Published in final edited form as:

Neuroradiology. 2017 December ; 59(12): 1241–1250. doi:10.1007/s00234-017-1904-1.

Disease Stage Dependent Relationship between Diffusion Tensor Imaging and Electrophysiology of the Visual System in a Murine Model of Multiple Sclerosis

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Abstract

Purpose: Diffusion Tensor Imaging (DTI) is commonly used to evaluate white matter integrity in Multiple Sclerosis (MS), but the relationship between DTI measures and functional changes during disease remain ambiguous. Using a mouse model of MS, We tested the hypothesis that DTI measures would correlate to the visual evoked potential (VEPs) dynamically at different disease stages.

Methods: In vivo DTI, Gadolinium-enhanced T1WI (Gd-T1WI) and VEPs were performed in 5 control and 25 mice after 2–12 weeks of Experimental Autoimmune Encephalomyelitis (EAE). DTI indices, including Fractional Anisotropy (FA), Axial and Radial diffusivities (AD and RD), and Gd-T1WI enhancement were measured in the optic nerve and tract (ON and OT), which were compared with measured VEPs.

Results: Gd-T1WI showed a 3–4 fold enhancement over controls beginning after 2 weeks of EAE. Across the timecourse, we found progressive reductions in FA and increases in RD paralleled by increases in VEP latency and reductions in amplitude. Significant correlations between DTI (FA and RD) and VEP evolved - in control / early asymptomatic EAE mice, both FA and RD were highly correlated with VEP latency (but not amplitude), while in late EAE, both DTI indices were highly correlated with VEP amplitude (but not latency).

Conclusions: DTI measures FA and RD are associated to VEP latency in early stages of EAE, but associated to VEP amplitude in later stages, suggesting that the patterns of DTI related to the functional decline may depend on the stage of disease progression.

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Compliance with ethical standards

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed consent: Statement of informed consent was not applicable since the manuscript does not contain any patient data.

Keywords

Diffusion Tensor Imaging (DTI); Visual Evoked Potential (VEP); Gd-enhanced T1WI (Gd-T1WI); experimental autoimmune encephalomyelitis (EAE); Multiple Sclerosis (MS)

Introduction

Multiple Sclerosis (MS) is inflammatory demyelinating neurodegenerative disease affecting primarily white matter of the central nervous system. Magnetic Resonance Imaging (MRI) provides critical information to assess MS [1]. Active lesions can be detected using Gd-enhanced T1-weighted imaging (Gd-T1WI), which allows visualization of blood-brain barrier (BBB) permeability [1]. Diffusion Tensor imaging (DTI) is sensitive to microstructure changes in white matter tracts. DTI indices including axial diffusivity (AD), radial diffusivity (RD), and Fractional Anisotropy (FA), derived from DTI can be used to reveal different characteristics of white matter damage including axonal damage and demyelination [2–5]. While these tools are useful for probing the structural characteristics and properties in white matter during MS, it is relatively unclear how they relate to functional outcomes across the spectrum of disease.

The visual system is an ideal model to simulate the structural and functional changes that occur in white matter during MS. The connectivity and electrophysiological properties of the visual system are well characterized [6]. Additionally, the system is frequently affected in MS, especially during bouts of optic neuritis [3–5,7]. Damage to the visual system can be detected noninvasively by MRI as well as functionally assessed using the visual evoked potential (VEP) [8–10]. However, findings from the relationship between MRI and VEPs have not been consistent. Naismith et al reported significant correlation between DTI metrics (FA, AD and RD) and both VEP amplitude and latency [4]. In contrast, two other studies from Trip et al and Kolbe et al independently showed significant correlations of DTI only with the VEP amplitude but not with the VEP latency [3,5]. This discrepancy may be explained by differences in patient populations between these studies; Naismith et al. included patients with optic neuritis incidents within 6 months, while studies by Kolbe et al and Trip et al. included populations with a mean time since optic neuritis of 4 and 3.1 years, respectively. This difference suggests that DTI measures in white matter may have varying sensitivity to functional nerve properties, depending upon time after initial injury or disease stage.

