Longitudinal Anterior Visual Pathway Evaluation of Patients with Multiple Sclerosis Using Multifocal Visual Evoked Potential and Optical Coherence Tomography

By

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DISSERTATION

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General Abstract

Purpose: Multiple sclerosis (MS) is a chronic disease of the central nervous system involving inflammation, demyelination and neurodegeneration. Optic neuritis (ON) is acute inflammatory, demyelination of the optic nerve. The anterior visual pathway serves as a good model to track MS related disease changes. Multifocal visual evoked potential (mfVEP) is an objective, non-invasive technique that provides spatially localized, topographic information on visual function. MfVEP response amplitude and latency reflects integrity of axons and myelin, respectively. Optical coherence tomography (OCT), provides high-resolution, cross-sectional images of the retina and is a sensitive technique for assessing neurodegeneration. The purpose of this dissertation was to assess functional and structural changes over time in the visual system of relapsing-remitting MS (RRMS) patients.

Methods: 1) In the first experiment, to assess reproducibility, mfVEP was recorded twice within a month in 40 normals and 40 RRMS patients (25 eyes with last ON ≥ 6 months (mo), 34 non-ON). Global and 9 regional mfVEP amplitudes (logSNR) and latency (ms) were calculated. Traditional pattern VEP (TVEP) was recorded (15', 60', 120' checks) in subsets of 34 normals and 30 RRMS patients. Reproducibility was evaluated using intraclass correlation coefficient (ICC) and test-retest variability (TRV) to establish 95% tolerance limits. 2) In the second experiment to evaluate longitudinal changes in visual

function, mfVEP, contrast sensitivity (CS) and Humphrey visual fields (HVF) were obtained at two different visits (mean follow-up: 1.5 ± 0.9 years) in 57 RRMS patients (53 eyes with optic neuritis (ON): 14 with ON within 6 months (mo) of first visit (ON < 6mo), 39 with ON ≥ 6mo, 57 non-ON). Longitudinal changes were assessed using mfVEP amplitude, latency, log CS and HVF mean deviation (MD) based on 95% tolerance limits of TRV established in experiment 1. 3) In the third experiment, to assess neurodegenerative changes over time in MS eyes, retinal nerve fiver layer thickness (RNFLT) and ganglion cell-inner plexiform layer thickness (GCIPT) were measured using Cirrus OCT in 133 RRMS patients (149 non-ON, 97 ON ≥ 6 mo eyes). 93 patients were scanned at two visits. Percents of abnormal GCIPT vs RNFLT (< 5% of machine norms) in cross-sectional data were compared. Relations between RNFLT/GCIPT and MS duration (cross-sectional) and follow-up time (longitudinal) were assessed.

Results: 1) ICCs for global and regional mfVEP amplitude and latency were all > 0.80 indicating good intervisit agreement. ICCs for tVEP ranged from 0.52 to 0.86, being lowest for ON latency (0.52 to 0.68). TRV for amplitude (mfVEP and tVEP) was similar across groups. TRV for latency, greater for tVEP than mfVEP in all groups, was larger in ON (5.3/9.2 ms for mfVEP global/60’ tVEP) compared to non-ON (3.1/5.6, $p = 0.003$/$p = 0.02$) and normals (2.3/4.1, $p = 0.0001$/$p < 0.01$). 2) A significant percentage of ON < 6mo eyes exceeded 95% tolerance limits for mfVEP amplitude (21%, $p < 0.05$), latency (35%, $p < 0.01$) and for CS (31% $p < 0.001$); more improved than worsened. MfVEP latency shortened in

11% non-ON, 10% ON ≥ 6mo, lengthened in 21%, and 10%, respectively ($p < 0.01$ for all). Latency changes correlated negatively with baseline latency ($r = -0.43, -0.45$ for non-ON, ON ≥ 6mo; $p = 0.0008$). Although an insignificant number of non-ON and ON ≥ 6mo eyes exceeded tolerance limits for amplitude, CS or HVF; amplitude and latency changes were correlated, and both measures correlated with changes in CS ($r = 0.47$ to 0.79, $p < 0.01$). 3) GCIPT was abnormal in more eyes than RNFLT (27% vs 16% $p = 0.004$ in non-ON, 82% vs 72% $p = 0.007$ in ON ≥ 6 mo). RNFLT and GCIPT decreased with MS duration by - 0.49 µm/yr ($p = 0.0001$) and - 0.36 ($p = 0.005$) for non-ON; - 0.52 ($p = 0.003$) and - 0.41 ($p = 0.007$) for ON ≥ 6 mo. RNFLT and GCIPT decreased with follow-up time by - 1.49 µm/yr ($p < 0.0001$) and - 0.53 ($p = 0.004$) for non-ON, - 1.27 ($p = 0.002$) and - 0.49 ($p = 0.04$) for ON ≥ 6 mo.

Conclusions: MfVEP showed better reproducibility than tVEP in normals and RRMS patients. MfVEP, and particularly latency, can be used to track visual functional changes in individual RRMS eyes. Progressive loss of RNFLT and GCIPT occurs even in the absence of clinically-evident inflammation in RRMS. MfVEP and OCT are potentially useful for assessing therapeutic effects of novel remyelinating and neuroprotective strategies in RRMS.

Chapter 1 : General Introduction

Multiple Sclerosis

Multiple sclerosis (MS), a chronic disease of the central nervous system (CNS), is the most common cause of permanent neurological disability in young adults. (Tremlett et al. 2010; Koch-Henriksen and Sorensen 2010) MS affects approximately 400,000 people in the United States and about 2.5 million worldwide. (Tullman 2013; Scalfari et al. 2010) Diagnosis is typically made between 20 to 40 years of age with three times higher incidence in females than in males. (Compston and Coles 2008; Kremenchutzky et al. 2006; Weinshenker et al. 1991) The average life expectancy in MS patients is about 5 to 10 years lower than unaffected population. (Compston and Coles 2008) Risk factors for MS include environmental triggers and genetic susceptibility. Incidence and prevalence of MS varies geographically and is high in regions that are far from the equator. Sunlight exposure and vitamin D status is believed to be important environmental factors, with risk of MS being high in people with vitamin D deficiency. (Ramagopalan et al. 2010) The recurrence risk for multiple sclerosis in families increases from less than 5% in siblings to 30 to 35% in monozygotic twins. (Compston and Coles 2008) Genetical changes in the HLA (Human Leukocyte Antigen) region on chromosome 6, that encodes for major histocompatability complex (MHC), has been linked with increased risk of MS. (Compston and Coles 2008)

Traditionally, it is believed that MS is auto-immune in origin, in which inflammatory T-cells, primarily Th1 and Th17, are abnormally activated in the periphery and migrate across the blood brain barrier. Here, a cascade of immunopathogenic events is initiated targeting the CNS nerve fibers, thereby causing demyelination and axonal degeneration. (Compston and Coles 2008; Lopez-Diego and Weiner 2008) Recent studies have raised inconsistencies in this primary auto-immune theory and have alternatively proposed cytodegeneration of CNS nerve fibers as the primary event, eliciting secondary immune response. (Stys et al. 2012; Stys 2013) Thus, etiology of MS is currently debatable (Trapp and Nave 2008) (refer to chapter 5 for a discussion on the two theories of MS). Regardless, there is a general consensus that pathological outcome of the disease process is formation of multiple sclerotic plaques as a result of inflammation, demyelination and axonal degeneration. (Henderson et al. 2009; Lassmann 2010; Trapp et al. 1998; Bjartmar and Trapp 2003)

About 80 to 90 % of MS patients initially begin with a relapsing-remitting course (RRMS), characterized by alternating episodes of neurological deficits (relapses) and asymptomatic periods of remission. (Ebers et al. 2000; Confavreux et al. 2003) The natural history of MS suggests that 15 to 25 years after the disease onset, 50% to 90% of RRMS patients convert to a secondary-progressive phase (SPMS), with the percentage being higher in studies with longer follow-up time. (Amato and Ponziani 2000; Tremlett et al. 2008; Kremenchutzky et al. 1999; Kurtzke et al. 1977) The SPMS phase is characterized by infrequent relapses, steady decline in neurological function and

accumulation of disability. (Tremlett et al. 2010; Rovaris et al. 2006) About 10% of MS patients are of the primary progressive form (PPMS), in whom the disease progresses from the onset with no distinct relapses. (Vukusic and Confavreux 2003) The Expanded Disability Status Scale (EDSS) is commonly used to assess the extent of disability and disease progression in MS patients. (Kurtzke 1983) EDSS is graded by assessing neurological impairment in eight functional systems (FS) including pyramidal, cerebellar, brain stem, sensory, bowel and bladder, visual, cerebral (mental) functions, and other MS-related neurological findings; and assigning a score from 0 to 10 in 0.5 increments. A score of 0 indicates normal neurological status and 10 indicates death due to MS (Meyer-Moock et al. 2014). Disability is defined as irreversible when a given EDSS score has persisted for at least six months. (Confavreux and Vukusic 2006) The majority of RRMS patients have an EDSS score of less than 3. EDSS of 3 indicates the patient is fully ambulatory with moderate disability in one FS (severe nystagmus is an example of moderate disability of brain stem function) or mild disability in 3 or 4 FS (mild urinary urgency is an example of mild disability of bowel and bladder function). (Kurtzke 1983) It is estimated that patients with an initial RRMS course will reach an EDSS score of 6 (i.e. require intermittent or constant assistance of a cane or crutch to walk 100 meters) after 23 years and a score of 7 (essentially restricted to a wheelchair) after 33 years of onset.

Currently there are ten FDA approved drugs for MS. Nine of them, including four interferon beta preparations (Avonex, Betaseron, Extavia, Rebif), glatiramer acetate (Copaxone), natalizumab (Tysabri), fingolimod (Gilenya),

teriflunomide (Aubagio), dimethyl fumarate (Tecfidera) are indicated for RRMS. Mitoxantrone (Novantrone) is indicated for progressive MS. (K Costello 2013) Although the exact mode of actions for individual drugs is not clear, most of them work by immuno-modulation such as exerting anti-proliferative activity on lymphocytes. For example, it is believed that drug natalizumab exerts immune-modulatory effects by impairing the VLA4 adhesion complex, an integrin critical for T cells to cross the blood brain barrier. (Lopez-Diego and Weiner 2008) Mitoxantrone is an immune suppressor that exerts a broad range of actions by suppressing proliferation of T cells, B cells and macrophages and can also induce apoptosis in lymphocytes. (K Costello 2013; Fox 2004; Neuhaus et al. 2004; Wingerchuk and Carter 2014). The PRISMS (Prevention of Relapses and disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) study was a two year, double-blinded placebo-controlled clinical trial to assess therapeutic effects of interferon β-1a in RRMS patients. (PRISMS study group 1998) The results of this trial showed that the interferon group (44 µg dose) showed a time delay of 5 months to first relapse and had 33% fewer relapses when compared to the placebo group. Similarly, the time to sustained disease progression, which was defined as increase in EDSS of at least one point that sustained for at least 3 months, was also significantly longer in the interferon group (21.3 months) when compared to the placebo group (11.9 months). Despite showing robust treatment effects in RRMS, the efficacy of current DMTs in SPMS patients has been less promising. A three year clinical trial that assessed effects of interferon β-1a in SPMS patients showed that although the patients in the interferon group

had significantly fewer relapses than the placebo group, the time to sustained disability remained the same in both the groups. (SPECTRIMS study group 2001) A recent study to assess long term effects of DMT showed that despite being continuously treated with glatiramer acetate for 15 years, 35% of RRMS patients still transitioned into SPMS. (Ford et al. 2010) These results highlight that although current DMTs are effective in reducing relapses, inflammation, formation of new lesions on magnetic resonance imaging, (Castro-Borrero et al. 2012; Cree 2014; Luessi et al. 2012) they are still unable to completely halt disease progression. (Ford et al. 2010). Disease progression and permanent disability in MS is believed to result from irreversible neurodegeneration. (Trapp et al. 1999; Trapp and Stys 2009) Thus, in addition to current immuno-modulatory DMTs, there is a need for novel neuroprotective therapies in MS. (Stys et al. 2012; Maghzi et al. 2013)

Visual system in multiple sclerosis

The visual system can serve as a good model to study MS. Visual deficits are typically symptomatic, promoting patients to seek immediate medical attention. Further, there are several clinically available functional and structural tests, such as visual evoked potential (VEP), contrast sensitivity (CS), Humphrey visual fields (HVF) and optical coherence tomography (OCT), that can be used to evaluate visual defects. (Frohman et al. 2008a; F Costello 2013)

The optic nerve is a small compartment within the CNS that is a frequent site of damage in MS. (Frohman et al. 2008a; F Costello 2013). Optic neuritis

(ON), an acute inflammatory demyelination of the optic nerve, is the initial manifestation of MS in 38% of patients and affects more than 50% of MS patients at some point during the disease course. (Optic Neuritis Study Group 2008) Typically, ON is characterized by acute loss of vision in the affected eye with spontaneous recovery, hastened by the use of steroids, within several weeks to months. (Optic Neuritis Study Group 1991) This initial recovery of visual function is due to subsiding of inflammation, re-organization of ion-channels along the demyelinated regions, and the reparative process of remyelination as well. Within the first 3 to 6 months following acute ON, significant optic nerve atrophy (Hickman et al. 2002a) and reduction in retinal nerve fiber thickness (RNFLT) and ganglion cell-inner plexiform layer thickness (GCIPT) occurs, (Costello et al. 2008; Syc et al. 2012; Henderson et al. 2010a) resulting in permanent visual deficits.

MS eyes without a history of optic neuritis (non-ON eyes) are not normal either. Post-mortem analysis revealed optic nerve lesions in more than 90% of MS patients, irrespective of ON history. (Ikuta and Zimmerman 1976; Toussaint et al. 1983) Further, non-ON eyes have significant abnormalities in visual function (Brusa et al. 1999; Laron et al. 2009; Wang et al. 2012) and also exhibit significant axonal loss. (Petzold et al. 2010; Laron et al. 2010; Quelly et al. 2010) It is not clear if the pathological changes observed in non-ON eyes are due to subclinical inflammatory episodes, primary degenerative process or transynaptic degeneration from post-geniculate lesions.

Functional and structural tests to evaluate visual system in multiple sclerosis

This section provides a brief description of functional and structural tests used in this dissertation.

Functional tests

Functional tests can be broadly classified as subjective or objective. Subjective tests require an active response from the patient to perform the test reliably. Objective tests can be performed with minimal response from the patient.

Subjective functional tests

Pelli-Robson Contrast sensitivity

The Pelli-Robson contrast sensitivity (CS) is a letter chart in which letters decrease in contrast but not in size. The letters are arranged in groups of three, which decrease in contrast with successive groups. A subject's CS is measured as the lowest contrast for which at least two letters in a group are read correctly (see methods in chapter 2 for details). Results from optic neuritis treatment trial (ONTT) showed that among eyes with 20/20 or better visual acuity, 87% had abnormal CS. (Optic Neuritis Study group 1991) Evidence from several studies

suggests that CS is a sensitive measure of visual dysfunction in MS. (Balcer et al. 2000)

Standard automated perimetry

Standard automated perimetry performed using Humphrey visual fields (HVF) provide topographic information on visual sensitivity. In ONTT, at baseline, 100% affected eyes and 75% fellow eyes showed abnormality in HVF 30-2 test. (Keltner et al. 2010) The frequency of abnormal visual field was around 50% in affected eyes, and 40 to 36% in fellow eyes for years 1 through 15 after ON. Diffuse and central loss was predominant (66%) in affected eyes at baseline, which decreased to about 8 to 10% for years 1 through 15. In fellow eyes, field defects were typically localized. (Keltner et al. 2010)

Objective functional tests

Visual evoked potential

Pattern-reversal visual evoked potential (VEP) is a non-invasive technique to record electrical responses generated by the visual cortex in response to visual stimulation. (Sokol 1976) The VEP reflects functional integrity of the entire visual pathway. VEP amplitudes reflect the number of functional fibers and the

degree of synaptic activity at the visual cortex. Conduction block due to inflammation during acute ON episodes and/or loss of optic nerve fibers causes reduction in VEP amplitudes. (Klistorner et al. 2010a) The VEP time response, referred to as latency, reflects the integrity of myelin and is delayed when travelling through demyelinated fibers. (Halliday et al. 1972). VEP amplitude and latency provide objective measures of axonal and myelin integrity of the visual pathway.

Traditional pattern-reversal visual evoked potential

The traditional pattern-reversal VEP (tVEP) is recorded using a black and white checkerboard pattern that reverses in contrast, and is recorded for different check sizes. Typically, a single channel recording is performed with placement of a midline occipital active electrode, a reference electrode, and a ground electrode. For a given check size, the response is a single waveform averaged across multiple sweeps (at least 64 recommended (Odom et al. 2009)). A tVEP waveform (Figure 1-1) comprises three prominent landmarks, N75, P100 and N135; designated as negative ‘N’ or positive ‘P’ followed by the typical mean latency in normal subjects. The P100 amplitude (AMP) is measured from the N75 trough to P100 peak; the P100 latency (LAT) from stimulus onset to P100 peak. (Odom et al. 2009)

Figure 1-1 near here

Demyelination causes delays in VEP signals. (Halliday et al. 1972) TVEP P100 latency is widely used in the clinic to confirm previous history of demyelinating ON (Hammond and Yiannikas 1986) or to demonstrate subclinical demyelination in the visual system of MS patients or MS suspects. (Matthews et al. 1977). A number of studies on MS patients have used tVEP latency as a surrogate measure to estimate the extent of demyelination and to provide evidence for remyelination. (Brusa et al. 1999; Brusa et al. 2001) Although the P100 amplitude provides information on axonal integrity, its clinical usage has been limited due to the high inter-subject variability of monocular amplitudes. (Shahrokhi et al. 1978) Interocular comparisons are sensitive in detecting unilateral loss of function, but can be reliably used only when the contralateral eye remains unaffected. A limitation of tVEP is that it sums responses from a number of normal and abnormal neurons over a large field and is heavily dominated by response from macular region, hence can miss localized defects. (Fortune and Hood 2003; Klistorner et al. 2008)

Multifocal visual evoked potential

The multifocal VEP (mfVEP) stimulus compromises a 60-sector dartboard pattern (Figure 1-2 a). (Hood and Greenstein 2003) Each sector consists of 16 checks, 8 black and 8 white. The sectors and the checks are scaled to be of equal effectiveness, based on cortical magnification factors. (Baseler et al. 1994). Checks within each sector reverse in contrast based on a pseudo random m-

sequence. For multi-channel recordings, three active electrodes, a reference electrode and a ground electrode are used. Recordings are obtained from three channels and three more are derived by software analysis post recording. Each recording elicits 60 local VEPs simultaneously from a field of 22.2° radius (Figure 1-2 b). Using customized Matlab software, (Hood and Greenstein 2003; Hood et al. 2004; Fortune et al. 2004) mfVEP amplitude and latency measures are calculated from each location. The monocular amplitude measured as the signal to noise ratio (SNR), is calculated as the root mean square (RMS) of the sector's waveform in the signal window (45-150 ms) divided by the mean RMS from the noise windows (325-430 ms) of all 60 sectors (Hood and Greenstein 2003). The monocular relative latency is calculated using cross correlation between the waveform and a template derived from the Portland 100 normal controls (Devers Eye Institute, Portland, OR) (Hood et al. 2004), and is the shift in milliseconds (ms) needed to achieve the best cross correlation determined by the 'xcorr' function in MATLAB (The MathWorks Inc. Natick, MA). Thus, the monocular latency that is derived based on this template method is a 'relative latency' measure. MfVEP measures showed high sensitivity (> 90%) in detecting visual function abnormalities in MS (Laron et al. 2009) and glaucoma patients (Balachandran et al. 2006) MfVEP detected 10 to 15% more abnormalities when compared to tVEP in ON/MS. (Klistorner et al. 2008; Grover et al. 2008) In addition, mfVEP amplitude (Klistorner et al. 2010a; Hood et al. 2000) and latency (Yang et al. 2007; Klistorner et al. 2010b) measures showed promise in assessing visual function in longitudinal studies.

Figure 1-2 near here

Structural test

Optical coherence tomography

Optical coherence tomography *(*OCT) is a non-invasive technique that can be used to obtain high resolution, cross-sectional images of the retina. (Huang et al. 1991). The retina is a unique structure of the CNS, in which measurements of axonal and neuronal integrity could be made, in the absence of myelin. Following an ON episode, retrograde degeneration leads to loss of retinal ganglion cell axons and their cell bodies, which can be detected as thinning of the retinal nerve fiber layer (RNFL) and ganglion cell layer-inner plexiform layer (GCIP) in OCT.

In a meta-analysis of 27 MS studies that used time domain Stratus OCT, (Petzold et al. 2010) it was observed that RNFL thickness (RNFLT) in ON eyes was about 20 µm thinner than that in normals. Significant loss of RNFLT was also observed in non-ON MS eyes (about 7 µm thinner than normals). It is also widely accepted that a reduction in RNFLT due to axonal loss is apparent within 3 to 6 months following an acute ON attack. (Costello et al. 2008; Henderson et al. 2010a; Klistorner et al. 2010a; Hickman et al. 2004) The literature on axonal loss beyond the post-inflammatory period (6 months after onset of ON) is more limited and less consistent. Some studies have shown stabilization of RNFLT by

6 months after ON (Costello et al. 2008; Henderson et al. 2010a) while others reported progressive RNFL thinning during the 12 months observed (Klistorner et al. 2010a) and continuous optic nerve atrophy, detected by MRI, 2.5 years after ON. (Hickman et al. 2002a) Even among recent OCT studies using spectral-domain (SD) OCT), there are conflicting reports ranging from no changes in RNFLT (Henderson et al. 2010b; Serbecic et al. 2011; Ratchford et al. 2013) to progressive thinning of RNFL within two to three years of follow-up in MS eyes. (Talman et al. 2010; Garcia-Martin et al. 2011; Herrero et al. 2012) It is also not clear if continuous loss of RNFL occurs in non-ON eyes, as observed by some (Talman et al. 2010; Garcia-Martin et al. 2011) but not by others. (Costello et al. 2008; Klistorner et al. 2010a). A recent autopsy study on MS lesions suggested that compared to RNFL, the ganglion cell layer might be less confounded by perivascular inflammation thus more suitable for assessing neurodegeneration. (Green et al. 2010)

A brief over view of experiments described in chapters 2 to 5

The objective of this dissertation was to establish methodologies that could be used to longitudinally track functional and structural changes in the visual system of MS patients. MS eyes were segregated into groups based on previous history of ON (Hickman et al. 2002b): ON eyes for those with and non-ON eyes for those without a history. ON eyes were further divided based on the time since last ON episode: within 6 months of test date (ON < 6 months (mo))

and ON ≥ 6 mo eyes. The 6 months waiting time is to ensure resolution of edema from acute ON and complete retrograde axonal degeneration in RNFL. (Costello et al. 2008)

Chapter 2 describes experiment 1. The purpose of experiment 1 was to establish reproducibility of functional tests such as mfVEP, tVEP and Pelli-Robson CS using intra class correlation coefficients (ICC) and test retest variability (TRV) in normal and non-ON and ON ≥ 6 mo eyes. Reproducibility of a technique needs to be known prior to its application in longitudinal analysis. The tVEP is quick to perform, easy to interpret, but only offers summed information. The mfVEP provides localized information but takes twice as much time to record and requires sophisticated waveform analysis. Hence reproducibility of tVEP and mfVEP was compared in this experiment. Based on the TRV values calculated, 95% tolerance limits was estimated for each measure and used in experiment 2 for longitudinal analysis.

Chapter 3 describes experiment 2. The purpose of experiment 2 was to assess longitudinal changes in visual function for mfVEP amplitude, latency, Pelli-Robson CS and HVF based on established 95% tolerance limits of TRV and correlation among changes in various measures, in three groups of MS eyes (ON < 6 mo, ON ≥ 6 mo and non-ON). The majority of previous studies have used sample averages at different times to report longitudinal changes over time. Our preliminary data indicated that heterogeneous variation in functional measures

could be expected in MS patients. Since the disease process in MS does not follow a homogenous pattern in all patients, we hypothesized that tracking individual patients might reveal changes that could be masked by group averaging.

Chapter 4 describes experiment 3. The purpose of experiment 3 was to determine whether progressive neuronal degeneration occurred over time in RRMS eyes in the absence of clinically evident ON attacks. Results from current literature are ambiguous if axonal/neuronal loss stabilizes or continuously progresses after resolution of acute inflammation (i.e. 6 months after an ON episode). Similarly, it is unclear if progressive neurodegeneration occurs over time in non-ON eyes. For this experiment, SD-OCT measures of RNFL and GCIP thicknesses were analyzed over time using both cross-sectional and longitudinal analysis in non-ON and ON ≥ 6 mo eyes.

Chapter 5 reported additional analysis not reported in previous chapters including percentage of abnormalities detected by various structural and functional tests, and correlation between functional and structural measures. The implication of results from chapters 2 to 4, two most popular theories of primary event in MS, and novel therapeutic strategies in MS are also discussed.

Figures

Figure 1-1: TVEP waveform (normal)

The tVEP waveform from a normal subject with N75, P100 and N135 marked. AMP is measured from the trough of N75 to the peak of P100. LAT is measured from stimulus onset to P100 peak.

