visual acuity (VA) and optical imaging of spontaneous hemodynamic activity both before and after inducing ON. We observed a significant decline in VA following the induction of ON. Using fcOIS, we observed reductions in bilateral FC in the visual cortex in mice with ON. These results provide technical and mechanistic insight into changes of cortical function due to ON in an experimental animal model system. As such, these results could facilitate the testing of potential interventional strategies for inflammatory demyelinating diseases of the CNS.

2. Materials and methods

2.1. Mice

Male and female C57BL/6 (B6) and 2D2 mice (Bettelli et al., 2003) were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were housed in specific pathogen-free conditions. All animal experiments were performed in compliance with regulations specified by the Washington University in St. Louis Animal Studies Committee.

2.2. Optic neuritis induction and evaluation

To determine whether connectivity alterations are observed in murine ON, we examined 2D2 mice which are highly susceptible to ON (Bettelli et al., 2003). Because spontaneous demyelination in rodents is quite variable, the frequency of ON in 2D2 mice was enhanced by immunization of a cohort of 16 2D2 mice with a low dose of the 35–55 peptide fragment of MOG (MOG_{35-55}) in Complete Freunds Adjuvant (CFA) (Bettelli et al., 2003). Approximately four weeks later (range: 24–28 days), ON was assessed histologically and detected in nine 2D2 mice ("ON+"). Optic nerves from all mice were dissected and frozen in OCT (Tissue-Tek) before sectioning at 4 μm. Tissue was stained with Hematoxylin and Eosin and scored for inflammatory infiltrates according to an established protocol (Wu et al., 2011). Spatial visual acuity was performed using a virtual optomotor system (OptoMotry; CerebralMechanics) as described (Douglas et al., 2005). All mice were tested with both clockwise and counter-clockwise variable spatial frequency sine wave patterns. Visual thresholds for each animal were defined as the highest spatial frequency the animal could track as determined by the same observer masked to genotype. A cohort of seven 2D2 mice that did not develop ON served as controls. Additionally, because naive 2D2 mice are genetically identical to B6 mice except for the T cell receptor transgene directing T cell recognition of MOG, four B6 mice immunized with low-dose MOG_{35-55} that did not develop ON were added to the 2D2 cohort without ON, producing a total of 11 mice without ON ("ON−"), (Fig. 1A).

2.3. Connectivity imaging

FcOIS acquisition was carried out using a previously described imaging system and approach (White et al., 2011). Following intraperitoneal injection of 86.9 mg/kg of Ketamine and 13.4 mg/kg of Xylazine for anesthesia along with local Lidocaine injection and resection of the scalp, mice were placed on a heating pad maintained at 37 °C (mTCII, Cell Microcontrols) while their heads were secured in a stereotactic frame. Sequential illumination was provided by LEDs at four wavelengths (LEDs; 478 nm, 588 nm, 610 nm, and 625 nm; RLS-5B475-S, B5B-4343-TY, B5B435-30S, and OSCR5111A-WY, respectively, Roithner Lasertechnik) and a cooled, frame-transfer EMCCD camera (iXon 897, Andor Technologies) was used for image detection with a field of view of approximately 1 cm^2, The LEDs and camera were time synchronized and externally triggered using custom software (MATLAB, Mathworks). A frame-rate of 120 Hz was used to acquire images at 30 Hz with four temporally encoded wavelengths. To minimize specular reflection from the surface of the mouse skull, crossed linear polarizers were placed immediately in front of the LEDs and the camera lens.

2.4. Image processing and FC analysis

Image processing and analysis approaches have been previously described (White et al., 2011). In brief, images were spatially binned from full frame 512 × 512 pixels to 128 × 128 pixels. Changes in light reflectance were converted to differential changes in concentration of oxyhemoglobin and deoxyhemoglobin using the Modified Beer-Lambert Law. Images were then temporally filtered over the canonical FC frequency band (0.009 Hz–0.08 Hz), spatially smoothed with a 5 × 5 Gaussian kernel, and down-sampled from ~30 Hz to 1 Hz. Finally, the global signal (mean time series across the skull) was regressed from the time series at every pixel to remove global sources of shared variance. Using the Paxinos histological atlas as a reference, cortical fcOIS was measured based on 0.5 mm diameter circles. These seeds sampled the time series from the left and right visual, motor, retrosplenial, somatosensory, parietal, cingulate, and auditory networks (White et al., 2011). All time series within a seed were averaged and were correlated against the time series at every pixel over the brain to construct seed-based FC maps. FC maps from each imaging session were Fisher's z-transformed before being averaged within and across mice and subsequently back-transformed for plotting.

2.5. Statistical analysis

Once determination of ON was histologically determined at the conclusion of the experiment, OptoMotry scores and imaging data were segregated based on extent of optic nerve inflammation. A given mouse was classified with ON if at least one optic nerve had an inflammation score ≥2 (Wu et al., 2011). Post-hoc Welch's unequal variances *t*-test and paired Student's *t*-tests were used to examine longitudinal changes in OptoMotry performance and bilateral FC scores. All FC correlation values were first Fisher's z-transformed before all statistical analysis.

3. Results

3.1. ON induction in 2D2 mice

We examined 2D2 mice with high susceptibility of ON for FC alterations. In total, we immunized 16 2D2 mice (Fig. 1A), of which nine exhibited evidence of ON ("ON+") by histologic assessment four weeks following immunization as reflected by inflammatory infiltrates and perivascular cuffing (Fig. 1B). Seven 2D2 mice along with four B6 mice also immunized with low-dose MOG peptide that did not develop ON served as controls, producing a total of 11 mice without ON ("ON−"), (Fig. 1A).

3.2. Connectivity alterations in 2D2 mice with ON

Experimental mice were assessed by fcOIS imaging in longitudinal fashion: before and after immunization with MOG_{35-55} (Fig. 1A). Both the ON− and ON+ groups were imaged in parallel using fcOIS. Comparison of all seven seed-based networks between WT ($n = 4$) and 2D2 ($n = 7$) mice without ON at both the pre- and post-immunization time points are shown in Supplementary Table 1. Because no significant differences were observed between these groups for any network at either time point, we pooled WT and 2D2 mice without ON to serve as the control cohort. At baseline, FC maps (Fig. 1C) were similar in the visual network (Fig. 1D) and other networks for the control and ON+ mice (Table 1). Additionally, OptoMotry was performed to assess the clinical