We hypothesized, based upon results from previous human data, that MRI data would have stronger sensitivity to functional nerve properties at early disease stages vs. late chronic stages. In order to fully appreciate the relationship between structural MRI data and functional changes measured through VEP, we evaluated each during different stages of disease in the commonly used experimental autoimmune encephalomyelitis (EAE) MS model. Using the animal model, the relationship between MRI and VEP was investigated at the acute and chronic stages of EAE. Using this model, we can eliminate much of the individual variability and focus on the manifestation of injury, which will allow a better understanding of how MRI changes correspond to functional decline.

Materials and Methods

Animal Preparation

This study was conducted in accordance with National Institutes of Health guidelines and Statement for the Use of Animals in Ophthalmic and Visual Research, and was approved by the Institutional Animal Care and Use Committee in Loma Linda University.

A total of 30 female C57Bl6/WldS mice were used. Five (20 weeks old) were used as controls and 25 (eight week old) were EAE induced. For EAE induction, animals were immunized with 60 µg MOG₃₅₋₅₅ emulsified (1:1) in incomplete Freund's adjuvant (IFA) [11–15]. Pertussis toxin (200 ng; PTX, List Laboratories, Campbell, CA) was injected intraperitoneally on the day of immunization and three days later. Five mice were used at each time-point for parallel DTI, Gd-T1WI and VEP at 2, 4, 8 and 12 weeks post EAE. Untreated control animals (N=5) were assessed using DTI, Gd-T1 and VEP at 20 weeks of age. An additional five were used to complete a longitudinal DTI study, with scans at baseline (immediately before immunization) and 4, 8 and 12 weeks post EAE induction. All animals were graded every two days for clinical neurological impairment on a scale of 0 – 5 [11].

MRI Acquisition

Mice were anesthetized by 1.5% isoflurane/oxygen using an isoflurane vaporizer (Vet Equip, Pleasanton, CA). Body temperature was maintained at 37°C using a warm water heating pad. Scans were collected using a Bruker 11.7T BioSpec small animal MRI instrument. The acquisition protocol used a slice thickness 0.5 mm, FOV of 1.5 cm x 1.5 cm and matrix 128 × 128 (zero filling to 256 × 256), TR 2.5 s, TE 29 ms, 20 ms, 8 3 ms, and 21-direction diffusion scheme with b-values of 0 and 0.85 ms/µm² [16]. Using FSL, raw DWIs were corrected for eddy current and motion distortions (<http://fsl.fmrib.ox.ac.uk/fsl/>). Corrected scans were then imported into 3D Slicer, where eigenvalues (λ_1 , λ_2 , and λ_3) derived from the diffusion tensor were used to calculate AD, RD, MD and FA, defined by the following equations:

$$AD = \lambda_1 \quad (1)$$

$$RD = (\lambda_2 + \lambda_3)/2 \quad (2)$$

$$MD = (\lambda_1 + \lambda_2 + \lambda_3)/3 \quad (3)$$

$$FA = \frac{\sqrt{3} \sqrt{(\lambda_1 - ADC)^2 + (\lambda_2 - ADC)^2 + (\lambda_3 - ADC)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \quad (4)$$

DTI measurements were made by manually selecting regions of interest (ROIs). The high anisotropy and low RD of the analyzed regions (optic nerve and tract) provided contrast against neighboring CSF and grey matter to accurately define the regions (Figure 1).

Blood Brain Barrier (BBB) permeability was assessed using gadolinium-enhanced T1-weighted imaging (TR = 0.5s and TE = 14.5ms). Mice were scanned before and 20 minutes after a 40 uL gadolinium contrast agent (Omniscan, Amersham Health) injection. Selection of the optic nerve and tract ROI was made using a T2-weighted Rapid Acquisition with Refocused Echoes (RARE) with TR 1s, echo train 4, and effective TE of 28 ms. Signal intensity in the optic nerve and tract were normalized using background signal from surrounding air and calculated as a percent increase over baseline scans.