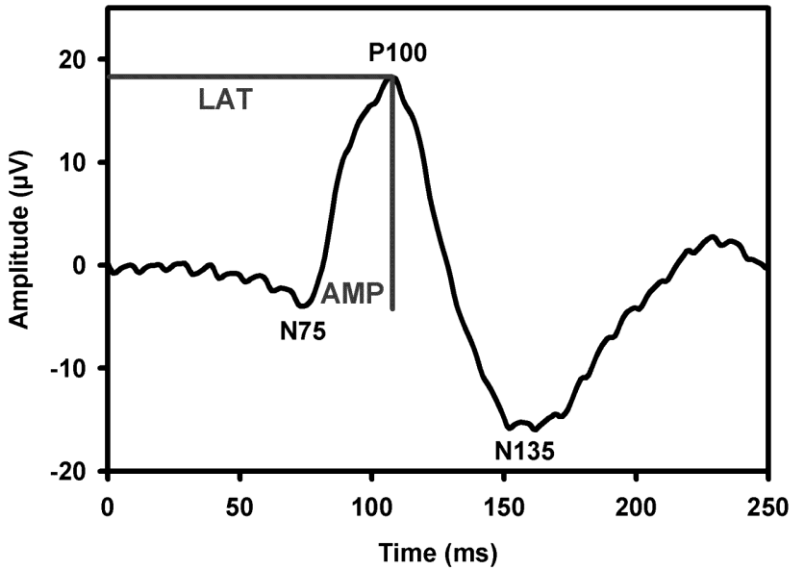
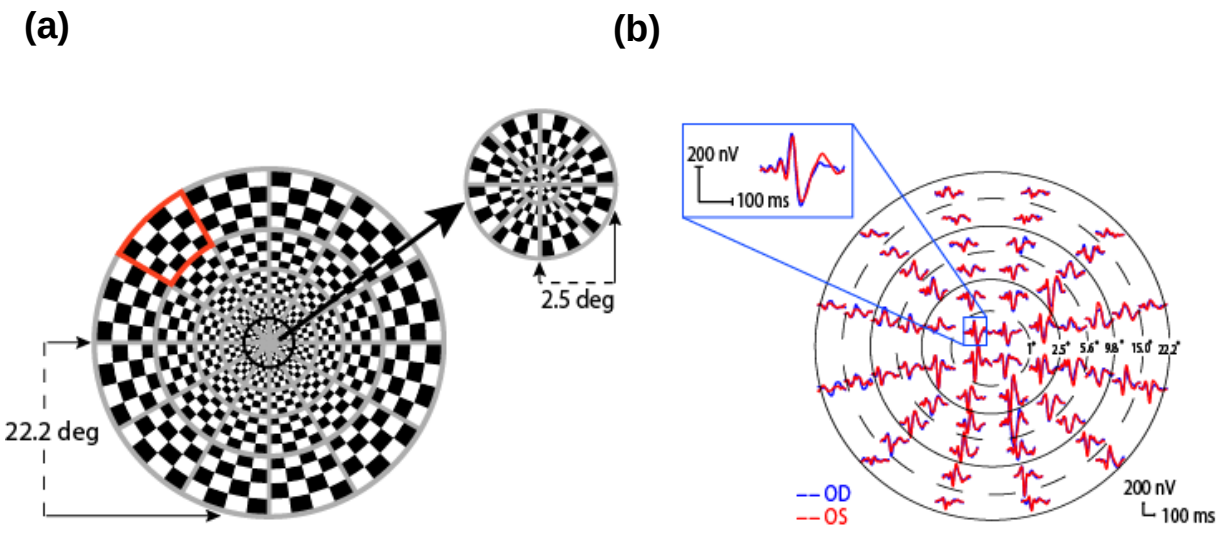


Figure 1-2: MfVEP stimulus and waveform (normal)

The mfVEP 60 sectors dartboard stimulus with one of the sector marked in red (a). MfVEP responses from two eyes of a normal subject. Blue and red traces represent responses from the right and left eyes respectively, with responses from one location enlarged in the inset (b).



Chapter 2 : Reproducibility of Multifocal Visual Evoked Potential and Traditional Visual Evoked Potential in Normal and Multiple Sclerosis Eyes

This chapter was submitted to Documenta Ophthalmologica on July 9th, 2014 and is currently under revision.

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Abstract

Background: Multifocal visual evoked potential (mfVEP) provides topographic information on visual function.

Purpose: The purpose of this paper is to establish reproducibility of multifocal visual evoked potential (mfVEP) amplitude/latency, and compare it to traditional pattern-reversal VEP (tVEP) in normals and relapsing-remitting multiple sclerosis (RRMS) patients.

Methods: MfVEP (60-sector dartboard stimuli) was recorded twice within a month in 40 normals and 40 RRMS patients (25 eyes, last optic neuritis (ON) ≥ 6 months, 34 non-ON). Global and 9 regional mfVEP amplitudes (logSNR) and latency (ms) were calculated. TVEP was recorded (15', 60', 120' checks) in subsets of 34 normals and 30 RRMS patients. Reproducibility was evaluated using intraclass correlation coefficient (ICC) and test-retest variability (TRV).

Results: ICCs for global and regional mfVEP amplitude and latency were all > 0.80 suggesting good intervisit agreement. ICCs for tVEP ranged from 0.52 to 0.86, being lowest for ON latency (0.52 to 0.68). TRV for amplitude (mfVEP and tVEP) was similar among all groups. TRV for latency, greater for tVEP than mfVEP in all groups, was larger in ON (5.3/9.2 ms for mfVEP global/60' tVEP) compared to non-ON (3.1/5.6, $p = 0.003$/$p = 0.02$) and normals (2.3/4.1, $p = 0.0001$/$p < 0.01$).

Conclusions: MfVEP showed better reproducibility than tVEP. TRV for mfVEP latency was about half the respective values for tVEP in normal and RRMS eyes.

Introduction

In multiple sclerosis (MS), irreversible axonal damage is a major substrate for permanent disability. (Dutta and Trapp 2011) Remyelination of nerve fibers is believed to play a protective role in preventing further axonal degeneration. (Irvine and Blakemore 2008) Optic neuritis (ON), an acute inflammatory demyelination of the optic nerve, occurs in more than 50% of MS patients, leading to retrograde degeneration in the retinal nerve fiber layer (RNFL)/ ganglion cell-inner plexiform layer (GCIP) (Syc et al. 2012; Narayanan et al. 2014) and persistent visual dysfunction.

The visual evoked potential (VEP) provides an objective, non-invasive measure of visual function. VEP amplitude reflects the number of functional fibers reaching the visual cortex and is reduced due to transient conduction block during acute ON and/or permanent axonal loss post-ON. (Klistorner et al. 2010a; Jones and Brusa 2003) VEP latency is unique in that it reflects the integrity of myelin and is potentially useful in measuring the extent of demyelination/remyelination in the visual pathway. (Klistorner et al. 2010b; Jones and Brusa 2003) The traditional pattern-reversal VEP (tVEP) is dominated by responses from the macular region, and sums responses from normal and abnormal neurons over a field of at least 15° diameter. It therefore can miss localized defects in MS. (Klistorner et al. 2008; Hood and Greenstein 2003)

The multifocal visual evoked potential (mfVEP) includes multiple focal VEPs simultaneously across the field tested, providing spatially localized topographic information on response amplitude and latency. (Hood and

Greenstein 2003) The mfVEP is highly sensitive in detecting visual defects in MS eyes with or without a history of ON. (Laron et al. 2009; Klistorner et al. 2008; Fraser et al. 2006) The mfVEP has been shown to detect more abnormality in ON/MS compared to tVEP, (Klistorner et al. 2008; Grover et al. 2008) optical coherence tomography (OCT) or Humphrey visual fields (HVF). (Laron et al. 2010) Further, mfVEP amplitude (Klistorner et al. 2010a; Hood et al. 2000) and latency (Yang et al. 2007; Klistorner et al. 2010b) have shown promise in tracking changes in local optic nerve function in MS.

For the mfVEP to detect and monitor MS-related changes, it is crucial to define its reproducibility. Reproducibility refers to the variability in repeated measurements when one or more factors such as observer or time is varied while repeatability is the variability in measurements by one observer when all other factors are constant. (McAlinden et al. 2011) Both terms have been used interchangeably in different studies to determine the precision of a technique in obtaining a measurement. Good reproducibility of mfVEP amplitude has been demonstrated in normal (Fortune et al. 2006) and glaucomatous patients (Chen et al. 2003) but limited literature is available on reproducibility of mfVEP latency. (Sriram et al. 2012) Reproducibility of mfVEP in MS patients is unknown. In MS, factors such as attention deficits, fatigue, (Compston and Coles 2008) transient worsening of symptoms due to changes in core body temperature (Uhthoff's phenomenon) may potentially influence a test's outcome. Disease severity may impact reproducibility. For example, variability of HVF sensitivity was found to be higher in ON than in normal eyes (Wall et al. 1998) and increased with severity in

glaucoma. (Chauhan and Johnson 1999) In this study, we determined the reproducibility of mfVEP amplitude and latency in normal eyes, MS eyes with the last ON event at least 6 months prior (ON) and those without a history of ON (non-ON). For comparison, reproducibility of tVEP was determined for the same groups of eyes. Some of the findings in this study have been reported in abstract form (Narayanan D, et al. *IOVS* 2013; 54: E-Abstract 5131).

Materials and Methods

Subjects

MfVEPs were recorded in two visits (intervisit time 1 to 35 days) for 40 RRMS (Polman et al. 2005a) and 40 age-matched normal subjects (Table 2-1). MS patients recruited from the University of Houston MS Eye CARE clinic had comprehensive eye exams by an experienced neuro-ophthalmologist. Patients with ocular/systemic diseases other than ON/MS were not included. Eyes with an ON attack within 6 months (acute ON) or between visits were excluded. Monocular contrast sensitivity was assessed monocularly using the Pelli-Robson CS chart at 1 m. The chart comprises 16 triplets of letters each subtending 2.8°. Letters within a triplet have the same contrast and successive triplets decreases in contrast from 0 to 2.25 log units in 0.15 steps. Each letter read correctly was counted as 0.05 log unit and the test was terminated when a subject read 2 letters in the triplet incorrectly. (Arditi 2005) Normal subjects will have a score of

about 1.6 out of a theoretically possible score of 2.40. When both eyes of a subject belonged to the same group (i.e. non-ON or ON), one eye was randomly selected. MfVEP analysis included 40 normal, 34 non-ON and 25 ON eyes. TVEP analysis included subsets of 34 normal subjects and 30 MS patients with 34 normal, 24 non-ON and 20 ON eyes.

Table 2-1 near here

All procedures adhered to the tenets of Declaration of Helsinki. The protocol was approved by the University of Houston Committee for the Protection of Human Subjects. Informed consent was obtained from all subjects.

MfVEP procedures and analysis

MfVEP recordings and analysis were performed as previously described (Laron et al. 2009) using a 22.2° 60-sector cortically scaled dartboard pattern (VERIS 5.1, 66 cd/m^2 mean luminance, 95% contrast) (Hood and Greenstein 2003) (Figure 2-1 a and 2-1 b). Electrodes included a ground on the forehead, reference at inion, and three active electrodes (one 4 cm above inion, two 1 cm above and 4 cm on either side of the inion). Two 7-minute recordings from each eye were obtained and averaged for analysis.

For each sector, monocular amplitude, measured as log signal-to-noise ratio (logSNR), and monocular latency (ms) were derived using a customized MATLAB program. (Hood and Greenstein 2003; Hood et al. 2004; Fortune et al. 2004) Monocular latency was a relative latency (ms), calculated as the shift needed to achieve the best cross correlation between the subject's waveform and a normative template. (Hood et al. 2004) Individual sectors were grouped into 9 regions (Figure 2-1 c). MfVEP amplitude and latency were calculated as the mean logSNR and median latency from all 60 sectors (global), central 5.6°, and 9 regions. The central 5.6° is potentially useful for future structural-functional studies comparing mfVEP and thickness of cellular layers in the macula, measured by OCT. Monocular amplitude (MAMP) and latency (MLAT) probability plots were obtained by comparing each sector's response to the normative data at corresponding locations and assigning a probability value (Figure 2-1 d). (Hood and Greenstein 2003; Hood et al. 2004; Fortune et al. 2004) Adjacent abnormal points (i.e. $p < 0.05$) were defined as a cluster when they met cluster criteria with 95% specificity: (Laron et al. 2009) ≥ 5 points with $p < 0.05$ for MAMP, ≥ 4 points with $p < 0.01$ or a cluster > 7 for MLAT. For quantifying abnormality, total number of abnormal points and the number of abnormal points within a cluster (cluster size) were counted, then each divided by the total number of measurable points (always 60 for MAMP) and expressed as percentage.

Figure 2-1 near here

TVEP procedures and analysis

TVEPs were recorded (Odom et al. 2009) using a 22° x 22° pattern-reversal stimulus (51.2 cd/m^2 mean luminance, 95% contrast) (Espion; Diagnosys LLC, Lowell, MA). We used 15', 60' checks as recommended by the ISCEV standards (Odom et al. 2009) and additionally a 120' check to elicit responses from eyes with poor visual acuity. A ground electrode was placed on the forehead, a reference at Fz, and an active electrode 4 cm above inion. Two recordings were performed and averaged for each eye.

N75 was identified as the trough between 60 to 110 ms, P100, the peak between 75 and 180 ms. (Grippo et al. 2006) TVEP amplitude was measured from N75 to P100, and latency from stimulus onset to P100 (peak method). In three ON eyes amplitudes were too small to discern trough/peak and were excluded from analysis. To directly compare tVEP and mfVEP latency, tVEP relative latency was calculated by cross-correlating a waveform and the tVEP normative template (averaged normal response for each check size) using the MATLAB 'xcorr' function (cross-correlation method). For both mfVEP and tVEP, the distance between inion and nasion was kept constant for both visits.

Statistical analysis

Statistical analyses were performed on SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) and Tukey post-hoc analysis were used to compare mean amplitude and latency among groups. Paired t-test was used to compare measures between two visits. F-test was used to compare variances.

Reproducibility was assessed using two different methods: intraclass correlation coefficient (ICC) and test retest variability (TRV). ICC measures the intervisit agreement, which is considered good for ICC ≥ 0.75. (Lee et al. 1989) TRV calculation followed Bland and Altman's method as briefly described below. (Bland and Altman 1996) Standard deviation (SD) of repeated measurements on the same subject (called subject SD) represents measurement error. When subject SD is unrelated to magnitude of the measurement (for example, Figure 2-2, $p > 0.05$ for all groups), the common SD for all subjects (i.e. within-subject standard deviation, s_w), can be obtained by averaging variances (the squares of subject SDs) across all subjects. In the case that a subject has two repeated measurements:

$$s_w^2 = \frac{1}{2n}\sum d_i^2 \qquad \text{Eq. 1}$$

where n is the number of subjects, d_i is the difference between the two repeated measurements for the subject i. For 95% of observations, the difference between a subject's measurement and the true value will be less than $1.96*s_w$. TRV was calculated as $1.96*s_w$. (Budenz et al. 2008) Relation between subject

SD and response magnitude was verified before calculating TRV in each analysis.

TRV is also commonly reported in terms of limits of agreement (LoA) (i.e. 1.96*SD, where SD is $\sqrt{\frac{\sum di^2}{n-1}}$ and di is intervisit difference, assuming mean difference of repeated measurements is zero). For comparison, LoA values from other studies were converted to our TRV ($1.96s_w$) using the following equation

$$s_w = \frac{SD * \sqrt{n-1}}{\sqrt{2n}} \qquad \text{Eq. 2}$$

Figure 2-2 near here

Additionally for mfVEP probability plots, intervisit agreement in classifying an eye as normal or abnormal using cluster criteria was reported in percentage and corrected for chance using AC1 statistic (Gwet et al. 2002). Compared to a commonly used Kappa statistic, AC1 is less dependent on the prevalence of a trait. AC1 = $\frac{p_a - p_e}{1 - p_e}$ where p_a is the observed agreement and p_e is the agreement expected by chance (see Cheng et al for details). (Cheng et al. 2007)

Results

MfVEP Results

Global mfVEP amplitude and relative latency

Mean mfVEP global amplitude (logSNR) and latency for three groups (40 normal, 34 non-ON and 25 ON eyes) are summarized in Table 2-2. Figure 2-3 shows a scatter plot of logSNR (a) and latency (b) for visit 1 and visit 2 in normal, non-ON and ON eyes. Mean global logSNR was reduced for non-ON and ON compared to normals ($p < 0.001$ for all, ANOVA, Tukey post-hoc). LogSNR was slightly smaller in the second visit in all three groups but the difference was not significant ($p > 0.05$ for all, paired t-test). Mean global latency (ms) was delayed in non-ON ($p < 0.01$ for both visits) and ON ($p < 0.0001$, ANOVA, Tukey post-hoc) compared to normal eyes. Latency was not different between the two visits for all groups ($p > 0.05$ for all, paired t-test).

Table 2-2 near here

Figure 2-3 near here

MfVEP intraclass correlation coefficient (ICC) and test retest variability (TRV)

ICC for global, regional amplitude and latency were all above 0.80 (Table 2-3), suggesting good intervisit agreement (Lee et al. 1989) of amplitude and latency in normal, non-ON and ON eyes.

Table 2-3 near here

TRV ($1.96*s_{w)}$ for global amplitude was similar to normal (0.11) in non-ON (0.11, p = 0.14) and ON (0.10, p = 0.68, F-test). TRV for global latency (ms) was higher in ON (5.3) than normal (2.3, p = 0.0001) and non-ON (3.1, p = 0.003), the latter two not different from each other (p = 0.13, F-test) (Table 2-4). TRV for regional amplitude was similar across all regions (Figure 2-4a) while for latency, TRV was lowest in central 2.5° and about 50% higher in peripheral regions (Figure 2-4b) in normal, non-ON and ON eyes.

Table 2-4 near here

Figure 2-4 near here

MfVEP probability plots: abnormal points and cluster size

Abnormalities of individual sectors could be assessed using monocular probability plots (Figure 1-1d) and each eye was classified as normal or abnormal based on cluster criteria (see methods). There was very good agreement between the two visits in classifying an eye as normal or abnormal based on cluster criteria. For both amplitude and latency, agreement was 90% or greater (AC1 values 0.81 to 0.97) for all three groups (Table 2-5). Figure 2-5 shows examples of ON eyes that showed good agreement and poor agreement on intervisit probability plots, respectively. Further, we compared between the two visits the total number of abnormal points (for eyes with at least one abnormal point) and cluster size for those with a valid cluster in either visit, both expressed as percentages of measurable points. In normals, only abnormal points were calculated as valid clusters were present only in 1 eye for MAMP and 3 eyes for MLAT. The means for visit 1 were about the same as those for visit 2 ($p > 0.05$ for all, paired t-test, Table 2-2). TRV for total number of abnormal points (%) was 6, 11, 11 for MAMP and 6, 9, 14 for MLAT in normal, non-ON, and ON, respectively. For cluster size (%), TRV was 12 for MAMP in both groups and 12 and 18 for MLAT in non-ON and ON (Table 2-2).

Table 2-5 near here

Figure 2-5 near here

Source of variability in mfVEP

To estimate contributions of factors such as electrode position, subclinical disease change to the overall intervisit variability, we calculated intravisit TRV based on the two 7 minute runs from visit 1, assuming changes in these factors were minimal on a given day. For comparable measures for this analysis, intervisit TRV was reanalyzed using only the first runs from visit 1 and visit 2. For amplitude, more than 60% of intervisit TRV was reflected in intravisit for all groups. For latency, more than 85% intervisit TRV was reflected in intravisit in normal and non-ON but only about 50% in ON, indicating greater variability of latency in ON eyes between the two visits (Table 2-6).

To determine whether latency variability was associated with magnitude of response amplitude, an eye's SD of global latency from the two visits was plotted against its mean global logSNR from the two visits. Larger SD in latency was observed in eyes with smaller amplitudes ($p = 0.006$, Spearman's correlation). (Figure 2-6).

Figure 2-6 near here

TVEP Results

TVEP amplitude and latency

TVEP was assessed in 34 normal, 24 non-ON and 19 ON eyes (Table 2-2). Figure 2-3 shows a scatter plot of tVEP P100 amplitude (c) and latency (d) for 60'check size for visit 1 and visit 2 in normal, non-ON and ON eyes. TVEP amplitudes were similar in non-ON and ON ($p > 0.05$ for all); both smaller than normals for all check sizes in both visits ($p < 0.05$ for all, ANOVA, Tukey post-hoc) except for non-ON visit 1 60' ($p = 0.09$) and visit 1 120' ($p = 0.08$). P100 latency was delayed in non-ON (except for 60' in visit 1 and 120' in both visits for peak and cross-correlation methods) and ON compared to normals ($p < 0.05$ for all, ANOVA, Tukey post-hoc). No difference between the two visits was found in amplitude or latency for all check sizes in all groups ($P > 0.05$, paired t-test).

TVEP ICC and TRV

ICC for tVEP amplitude and latency ranged from 0.81 to 0.86 in normals, suggesting good intervisit agreement. ICC ranged from 0.61 to 0.78 for non-ON amplitude/latency and ON amplitude, and was lowest (0.52 to 0.57) for ON

latency (peak method). ICC was slightly better for tVEP latency with cross-correlation method (Table 2-3).

For all 3 check sizes, amplitude TRV was similar across all groups ($P > 0.05$ for all). Latency TRV (peak method) was higher in non-ON and ON than in normal eyes for all checks ($p < 0.01$ for all except, $p = 0.28$ for 60' non-ON vs normal, F-test). With cross-correlation method, latency TRV in non-ON eyes improved, becoming similar to normal ($p > 0.05$ for all checks), but TRV in ON eyes remained larger than normal ($p < 0.01$ for all checks) (Table 2-4).

TRV of Pelli-Robson CS

For Pelli-Robson CS, TRV was not significantly different among normal (0.13), non-ON (0.14) and ON eyes (0.16) ($p > 0.05$, F test).

Discussion

In this study we assessed reproducibility of mfVEP and tVEP amplitude and latency in three groups (normal, non-ON and ON eyes), using ICC and TRV.

The high ICC values for global and regional measures that we observed for mfVEP amplitude indicate good reproducibility in all three groups. The TRV for mfVEP amplitude in our normals is similar to that reported by Fortune et al (Fortune et al. 2006) when adjusted for differences in methods (mean TRV for

individual sectors was 1.9 dB in our study and 2.1 dB in Fortune's). TRV for MAMP abnormal points (11%) and cluster size (12%) in our study was also similar to that reported for glaucoma eyes (6 sectors, i.e., 10%). (Wangsupadilok et al. 2009; Bjerre et al. 2004) Reproducibility for mfVEP amplitude was similar across all regions and groups, as indicated by similar ICC and TRV. This agrees with a previous report of reproducibility being similar across eyes/locations with different amplitudes in normals. (Fortune et al. 2006) This highlights that reproducibility of mfVEP amplitude does not depend on magnitude of amplitude unlike HVF in glaucoma for which variability is larger in eyes/locations with greater damage. (Chauhan and Johnson 1999) However, due to the limited dynamic range of mfVEP amplitude, HVF might be better for tracking progression in those subjects with small amplitudes. (Fortune et al. 2006) For tVEP amplitude, TRV was similar in all groups. However, lower ICCs in MS eyes for all check sizes suggests that reproducibility for tVEP amplitude is decreased in MS eyes compared to normals.

Good reproducibility of mfVEP latency was observed in our study with high ICC values in all regions and groups. TRV for global mfVEP latency (ms) was largest in ON eyes, about two times the value in normal eyes. In all groups, TRV for regional mfVEP latency was smallest in central 2.5° and about 50% larger in regions beyond 9.8° eccentricity. Our TRV of 2.3 ms in normals was much lower than 6.4 ms reported by Sriram et al, (Sriram et al. 2012) but their recording and data analysis methods differed from ours. Since publications reporting mfVEP latency reproducibility in MS were not available, we could only

compare our tVEP latency reproducibility to that previously reported. TRV for tVEP latency (ms) in our normals was similar to that reported in normal (Hammond et al. 1987) and MS eyes. (Thomae et al. 2010) Consistent with our mfVEP results, tVEP latency showed worse reproducibility in MS than in normal eyes. This was evident from lower ICC and larger TRV values (peak method) in ON and non-ON eyes, compared to normal eyes. When a cross-correlation method was used, TRV in non-ON improved but not in ON eyes. There was no trend for variation in reproducibility across different check sizes for tVEP amplitude and latency in any groups.

MfVEP measures generally showed better reproducibility than tVEP measures. ICC was greater for mfVEP than tVEP amplitude in MS eyes. For latency, both normal and MS eyes showed higher ICCs for mfVEP than tVEP. Most importantly, latency TRVs for tVEP in normal, non-ON and ON eyes were about twice the respective global values for mfVEP. This is not due to magnitude of measurements being different (mfVEP 'relative latency' vs tVEP 'peak latency'). For mfVEP, an individual waveform's latency measured from stimulus onset to the peak amplitude would be equal to a constant (based on the sector's normative template) adding to the relative latency; and this constant, being the same for both visits, would cancel out in TRV calculations. Therefore, TRV based on relative latency would not differ fundamentally from that measured from stimulus onset to peak. This is evident from the fact that tVEP latency TRV was similar for peak and cross-correlation methods (relative

latency). A main reason for lower TRV in mfVEP could be that our global mfVEP latency was obtained by averaging individual sectors

When TRV of individual runs from the first visit was compared to TRV from two visits, we found that intra-visit variability had a large contribution for normal and non-ON eyes, similar to the findings of Chen et al. (Chen et al. 2003) In other words, the majority of inter-visit variability is attributed to factors such as neck muscle tension and/or α waves during recording. ON eyes however, showed higher latency variability between visits. ON eyes could be more vulnerable to subclinical inflammation, demyelination and remyelination than non-ON eyes. Although reduced amplitudes and/or distorted waveforms might contribute to increased latency variability in ON, the fact that intravisit latency TRV in ON eyes was similar to that in normal and non-ON eyes supports subclinical changes in ON eyes even within a short time interval, causing larger intervisit variability.

Conclusions

Good reproducibility of mfVEP amplitude and latency was observed in normal and MS eyes. For mfVEP amplitudes, TRV was similar in normal, non-ON and ON eyes; while for latency, ON eyes showed larger variability than non-ON and normal eyes. For all groups, TRV for mfVEP latency was only half the respective values for tVEP, suggesting better detection of latency changes using mfVEP. MfVEP is useful in detecting local functional changes related to axon and

myelin of optic nerve in MS, and potentially can be used as an outcome measurement for novel neuroprotective and remyelination strategies.