VEP Recordings

VEP recordings were performed as previously described [10]. Briefly, animals were anesthetized with a mixture of oxygen and isoflurane (1.5%). Body temperature was maintained at 37°C throughout the experiment using a water-circulating heating pad. The mouse was mounted into a stereotaxic rig and a midline incision was made through the skin to expose the skull. Two small holes were drilled above the visual cortex (0.5mm rostral, 2mm lateral to lambda) and cerebellum (1mm caudal to lambda). Care was taken to make minimum disturbance to the underlying brain tissue. For VEP recordings, a small loop of silver wire was overlaid on the surface of the visual cortex. Before recording, mice were dark-adapted for 10 minutes. Light stimulation was applied to the eye contralateral to the recording site using an LED light source at frequency of 0.2 Hz with a duration of 5 ms. Field potentials were recorded using a CyberAmp380 amplifier (Molecular Devices, Sunnyvale, CA) and a Digidata1440A interface (Molecular Devices, Sunnyvale, CA), with a sampling rate of 20 KHz and a bandpass filter of 0.1–300 Hz, controlled by Clampex 10.2 (Molecular Devices, Sunnyvale, CA). Averaged data from 100 continuous traces was used to calculate VEP endpoints, including N1 and P1 latency and amplitude. Latency and amplitude of the principal components of the VEP signal (N1 and P1) were measured using Clampfit.

Statistics

Group data are shown as mean ± standard deviation. A one way ANOVA was used to compare group means, which was followed by a post-hoc Tukey test. Statistical comparisons were considered significant at $P < 0.05$. All correlations between DTI data, VEP measures and Gd-Enhancement were evaluated using a Pearson's correlation coefficient. Correlations between average EAE scores and DTI, VEP and Gd-enhancement measures were performed using Spearman's r . Data manipulation and statistical analysis were carried out on Prism Graphpad v6.0.

Results

Clinical Score

The clinical scores were summarized in Figure 2. After EAE induction, mice started to develop noticeable behavioral deficits after 2–3 weeks. The earliest symptom was a limp tail (score 1), which progressed to hindlimb weakness (score 2) and eventual hindlimb paralysis (unilateral-score 3, bilateral-score 4). Average EAE scores were calculated for each mouse in the two weeks preceding time of sacrifice.

DTI findings

After EAE induction, evidence of damage in the visual pathway was discernible on DTI maps (Figure 1). A summary of our DTI data in the ON and OT across the time course is shown in Figure 3. FA trended down while RD trended up. Beginning at 4 weeks, significant DTI changes were observed with a 6.5% reduction of FA in ON ($p = 0.042$) a 22.3% increase of RD in ON ($p = 0.021$), and 17.6% increase of RD in OT ($p = 0.027$). Changes in AD were mixed; only at 8 weeks a 10% reduction in ON was significant ($p = 0.005$).

Visual evoked potential (VEP) results

Averaged VEP waveforms from each cohort across the time course are shown in Figure 4. The sharp VEP waveform gradually became less defined over the span of EAE. Despite this feature, the first two major peaks, N1 and P1, were easily identified. The latencies and the amplitude of N1 and P1 were quantified, showing gradual increases in latency and gradual reductions in amplitude with longer durations of disease (Figure 4). Both measures showed significance relative to the controls beginning at 4 weeks with a 28.1% reduction in amplitude ($p = 0.003$) and a 14.0% increase in latency ($p = 0.045$), which reached a 49.6% reduction in amplitude ($p = 0.0004$) and a 28.2% increase in latency ($p = 0.01$) after 12 weeks.

Gadolinium-Enhancement

Blood-brain barrier integrity was assessed using Gd-T1WI. A significant 3-to-4-fold signal enhancement ($p = 0.017$ – 0.002) was found in the ON at all time-points beginning 2 weeks after EAE induction (Figure 5), while there was no significant changes in enhancement in the OT.

VEP and DTI Correlation Analysis

For comparisons between VEP and DTI data, DTI of each side of the visual pathway (averaged between ON and contralateral OT) was used to compare with acquired VEP signal. The comparisons between VEP and DTI data revealed several significant linear correlations (Table 1 and Figure 6). We found striking differences between control mice and mice with very early EAE (control and 2-week EAE mice) vs. mice at late stages of EAE (8 and 12 week EAE mice). Control and 2-week-EAE mice showed strong relationships between VEP latency and FA ($r = -0.79$, $p = 0.0001$) as well as RD ($r = 0.85$ and $p = 0.0003$) (Table 1 and Figure 6). Interestingly, these same mice showed no significant relationship between VEP amplitude and DTI measures. Conversely, mice at late stages of

EAE showed strong relationships between reduced VEP amplitude and decreased FA ($r = 0.74$ and $p = 0.0002$) and increased RD ($r = -0.73$ and $p = 0.0003$) (Table 1 and Figure 6). In these mice however, VEP latency did not correlate with DTI indices.

VEP and Gadolinium Enhancement Correlation

As shown in Table 1, across all mice we observed a significant negative relationship between VEP amplitude and Gd-enhancement ($r = -0.497$, $p = 0.0004$). This relationship was significant both during early (2wk) and later stages (8wk) of EAE (Figure 7B). We saw no significant relationship between enhancement and latency ($r = 0.207$, $p = 0.16$). We also did not observe any significant relationships between Gd-enhancement and any DTI indices.

Clinical Evaluations correlated to VEP, Gadolinium Enhancement, and DTI Measures

Average EAE scores in mice with appreciable disease activity (4, 8 and 12wk cohorts) were compared with all DTI, VEP and Gd data (Table 2). Significant correlations were found between average EAE score and RD ($r = 0.387$, $p = 0.029$) as well as FA at 4wks ($r = -0.714$, $p = 0.021$).

Discussion

In this study, we demonstrate parallel changes of DTI, Gd-T1WI, and VEPs to characterize disease progression through the visual pathway in mice affected by EAE. Our findings are largely in agreement with data from human MS studies [3–5]. In MS, Gd-T1WI is a powerful tool for assessing disease activity [17–19], and our study showed that significant changes in Gd-enhancement was the first indication of disease activity, preceding overt behavioral, functional or diffusional differences. After the onset of EAE, we found continuous reductions in FA and increases in RD within the visual pathway white matter which paralleled gradual decreases in VEP amplitude and increases in VEP latency.

A novel finding from our data is the differentiation of the DTI-VEP relationship in animals at early and late stage of disease. In both control and asymptomatic mice 2 weeks after EAE induction, a significant correlation exists between VEP latency (but not the amplitude) and DTI metrics FA and RD. This is in contrast to data at late disease stages, where significant correlations are between VEP amplitude (instead of the latency) and DTI metrics FA and RD. We interpret our finding to suggest that during basal conditions, FA and RD may be especially sensitive to microstructural differences in myelination, which could explain tight correlation to VEP latency. Our observations may be related to data obtained from human infants, in which VEP latency, instead of amplitude, shows significant correlation to DTI metrics [20]. VEP latency is highly affected by changes in myelination, both in development [21] and during demyelinating conditions [22]. Additionally, mouse models of demyelination have suggested that FA and RD are indeed very sensitive to small changes in myelination [23,24]. At the later stages of EAE, cumulative demyelination, inflammation, and axonal damage become prominent [25–27], which affect VEP amplitude and may sensitize DTI measures to differences in axon density. These findings help explain inconsistent literature concerning the relationship between DTI data and VEP signal latency

and amplitude [3–5,7]. Our experiment suggests that the ability of DTI to predict neurological functions may be disease-stage dependent in MS.

Several studies have been conducted using human tissue to examine the connection between DTI data and histology outcomes in MS brain and spinal cord samples [28–31]. These studies may shed light on the pathological factors that influence the DTI signal. Results from this work have demonstrated that DTI parameters, especially FA and RD are sensitive to demyelination [29–31]. Less clear is the relationship between axonal loss and DTI parameters, which has been associated with AD in MS mouse models [32,33], but not generally observed in human tissue [30]. FA, RD and MD have been shown to correlate with axonal counts in white matter [30,28,29], though work by Schmierer et al. suggests this correlation may be driven primarily by differences in myelin content [28,29]. Overall, these data show that DTI appears to be an accurate surrogate measure of demyelination and potentially axonal losses, though the results must be interpreted with several caveats. Importantly, these studies employed different imaging parameters, such as diffusion time and direction encoding schemes. For imaging of tissue specimens the fixation processes may also affect the DTI signal [34]. Additionally, these studies include extremely varied MS populations, making any distinctions between histological patterns, disease stage and DTI difficult. In light of our mouse data, it may be interesting to reexamine the connection between DTI metrics and histology in newer vs older lesions.