Acknowledgements

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Figures and Tables

Figure 2-1: MfVEP stimulus and response (MS)

The 60-sector mfVEP dartboard stimulus with one sector marked in red and 2.5°, 9.8°, 22.2° eccentricities marked in blue (a). MfVEP response traces from an ON eye (red) and the normative template (black) (b). (Hood et al. 2004) Traces from one sector are enlarged. Nine locations for regional analysis of mfVEP amplitude and latency reproducibility (c). MLAT probability plot of the ON eye from (b). Each sector is marked as ‘normal’ in black ($p > 0.05$), ‘abnormal’ in red (desaturated for $p < 0.05$, saturated for $p < 0.01$) and un-measureable in gray (d). (Hood et al. 2004)

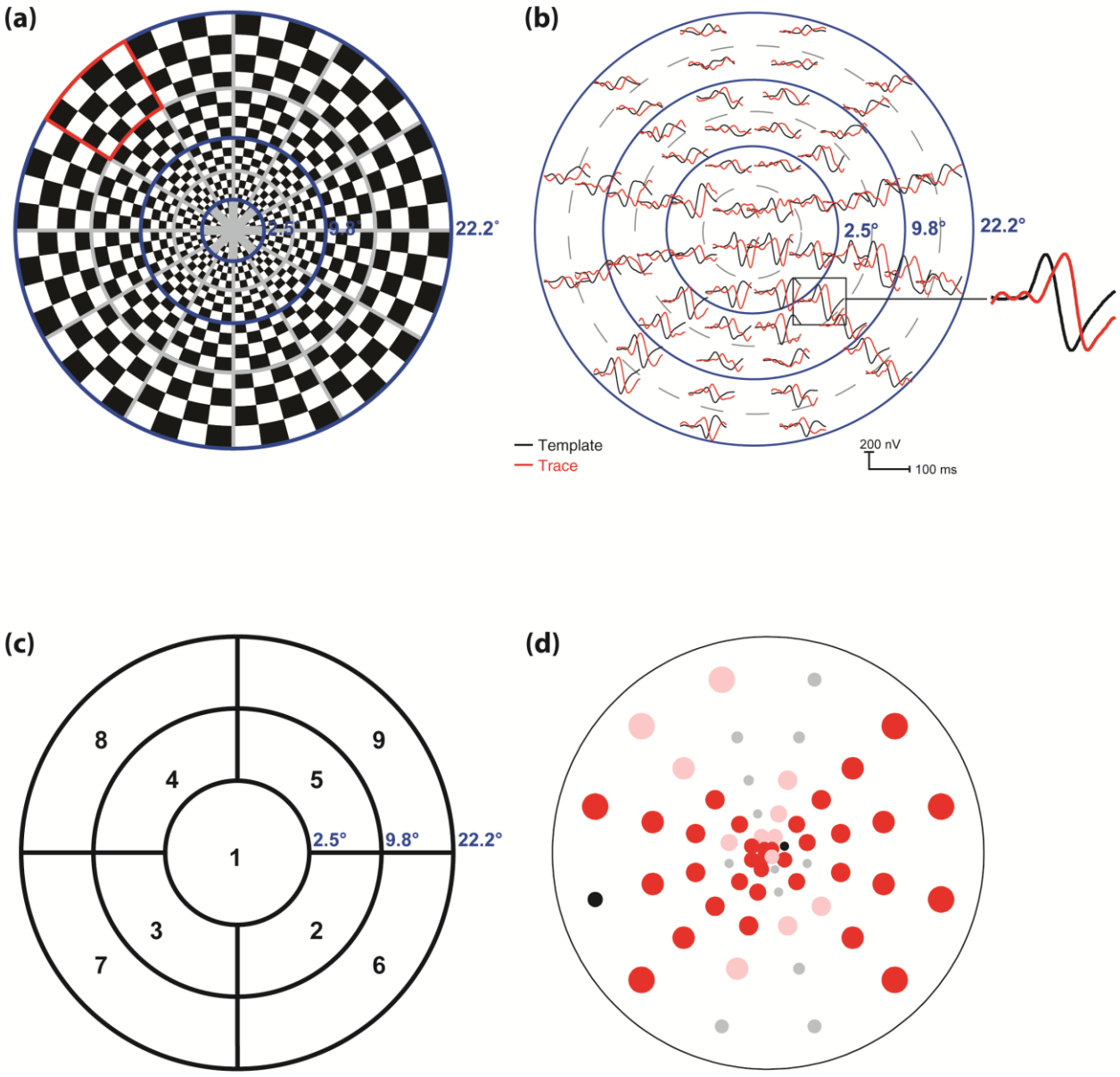
(a)
2.5°
9.8°
22.2°
(b)
2.5°
9.8°
22.2°
Template
Trace
200 nV
100 ms
(c)
8
9
4
5
1
2.5°
9.8°
22.2°
3
2
7
6
(d)

Figure 2-2: Subject mean vs subject SD

Individual subject's SD is independent of the mean for mfVEP global amplitude (a) and global relative latency (b) ($p > 0.05$).

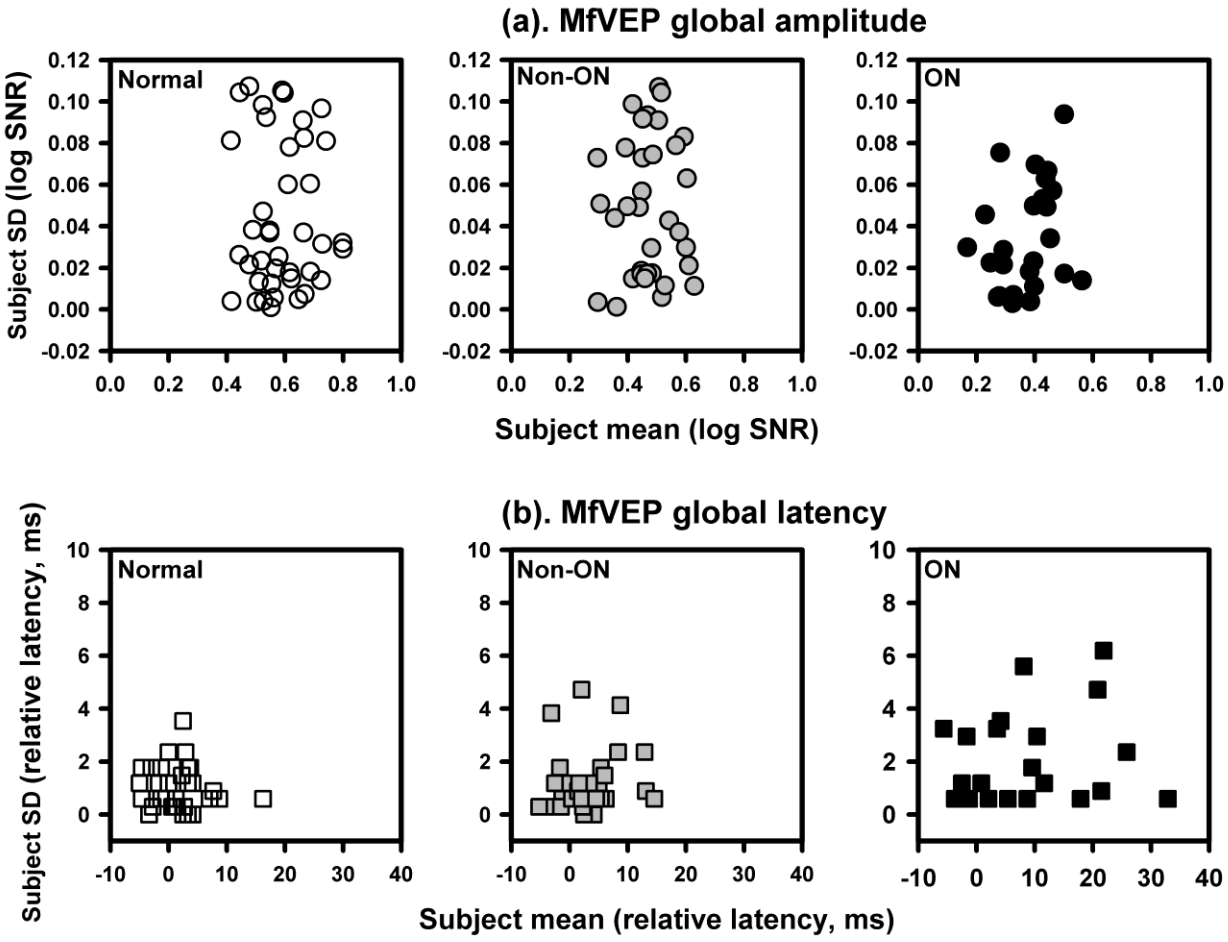

Figure 2-3: Responses from visit 1 vs visit 2

Monocular VEP responses from visit 1 are plotted against those from visit 2 for mfVEP global logSNR (a) mfVEP relative latency (b), tVEP 60’ P100 amplitude (c) and tVEP 60’ P100 latency (d). The dashed line has a slope of 1. The inward ticks (dotted for normal, gray for non-ON and black for ON eyes) indicate the group means from each visit.

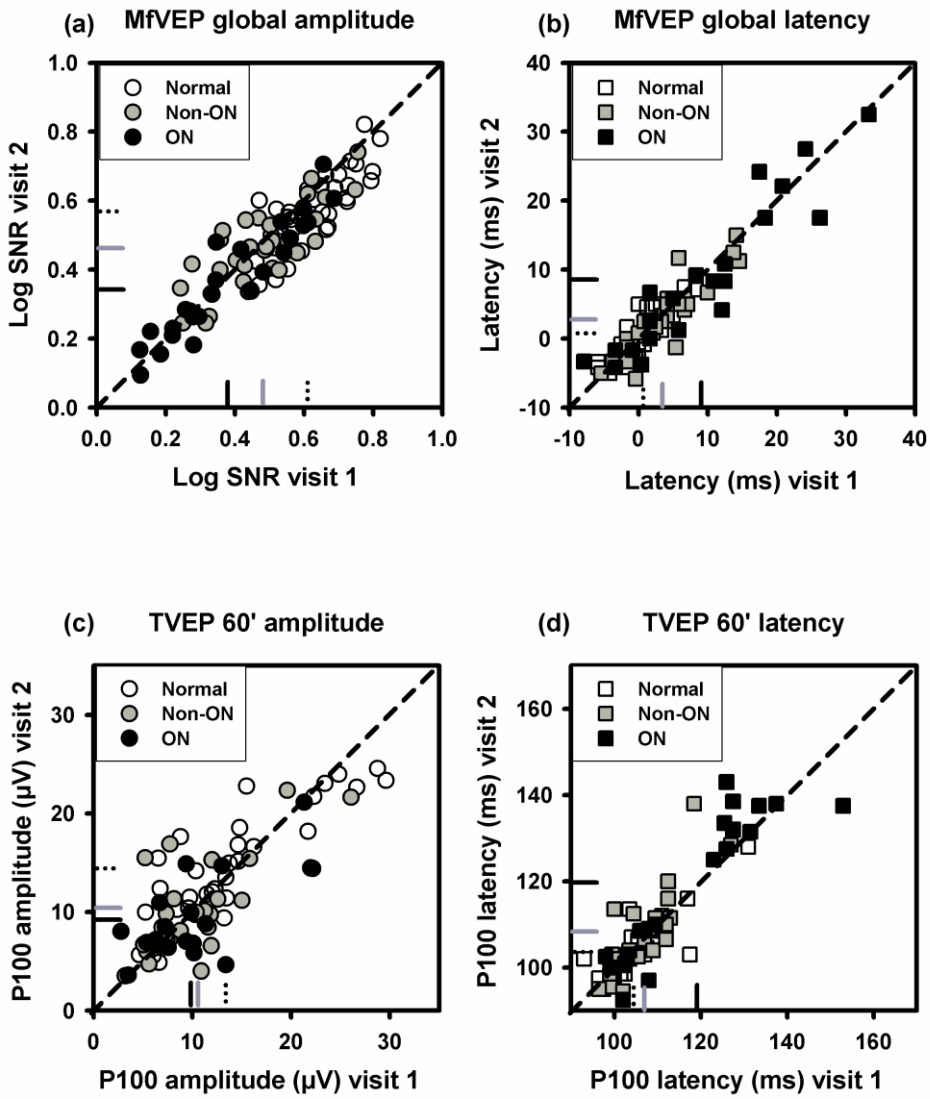
(a) MfVEP global amplitude
Normal
Non-ON
ON
Log SNR visit 2
Log SNR visit 1
0.0
0.2
0.4
0.6
0.8
1.0
(b) MfVEP global latency
Normal
Non-ON
ON
Latency (ms) visit 2
Latency (ms) visit 1
-10
0
10
20
30
40
(c) TVEP 60' amplitude
Normal
Non-ON
ON
P100 amplitude (µV) visit 2
P100 amplitude (µV) visit 1
0
10
20
30
(d) TVEP 60' latency
Normal
Non-ON
ON
P100 latency (ms) visit 2
P100 latency (ms) visit 1
100
120
140
160

Figure 2-4: Intervisit TRV for regional amplitude and latency

Intervisit TRV for mfVEP regional amplitude was similar for all groups and regions (a). Intervisit TRV for regional latency was the best in the central 2.5° and about 50% higher in peripheral regions for all groups (b).

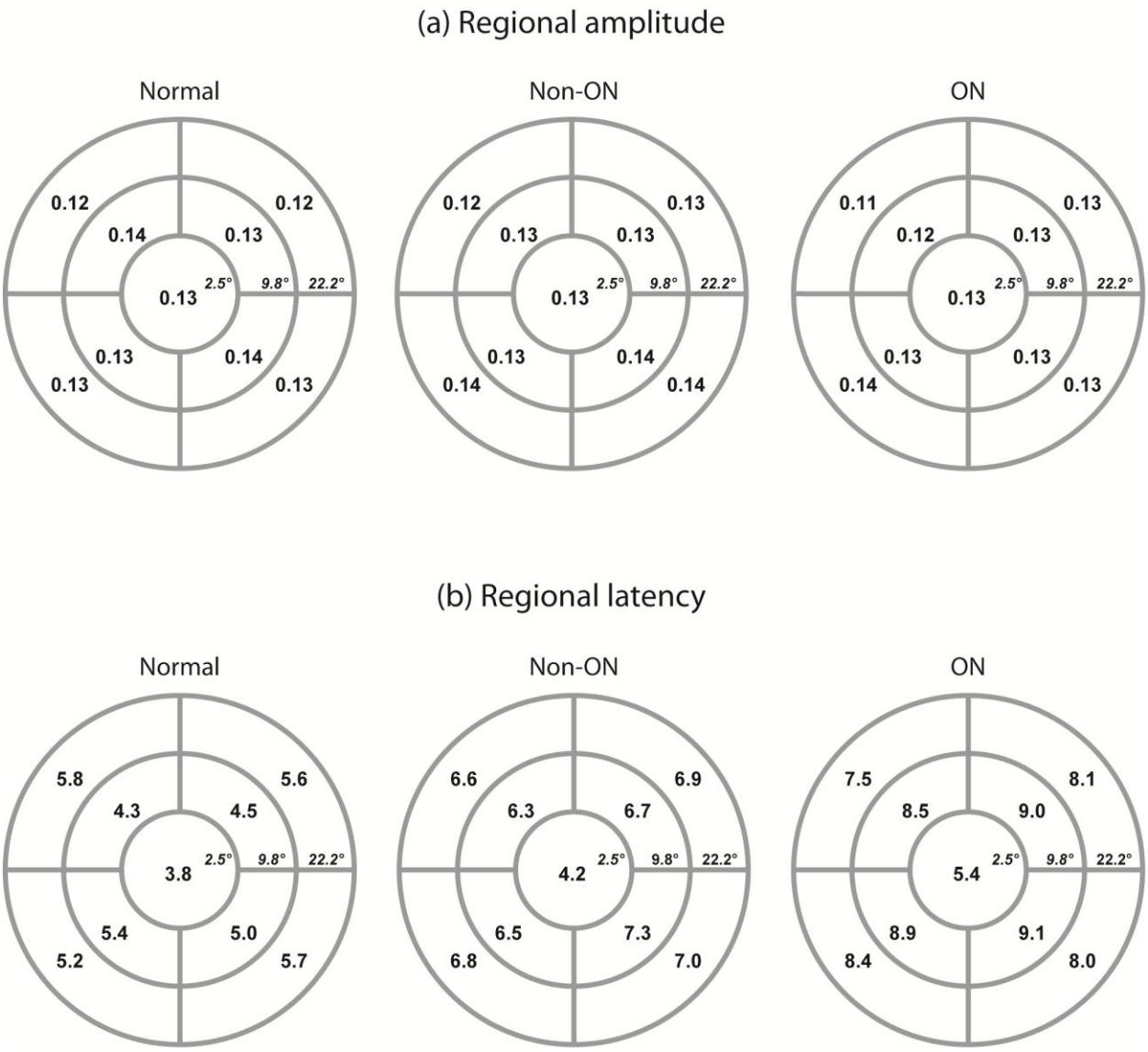

Figure 2-5: Examples of probability plots

Monocular latency probability plots from ON ≥ 6 mo eyes that showed good intervisit agreement (a) (cluster size in visit 1: 86%, cluster size in visit 2: 85%) and poor intervisit agreement (b) (cluster size in visit 1: 33%, cluster size in visit 2: 0%) in classifying an eye as abnormal based on cluster criteria. (Laron et al. 2009)

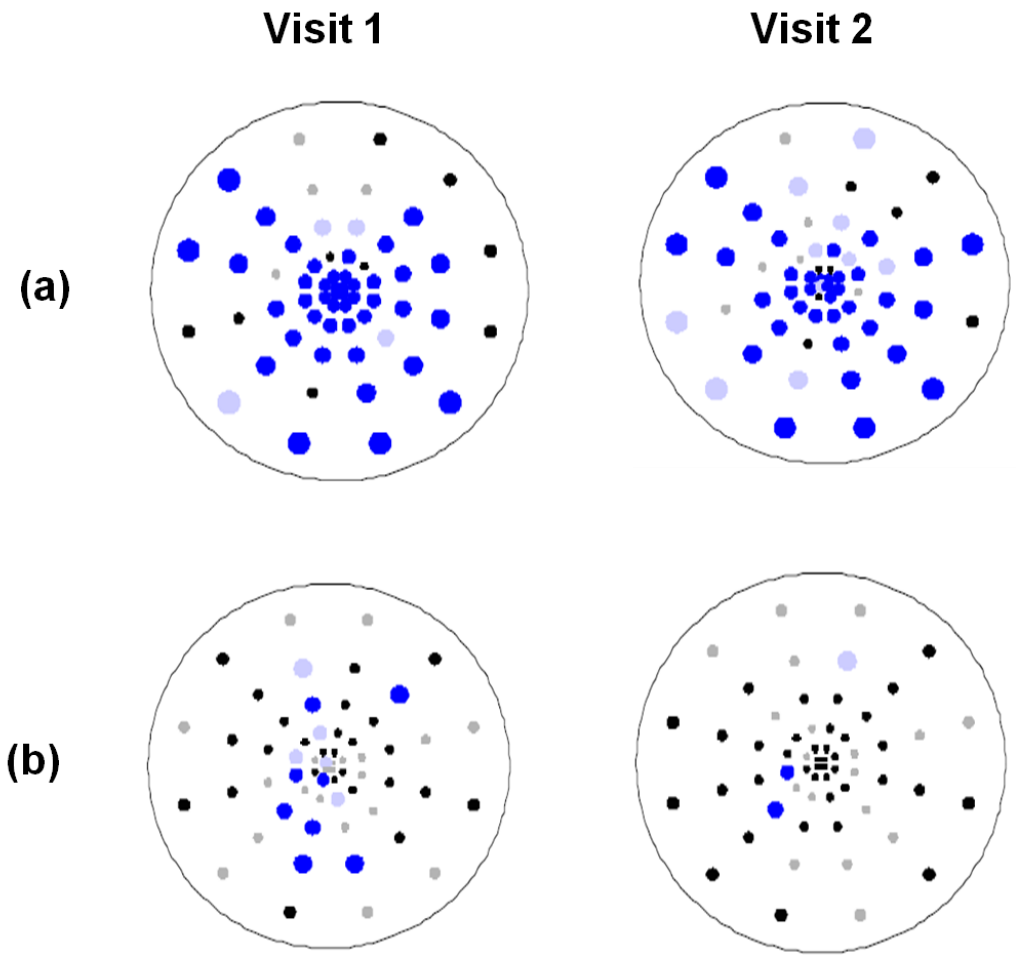

Figure 2-6: Mean logSNR vs latency SD

Mean global logSNR plotted against SD of global latency from two visits. Larger SD in latency was observed for eyes with smaller response amplitudes (p = 0.006, Spearman's correlation).

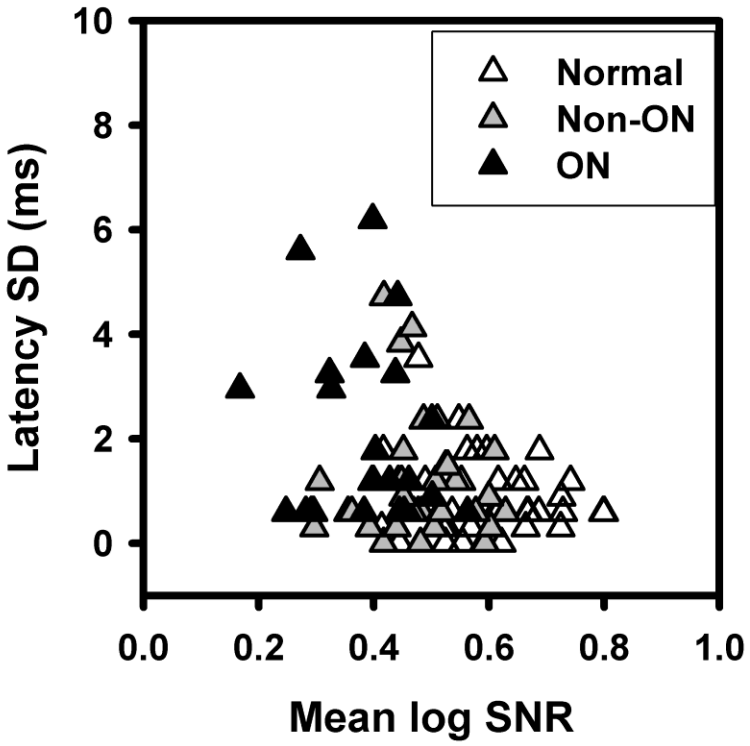

Table 2-1: Baseline demographic and clinical characteristics

	Normal subjects (n=40)	**MS subjects (n=40)**	
Age (years)	37.0±15.2	39.5±9.7	
F:M	3:1	3:1	
Intervisit time (days)	12.8±9.9	15.9±8.0	
MS duration (years)	NA	6.6±7.5	
	Normal eyes (n=40)	**Non-ON eyes (n=34)**	**ON eyes (n=25)**
Time from last ON (years)	NA	NA	4.8±6.4
HVF MD (dB)	NA	-1.1±1.5	-2.8±2.8
VA 20/20 or better* (%)	40(100%)	32(94%)	17(68%)
VA range	20/20 to 20/15	20/30 to 20/15	20/60 to 20/20
Contrast sensitivity*	1.6±0.1	1.5±0.1	1.4±0.2
Contrast sensitivity range	1.50 to 1.80	1.35 to 1.65	0.75 to 1.65

*Visual acuity and contrast sensitivity values were same for both visit

Table 2-2: Amplitude and latency for mfVEP and tVEP

	Normal		Non-ON		ON	
	Visit 1	**Visit 2**	**Visit 1**	**Visit 2**	**Visit 1**	**Visit 2**
mfVEP global logSNR	0.61±0.02	0.57±0.02	0.48±0.02	0.46±0.02	0.38±0.02	0.36±0.03
mfVEP global relative latency (ms)	0.7±0.5	0.9±0.5	3.5±0.8	2.8±0.8	9.1±2.2	8.5±2.1
MAMP abnormal points/cluster size (%)	3/NA	5/NA	14/18	15/19	33/48	32/46
MLAT abnormal points/cluster size (%)	8/NA	7/NA	17/34	16/27	40/62	33/55
tVEP P100 amplitude (μV)						
15’	16.6±1.4	16.5±1.4	11.2±1.1	11.7±1.1	10.0±1.3	10.0±1.2
60’	13.5±1.3	14.4±1.0	10.4±1.0	10.6±1.0	9.8±1.2	9.2±1.0
120’	14.3±1.1	14.3±1.1	10.9±1.0	11.5±1.0	9.8±1.2	10.4±1.1
tVEP latency, peak method (ms)						
15’	106.9±1.2	107.0±1.3	112.4±2.0	115.1±1.9	124.7±3.4	124.2±3.2
60’	104.4±1.2	103.8±1.1	107.1±1.4	108.4±2.1	118.9±3.6	119.9±4.0
120’	105.7±1.0	105.5±1.1	108.1±2.2	107.4±2.3	120.6±3.7	120.5±3.8
tVEP latency, cross-correlation method (ms)						
15’	0.7±1.1	1.1±0.9	3.8±0.8	4.7±0.9	16.7±3.8	14.9±3.4
60’	0.9±1.1	0.8±1.2	3.3±0.7	3.6±0.7	11.8±2.7	15.3±3.5
120’	0.9±0.9	1.2±0.9	2.5±0.5	4.0±0.8	14.5±3.3	15.7±3.6

Table 2-3: ICC for mfVEP, tVEP amplitude and latency and Pelli-Robson CS

	Normal	Non-ON	ON	Normal	Non-ON	ON
		MfVEP amplitude			**MfVEP latency**	
Global	0.85	0.88	0.95	0.96	0.94	0.97
Central 5.6°	0.90	0.91	0.93	0.95	0.95	0.96
Central 2.5° (region 1)	0.93	0.92	0.93	0.94	0.94	0.98
2.5° to 9.8° (region 2 to 5)	0.85 to 0.89	0.82 to 0.90	0.90 to 0.95	0.93 to 0.94	0.82 to 0.92	0.90 to 0.96
9.8° to 22.2° (region 6 to 9)	0.85 to 0.87	0.84 to 0.89	0.86 to 0.92	0.85 to 0.87	0.84 to 0.89	0.86 to 0.95
Check size		**tVEP amplitude**		**tVEP latency (peak/cross-correlation method)**		
15’	0.86	0.78	0.61	0.85/0.84	0.77/0.81	0.52/0.67
60’	0.84	0.76	0.77	0.84/0.81	0.75/0.84	0.57/0.68
120’	0.81	0.78	0.77	0.85/0.85	0.75/0.83	0.56/0.63
		Pelli-Robson CS				
	0.92	0.92	0.91			

Table 2-4: Intervisit TRV for mfVEP, tVEP and CS

	Normal	Non-ON	ON	Normal	Non-ON	ON
	MfVEP amplitude (logSNR)			**MfVEP relative latency (ms)**		
Global	0.11	0.11	0.10	2.3	3.1	5.3
Central 5.6°	0.12	0.11	0.11	2.9	3.3	5.0
Check size	**tVEP amplitude (μV)**			**tVEP latency (ms) (peak/cross-correlation method)**		
15’	6.3	5.3	6.4	4.4/5.7	7.8/5.1	10.5/10.3
60’	5.7	5.2	5.4	5.7/4.1	8.3/5.6	10.3/9.2
120’	4.8	5.3	5.3	4.4/5.2	10.9/7.4	9.1/10.8
	Pelli-Robson CS					
	0.13	0.14	0.16			

Table 2-5: Percent of agreement (AC1 statistic) in classifying an eye as normal or abnormal

	Normal	Non-ON	ON
Amplitude	98% (0.97)	90% (0.80)	96% (0.92)
Latency	98% (0.97)	90% (0.83)	90% (0.81)

Table 2-6: Percentage of intervisit variability reflected in intravisit variability

(a)	Normal	Non-ON	ON	Normal	Non-ON	ON
	Global amplitude			Regional amplitude*		
Intervisit TRV	0.11	0.11	0.12	0.12	0.13	0.14
Intravisit TRV	0.07	0.07	0.07	0.08	0.09	0.10
Percentage	61	60	59	78	77	71

(b)	Normal	Non-ON	ON	Normal	Non-ON	ON
	Global latency			Regional latency*		
Intervisit TRV	2.6	3.5	6.1	5.3	7.2	13.8
Intravisit TRV	2.2	2.9	3.2	4.8	6.2	6.0
Percentage	87	85	52	88	85	42

* Median of 9 regional TRVs

Chapter 3 : Longitudinal Evaluation of Visual Function in Patients with Multiple Sclerosis using Multifocal Visual Evoked Potential

Abstract

Background: Multifocal visual evoked potential (mfVEP) amplitude and latency reflect axonal and myelin health.