In addition to indicating damage in the visual pathways, VEPs may correlate broadly with changes in the brain. A study by Lobsien et. al. using Tract-Based Spatial Statistics (TBSS) examined white matter across the brain (not including the optic nerve) and compared with VEP signal [35]. They found significant correlation between white matter microstructure alterations and VEP latency in several regions, including regions with no direct role in the visual pathway such as the fornix, as well as structures with a well defined role for visual pathway, as in the optic radiation. Their work suggests that functional deficits in the visual system may signal more general white matter damage across the brain in MS.

To our knowledge, this study is among the first to define the DTI-VEP relationship using an animal model of MS. Although there is a lack of journal articles, studies with preliminary results have been presented in conference settings [36,37]. However, none of these presentations have demonstrated the importance of disease stage in determining the relationship between functional and microstructure data.

In conclusion, we compared measurements from DTI, Gd-T1WI, and VEPs to quantify structural and functional deficits in mice affected by EAE. Gd-T1WI detects early alterations in BBB integrity, while DTI changes (especially RD and FA) correlate with increasing VEP latency and reduced amplitude. The ability of DTI to predict VEP latency and amplitude depends upon the stage of disease. The data suggests that information about disease stage may advance the use of DTI data to predict functional changes in the nervous system during MS.

Acknowledgment

We thank the insightful discussion with Dr. Wei-Xing Shi, Pharmaceutical Science, Loma Linda University.

Funding: This work was funded in part by NIH R01 NS062830 and the National Space Biomedical Research Institute (RE03701) through the National Aeronautics and Space Administration NCC 9-58.

Abbreviations

DTI	Diffusion Tensor Imaging
VEP	Visual Evoked Potential
Gd-T1WI	Gd-enhanced T1-weighted imaging
EAE	experimental autoimmune encephalomyelitis
MS	Multiple Sclerosis
FA	Fractional anisotropy
MD	Mean diffusivity
AD	Axial diffusivity
RD	Radial diffusivity

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Figure 1.

DTI maps from the Optic Nerve and Optic Tract. FA maps show the location of the ON and OT (left), with individual diffusion index maps show on the right. Diffusion maps are pseudocolored to show changes in each metric, with ON and OT regions of interests outlined in white. After 12 weeks of EAE, the ON has reductions in FA and increases in RD vs. controls. Alterations in the OT are less obvious, with only small reductions in anisotropy and increases in RD.

Figure 2.
Clinical scores of mice affected by EAE. Mice began to show symptoms 2–3 weeks after EAE induction.

Figure 3.
DTI measurements from the Optic Nerve and Tract. With increasing durations of EAE, the ON and OT have reductions in FA, increases in RD, and inconsistent changes in AD. “*” indicates a p<0.05 compared to controls.

Figure 4. Averaged VEP waveforms from each experimental cohort. VEPs are increased in latency and reduced in amplitude with longer durations of EAE. “*” indicates a $p < 0.05$ compared to controls.

Figure 5. Gadolinium Enhancement reveals potential BBB leakage in the ON.

Top left, RARE image shows coronal slice with optic nerves outlined in white (right).

Bottom left, T1 images show signal enhancement after gadolinium. Quantification (right) of enhancement in the ON and OT after Gd-injection shows selective increases above control levels in the ON but not OT. “*” indicates a $p < 0.05$ compared to controls.

Figure 6.

Significant relationships between VEP and DTI data. VEP latency and amplitude are correlated with DTI data at different stages of disease severity. **A.** VEP latency is closely correlated to FA and RD measures in control and 2wk EAE mice, without any significant linear relationship at later stages of EAE (8 and 12 weeks). **B.** In contrast, VEP amplitude shows little relation to DTI measures during early stages of disease, but correlates well after 8 and 12 weeks of EAE. Each mouse includes two independent datapoints (ex. N=5 mice, 10 optic nerves/tracts). The 2wk time-point includes N=4 mice due to early death of one mouse.

Figure 7.

Relationships between VEP measurements and ON-Gd enhancement. **A.** Blood-brain barrier permeability within the ON did not correlate with VEP latency in Control / 2wk EAE groups or 8 / 12wk EAE groups. **B.** Gd-enhancement did show significant relation to VEP amplitude at several stages of EAE, in 2 wk and 8 wk EAE groups.

Table 1.
DTI, VEP & Gd Data Correlation

Summary of DTI, VEP and Gd data correlations. Total numbers of mice from each condition are listed. Each mouse includes two independent datapoints (ex. N=5 mice, 10 optic nerves/tracts).

Cohort		N1 Latency		N1-P1 Amplitude		Gd Enhancement	
		Pearson r	<i>p value</i>	Pearson r	<i>p value</i>	Pearson r	<i>p value</i>
All Mice N=24	FA	-0.405	0.0039	0.555	<0.0001	-0.241	0.0996
	RD	0.488	0.0004	-0.518	0.0002	0.183	0.213
	AD	0.017	0.9088	0.237	0.1054	-0.183	0.212
	Gd	0.207	0.1627	-0.497	0.0004	x	x
Control N=5	FA	-0.843	0.0022	-0.458	0.1833	0.259	0.4702
	RD	0.916	0.0002	0.502	0.1392	-0.469	0.1721
	AD	-0.115	0.7518	-0.115	0.7518	-0.287	0.422
	Gd	-0.355	0.3143	-0.591	0.0722	x	x
2Wk N=4 *	FA	-0.719	0.0443	0.206	0.6251	0.167	0.6935
	RD	0.694	0.0560	-0.207	0.6231	-0.043	0.919
	AD	-0.029	0.9455	-0.029	0.9455	0.489	0.218
	Gd	0.236	0.574	-0.809	0.015	x	x
4Wk N=5	FA	0.180	0.6189	0.030	0.9341	-0.701	0.0525
	RD	-0.044	0.9036	0.175	0.6281	0.581	0.1311
	AD	0.402	0.2499	0.401	0.2499	-0.171	0.685
	Gd	-0.389	0.3396	0.0583	0.8909	x	x
8Wk N=5	FA	0.323	0.3629	0.599	0.0673	-0.0906	0.8035
	RD	-0.370	0.2921	-0.557	0.0945	-0.0294	0.9358
	AD	-0.046	0.8997	-0.046	0.8997	-0.363	0.302
	Gd	0.588	0.0741	-0.67	0.0339	x	x
12Wk N=5	FA	-0.320	0.3381	0.782	0.0075	-0.35	0.2648
	RD	0.441	0.1739	-0.778	0.0081	0.372	0.2333
	AD	-0.130	0.7031	-0.130	0.7031	-0.218	0.497
	Gd	0.332	0.3193	-0.155	0.669	x	x

* The 2wk time-point includes N=4 mice due to early death of one mouse. Significant correlations at $p < 0.05$ shown in bold.

Table 2.
EAE Score correlation to DTI, Gd and VEP

Correlations between average EAE scores and DTI, VEP and Gd data. Average EAE scores were calculated as an average of the previous two weeks' score. The 2wk EAE group was omitted from analysis due to the lack of an appreciable score.

Cohort		Spearman r	p value	Cohort		Spearman r	p value
4,8,12 week N=15	FA	-0.315	0.078	8wk N=5	FA	-0.055	0.608
	RD	0.387	0.029		RD	-0.028	0.667
	AD	0.113	0.54		AD	-0.413	0.114
	Gd	0.293	0.116		Gd	0.29	0.416
	N1 Latency	-0.12	0.949		N1 Latency	-0.028	0.667
	N1-P1 Amp	0.017	0.928		N1-P1 Amp	-0.275	0.246
4wk N=5	FA	-0.714	0.0211	12wk N=5	FA	0.086	0.795
	RD	0.591	0.0831		RD	-0.279	0.329
	AD	0.098	0.81		AD	-0.093	0.709
	Gd	0.683	0.083		Gd	0.394	0.206
	N1 Latency	-0.443	0.183		N1 Latency	-0.328	0.275
	N1-P1 Amp	0.197	0.602		N1-P1 Amp	0.44	0.204