Objective: To evaluate longitudinal changes of visual function in relapsing-remitting multiple sclerosis (RRMS).

Methods: MfVEP, contrast sensitivity (CS) and Humphrey visual fields (HVF) were obtained at two visits (mean follow-up:1.5 ± 0.9 years) in 57 RRMS patients (53 eyes with optic neuritis (ON): 14 with ON within 6 months (mo) of first visit (ON < 6 mo), 39 with ON ≥ 6 mo, 57 non-ON). Longitudinal changes were assessed using mfVEP amplitude, latency, CS and HVF mean deviation based on established 95% tolerance limits of test-retest variability.

Results: A significant percentage of ON < 6 mo eyes exceeded the 95% tolerance limits for mfVEP amplitude (21%, $p < 0.05$), latency (35%, $p < 0.01$) and CS (31% $p < 0.001$); more improved than worsened. MfVEP latency shortened in 11% non-ON, 10% ON ≥ 6 mo, lengthened in 21%, and 10%, respectively ($p < 0.01$ for all). Latency changes correlated negatively with baseline latency ($r = - 0.43, - 0.45$ for non-ON, ON ≥ 6 mo; $p = 0.0008$). Although an insignificant number of non-ON and ON ≥ 6 mo eyes exceeded tolerance limits for amplitude, CS or HVF; amplitude and latency changes correlated, both measures correlated with changes in CS ($r = 0.47$ to 0.79, $p < 0.01$).

Conclusions: MfVEP, and particularly the latency, is potentially useful for assessing neuroprotective and remyelinating strategies in RRMS.

Introduction

The pathological hallmark in multiple sclerosis (MS) is formation of sclerotic plaques in the central nervous system (CNS) as a result of inflammatory demyelination of the nerve fibers (Compston and Coles 2008) and degeneration of the underlying axons. The strong inflammatory-immune character of the disease, indicated by transient enhancement of gadolinium lesions on magnetic resonance imaging (MRI), inflammatory infiltrates in lesions examined post-mortem, lead to the development of several immune-modulators as disease modifying therapies (DMT) for MS. (Menge et al. 2008) Current DMTs are effective in reducing inflammation, clinical relapses, and new lesions detected by MRI in relapsing-remitting MS (RRMS) (Castro-Borrero et al. 2012), but are not completely effective in preventing disease progression.(Ford et al. 2010) It is believed that progressive neurological disability in MS is a result of continuous neuronal loss (Trapp and Stys 2009) that occurs even in the absence of clincally-evident inflammation. (Narayanan et al. 2014)

Neuroprotective therapies such as anti-oxidative agents(Nikic et al. 2011), sodium channel blockers (Bechtold et al. 2006) and remyelination strategies (Bruce et al. 2010) are shown to reduce axonal degeneration in animal models of MS. Remyelination, though limited and often incomplete in human CNS, can improve neurological function by restoring conduction, (Smith et al. 1979; Duncan et al. 2009) providing trophic support and preventing further axonal loss. (Irvine and Blakemore 2008) Agents such as anti-lingo-1 antibody (Mi et al. 2009) and human monoclonal IgM antibody 22 (Jolanda Munzel and Williams 2013)

have shown promise in promoting remyelination in animal models of MS and are currently in early stages of clinical trials. (Franklin et al. 2012)

The anterior visual pathway, an accessible site of MS damage, can potentially serve as a good model for tracking neurodegenerative changes and/or neuroprotective effects. (F Costello 2013; Frohman et al. 2008b) Optic neuritis (ON) is an acute inflammatory demyelination of the optic nerve that affects more than 50% of MS patients. Structural and functional visual deficits are observed in MS eyes with and without ON history (non-ON). (F Costello 2013) Previous studies reported that following an ON episode, visual function such as visual evoked potential (VEP) latency (Brusa et al. 2001; Klistorner et al. 2010b) and contrast sensitivity (CS) (Brusa et al. 1999) improves in the affected eyes, with the majority of changes occurring within first 6 months. For the unaffected non-ON eyes, lengthening of VEP latency was observed by some (Brusa et al. 1999; Brusa et al. 2001) but not by others. (Klistorner et al. 2010b) Similarly, contrasting results of no change in VEP amplitudes(Brusa et al. 1999; Brusa et al. 2001) or progressive improvement between 6 and 12 months after ON episode (Klistorner et al. 2010a) were reported. The discrepancies among different studies may be partially due to differences in the study population (e.g. MS versus isolated ON). Further, Brusa et al used traditional pattern-reversal VEP (tVEP) while Klistorner et al used multifocal VEP (mfVEP). In contrast to tVEP that provides a summed response dominated by the macular region, the mfVEP provides topographic information on local amplitude and latency. (Hood and Greenstein 2003)

In order to evaluate the efficacy of novel neuroprotective and remyelination strategies, it is important to understand the extent of functional change that occurs over time in MS eyes. Given the large individual variations in the location/extent of lesions, recovery process, and response to treatment, using group means as previous studies did, (Brusa et al. 1999; Klistorner et al. 2010a; Klistorner et al. 2010b) to estimate changes overtime might mask changes in individuals. Instead, evaluating changes in individual MS eyes (Yang et al. 2007) might offer more valuable information. In the current study, we evaluated changes in visual function over time in individual non-ON and ON eyes using the test-retest variability (TRV) established in the previous chapter. Visual function was assessed with mfVEP amplitude and latency, Pelli-Robson contrast sensitivity (CS) and Humphrey visual fields (HVF). Some of the findings in this study have been reported in abstract form (Narayanan D, et al. *IOVS* 2011; 52: E-Abstract 6089).

Methods

Subjects

Visual function was evaluated on two visits in 57 RRMS patients (F : M = 4.7 : 1, mean age at follow-up: 42.4 ± 10.5 years). Follow-up time ranged from 6 months to 4.2 years (mean 1.5 ± 0.9 years). Mean MS duration at follow-up was 5.6 ± 7.0 years. Eighty-seven percent of patients were on DMT during both visits. All patients underwent a comprehensive eye examination at the MS Eye CARE

clinic, University of Houston. Patients with systemic conditions such as diabetes or other ocular conditions such as glaucoma, retinal anomalies, or ON events between the visits were not included in the study. Fifty-seven non-ON and 53 ON eyes were included; ON eyes were subdivided into 14 eyes with last ON event within 6 months (mo) of the first visit (ON<6 mo) and 39 eyes with ON ≥ 6 mo. Three eyes with other ocular anomalies and one eye with an ON attack between visits were excluded. Time since last ON and visit 1 was 1.3 ± 1.5 months for ON < 6 mo and 6.7 ± 8.0 years for ON ≥ 6 mo. All procedures adhered to the tenets of Declaration of Helsinki, and the protocol was approved by the University of Houston Committee for the Protection of Human Subjects. Informed consent was obtained from all study subjects.

MfVEP procedures and analysis

MfVEP recordings and analysis were as previously described. (Laron et al. 2009) The mfVEP stimulus was a 60-sector cortically-scaled dartboard pattern (VERIS 5.1, 66 cd/m^2 mean luminance, 95% contrast) (Hood and Greenstein 2003) (Figure 3-1 a & b). A ground electrode on forehead, a reference at inion, and three active electrodes (one 4 cm above inion, two 1 cm above and 4 cm on either side of the inion) were attached. Two 7-minute recordings from each eye was obtained and averaged for analysis.

MfVEP amplitude, measured as log signal-to-noise ratio (logSNR), and relative latency (ms) from each sector were derived using a customized MATLAB program.(Hood and Greenstein 2003; Hood et al. 2004; Fortune et al.

2004) Relative latency was calculated as the shift needed to achieve the best cross correlation between the subject's waveform and a normative template for each sector.(Hood et al. 2004) Mean logSNR and median latency for global and 9 regions were calculated (Figure 3-1 c). Monocular amplitude (MAMP) and latency (MLAT) probability plots were generated in which response from each sector was compared to the normative at corresponding locations and assigned a probability value (Figure 3-1 d & e). (Hood and Greenstein 2003; Hood et al. 2004; Fortune et al. 2004) Adjacent abnormal points (i.e. $p < 0.05$) were defined as a cluster when they met cluster criteria with 95% specificity (Laron et al. 2009): ≥ 5 points with $p < 0.05$ for MAMP, ≥ 4 points with $p < 0.01$ or a cluster > 7 for MLAT. Total number of abnormal points and cluster size (number of abnormal points within a cluster) were counted then each divided by the total number of measurable points (always 60 for MAMP) and expressed in percentage.

Pelli-Robson CS

Monocular contrast sensitivity (CS) was measured using a Pelli-Robson chart at 1m for all eyes during both visits as described in chapter 2. Briefly, the Pelli-Robson chart measures contrast from 0 to 2.25 log units in 0.15 log unit steps. Each letter read correctly was counted as 0.05 log unit. (Arditi 2005) Normal subjects will have a score of about 1.6 out of a theoretically possible score of 2.40.

Table 3-1 near here

HVF

HVF (Carl Zeiss Meditec, Inc.) was performed using the SITA (Swedish interactive threshold algorithm) 24-2 or 30-2 protocols. Unreliable tests i.e. fixation losses, false positives or false negatives > 33% were excluded. The mean deviation (MD) was recorded.

Statistical analysis

Analysis of variance (ANOVA) and Tukey post-hoc analysis were used to compare mean amplitude and latency across groups. Paired t-test was used to compare means between two visits. We previously established the test-retest variability (TRV) for mfVEP and CS using $1.96*s_w$. When two measurements are compared, the 95% tolerance limit for detecting a change should be $\sqrt{2}*1.96*s_w$. (Bland and Altman 1996) This is to account for, for instance, the possibility that the baseline measurement may be randomly on the high side whereas the follow-up measurement on the low side of the true value (for more details, see appendix from Budenz et al (Budenz et al. 2008)). The 95% tolerance limits estimated for each parameter for normal, non-ON and ON eyes are listed in Table 3-1. For mfVEP logSNR and Pelli-Robson CS, TRV was not significantly different for normal, non-ON and ON eyes; hence normal limits were used for non-ON and ON eyes. For mfVEP global latency, TRV was significantly higher in ON eyes than in normal and non-ON eyes, which were not different from each other;

normal limits were used for non-ON, and ON limits for ON eyes. For MAMP and MLAT probability plots, TRV for abnormal points was significantly higher in non-ON and ON eyes than normal, and TRV for cluster size could not be estimated in normals due to the low prevalence of valid clusters; thus for abnormal points and cluster size, limits estimated from non-ON and ON eyes were used for the respective groups.

Results

Comparing mean measurements among different groups and between the two visits

Table 3-2 summarizes the group means for all visual parameters measured. For both visits, all three groups (non-ON, ON ≥ 6 mo and ON < 6 mo) had significantly reduced mfVEP amplitude and delayed latency compared to normals ($p < 0.05$), with both ON groups worse than non-ON ($p < 0.05$). Similarly, HVF MD in non-ON and ON eyes were significantly lower than normative values reported ($p < 0.05$), and the reduction was more in ON than non-ON ($p < 0.05$). Pelli-Robson CS was reduced in ON ≥ 6 mo and ON < 6 mo compared to normal and non-ON ($p < 0.05$), but CS in non-ON was not different from that in normal ($p > 0.05$). In non-ON and ON ≥ 6 mo groups, on average, none of the measures from visit 1 were different from those of visit 2 ($p > 0.05$, paired t-test). In contrast, in ON < 6 mo eyes, all functional measures showed significant improvement in visit 2 when compared to visit 1 ($p < 0.05$, paired t-test). This

finding is consistent with the recovery from transient inflammation that is well documented in other studies. (Beck and Cleary 1993)

Table 3-2 near here

Changes in individual MS eyes based on 95% tolerance limits of TRV

For mfVEP, changes in individual eyes were assessed for (1) global and regional amplitude and latency, and (2) number of abnormal points and clusters on probability plots (see Methods).

MfVEP amplitude As shown in Figure 3-2 a, changes were heterogeneous, with some improving some worsening, but only four of 57 (4/57, 7%) non-ON and 3/39 (8%) ON ≥ 6 mo eyes falling outside the 95% tolerance limits of TRV for global logSNR, which was not statistically different from predicted false positive rate of 5% for either group ($p = 0.53$ for non-ON, $p = 0.64$ for ON ≥ 6 mo). Consistent with the findings for global logSNR, neither group had significant number of eyes exceeding tolerance limits based on MAMP abnormal points and cluster sizes from probability plots (Figure 3-2 b & c) or the nine separate mfVEP regions (Table 3-3 a & b). In contrast, in the ON < 6 mo group, a significant number of eyes fell outside of tolerance limits in global logSNR 3/14 (21%) and MAMP abnormal points/cluster size 5/14 (35%) ($p < 0.05$ for all), with more eyes improved than worsened (Table 3-3 a).

Figure 3-2 near here

Table 3-3 a & b near here

MfVEP latency For global latency, 18/57 (32%) non-ON, 8/39 (20%) ON ≥ 6 mo and 5/14 (35%) ON < 6 mo eyes fell outside of 95% tolerance limits, significantly more than 5% ($p < 0.01$ for all) (Figure 3-2 d). As observed for amplitude, changes in latency were also heterogeneous (Table 3-3 a). If tolerance limits based on the normal subjects were used, more ON eyes would have exceeded the limits (see discussion). Diverse changes in regional latency and probability plots (Figure 3-2 e & f, Table 3-3 a & b) were also observed. Global latency changes correlated significantly with baseline latency for non-ON ($r = - 0.43$) and ON ($r = - 0.45$) ($p = 0.0008$ for both) (Figure 3-3 a & b). Although our results were based on two visits, observed changes in the latency could not be attributed to random variability of the measurement, as generally consistent trends for individuals were seen in a small subset of 10 patients (13 non-ON and 7 ON eyes) with more than two visits (Figure 3 c & d).

Figure 3-3 and 3-4 illustrates examples of MS eyes that remained stable, improved and worsened in MAMP and MLAT probability plot from the two visits, respectively.

Figure 3-3 near here

Figure 3-4 near here

Pelli-Robson CS and HVF MD For CS, only 6/51 (12%) non-ON and 6/33 (18%) ON ≥ 6 mo eyes exceeded 95% tolerance limits, not significantly different from 5% for both, and equal numbers improved and worsened. In ON < 6 mo, a significant percentage (4/13, 31%) showed improvement in CS ($p < 0.001$) (Figure 3-5 a). For HVF MD, none of the non-ON, and only 2/14 (11%) of ON < 6 mo and 1/27 (4%) of ON ≥ 6 mo exceeded the limits, both in the direction of improvement, but the percentages were not significantly different from 5% for either group (Figure 3-5 b).

No relationship was observed between changes in functional measures and follow-up time for all tests ($p > 0.05$).

Figure 3-5 near here

Correlation between the changes in mfVEP global logSNR, latency , Pelli-Robson CS and HVF MD

Pearson correlation (r) was used to assess if changes in mfVEP global logSNR and latency were associated with changes in other functional tests. To avoid transient effects of acute inflammation, only non-ON and ON ≥ 6 mo eyes were included for this analysis. When both eyes of a patient belonged to the same group, i.e., non-ON or ON ≥ 6 mo, only one eye was randomly selected for analysis, resulting in a total of 46 non-ON and 35 ON ≥ 6 mo eyes. Changes in

mfVEP global logSNR and global latency correlated significantly with changes in CS in both non-ON and ON ≥ 6 mo eyes ($r = 0.47$ to 0.79, $p < 0.01$) (Figure 3-6 a and b). Global logSNR changes also showed significant correlation with global latency in non-ON ($r = 0.54$, $p = 0.001$) and ON ≥ 6 mo groups ($r = 0.74$, $p < 0.0001$) (Figure 3-6 e). About 71% to 78% of eyes fell within the gray shaded regions indicating that changes were in the same direction (greater or less than zero) for the two parameters compared (Figure 3-6 a, b, e). In fact, 70% non-ON and 74% ON ≥ 6 mo eyes showed changes in the same direction for all three parameters Neither mfVEP logSNR or latency change showed correlation with change in HVF MD ($p > 0.05$ for all) (Figure 3-6 c and d).

Figure 3-6 near here

For all measures, results from unilateral non-ON eyes (i.e., unaffected fellow eyes of ON) were similar to those from biltateral non-ON eyes, suggesting that the changes observed in non-ON is not due to effects of ON in the fellow eye.

Discussion

We evaluated longitudinal changes in visual function in RRMS patients using mfVEP amplitude, latency, CS and HVF. Our study design was unique in

that we were able to track changes in individual MS eyes. Several previous studies reported longitudinal changes after averaging values across patients. (Brusa et al. 1999; Klistorner et al. 2010a; Brusa et al. 2001) Given the diverse nature of MS-related lesions, averaging may mask individual changes. This is evident from our data, in which for non-ON and ON ≥ 6 mo eyes, group means from the two visits were not different but, changes in individual eyes were revealed by the 95% tolerance limits of TRV for latency, and correlation among mfVEP logSNR, latency and CS.

For mfVEP latency, about one-third of MS eyes fell outside of tolerance limits with some shortening, and some lengthening. The changes were not random variations between two visits because a consistent trend was observed in a small subset of eyes with multiple visits. These findings are consistent with earlier mfVEP studies in which diverse variations in latency changes were observed across patients. (Yang et al. 2007; Klistorner et al. 2007) It is known from post-mortem studies that the extent of demyelination and/or remyelination can largely vary from lesion to lesion, and balance between the two processes determines how a lesion evolves overtime in MS. (Prineas et al. 1984) In both non-ON and ON eyes, latency changes were significantly and inversely correlated with baseline latency (Figure 3-3 a & b), specifically, MS eyes that recovered to shorter delays were those with prolonged baseline latency in the first visit, whereas those for which delays became longer, had shorter latency in the first visit.

In our previous reproducibility study, we found that variability of mfVEP latency was significantly higher in ON eyes compared to normal and non-ON eyes, which were not different from each other. Thus, we used 95% tolerance limits of TRV estimated from normal and ON groups to detect longitudinal latency changes in non-ON and ON eyes, respectively. Small amplitudes in ON eyes could attribute to high variability observed. However, comparison of intra-visit vs inter-visit analysis suggested that subclinical disease changes might be occurring in ON eyes even within a short time interval. Thus, it is debatable if longitudinal studies should use normative or ON limits to estimate true changes over time. Our conservative approach to use ON limits probably underestimated the true change. For example, 22% of all ON eyes in our study exceeded ON limits while 46% exceeded normal limits for global latency.

For mfVEP amplitude, CS and HVF MD, there were not a significant number of non-ON and ON ≥ 6 mo eyes exceeding the 95% tolerance limits. However, global logSNR changes correlated significantly with changes in global latency. MfVEP amplitude and latency changes also significantly correlated with changes in Pelli-Robson CS, a sensitive indicator of visual dysfunction in MS/optic neuritis, used in clinical trials such as Optic Neuritis Treatment Trial. (Beck and Cleary 1993) In more than 70% of eyes, the changes occurred in the same direction for mfVEP logSNR, latency and CS. These findings suggest that individual eyes that did not exceed the tolerance limits were probably changing and methods to reduce variability of individual tests might enable better detection

of disease progression. Correlation between latency and logSNR changes also support that myelin and axonal health are related.

In future studies, individual MS eyes should be tracked at frequent intervals for long follow-up time periods, using both functional and structural measures. Our study suggests that mfVEP and Pelli-Robson CS are sensitive measures to detect visual functional changes in MS. Recent spectral-domain optical coherence tomography studies suggest that retinal nerve fiber layer thickness and ganglion cell-inner plexiform layer thickness are both sensitive structural measures useful for tracking neurodegeneration in MS eyes. (Ratchford et al. 2013; Narayanan et al. 2014; Saidha et al. 2011) In the present study time domain OCT was used from initial visits in many cases, so longitudinal assessment was not possible.

Conclusions

In summary, among the functional tests that we studied, mfVEP latency detected the greatest changes in MS eyes, with some worsening, some improving and others being stable. Considering the heterogeneous nature of latency changes and the variable extent of myelin loss/recovery in different lesions, it is likely that treatment outcomes using novel therapeutics will show considerable variations across patients. MfVEP can serve as an effective tool for longitudinal assessment of visual function in individual patients and can

potentially be used as an outcome measure to evaluate efficacy of novel remyelinating therapies in RRMS.

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Figures and Tables

Figure 3-1: MfVEP stimulus, response and probability plots (MS)

The mfVEP 60-sector dartboard stimulus with one sector marked in red and 2.5°, 9.8°, 22.2° eccentricities marked in blue (a). MfVEP response traces from a non-ON eye (red) and the normative template (black). (Hood et al. 2004) Traces from one sector are enlarged (b). Nine locations for regional mfVEP analysis (c). MLAT probability plots with each sector marked as ‘normal’ in black ($p > 0.05$), ‘abnormal’ in red (desaturated for $p < 0.05$, saturated for $p < 0.01$) and un-measureable in gray (d & e). More sectors show abnormalities in visit 2 (e) when compared to visit 1 (d).

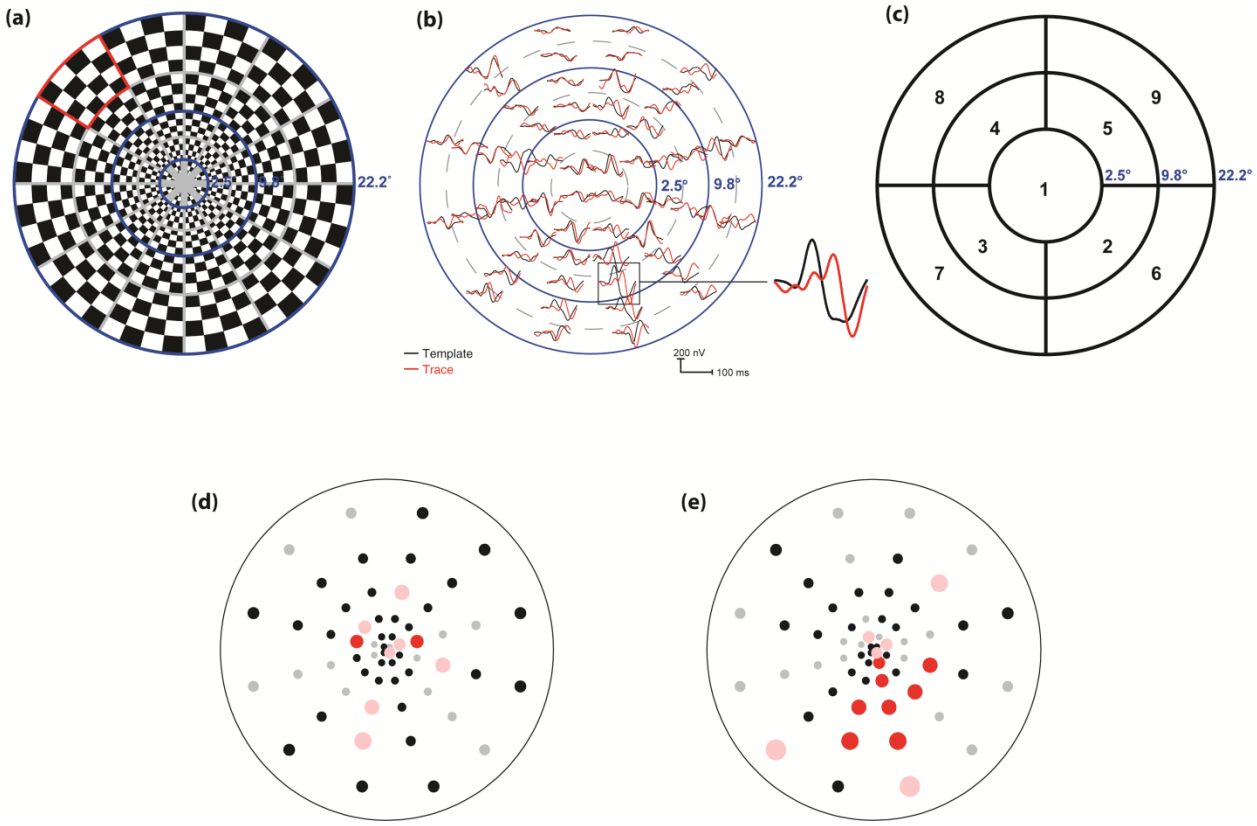
(a)
22.2°
(b)
2.5°
9.8°
22.2°
Template
Trace
200 nV
100 ms
(c)
1
2
3
4
5
6
7
8
9
2.5°
9.8°
22.2°
(d)
(e)

Figure 3-2: Intervisit difference for mfVEP amplitude and latency

Intervisit difference (visit 2 – visit 1) for mfVEP amplitude (a, b, c) and latency (d, e, f) for individual MS eyes is plotted against the follow-up time. The solid and the dashed lines represent the 95% tolerance limits of TRV estimated from normal and ON eyes, respectively.

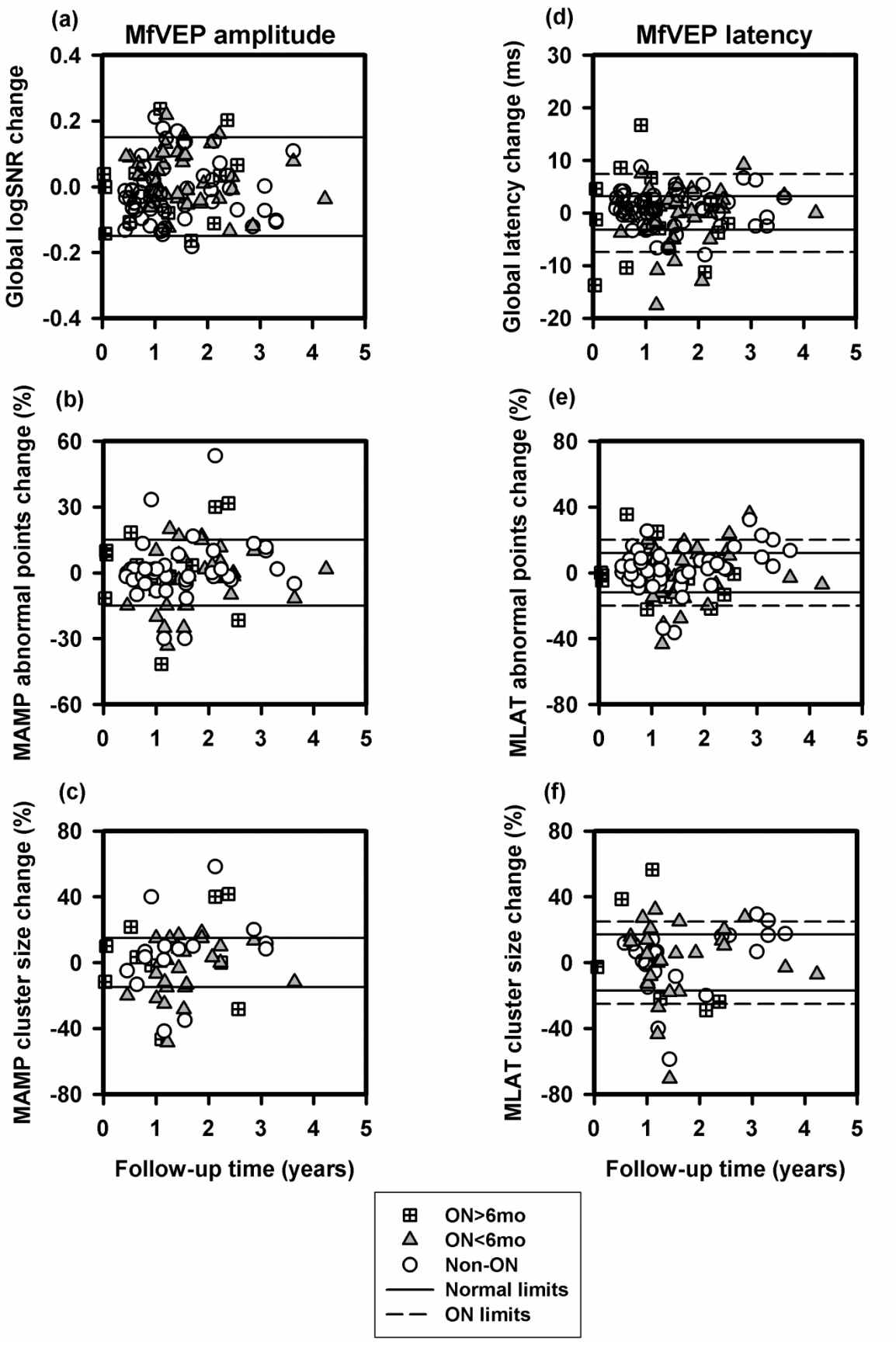
(a)
MfVEP amplitude
Global logSNR change
0.4
0.2
0.0
-0.2
-0.4
(d)
MfVEP latency
Global latency change (ms)
30
20
10
0
-10
-20
(b)
MAMP abnormal points change (%)
60
30
0
-30
-60
(e)
MLAT abnormal points change (%)
80
40
0
-40
-80
(c)
MAMP cluster size change (%)
80
40
0
-40
-80
(f)
MLAT cluster size change (%)
80
40
0
-40
-80
0 1 2 3 4 5
Follow-up time (years)
Follow-up time (years)
ON>6mo
ON<6mo
Non-ON
Normal limits
ON limits

Figure 3-3: Examples of intervisit change in MAMP probability plot

Examples of MS eyes that remained stable, improved, worsened in MAMP probability plots in the second visit when compared to the first visit

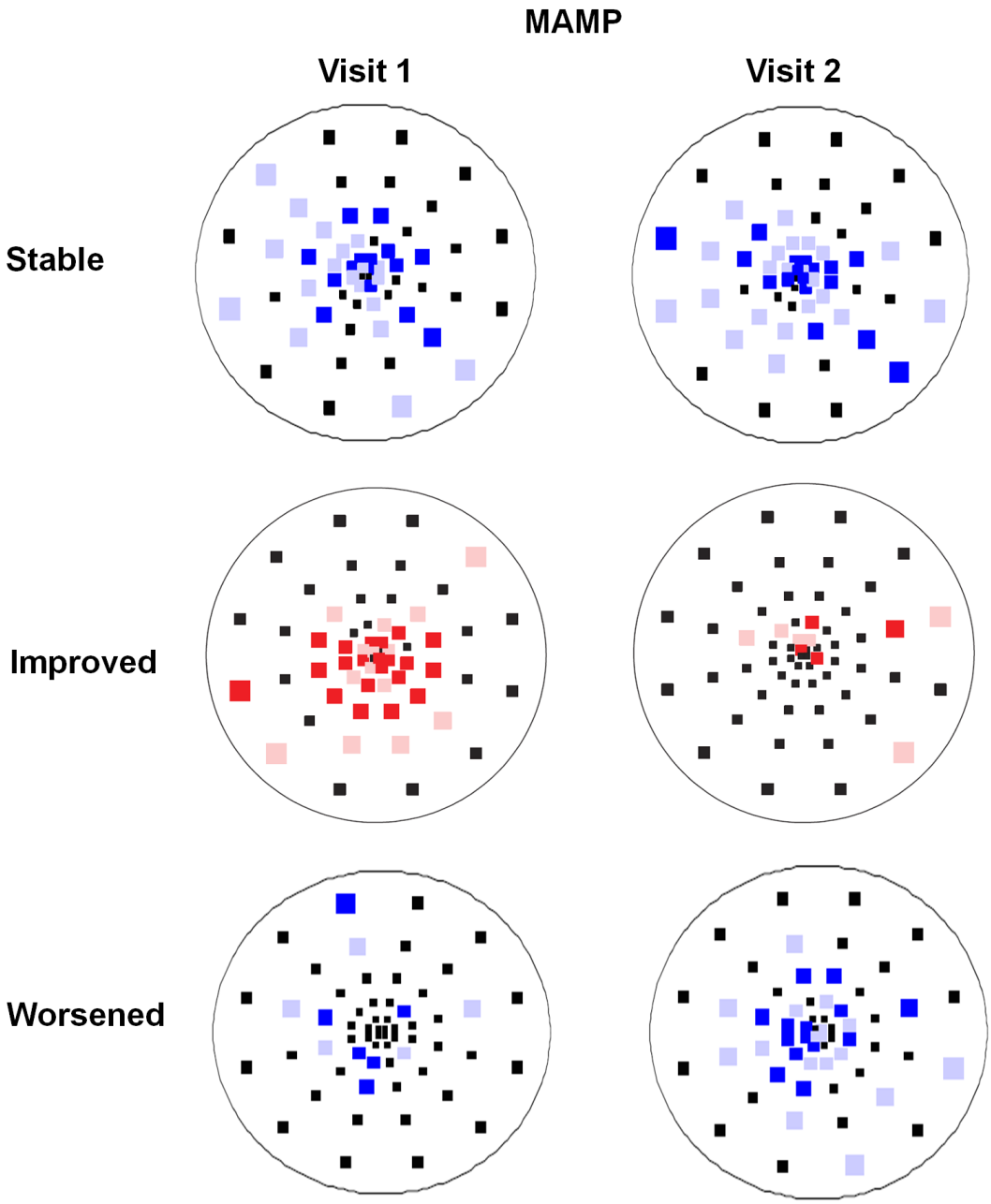
MAMP
Visit 1
Visit 2
Stable
Improved
Worsened

Figure 3-4: Examples of intervisit change in MLAT probability plot

Examples of MS eyes that remained stable, improved, worsened in MLAT probability plots in the second visit when compared to the first visit

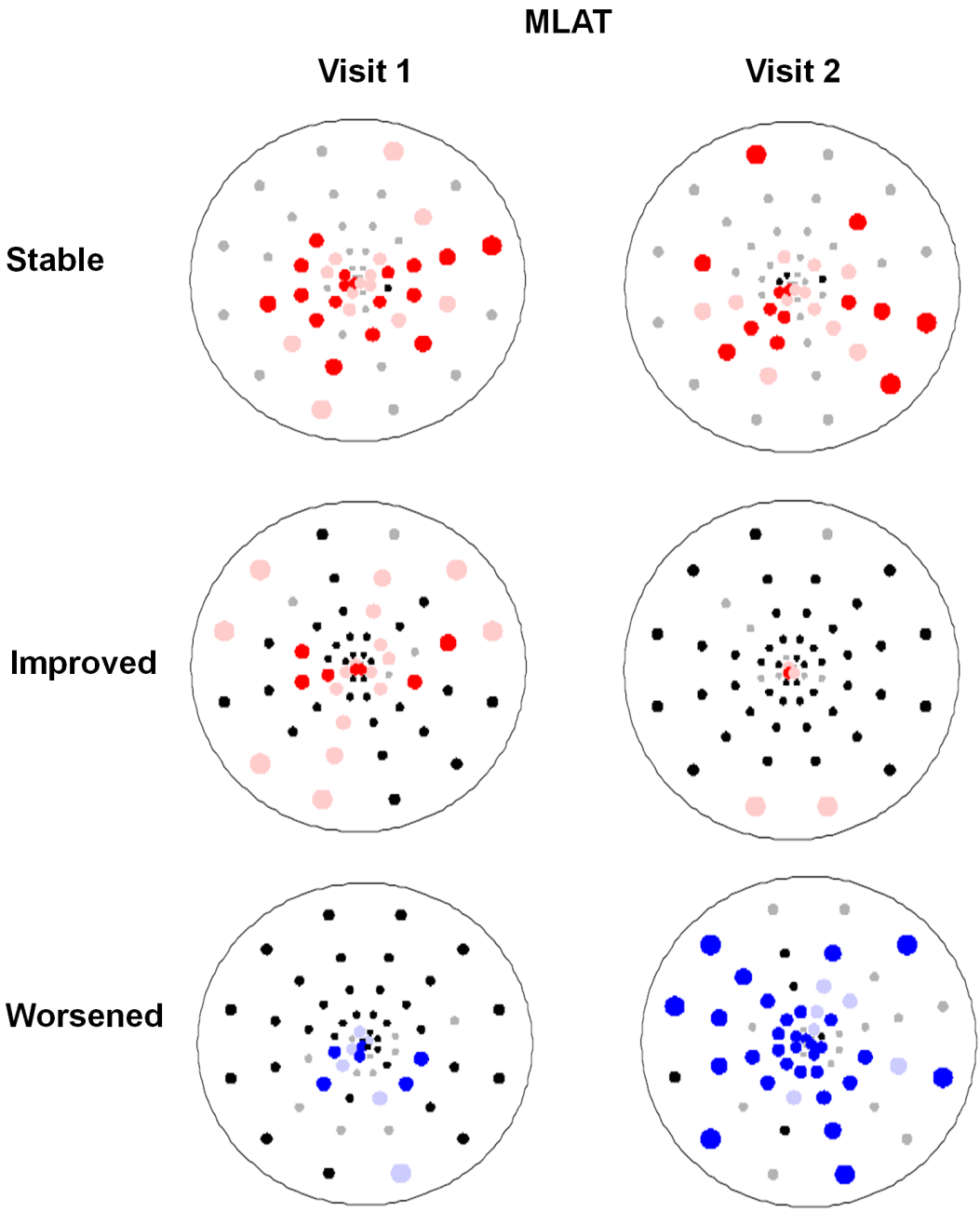
MLAT
Visit 1
Visit 2
Stable
Improved
Worsened

Figure 3-5: Latency change vs baseline latency and latency from multiple visits

Change in global latency (visit 2 – visit 1) is significantly correlated with baseline latency in non-ON (a) and ON (b) eyes. The solid lines in a & b are the fitted linear regression lines. MfVEP global latency from multiple visits in a small subset of non-ON (c) and ON eyes (d) depicts that latency changes in individual eyes generally follow a consistent trend instead of random variations.

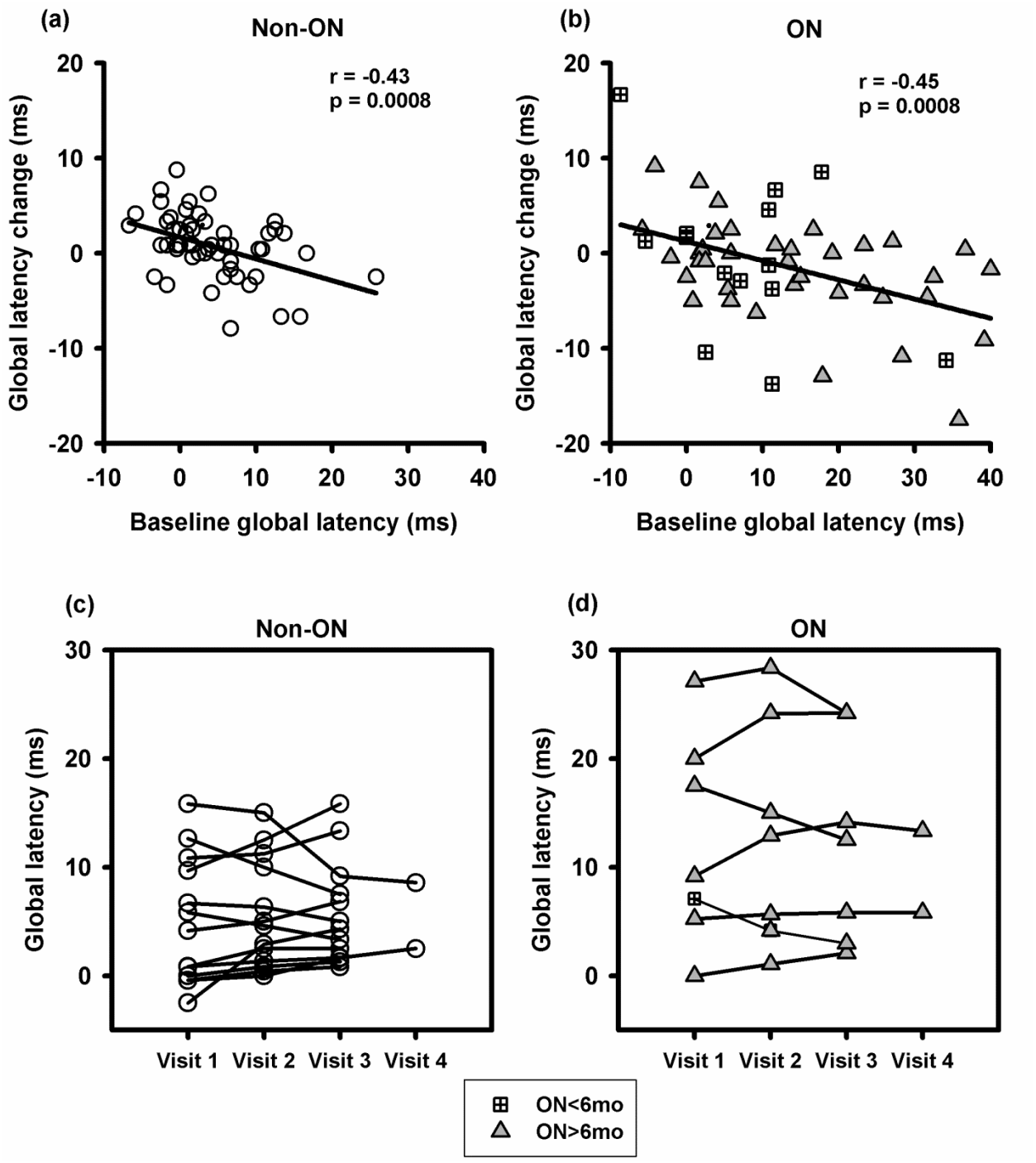
(a)
Non-ON
r = -0.43
p = 0.0008
Global latency change (ms)
Baseline global latency (ms)
(b)
ON
r = -0.45
p = 0.0008
Global latency change (ms)
Baseline global latency (ms)
(c)
Non-ON
Global latency (ms)
Visit 1 Visit 2 Visit 3 Visit 4
(d)
ON
Global latency (ms)
Visit 1 Visit 2 Visit 3 Visit 4
ON<6mo
ON>6mo

Figure 3-6: Intervisit difference for Pelli-Robson CS and HVF MD

Intervisit difference (visit 2 – visit 1) for Pelli-Robson CS (a) and HVF MD (b) for each MS eye is plotted against the follow-up time. The solid lines represent the 95% tolerance limits of TRV for each test.

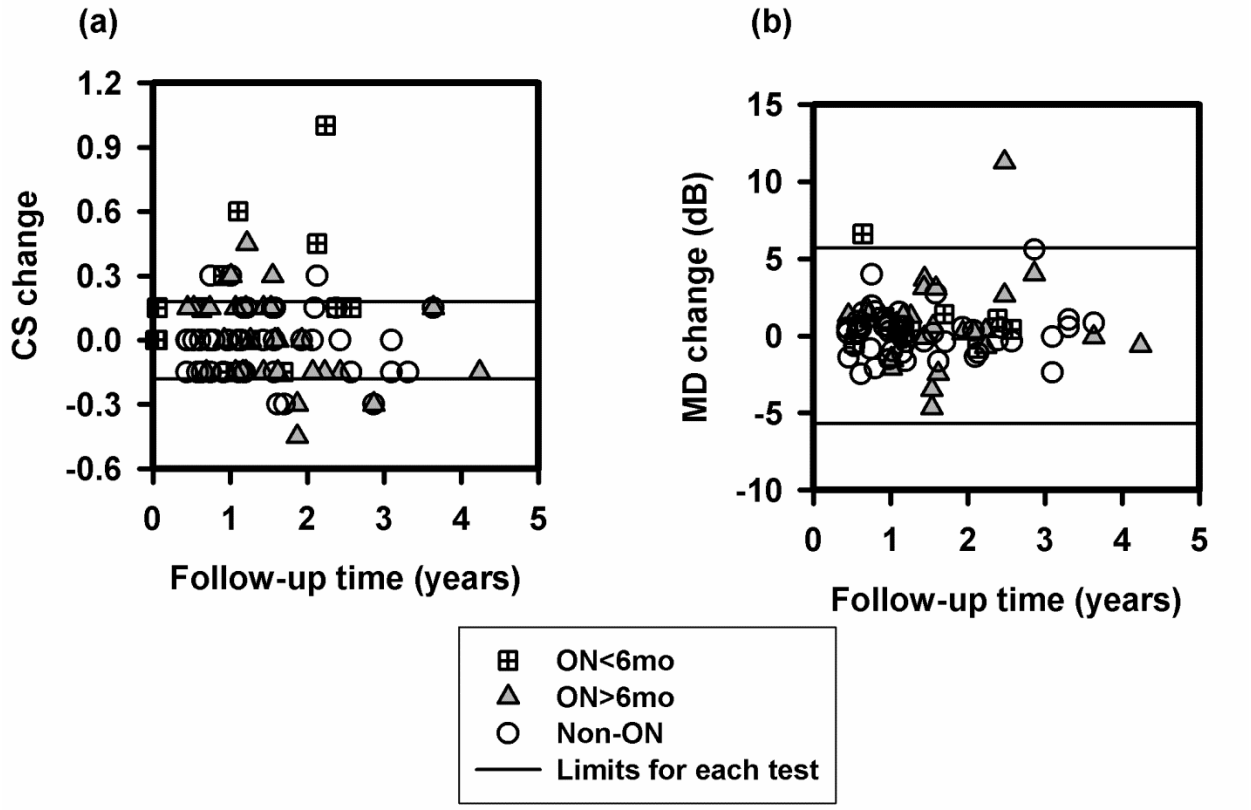

Figure 3-7: Correlation among functional measures

Changes in mfVEP global logSNR and latency correlated significantly with Pelli-Robson CS (a & b), but not with HVF MD (c & d).Change in global logSNR also correlated with change in global latency (e). The black solid and gray dashed lines represent fitted regression lines for non-ON and ON eyes respectively. Data points (71% to 78% eyes) within the gray shaded regions exhibited changes in the same direction (greater or less than zero) for the two parameters compared.

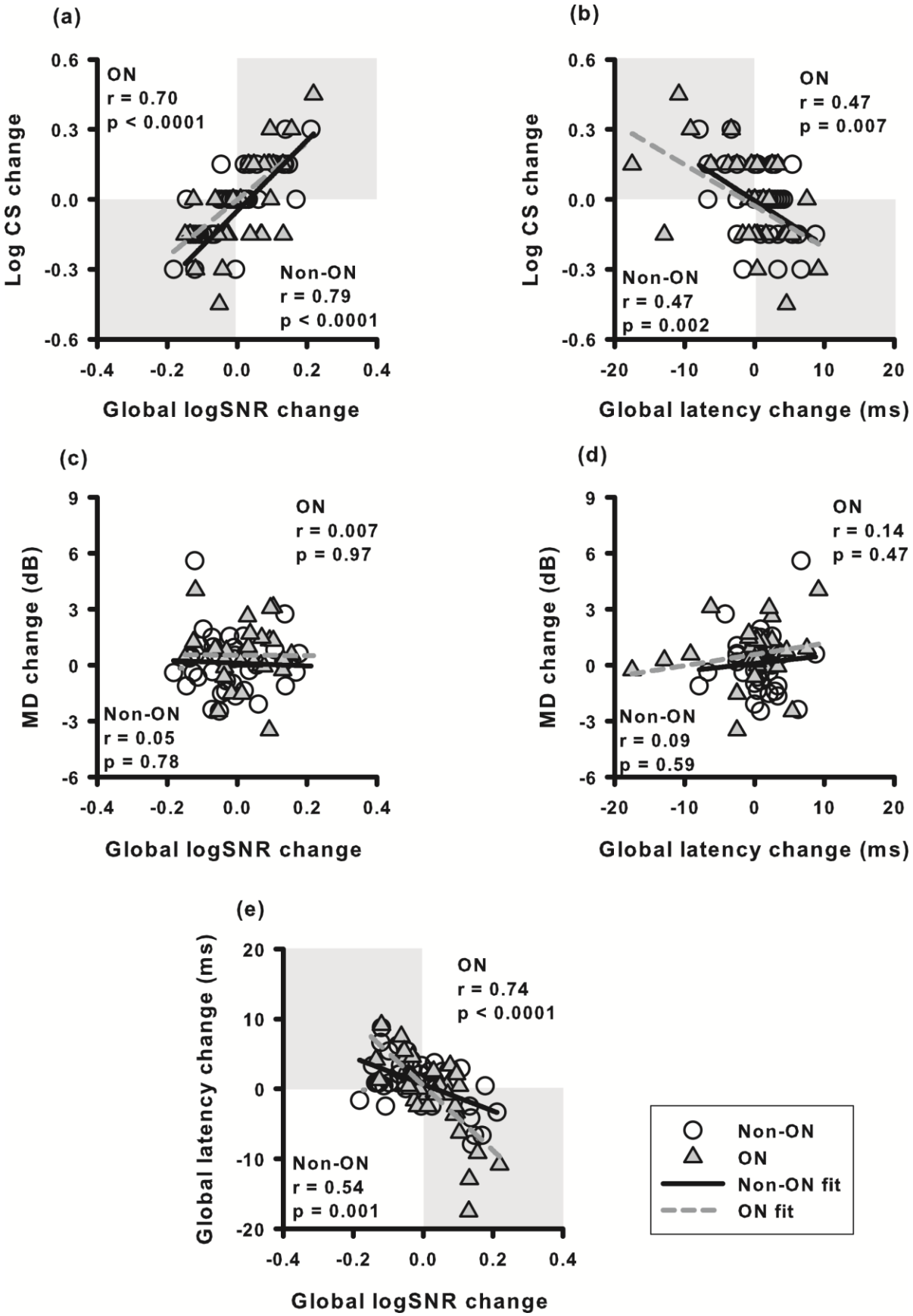
(a)
ON
r = 0.70
p < 0.0001
Non-ON
r = 0.79
p < 0.0001
Log CS change
Global logSNR change
(b)
ON
r = 0.47
p = 0.007
Non-ON
r = 0.47
p = 0.002
Log CS change
Global latency change (ms)
(c)
ON
r = 0.007
p = 0.97
Non-ON
r = 0.05
p = 0.78
MD change (dB)
Global logSNR change
(d)
ON
r = 0.14
p = 0.47
Non-ON
r = 0.09
p = 0.59
MD change (dB)
Global latency change (ms)
(e)
ON
r = 0.74
p < 0.0001
Non-ON
r = 0.54
p = 0.001
Global latency change (ms)
Global logSNR change
Non-ON
ON
Non-ON fit
ON fit

Table 3-1: 95% tolerance limits of TRV for various tests

	Normal	Non-ON	ON
MfVEP amplitude			
Global logSNR	±0.15	±0.15	±0.14
MAMP abnormal points (%)	±8	±15	±15
MAMP cluster size (%)	N/A	±15	±15
MfVEP latency			
Global relative latency (ms)	±3.3	±4.4	±7.4
MLAT abnormal points (%)	±8	±12	±20
MLAT cluster size (%)	N/A	±17	±25
Pelli-Robson CS (log unit)	±0.18	±0.19	±0.22
HVF 24-2 or 30-2 MD (dB)*	N/A	±5.7	±5.7

$s_{w:}$ within-subject standard deviation (Bland and Altman 1996)

* calculated from Wall et al 1998(Wall et al. 1998)

Table 3-2: Group means for various measurements

	Normals	Non-ON (n=57)		ON≥6 mo (n=39)		ON<6 mo (n=14)	
		Visit 1	Visit 2	Visit 1	Visit 2	Visit 1	Visit 2
MfVEP amplitude*							
Global logSNR	0.6±0.02	0.5±0.01[a]	0.5±0.01[a]	0.4±0.03[a,b]	0.4±0.03[a,b]	0.3±0.03[a,b]	0.4±0.02[a,b,c]
MAMP abnormal points (%)	2.8±0.4	10.1±1.8[a]	11.1±2.4[a]	33.0±4.1[a,b]	30.8±4.2[a,b]	39.2±4.4[a,b]	29.1±4.8[a,b,c]
MAMP cluster size (%)	NA	11.0±4.0	16.6±5.1	40.8±4.5[b]	37.2±4.9[b]	49.6±4.3[b]	39.5±4.3[b,c]
MfVEP latency*							
Global relative latency (ms)	0.7±0.5	4.1±0.8[a]	4.9±0.7[a]	15.2±2.2[a,b]	14.4±2.1[a,b]	12.2±2.8[a,b]	7.4±2.9[a,b,c]
MLAT abnormal points (%)	7.1±1.4	17.1±3.0[a]	19.1±2.6[a]	39.8±5.6[a,b]	41.1±5.6[a,b]	42.2±5.3[a,b]	32.6±5.6[a,b,c]
MLAT cluster size (%)	NA	24.0±5.9	25.3±4.8	47.9±6.8[b]	48.6±5.8[b]	50.5±4.8[b]	42.6±4.3[b,c]
CS*	1.6±0.06	1.6±0.03	1.6±0.03	1.4±0.06[a,b]	1.4±0.05[a,b]	1.0±0.01[a,b]	1.4±0.01[a,b,c]
HVF MD (dB)†	0.5±1.1	-1.8±0.3[a]	-1.7±0.3[a]	-5.1±1.0[a,b]	-4.4±0.9[a,b]	-6.2±1.1[a,b]	-3.3±1.1[a,b,c]

[a]p<0.05 compared to normal eyes
[b]p<0.05 compared to non-ON eyes
[c]p<0.05 comparison between visit 1 and visit 2
*Normative data from our lab
†Normative values reported by Wall et al(Wall et al. 1998)

Table 3-3: Percentage of eyes that exceeded tolerance limits for various tests

	Non-ON		ON≥6 mo only		ON<6 mo only	
	Improved	Worsened	Improved	Worsened	Improved	Worsened
MfVEP global logSNR	5%	2%	8%	0%	14%	7%
MAMP abnormal points	5%	4%	8%	10%	21%	14%
MAMP cluster size	6%	4%	5%	13%	21%	14%
MfVEP global latency	11%	21%	10%	10%	21%	14%
MLAT abnormal points	5%	16%	9%	8%	14%	6%
MLAT cluster size	4%	15%	10%	8%	14%	7%
CS	6%	6%	9%	9%	31%	0%
HVF MD	0%	0%	4%	0%	11%	0%

Table 3-4: Percentage of eyes that exceeded tolerance limits in mfVEP regions

	LogSNR			Latency		
	Improved/Worsened (%)			Shortened/Lengthened (%)		
Regions	**Non-ON**	**ON≥6 mo**	**ON<6 mo**	**Non-ON**	**ON≥6 mo**	**ON<6 mo**
1	7/11	3/0	21/7	5/16	16/14	31/14
2	5/4	5/0	21/7	5/18	13/15	15/8
3	7/9	5/0	14/7	4/23	18/16	14/7
4	0/4	3/0	14/0	11/13	9/12	18/9
5	5/7	3/0	14/0	18/14	11/14	14/4
6	0/0	3/0	7/0	12/11	8/11	23/0
7	2/2	0/0	7/0	12/11	8/13	18/0
8	2/7	3/0	7/0	13/3	16/16	17/8
9	0/2	0/0	7/0	7/16	16/16	20/4

Chapter 4 : Tracking Changes Over Time in Retinal Nerve Fiber Layer and Ganglion Cell Inner Plexiform Layer Thickness in Multiple Sclerosis

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Abstract

Background: Neurodegeneration plays an important role in permanent disability in multiple sclerosis (MS).

Objective: To determine whether progressive neurodegeneration occurs in MS eyes without clinically-evident inflammation.

Methods: Retinal nerve fiver layer thickness (RNFLT) and ganglion cell-inner plexiform layer thickness (GCIPT) were measured using Cirrus optical coherence tomography (OCT) in 133 relapsing-remitting MS (RRMS) patients (149 non-ON, 97 ON eyes, last optic neuritis (ON) ≥ 6 months). 93 patients were scanned at two visits. Percents of abnormal GCIPT vs RNFLT (< 5% of machine norms) in cross-sectional data were compared. Relations between RNFLT/GCIPT and MS duration (cross-sectional) and follow-up time (longitudinal) were assessed.

Results: GCIPT was abnormal in more eyes than RNFLT (27% vs 16% p = 0.004 in non-ON, 82% vs 72% p = 0.007 in ON). RNFLT and GCIPT decreased with MS duration by -0.49 µm/yr (p = 0.0001) and - 0.36 µm/yr (p = 0.005) for non-ON; - 0.52 µm/yr (p = 0.003) and - 0.41 µm/yr (p = 0.007) for ON. RNFLT and GCIPT decreased with follow-up time by - 1.49 µm/yr (p < 0.0001) and - 0.53 µm/yr (p = 0.004) for non-ON, - 1.27 µm/yr (p = 0.002) and - 0.49 µm/yr (p = 0.04) for ON.

Conclusions: In RRMS eyes without clinically-evident inflammation, progressive loss of RNFLT and GCIPT occurred, supporting the need for neuroprotection in addition to suppression of auto-immune responses and inflammation.

Introduction

Multiple sclerosis (MS), a chronic demyelinating and degenerative disease of the central nervous system (CNS), is the leading cause of non-traumatic neurological disability in young adults. The natural history of MS suggests that about 85% of patients initially experience a relapsing-remitting course (RRMS) and within 25 years of onset a high percentage transition into a secondary progressive phase (SPMS) (Weinshenker et al. 1989; Noseworthy et al. 2000) with continuous neurological decline leading to permanent disability. (Dutta and Trapp 2011) Currently available immuno-modulatory disease modifying therapies (DMT) have been successful in reducing inflammation, relapses (Castro-Borrero et al. 2012) and in slowing disease progression in RRMS.(Rudick et al. 2005) However, a recent study of MS patients with 15 years continuous use of an immune-modulatory drug reduced, but did not eliminate progression to SPMS. (Ford et al. 2010) While it is generally agreed that permanent disability in MS is a consequence of irreversible axonal loss, (Dutta and Trapp 2011) the underlying causes of MS and progression of the disease remain unclear.

MS eyes provide a unique opportunity to study axonal degeneration. The retinal ganglion cells (RGC) and their naturally unmyelinated axons in the eye can be evaluated in vivo using spectral domain optical coherence tomography (OCT), a highly reproducible imaging technique. Inflammation of the optic nerve, i.e., optic neuritis (ON), is typically detectable with signs and symptoms such as eye pain, loss of vision, reduced color vision, swelling and presence of relative afferent pupillary defects In this study, retinal nerve fiber layer thickness (RNFLT)

and retinal ganglion cell-inner plexiform thickness (GCIPT) were measured in two groups of “clinically-silent” RRMS eyes: eyes without a history of ON (non-ON group) and those with a previous history of ON but the inflammatory event was at least 6 months prior to the onset of the present study (ON group). Separating the no-ON and ON groups allowed us to tease apart neurodegenerative effects that could be due to previous overt inflammatory episodes in ON eyes from those in non-ON eyes lacking a history of clinically-evident inflammation. The change of RNFLT and GCIPT over time was analyzed cross-sectionally and longitudinally.

Methods

Subjects

One hundred thirty-one RRMS patients (Polman et al. 2005b) from the University of Houston MS Eye CARE Clinic were included in the study. All patients underwent comprehensive eye examination by an experienced neuro-ophthalmologist. ON was diagnosed based on clinical signs and symptoms. (Optic Neuritis Study Group 1991) To minimize the effect of edema and other sequelae of acute inflammation, eyes with last ON attack within 6 months of the OCT measurement or between the baseline and follow-up measurements were excluded. Patients with ocular or systemic conditions other than ON/MS that could potentially influence OCT measures were excluded.

Two hundred forty-seven eyes of 131 RRMS patients (85% on DMT) were included for cross-sectional analysis (Table 4-1). Seven eyes with acute ON, 3

with unclear ON history, 3 with other ocular abnormalities and 2 with OCT signal strength < 7 were excluded. Twelve (7 non-ON and 5 ON) eyes did not have GCIPT. Among the 247 eyes, 241 had spherical equivalent refractive error (RE) less than - 6.0D, 13 worse than - 6.0D (range - 6 to - 15 D, median - 7.5 D) and 6 eyes with unknown RE. Excluding these 19 eyes did not change the results reported below.

Table 4-1 near here

Ninety-two RRMS patients (164 eyes) from the cross-sectional cohort had OCT at two different visits and were included for longitudinal analysis (Table 4-2). The time interval between the baseline and the follow-up visit ranged from 2 months to 3.8 years (median 1.0 years). For all eyes in the longitudinal cohort, OCT data from the second visit was reported in the cross-sectional analysis in order to include more eyes with longer duration of MS. Seven eyes with acute ON at the baseline, 5 with ON attack between two visits, 4 eyes with unclear ON history, 1 with other ocular abnormalities and 3 eyes with signal strength < 7 in OCT in either of the visits were excluded. Fourteen (9 non-ON and 5 ON) eyes did not have GCIPT measured in one or both visits.

Table 4-2 near here

Procedures adhered to the tenets of Declaration of Helsinki, and the protocol was approved by the University of Houston Committee for the Protection of Human Subjects.

Optical coherence tomography procedure

OCT was performed using Cirrus-HD OCT 4000 version 6.5 (Carl Zeiss Meditec, Inc., Dublin, CA) by a trained ophthalmic technician. Peripapillary RNFLT was acquired with the Optic Disc Cube 200 x 200 protocol that images the optic disc in a 6 mm x 6 mm region. The mean RNFLT and those from individual quadrants were obtained. Macular GCIPT was obtained using the Macular Cube 512 x 128 protocol that images a 6 mm x 6 mm area centered at the fovea. The GCIPT was derived automatically by the machine software over an elliptical annulus (2 mm x 2.4 mm radius), excluding the central foveal region (0.5 mm x 0.6 mm radius). In this paper, RNFLT and GCIPT refer to the overall mean unless a quadrant is specified. Only images with signal strength ≥ 7 and good centration were included. No OCT outputs had erroneous segmentation upon visual inspection.

Statistics

Statistical analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC, USA). For the cross-sectional data, RNFLT and GCIPT were each defined as abnormal if the values were below 5% of their respective age-matched

machine norms. The percent of eyes with abnormal RNFLT was compared to that of GCIPT using McNemar test. The relation between RNFLT/GCIPT and MS duration (cross-sectional data), RNFLT/GCIPT change and follow-up time (longitudinal data) were analyzed using the GEMOND procedure with generalized estimating equation (GEE) to account for age and intra-subject inter-eye correlation. The probability of an abnormal RNFLT and GCIPT as a function of MS duration was modeled using GEE logistic regression. P values less than 0.05 were considered statistically significant.

Results

Cross-sectional analysis: OCT

Reduced RNFLT and GCIPT in non-ON and ON eyes

One hundred thirty-one RRMS patients (149 non-ON, 98 ON eyes) with MS duration ranging from < 1 month to 32 years were included for cross-sectional analysis (Table 4-1). The mean (± SD, µm) RNFLT (75.0 ± 14.0) and GCIPT (65.5 ± 11.9) in ON were significantly thinner than in non-ON eyes (RNFLT: 88.6 ± 11.5, GCIPT: 76.5 ± 9.2, $p < 0.0001$ for both). Both non-ON and ON eyes had significantly thinner measures compared to normative RNFLT (92.8 ± 9.4 µm) and GCIPT (82.1 ± 6.2 µm) for Cirrus OCT (Mwanza et al. 2011a) ($p < 0.0001$ for all comparisons). Twenty-five percent of ON eyes (n = 25) had more than one ON episode in the same eye (recurrent ON) and the mean RNFLT and GCIPT (µm) in these eyes were significantly thinner (67.1 ± 10.9 and 59.4 ± 11.9)

than in eyes with single ON attacks (77.3 ± 14.1 and 67.3 ± 11.2, $p < 0.001$for both).

Linear regression between RNFLT/GCIPT and MS duration

To determine the effects of long term MS on neuronal loss, we performed linear regression between RNFLT/GCIPT and MS duration using GEE model to correct for age and intra-subject inter-eye correlation (Figure 4-1). RNFLT decreased at a mean rate (µm/yr) of - 0.49 ($p = 0.0001$, Figure 4-1a) in non-ON and - 0.52 ($p = 0.002$, Figure 4-1b) in ON, and these two slopes were not significantly different ($p = 0.90$). To examine whether RNFLT loss in non-ON eyes was influenced by an ON history in the fellow eye, we analyzed bilateral non-ON eyes ($n = 95$) separately; which showed a slope of - 0.37 µm/yr, not different from that for all non-ON eyes ($p = 0.56$). In ON eyes, thinner RNFLT with longer MS might be partially attributed to more recurrent ON events over time. Interestingly, eyes with only one episode of ON ($n = 73$) showed a slightly steeper slope (- 0.88 µm/yr) than that of the whole ON group (- 0.52 µm/yr) although they were not significantly different ($p = 0.17$). RNFLT in individual quadrants also decreased with MS duration in non-ON and ON eyes (Table 4-3, cross-sectional data on the left). GCIPT decreased with MS duration at a mean rate (µm/yr) of - 0.36 ($p = 0.005$, Figure 4-1c) in non-ON and - 0.41 ($p = 0.007$, Figure 4-1d) in ON, and these two slopes were not different ($p = 0.81$). GCIPT

slope (µm/yr) for bilateral non-ON eyes was - 0.33 ($p = 0.02$) and single ON eyes was - 0.67 ($p < 0.0001$).

Figure 4-1 near here

Table 4-3 near here

Abnormalities in GCIPT/ RNFLT and logistic regression

It is of clinical interest to detect neuronal loss on an individual basis; and to account for the effects of normal aging we examined RNFLT and GCIPT from individual eyes and classified them as abnormal if the values were below 5% of their respective age-matched machine norms. Notably, more eyes showed abnormal GCIPT than RNFLT (Table 4-4). Percent of eyes showing abnormal GCIPT vs RNFLT was 27% vs 16% ($p = 0.004$) in non-ON and 82% vs 72% ($p = 0.007$) in ON. GCIPT also was abnormal in more eyes than RNFLT when only the temporal quadrant of RNFLT, the receiving region for the macular fibers, was considered, 27% vs 15% ($p = 0.008$) in non-ON and 82% vs 66% ($p = 0.003$) in ON eyes. To examine whether more abnormal RNFLT/GCIPT existed in eyes with longer MS duration, we performed a logistic regression using MS duration as a continuous independent variable and the status of RNFLT/GCIPT (normal or abnormal) as a categorical dependent variable. In non-ON eyes, the probability of abnormal GCIPT increased significantly with MS duration ($p = 0.03$, Figure 4-2 a), however, for RNFLT the relation failed to reach statistical significance ($p = 0.56$). In ON eyes, the probability of abnormal RNFLT (Figure 4-2 b) and GCIPT

(Figure 4-2 c) both increased significantly with MS duration (p = 0.01 for RNFLT, p = 0.02 for GCIPT).

Figure 4-2 near here

Table 4-4 near here

Longitudinal analysis: OCT

RNFLT and GCIPT change as a function of follow-up time

Ninety-two RRMS patients (96 non-ON, 68 ON eyes) had two OCT measurements separated by a follow-up time up to 3.8 years (median 1 year) (Table 4-2). The rate of change was obtained by performing linear regression between the inter-visit change in RNFLT/GCIPT and follow-up time for non-ON and ON groups. RNFLT decreased with increase in follow-up time at a rate (µm/yr) of - 1.49 ($p < 0.001$) in non-ON (Figure 4-3 a) and - 1.27 (p = 0.002) in ON (Figure 4-3 b) and the slopes were not different (p = 0.64). To eliminate any possible effect of having ON history in the fellow eye, we analyzed bilateral non-ON eyes (n = 53) separately. The rate of RNFL loss was - 1.6, not different from that (- 1.49) for all non-ON eyes (p = 0.83). RNFLT in individual quadrants also decreased with follow-up time in non-ON or ON eyes (nasal and temporal quadrant in ON did not reach statistical significance) (Table 4-3, longitudinal data on the right). GCIPT decreased with follow-up time at a rate (µm/yr) of - 0.53 (p = 0.004) in non-ON (Figure 4-3 c) and - 0.49 (p = 0.04) in ON (Figure 4-3 d) and

the slopes were not different (p = 0.90). The rate of GCIPT loss in bilateral non-ON eyes was - 0.69, not different from that (- 0.53) for all non-ON eyes (p = 0.56). The association between RNFLT/GCIPT reduction and follow-up time remained significant for non-ON and ON eyes even after accounting for other co-variables such as age, MS duration, baseline RNFLT and time from last ON event (for ON eyes only) (Table 4-5).

Figure 4-3 near here

Table 4-5 near here

Humphrey visual field (HVF) in cross-sectional and longitudinal analyses

No association was found between HVF mean deviation (MD) and MS duration in cross-sectional analysis (Figure 4-4 a and b: slope = - 0.05 dB/yr, p = 0.42 in non-ON; slope = - 0.004 dB/yr, p = 0.95 in ON), or inter-vist change in MD and follow-up time in longitudinal analysis (Figure 4-4 c and d: slope = - 0.25 dB/yr, p = 0.15 in non-ON; slope = -0.24 dB/yr, p = 0.62 in ON). In the longitudinal cohort, the mean MD was similar during baseline and follow-up visits for non-ON (- 1.3 dB vs - 1.0 dB, p = 0.26) and ON (- 2.9 dB vs - 2.7 dB, p = 0.97) (Table 4-2). When the slope of MD vs follow-up time was calculated for individual eyes, the mean (±SE) of individual slopes was - 0.12 ± 0.20 dB/yr for non-ON and - 0.13 ± 0.23 for ON. HVF MD probably did not show a significant association with duration of disease or follow-up time because of the large inter-

subject variability in our study (Figure 4-4 a and b) and the previously documented poor repeatability in ON eyes. (Wall et al. 1998)

Figure 4-4 near here

Discussion

Our results clearly indicate that progressive loss of RNFLT and GCIPT occurs in the absence of clinically-evident inflammation in RRMS eyes. Progressive neuronal loss observed in non-ON eyes was not due to effects from ON in the contralateral eye as indicated by similar results in bilateral non-ON eyes. In the current study, the rates of change in RNFLT in cross-sectional (around - 0.5 µm/yr for both non-ON and ON eyes) and longitudinal data (- 1.49 µm/yr in non-ON and - 1.27 µm/yr in ON eyes) are both greater that of the normal age-related loss reported for Cirrus HD OCT: - 0.19 µm/yr in one cross-sectional study, (Knight et al. 2012) - 0.37 µm/yr in another, (Celebi and Mirza 2013) and - 0.52 µm/yr in a longitudinal study. (Leung et al. 2012) It is interesting to note that the rate of change in RNFLT obtained from cross-sectional analysis is less than that from longitudinal analysis for both normal and MS subjects. This emphasizes that rate comparison is only reasonable among studies with similar designs. The rate of change in RNFLT may not be a constant throughout the entire disease course therefore caution should be exercised in extrapolating results from a short time interval to long term effect. In our previous unpublished study using Stratus OCT, we obtained similar findings.

The rate of GCIPT loss in our cross-sectional data was about - 0.4 µm/yr for both non-ON and ON eyes, about 2.8 times the rate of normal aging (- 0.14 µm/yr) as reported in Mwanza's cross-sectional study of normal subjects between 18 and 84 years of age, their Figure 6a. (Mwanza et al. 2011a) The rate of GCIPT loss in our longitudinal analysis (- 0.49 µm/yr in non-ON and - 0.53 µm/yr in ON eyes), was about 60% greater than - 0.32 µm/yr reported by Leung et al (Leung et al. 2012) in normal subjects. Similarly Ratchford et al (Ratchford et al. 2013) reported GCIPT loss of - 0.37 µm/yr in MS and - 0.20 µm/yr in normals. As previously reported, (Garcia-Martin et al. 2011) the rate of neuronal loss was similar for non-ON and ON eyes, indicating that documented acute inflammatory episodes had little impact on the rate of progression six months beyond the event.

Consistent with linear regression, our logistic regression analysis showed that proportion of abnormal eyes with respect to GCIPT significantly increased with MS duration. By 30 years of MS, more than 50% of the no-ON eyes and 100% of the ON eyes would have significantly thinner GCIPT compared to age-matched normal individuals.

MS is traditionally viewed as a primary autoimmune disease in which inflammatory T cells cross the blood brain barrier and attack the central nervous system (CNS), causing demyelination and axonal degeneration. (Compston and Coles 2008) Recently, Stys et al (Stys et al. 2012) proposed that cytodegeneration of oligodendrocyte-myelin complex and underlying axons could be the primary event, and antigenic debris released as a consequence promotes

a secondary inflammatory immune response in a susceptible host. (Stys et al. 2012) Our finding that in the absence of clinically-evident inflammation, neurodegeneration progresses over time, supports the possibility of primary neurodegenerative process in MS. However, we could also explain our results based on the primary autoimmune model, considering that there might be undetectable subclinical episodes of ON and/or chronic low-level inflammation in optic nerve. Though there is evidence of subclinical demyelination as demonstrated by delayed latency of visual evoked potentials in non-ON MS eyes, (Laron et al. 2009) the presence of subclinical demyelination per se does not prove that the initiating trigger is autoimmune in nature, as it could also be interpreted as primary degeneration of myelin and underlying axons without sufficient antigenic myelin debris to initiate a secondary inflammatory immune response. (Stys et al. 2012) RGC axonal loss could also result from retrograde degeneration from lesions in optic tract and/or posterior visual pathway. In our study, none of the subjects showed homonymous visual field defects characteristic of optic tract lesion, although optic tract lesions could also be asymptomatic. (Davies et al. 1998) Lesions in the optic radiations could potentially lead to loss of RGC axons/neurons via retrograde transynaptic degeneration. Although there is some recent evidence of transynaptic degeneration in patients with acquired occipital lobe/optic radiation damage due to stroke, (Jindahra et al. 2012) this is yet to be demonstrated in MS.

In our study, more eyes showed abnormal GCIPT than mean or temporal RNFLT. GCIPT is likely more sensitive for detecting abnormal eyes (values

outside of the norms) because it has less normal variation than RNFLT. In the normal population, the distribution of GCIPT had smaller coefficient of variation (CoV) (7.6%) than mean RNFLT (10%) and temporal RNFLT (16%) (calculated from Mwanza et al 2011 (Mwanza et al. 2011a) and Knight et al 2012 (Knight et al. 2012)). Anatomically, the intersubject variability for RGC counts is lowest in the parafoveal region, but highest at far superior and inferior retina, predicting larger variability in peripapillary axons than central RGC counts. (Curcio and Allen 1990) For OCT, differences in optic nerve head size, blood vessel patterns and glial content across normal subjects may also contribute to higher variability in RNFLT. In fact in MS eyes it has been shown that GCIPT is less confounded by axonal edema and gliosis (Syc et al. 2012; Green et al. 2010) and therefore correlates better with visual dysfunction, (Saidha et al. 2011) clinical and radiological markers of disease activity (Ratchford et al. 2013) than RNFLT. Another possibility is that RGC somas atrophy before the axons even though in retrograde degeneration, axonal pathology/dysfunction precedes that of ganglion cell body. Fairless et al (Fairless et al. 2012) demonstrated in a rat model of MS, that at the time of significant RGC soma loss, the axons appeared to be intact in numbers but showed ultrastructural signs of degeneration. Recent studies in glaucoma demonstrated the value of measuring macular RGC. (Hood et al. 2013) Compared to temporal RNFLT, GCIPT measurements are more reproducible (Mwanza et al. 2010; Mwanza et al. 2011b) and might be a better measure to reflect macular damage. Therefore we believe that GCIPT is a great addition to tests for detecting and tracking neuronal changes in MS eyes.

(Ratchford et al. 2013; Saidha et al. 2011) In fact, in our longitudinal data, more non-ON eyes showed worsening based on GCIPT than RNFLT (see data points below the lower test-retest variability limits in Figure 4-3 a, c).

In summary our data showed increased loss of RNFLT and GCIPT with increase in MS duration and follow-up time. There were significantly more abnormal eyes in the GCIPT measurements than in the RNFLT measurements for both no-ON and ON eyes. Progressive neuronal loss, in the absence of clinically-evident inflammation, suggests a significant role for neurodegeneration in RRMS, traditionally viewed as an autoimmune disease. New therapeutic options should focus on remyelination and neuroprotection in addition to reducing inflammation and relapses.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

Figures and Tables

Figure 4-1: RNFLT vs MS duration and GCIPT vs MS duration

Scatter plot showing RNFLT vs MS duration (a and b) and GCIPT vs MS duration (c and d) for individual non-ON and ON eyes. Each symbol (circles for RNFLT and squares for GCIPT) represents an individual eye of a patient. Open symbols represent non-ON and filled symbols represent ON eyes. The solid lines are the fitted linear regression lines (GEE models). The dashed lines are the average reported normative values for a cohort between 18 and 84 years of age. (Mwanza et al. 2011a)

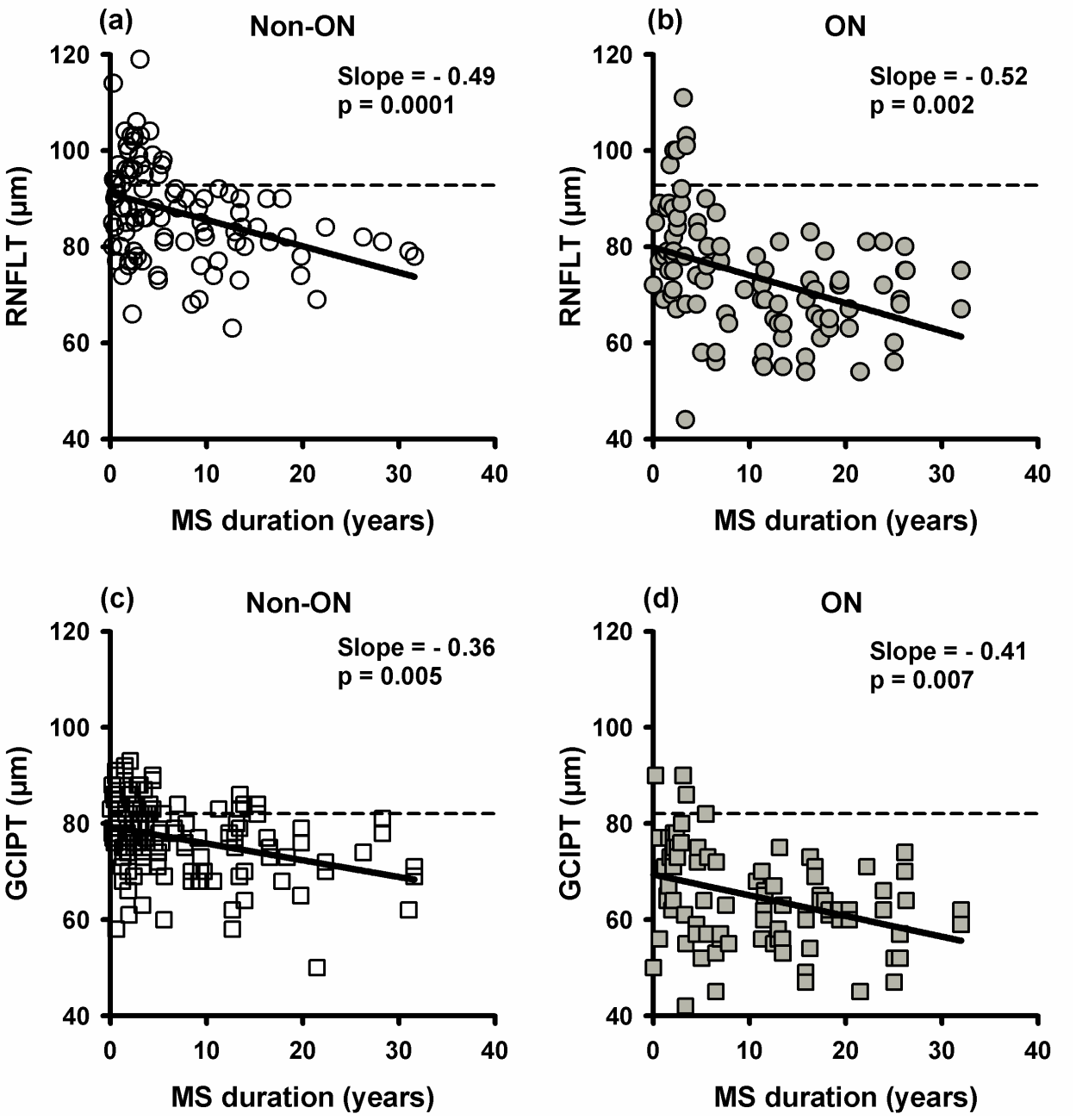
(a)
Non-ON
Slope = - 0.49
p = 0.0001
RNFLT (µm)
MS duration (years)
(b)
ON
Slope = - 0.52
p = 0.002
RNFLT (µm)
MS duration (years)
(c)
Non-ON
Slope = - 0.36
p = 0.005
GCIPT (µm)
MS duration (years)
(d)
ON
Slope = - 0.41
p = 0.007
GCIPT (µm)
MS duration (years)

Figure 4-2: Probability of abnormal RNFLT and GCIPT vs MS duration

Logistic regression analysis showing increase in the probability of abnormalities with MS duration for GCIPT non-ON (a), RNFLT ON (b) and GCIPT ON (c). Circles represent the raw data (0 for normal, 1 for abnormal). The line is the fitted logistic regression curve (GEE model).

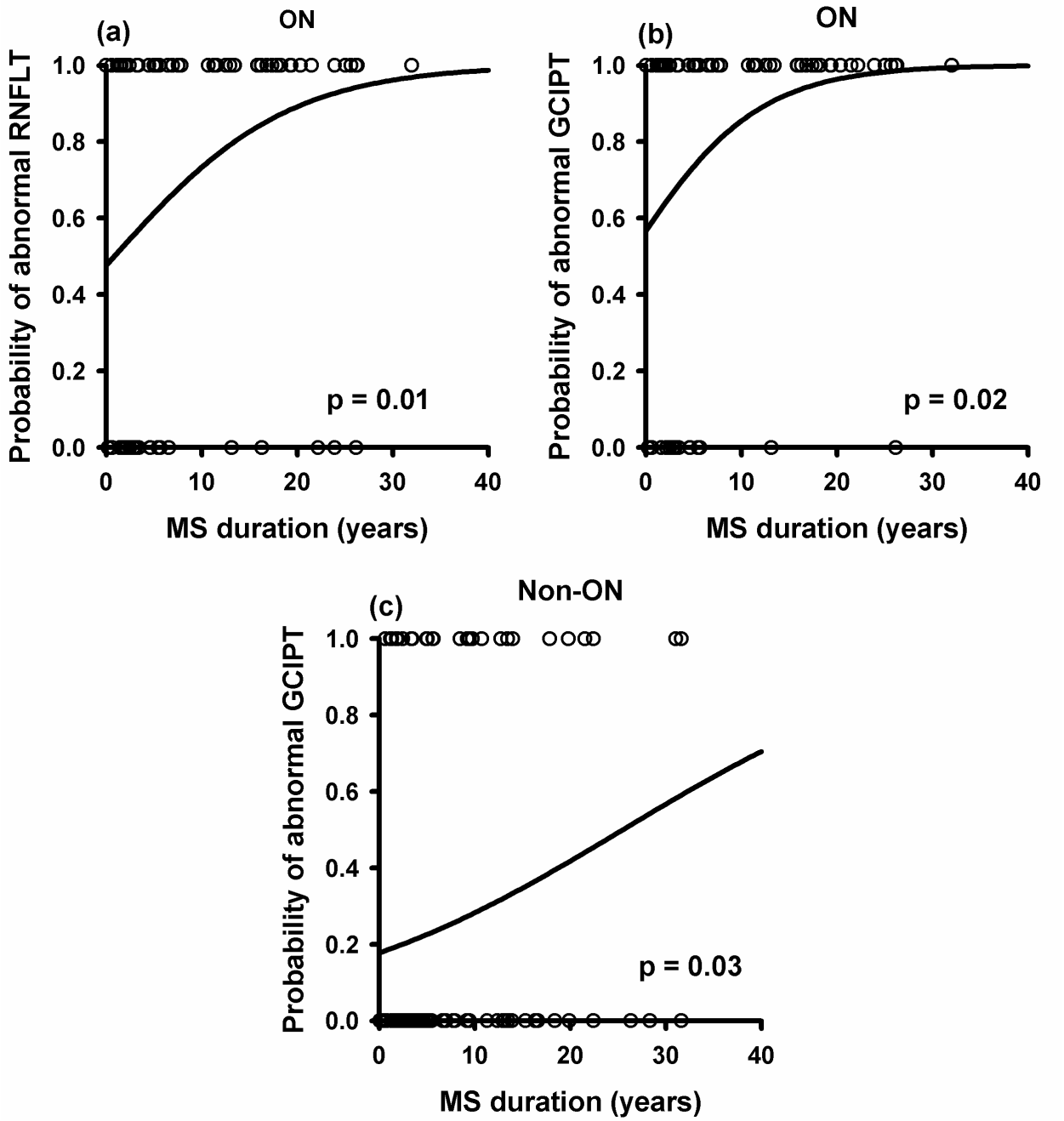
(a)
ON
Probability of abnormal RNFLT
1.0
0.8
0.6
0.4
0.2
0.0
p = 0.01
0
10
20
30
40
MS duration (years)
(b)
ON
Probability of abnormal GCIPT
1.0
0.8
0.6
0.4
0.2
0.0
p = 0.02
0
10
20
30
40
MS duration (years)
(c)
Non-ON
Probability of abnormal GCIPT
1.0
0.8
0.6
0.4
0.2
0.0
p = 0.03
0
10
20
30
40
MS duration (years)

Figure 4-3: RNFLT change vs follow-up time and GCIPT change vs follow-up time

Scatter plot showing relationship between the RNFLT change (a and b) and GCIPT change (c and d) with follow-up time for non-ON and ON eyes. Each symbol (circles for RNFLT and squares for GCIPT) represents an individual eye of a patient. Open symbols represent non-ON eyes and filled symbols represent ON eyes. The solid lines are the fitted linear regression lines (GEE models). The dashed lines are the test-retest variability limits reported for Cirrus OCT. (Mwanza et al. 2010; Mwanza et al. 2011b)

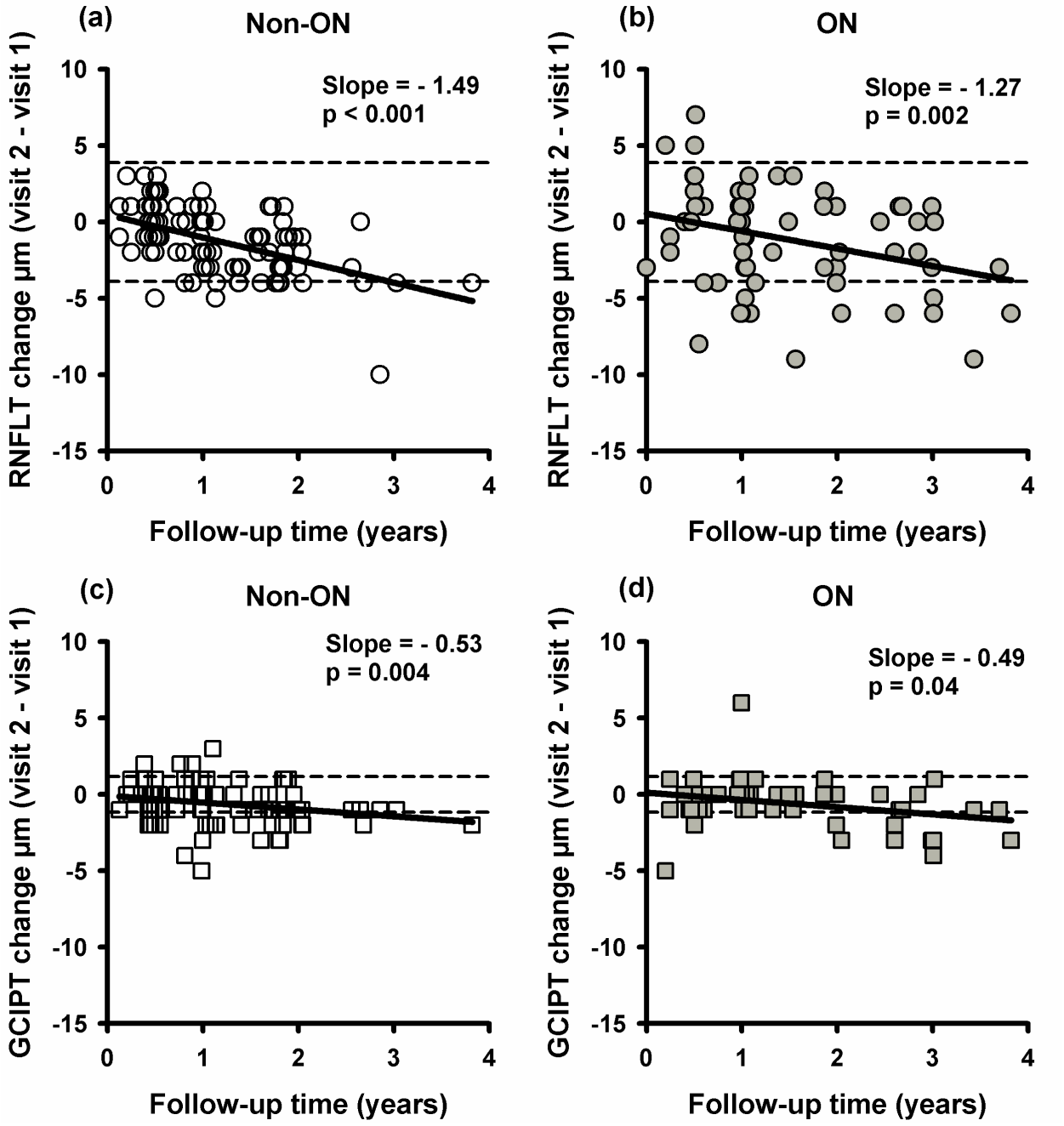
(a)
Non-ON
Slope = - 1.49
p < 0.001
RNFLT change µm (visit 2 - visit 1)
Follow-up time (years)
(b)
ON
Slope = - 1.27
p = 0.002
RNFLT change µm (visit 2 - visit 1)
Follow-up time (years)
(c)
Non-ON
Slope = - 0.53
p = 0.004
GCIPT change µm (visit 2 - visit 1)
Follow-up time (years)
(d)
ON
Slope = - 0.49
p = 0.04
GCIPT change µm (visit 2 - visit 1)
Follow-up time (years)

Figure 4-4: HVF MD vs MS duration and HVF MD vs follow-up time

Scatter plot of MD vs MS duration (a and b) and MD change vs follow-up time (c and d) for no-ON and ON eyes. Each symbol represents an individual eye of a patient. Open symbols represent no-ON eyes and filled symbols represent ON eyes. The solid lines are the fitted linear regression lines (GEE models).

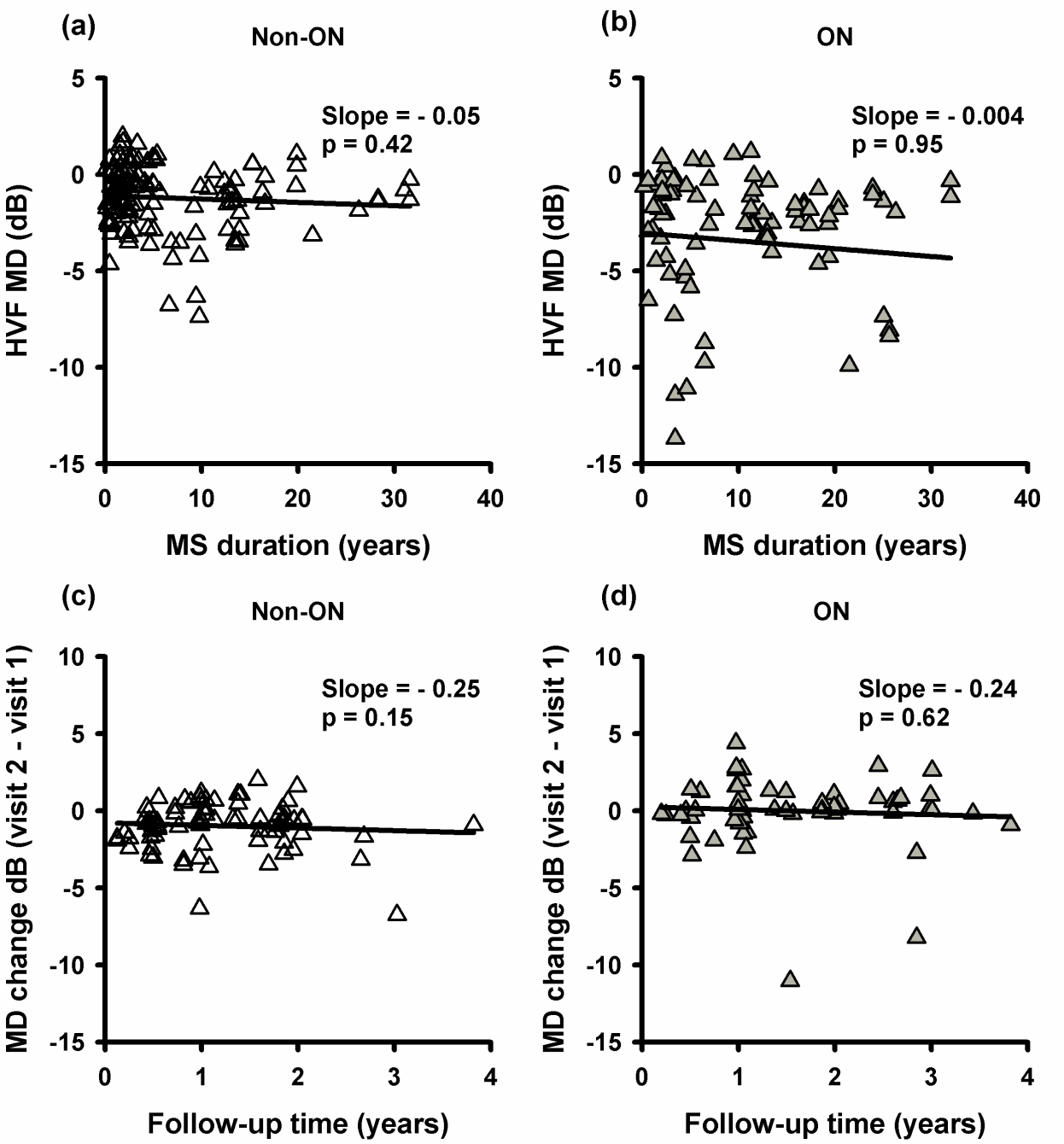
(a)
Non-ON
Slope = - 0.05
p = 0.42
HVF MD (dB)
MS duration (years)
(b)
ON
Slope = - 0.004
p = 0.95
HVF MD (dB)
MS duration (years)
(c)
Non-ON
Slope = - 0.25
p = 0.15
MD change dB (visit 2 - visit 1)
Follow-up time (years)
(d)
ON
Slope = - 0.24
p = 0.62
MD change dB (visit 2 - visit 1)
Follow-up time (years)

Table 4-1: Demographic and clinical characteristics (cross-sectional analysis)

Age (years, mean±SD, range)	43.4±11.1, 20.7 to 69.9	
F:M	4.3:1	
MS duration(years, mean±SD, range)	8.5±8.0, <1 month to 32	
	Non-ON eyes (n=149)	**ON eyes (n= 98)**
VA 20/20 or better (%)	140 (94%)	62 (63%)
HVF* MD±SE (dB)	-1.3±0.2	-3.2±0.5
Average RNFLT** (µm)	88.6±0.9	75.0±1.4
Superior RNFLT** (µm)	112.1±1.5	93.4±2.0
Nasal RNFLT** (µm)	67.2±0.9	61.2±1.1
Inferior RNFLT** (µm)	117.9±1.6	99.1±2.5
Temporal RNFLT** (µm)	56.9±1.1	47.1±1.4
Average GCIPT** (µm)	76.5±0.8	65.5±1.2
1 ON attack (%)	NA	73 (75%)
>1 ON attack (%)	NA	25 (25%)
Time since last ON (years, ±SD)	NA	8.5 ± 8.0

* HVF refers to Humphery visual field 30-2 or 24-2 SITA standard or SITA fast threshold tests with fixation loss, false positives and false negatives <33%.

** mean±SE

Table 4-2: Demographic and clinical characteristics (longitudinal analysis)

	Baseline		Follow-up	
Age (years, ±SD)	42.5±11.8		43.9±11.9	
F:M	4.4:1		4.4:1	
MS duration(years, ±SD)	7.2±7.6		8.6±7.7	
	Non-ON eyes (n=96)		**ON eyes (n=68)**	
	Baseline	**Follow-up**	**Baseline**	**Follow-up**
VA 20/20 or better (%)	94 (98%)	94 (98%)	49 (72%)	49 (72%)
HVF* MD±SE (dB)	-1.3±0.2	-1.0±0.2	-2.9±0.4	-2.7±0.5
Average RNFLT** (µm)	90.7±1.2	89.4±1.2	75.1±2.3	74.4±1.8
Superior RNFLT** (µm)	115.2±1.9	112.3±1.8	94.3±2.7	92.0±2.6
Nasal RNFLT** (µm)	69.9±1.4	68.7±1.3	62.8±1.6	61.8±1.5
Inferior RNFLT** (µm)	120.5±2.1	119.3±2.1	99.2±3.0	97.7±3.0
Temporal RNFLT** (µm)	57.3±1.3	56.1±1.3	46.7±1.6	46.1±1.7
Average GCIPT** (µm)	77.5±1.0	76.8±1.0	65.1±1.6	64.9±1.4
1 ON attack (%)	NA	NA	54 (79%)	54 (79%)
>1 ON attack (%)	NA	NA	14 (21%)	14 (21%)
Time since last ON (years, ±SD)	NA	NA	7.4±7.7	8.9±7.9

* HVF refers to Humphery visual field 30-2 or 24-2 SITA standard or SITA fast threshold tests with fixation loss, false positives and false negatives <33%.

** mean±SE

Table 4-3: Slope and p values from linear regression for RNFLT and GCIPT

	Cross-sectional Slope, μm/yr* (p value)		Longitudinal Slope, μm/yr** (p value)	
	Non-ON	**ON**	**Non-ON**	**ON**
Average RNFLT	-0.49 (0.001)	-0.52 (0.002)	-1.49 (<0.001)	-1.27 (0.002)
Superior RNFLT	-0.62(0.01)	-0.63 (0.01)	-2.18 (<0.001)	-2.47 (0.0006)
Nasal RNFLT	-0.24 (0.02)	-0.29 (0.01)	-1.58 (0.007)	-0.83 (0.23)
Inferior RNFLT	-0.54 (0.05)	-0.58 (0.05)	-1.41 (0.01)	-1.36 (0.01)
Temporal RNFLT	-0.58 (0.0001)	-0.54 (0.0008)	-1.54 (0.03)	-0.59 (0.15)
Average GCIPT	-0.36 (0.005)	-0.41(0.007)	-0.53 (0.004)	-0.49 (0.04)

*analysis of RNFLT vs MS duration

**analysis of RNFLT vs Follow-up time

Table 4-4: Percents of eyes with abnormal GCIPT and RNFLT

	Average GCIPT	Average RNFLT (p value)	Temporal RNFLT (p value)
Non-ON	27%	16% (0.004)	15% (0.008)
ON	82%	72% (0.007)	66% (0.003)

Table 4-5: Multivariate linear regression between RNFLT/GCIPT change and co-variables

	RNFLT Slope, μm/yr (p value)		GCIPT Slope, μm/yr (p value)	
Co-variables	**Non-ON**	**ON**	**Non-ON**	**ON**
Follow-up time	-1.42 (<0.001)	-1.0 (0.02)	-0.59 (0.01)	-0.57 (0.004)
Age at follow-up	0.02 (0.45)	0.03 (0.49)	0.02 (0.30)	0.05 (0.02)
MS duration at follow-up	-0.003 (0.93)	0.07 (0.12)	-0.02 (0.42)	-0.06 (0.20)
Baseline RNFLT	-0.03 (0.04)	-0.06 (0.06)	-0.03 (0.04)	0.001 (0.98)
Time from last ON event	NA	-0.08 (0.11)	NA	0.02 (0.71)

Chapter 5 : General Discussion

The purpose of this dissertation was longitudinal assessment of functional and structural changes in the anterior visual pathway of MS patients, using clinical tests such as mfVEP, tVEP, CS, HVF and Cirrus OCT.

The introductory chapter provided a general overview of MS disease, visual system involvement in MS patients and various functional and structural tests to evaluate changes in the visual system in MS. This was followed by chapters 2, 3 and 4 in which experiments 1, 2 and 3 were described, respectively. In experiment 1, reproducibility of functional measures such as mfVEP, tVEP and Pelli-Robson CS was assessed by repeating the tests with a month in normal, non-ON and ON ≥ 6 mo eyes. Reproducibility was assessed using ICC and TRV for each measure. Reproducibility was similar across the three groups for mfVEP amplitude, tVEP amplitude and CS. TRV for both mfVEP and tVEP latency was larger in ON ≥ 6 mo eyes compared to normal and non-ON eyes. In all three groups, mfVEP latency showed better reproducibility than tVEP with TRV for mfVEP being about half the respective values for tVEP. In experiment 2, 95% tolerance limits of TRV estimated from experiment 1, and correlation among various measures were used to assess functional changes over time in individual ON < 6 mo, ON ≥ 6 mo and non-ON eyes. A significant percentage of ON < 6 mo eyes exceeded the 95% tolerance limits for mfVEP amplitude, latency and CS; more eyes improved than worsened. In all three

groups, mfVEP latency showed significant changes with a heterogeneous pattern where some eyes shortened, some lengthened, and some remained unchanged. Although an insignificant number of non-ON and ON ≥ 6 mo eyes exceeded the tolerance limits for mfVEP amplitude, CS or HVF; changes in amplitude and latency correlated with each other and both measures correlated with changes in CS. These findings suggest that mfVEP amplitude, latency and CS in eyes that did not exceed the 95% tolerance limits were probably changing too. In experiment 3, changes in structural measures of RNFLT and GCIPT over time were assessed using cross-sectional and longitudinal analysis. RNFLT and GCIPT decreased significantly with increase in MS duration and follow-up time in both non-ON and ON ≥ 6 mo eyes. In both groups, GCIPT detected significantly more abnormal eyes than RNFLT.

Longitudinal changes in function (experiment 2) and structure (experiment 3) were revealed by different methods. In experiment 2, 95% tolerance limits and correlation among various functional changes showed that visual function changes were heterogeneous in individual eyes; and therefore, as a group, no general trend of functional changes was observed during the time studied. Heterogeneous changes in visual function are expected based on pathophysiology of MS. During an acute episode, saltatory propagation of action potentials in nerve fibers is impeded due to factors such as conduction block by inflammatory mediators, demyelination with intact axons or axonal transection. (Walker 2000; Bone 2000) All of these factors would cause impairment in visual

function. During recovery, resolution of edema, clearance of inflammatory mediators, removal of myelin debris by microglia to initiate remyelination, (Chang et al. 2002) redistribution of sodium channels along demyelinated axons (Coman et al. 2006) would promote signal transmission, leading to improvement in visual function. On the other hand, demyelinated axons lack trophic support from myelin and are directly exposed to harsh inflammatory infiltrates causing axonal degeneration (Compston and Coles 2008; Trapp et al. 1998) and worsening of visual function. Thus, functional outcome can either improve or worsen depending on how lesions evolve over time in individual patients. In contrast, changes in structural measures of neuronal integrity, as measured by RNFLT and GCIPT, can theoretically proceed only in one direction, i.e. thinning, unless intervened by clinical or sub-clinical inflammation, in which case nerve fibers could thicken due to edema. It is not surprising that in experiment 3, MS eyes without clinically evident inflammation, as a group, showed a general trend of significant thinning in RNFLT and GCIPT over time, despite the fact that few eyes exceeded the 95% tolerance limits.

Comparing structural and functional measurements in MS

Consistent with previous studies, results from our experiments confirmed that both non-ON and ON eyes have significant functional and structural abnormalities. Further, both functional and structural measures showed significant changes over time even in ‘clinically-silent’ MS eyes. In a previous study from our lab that compared functional and structural tests, (Laron et al.

2010) mfVEP detected significantly more abnormalities than either HVF or Stratus OCT in ON eyes. MfVEP logSNR also showed a moderate correlation with RNFLT from Stratus OCT. In order to compare and assess the relationship between various functional measures and structural measures from Cirrus OCT in the same population, we obtained mfVEP, Pelli-Robson CS, HVF and RNFLT, GCIPT from 90 RRMS patients. A subset of 30 patients also had tVEP.

Percent of abnormal eyes detected by functional and structural measures

In optic neuritis treatment trial (ONTT), at baseline (mean: 5.1 days after acute ON episode), 90% of eyes had high contrast visual acuity worse than 20/20.(1991) However at 1 year follow-up, majority of them showed good recovery with only 30% of eyes with visual acuity worse than 20/20 and only 10% worse than 20/40. (Beck and Cleary 1993) Among those with visual acuity 20/20 or better, 87% had abnormal results in Pelli-Robson CS and 70% had abnormal results in FM 100 color vision test. (Beck and Cleary 1993) These findings suggest that high contrast visual acuity might not be sensitive to pick subtle visual dysfunction and hence has limited use for tracking visual function changes over time in MS. (Balcer et al. 2000). Our aim was to compare measures used in experiments 1, 2 and 3 to identify the most sensitive functional and structural measure that detects abnormalities, not caused by acute inflammatory effects.

MfVEP, Pelli-Robson CS, HVF and Cirrus OCT were obtained from 90 RRMS patients (105 non-ON eyes and 58 ON ≥ 6 mo eyes). TVEP was obtained from a subset of 30 patients (30 non-ON eyes and 19 ON ≥ 6 mo). Percent of

abnormal eyes detected by each functional test was calculated. For mfVEP, CS and tVEP, an eye was classified as abnormal if < 5% of age matched norms. For HVF MD, RNFLT, and GCIPT, an eye was classified as abnormal if < 5% of respective machine norms.

Among functional measures, mfVEP amplitude (AMP) and latency (LAT) together (mfVEP AMP/LAT) detected the highest percent of abnormal eyes (47%) in non-ON eyes (Figure 5-1). MfVEP AMP (23%) and LAT (25%) alone also detected significantly more abnormal eyes when compared to tVEP AMP (3%) and LAT (10%) ($p < 0.01$ for both). More abnormal eyes were detected by HVF 24-2 (31%) and HVF 10-2 (36%) when compared to CS (8%) ($p < 0.01$). In ON ≥ 6 mo eyes, mfVEP AMP/LAT classified highest percent of eyes (80%) as abnormal (Figure 5-2). For AMP, more eyes showed abnormal mfVEP (59%) than tVEP (21%) ($p < 0.01$); for LAT, abnormality was similar for mfVEP (58%) and tVEP (63%) ($p > 0.05$). Percent of abnormal eyes detected by HVF 24-2 (50%) and 10-2 (47%) were not statistically different compared to CS (38%) ($p > 0.05$) in ON ≥ 6 mo .

Figure 5-1 near here

Figure 5-2 near here

Among structural measures, more eyes were classified as abnormal by GCIPT when compared to average RNFLT (ARNFLT) and temporal RNFLT (TRNFLT) in both non-ON and ON eyes ($p > 0.05$ for both) (Figure 5-3 and 5-4).

Percentage of eyes classified as abnormal by GCIPT, ARNFLT and TRNFLT were 20%, 11% and 13% in non-ON and 60%, 51% and 44% in ON ≥ 6 mo group.

Figure 5-3 near here

Figure 5-4 near here

In summary, among all functional tests mfVEP detected the highest percent of abnormalities in both non-ON and ON eyes. Among structural measures, GCIPT detected more abnormal eyes when compared to ARNFLT and TRNFLT.

Agreement between mfVEP AMP, LAT and GCIPT

Agreement between mfVEP AMP, LAT and GCIPT in classifying an eye as normal or abnormal was assessed using 2 x 2 agreement tables and was adjusted for chance using AC1, similar to methods described in chapter 2. In order to compare measurements from similar regions, mfVEP responses from the central 5.6° only was used for this analysis. This mfVEP region (7.3° horizontal and 6.0° vertical) when scaled for retinal ganglion cell displacement, (Drasdo et al. 2007) corresponds best with the GCIPT region measured by Cirrus OCT (8° horizontal and 6.7° vertical).

Moderate agreement was observed between GCIPT and mfVEP with AMP showing slightly better agreement than LAT in both non-ON and ON groups.

Agreement between MfVEP AMP and GCIPT was 68% (AC1 = 0.67) in non-ON and 78% (AC1 = 0.65) in ON eyes (Table 5-1). Agreement between MfVEP LAT and GCIPT was 63% (AC1 = 0.43) in non-ON and 70% (AC1 = 0.40) in ON eyes (Table 5-2). GCIPT and mfVEP may provide complimentary information in detecting structural and functional abnormalities in MS eyes.

Table 5-1 near here

Table 5-2 near here

Relationship between visual functional and structural measures

Relationship between various measures of visual function and structural measures of OCT was assessed. MfVEP, Pelli-Robson CS and Cirrus OCT from 90 RRMS patients used for the previous analysis were included. When both eyes of a patient belonged to the same group (i.e. ON or non-ON), one eye was randomly chosen for analysis. Forty-three ON eyes (last ON ≥ 6 months) and 73 non-ON eyes were included. Thirty-six ON eyes and 66 non-ON eyes had HVF 24-2 or 30-2; 15 ON and 10 non-ON eyes also had HVF 10-2. TVEP recordings were obtained from 16 ON and 22 non-ON eyes.

Pelli-Robson CS was recorded as described in chapters 2 and 3. For HVF, for each eye, individual deviations from the total deviation plot were unlogged and averaged to calculate relative visual sensitivity (RVS). MfVEP amplitude and latency were calculated as mean logSNR and median latency

from all 60 sectors for global and central 5.6° region. For tVEP, P100 amplitude and relative latency were derived as described in chapter 2. RNFLT and GCIPT measures from Cirrus OCT were estimated as described in chapter 4. Pearson correlation (r) was assessed between functional measures (CS, HVF mfVEP and tVEP) and structural measures (GCIPT, RNFLT).

As shown in Figure 5-5, all functional measures showed significant correlation with GCIPT in ON eyes. In non-ON eyes, CS (a) and mfVEP 5.6° logSNR (c) showed significant correlation, but 10-2 RVS (b) and tVEP amplitude (d) showed no correlation with GCIPT. Figure 5-6 shows the relationship between functional tests and RNFLT. CS (a) and mfVEP global logSNR (c) showed significant correlation while 24-2/30-2 RVS (b) and tVEP amplitude (d) showed no correlation with RNFLT in both ON and non-ON eyes. The relationship between mfVEP and tVEP latency and OCT measures are shown in Figure 5-7. MfVEP 5.6° and global latency showed significant correlation with GCIPT and RNFLT in both ON and non-ON eyes (a,c).TVEP latency showed significant correlation with GCIPT and RNFLT in ON but not in non-ON eyes (b, d). Pearson correlation (r) and p values are summarized in Table 5-3 and 5-4.

Table 5-3 and 5-4 near here

Figure 5-5, 5-6 and 5-7 near here

In summary, Pelli-Robson CS and mfVEP are more reflective of the structural alterations than HVF and tVEP, especially in non-ON eyes. Overall, all functional tests of vision correlated more strongly with GCIPT than RNFLT, especially in ON eyes, suggesting that GCIPT is a good measure for detecting neuronal degeneration in RRMS eyes.

Possible reasons for continuous progression in MS

DMTs are not effective in completely halting the disease process

Consolidating the results from experiments 1, 2 and 3, it could be inferred that functional and structural changes occur over time even when not intervened by acute inflammatory attacks. While visual function improved or worsened in different eyes, structural measures showed a general trend of worsening over time. For ON eyes, significant changes in latency were observed in longitudinal studies (experiment 2 and 3) where the follow-up was about 1 to 1. 5 years, and possibly in experiment 1 in which the follow-up was relatively short (about 2 weeks). These findings suggest that subclinical disease changes, both structural and functional, might be constantly occurring in the visual system of MS patients, even in the absence of clinically evident relapses. Interestingly, more than 85% of the patients were on immuno-modulatory DMTs during the entire period of follow-up. The majority of the ON eyes (> 80%) had experienced only a single episode of ON attack. Our results are consistent with previous findings that

current DMTs are effective in reducing relapses but less effective in completely preventing myelin, axonal or neuronal loss. (Castro-Borrero et al. 2012)

Subclinical inflammation, transynaptic degeneration

It is interesting that non-ON eyes also showed significant functional and structural changes over time. This was not driven by presence of ON in the fellow eye, as confirmed by analysis in subset of eyes with bilateral non-ON. Some possible underlying causes are low-level subclinical inflammatory episodes that remain asymptomatic. Autopsy studies have revealed presence of inflammatory infiltrates in MS lesions in patients with no clinical history of ON. (Green et al. 2010) Transynaptic retrograde degeneration could also explain neurodegeneration in non-ON eyes. This was demonstrated in patients with acquired hemianopia due to post-geniculate stroke (Jindahra et al. 2012). In a recent study, significant correlation was found between lesion volume in optic radiations and temporal RNFLT, in RRMS patients with no optic tract lesions. The authors suggest that loss of temporal RNFLT in non-ON eyes could be a consequence of transynaptic retrograde degeneration. (Klistorner et al. 2014)

MS pathology is ‘inside-out’, not ‘outside-in’

Primary cytodegeneration could also explain changes observed in non-ON eyes. MS is traditionally viewed as a primary autoimmune disease in which inflammatory T cells attack the CNS neurons, causing demyelination and axonal degeneration. (Compston and Coles 2008) However, there are some inconsistencies in this ‘outside-in’ model as discussed by Stys and colleagues in their recent review papers. (Stys et al. 2012; Stys 2013) First, careful histopathological examination of very early MS lesions revealed that subtle myelin abnormalities may begin in inner myelin sheaths in areas with little or no inflammatory infiltrates. (Rodriguez and Scheithauer 1994) More recently, reduced myelin and axonal density were noted in regions with no apparent inflammation. (Seewann et al. 2009) Second, immuno-modulators which have shown great promise in reducing both relapses and newly formed MRI lesions, are much less effective in halting progressive MS. (Hawker 2011) Even autologous haematopoietic stem-cell transplantation that aims at completely resetting the immune system, and is highly successful in reducing inflammation, (Mancardi and Saccardi 2008) is less effective in preventing progression of myelin loss or neuronal degeneration. (Metz et al. 2007)

An alternate ‘inside-out’ model proposes that cytodegeneration of oligodendrocyte-myelin complex and underlying axons could be the primary event, and antigenic debris released as a consequence promotes a secondary inflammatory immune response in a susceptible host. (Stys et al. 2012) According to this model, different MS subtypes could be a result of convolution

between underlying primary cytodegeneration and a dysfunctional immune system that is variable across hosts. (Stys et al. 2012) Uniform progression of disease process across MS subtypes (De Stefano et al. 2010) independent of inflammatory relapses (Scalfari et al. 2010) suggests that cytodegeneration is a plausible initiating event. Results from a recent study in a rat model of MS suggest the possibility that RGC somas could atrophy before the axons. (Fairless et al. 2012) This study demonstrated that at the time of significant RGC soma loss, the axons appeared to be intact in numbers but showed ultrastructural signs of degeneration.

Novel therapeutics in MS

Although the origin of MS remains unclear, it is evident that pathological outcome of the disease results in progressive neurodegeneration leading to permanent neurological disability. It is also evident that in addition to currently available immuno-modulatory DMTs, novel therapeutic strategies that can render remyelination and neuroprotection to enhance survival of neurons are needed. Lack of neuroprotective therapies in MS is partially due to limited availability of strategies to perform this function as well as lack of reliable outcome measures to test their efficacy. In addition, limited understanding of the complex interaction between immune-mediators, neurons and glials as well the unclear triggering factor has made it hard for researchers to identify potential targets of neuroprotection accurately. (Maghzi et al. 2013) In spite of all the complexities,

significant progress has been made in identifying and testing of few novel neuroprotective strategies as described below.

Sodium channel blockers

In normal myelinated fibers, Na^+ channels are concentrated at the nodes of Ranvier to allow saltatory conduction, facilitated by Na^+-K^+ ion exchange. (Ames 2000) In demyelinated fibers, Na^+ channels are redistributed diffusely along the demyelinated axon in order to restore conduction. However, this leads to increased energy demand and causes axonal Na^+ concentration to rise above the normal limits. (Trapp and Stys 2009; Waxman et al. 1992) This in turn causes Na^+/Ca^{2+} exchanger to operate in reverse mode which increases axoplasmic Ca^{2+} concentration and initiates Ca^{2+} mediated axonal degeneration. (Craner et al. 2004) Sodium channel blockers work by preventing excessive Na^+ influx into the axons. Drugs such as lamotrigine and phenytoin have shown neuroprotective effects in animal models of MS, where axonal degeneration was found to be significantly lesser in the treated group compared to placebo. (Bechtold et al. 2006) These drugs are currently in phase II clinical trial. (Franklin et al. 2012)

Calcium channel blockers

Similar to sodium channel blockers, calcium channel blockers work by preventing influx of Ca^{2+} into axons. Drugs such as nimodipine and nifedipine showed potential for neuroprotective effects by blocking the release of calcium

from intracellular sources in a rat spinal cord injury model. (Ouardouz et al. 2006) Although the pre- clinical results were encouraging, none of the calcium channel blockers are currently tested in clinical trials. (Maghzi et al. 2013)

Remyelination strategies

Myelin, formed by oligodendrocytes, plays an important role in promoting signal transmission, energy conservation and providing metabolic and trophic support to axons. Demyelination not only slows conduction and increases metabolic demand but also renders axons vulnerable to further degeneration. (Munzel and Williams 2013) Remyelination can serve as an important neuroprotective strategy. Animal model studies show that remyelination can restore clustering of sodium channels at nodes of Ranvier, (Howell et al. 2006) increase mitochondrial contents. (Zambonin et al. 2011) and improve neurological function. (Duncan et al. 2009; Murray et al. 2001) Post-mortem studies of brain tissues from MS patients also offer supportive evidence where remyelination was found to be extensive in a subset of patients (Patani et al. 2007; Patrikios et al. 2006) and axonal injury was less severe in remyelinated lesions than demyelinated ones. (Kuhlmann et al. 2002) A few potential candidate drugs that can promote remyelination are discussed below.

Anti-LINGO-1 antibodies One of the major reasons for remyelination failure in the CNS is attributed to mechanisms that inhibit differentiation of oligodendrocyte precursors cells (OPC) into mature myelianting

oligodendrocytes. (Chang et al. 2002) Inhibitors of OPC differentiation could serve as potential remyelination targets. (Givogri et al. 2002; Kotter et al. 2006) LINGO-1 (Leucine rich repeat and Ig domain containing NOGO receptor interacting protein 1) has been identified as a key inhibitor of oligodendrocyte differentiation and myelination during development. Suppression of LINGO-1 function caused enhanced OPC differentiation and myelination in vitro. Further, in vivo analysis in LINGO-1 knockout mice also showed early onset of myelination, supporting the important role of LINGO-1 antagonists in promoting myelination. (Mi et al. 2005) In a study using experimental autoimmune encephalomyelitis (EAE), a rat model of MS, animals treated with anti-LINGO-1 antibodies showed enhanced recovery of function. These animals also showed improved axonal integrity, as detected by MRI diffusion tensor imaging, and newly formed myelin sheath, as detected by electron microscopy. (Mi et al. 2007) A recent preclinical study on EAE (Cadavid et al. 2013) (published only as an abstract) demonstrated that, in the optic nerve of animals treated with anti-LINGO-1 antibodies, axonal loss was five times lesser when compared to control animals. A phase II clinical trial is currently in progress to test the safety, tolerability and efficacy of anti-LINGO-1 antibodies in MS patients. (Munzel and Williams 2013)

Human Monoclonal IgM Antibody 22 Monoclonal antibodies are naturally occurring antibodies as a part of human immunoglobulin repertoire. Their natural physiological function includes stimulation of cell processes and cleaning cell debris. (Rodriguez et al. 2009) The human monoclonal IgM antibody 22 is a

recombinant antibody which, when injected into animal models of MS, showed enhanced remyelination and protection of neurons. (Warrington et al. 2007; Warrington et al. 2000) It was found that this antibody was capable of inducing remyelination in the spinal cord of a demyelinating mice model. (Mitsunaga et al. 2002) Mice that received this antibody showed increased remyelination (60% compared to 16 % in control group). (Pirko et al. 2004) It is believed that the antibody works by promoting anti-apoptotic signaling in pre-myelinating oligodendrocytes. (Howe et al. 2004) A phase I clinical trial is currently in progress to assess the safety and tolerability of rHIgM22 in MS patients. (Munzel and Williams 2013)

Outcome measures for neuroprotective therapies

Most of the currently used outcome measures in MS clinical trials, such as number and volume of MRI lesions reflect inflammation with little information on underlying tissue loss. (Cohen et al. 2012) MRI lesions also have low histopathological specificity. (Barkhof et al. 2009) For example, brain atrophy measures overall loss of the tissue which could be influenced by a number of components such as axons, inflammatory cells, myelin and glial tissues. Although advanced MRI features such as magnetization transfer imaging and diffusion tensor imaging may better reflect tissue specific pathology, they are not routinely performed and not currently used as outcome measures in MS.(Maghzi et al. 2013) In contrast, OCT measures of RNFLT and GCIPT are highly

reproducible and are believed to reflect integrity of RGC axons and somas, in the absence of myelin. Although RNFLT measure is impacted by inflammation in the acute stage, it is believed that the GCIPT measure is less confounded by inflammation and gliosis and might better reflect true neuronal loss.(Ratchford et al. 2013; Green et al. 2010) In addition, OCT measures also show good correlation with standard MRI measures of brain volume, tissue loss(Gordon-Lipkin et al. 2007; Grazioli et al. 2008) and EDSS score.(Di Maggio et al. 2014)

MfVEP offers a unique advantage of providing information on the integrity of myelin in localized regions. Reproducibility of mfVEP responses and methods to reliably track individual eyes have been established in this dissertation. MfVEP detected more abnormalities compared to all other functional measures. Further, the strong correlation between mfVEP measures with OCT and Pelli-Robson CS supports that mfVEP has high sensitivity in detecting pathological changes in MS. In summary, mfVEP and OCT could serve as reliable techniques to track MS related disease changes and as outcome measures to assess therapeutic effects of novel neuroprotective strategies.

Limitations and future directions

Results from chapter 3 and 4 suggest that longitudinal changes in mfVEP and RNFLT/GCIPT measures occurred over time in MS eyes and mfVEP amplitude and latency showed significant correlation with both RNFLT and GCIPT (chapter 5). However, we were not able to obtain longitudinal data on mfVEP and Cirrus-OCT on the same patients since time domain OCT was used

from initial visits in many mfVEP cases. Our results were only based on two visits, although analysis on a small subset of patients showed a consistent trend cross multiple visits (chapter 3).

Future studies should use both mfVEP and Cirrus-OCT to longitudinally assess patients from early stages of MS at frequent time intervals for much longer follow-up time. This may reveal the temporal relationship between demyelination/remyelination and axonal loss, and help determine an optimal time window for new therapeutic treatments. Additional functional tests such as low-contrast letter acuity (LCLA) could also be added. Recent studies have shown that LCLA is a reliable and sensitivity test to capture visual impairment in MS and is currently used in clinical trials as an outcome measure.(Bermel and Balcer 2013)

Conclusions

MfVEP showed better reproducibility than tVEP. TRV for mfVEP latency was about half the respective values for tVEP in normal and RRMS eyes. Longitudinal changes in mfVEP amplitude and latency showed heterogeneous pattern with some eyes improving, some stable, and some worsening over time. MfVEP log SNR and latency changes correlated, and each showed good correlation with changes in Pelli-Robson CS. MfVEP, particularly the latency, can be used to track visual function changes in individual RRMS eyes. Using cirrus OCT, progressive loss of GCIPT and RNFLT occurred over time even in the

absence of clinically-evident inflammation. GCIPT detected significantly more abnormal eyes than RNFLT in RRMS. Among all functional tests mfVEP detected the highest percent of abnormal eyes in both non-ON and ON eyes. Pelli-Robson CS and mfVEP logSNR, latency correlated significantly with both GCIPT and RNFLT and hence are more reflective of the structural alterations when compared to HVF and tVEP, especially in non-ON eyes. All functional tests of vision correlated more strongly with GCIPT than RNFLT, especially in ON eyes. MfVEP and Cirrus OCT could be used to track functional and structural changes in the visual system and are potentially useful for assessing therapeutic effects of remyelinating and neuroprotective strategies in RRMS.

Figures and Tables

Figure 5-1: Percent of abnormal eyes detected by functional measures in non-ON eyes

The histogram shows the percentage of abnormal eyes detected by various functional measures in non-ON eyes. For subjective functional tests, HVF 24-2/30-2 and 10-2 detected significantly more abnormalities than CS. For objective functional tests, mfVEP detected significantly more abnormalities than tVEP.

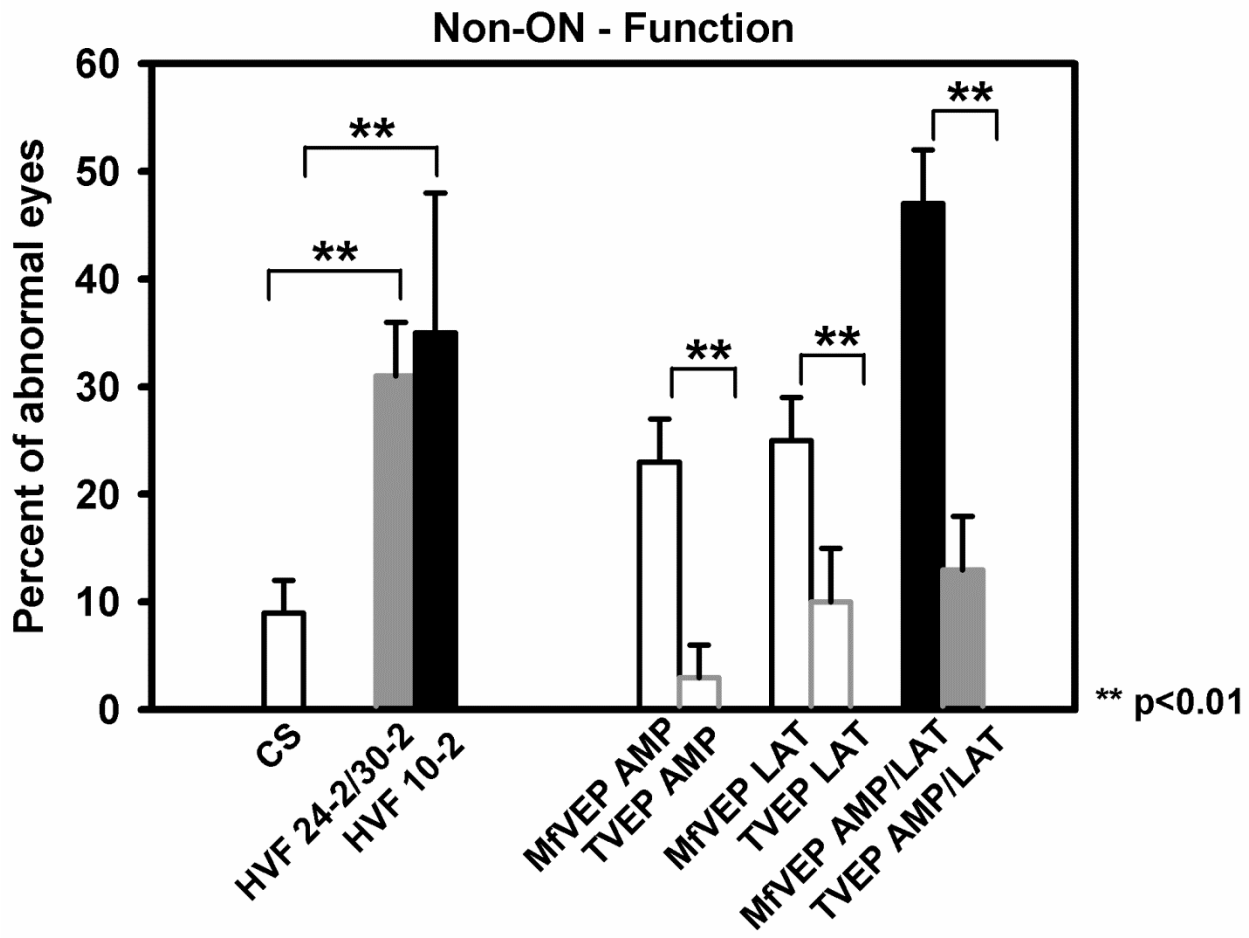

Figure 5-2: Percent of abnormal eyes detected by functional measures in ON eyes

The histogram shows the percentage of abnormal eyes detected by various functional measures in ON eyes. MfVEP AMP detected significantly more abnormalities than tVEP AMP. Percentage of abnormalities detected were not significantly different among HVF and CS or among LAT, AMP/LAT measures of mfVEP and tVEP

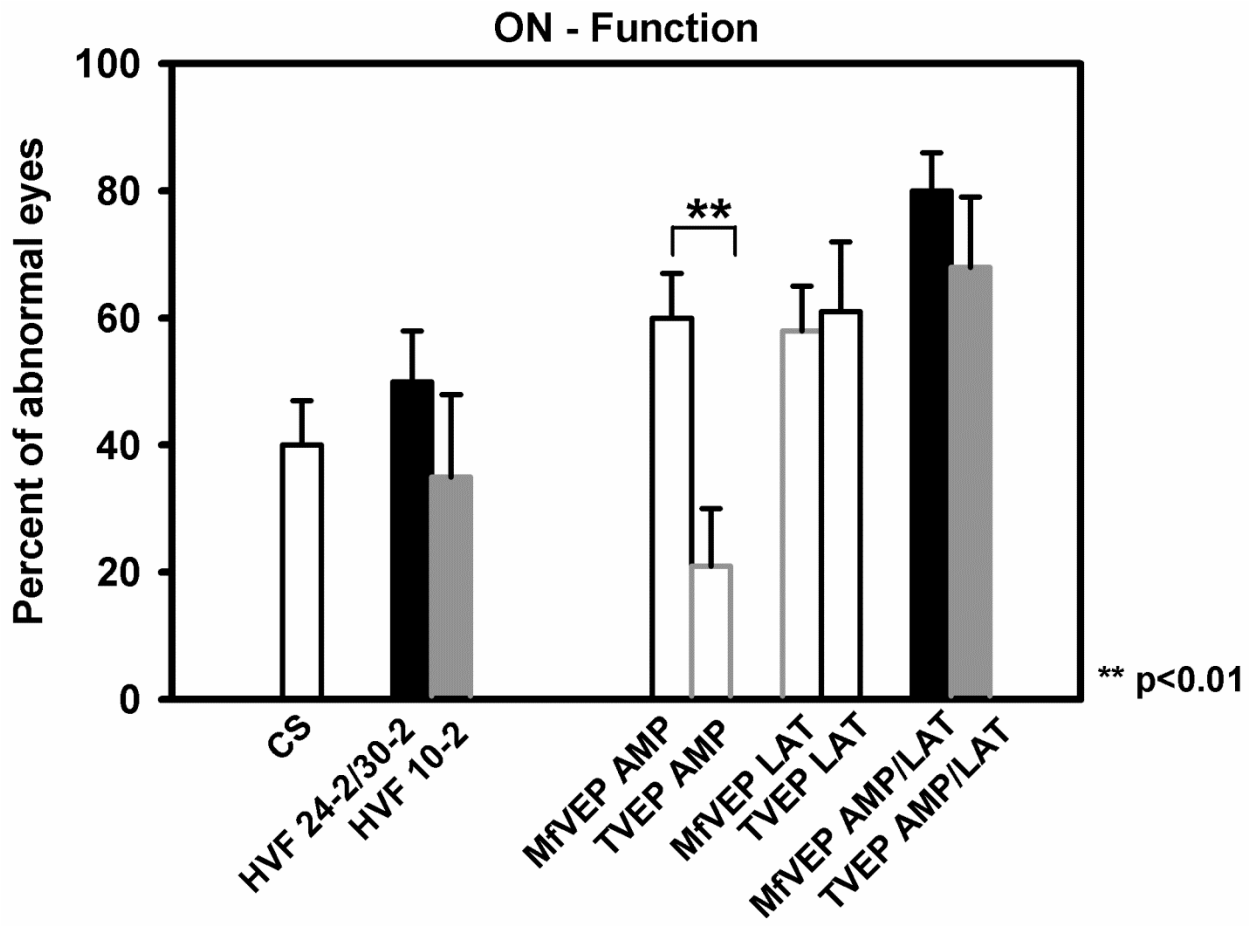

Figure 5-3: Percent of abnormal eyes detected by structural measures in non-ON eyes

The histogram shows the percentage of abnormal eyes detected by various structural measures in non-ON eyes. GCIPT detected significantly more abnormalities than ARNFLT and TRNFLT.

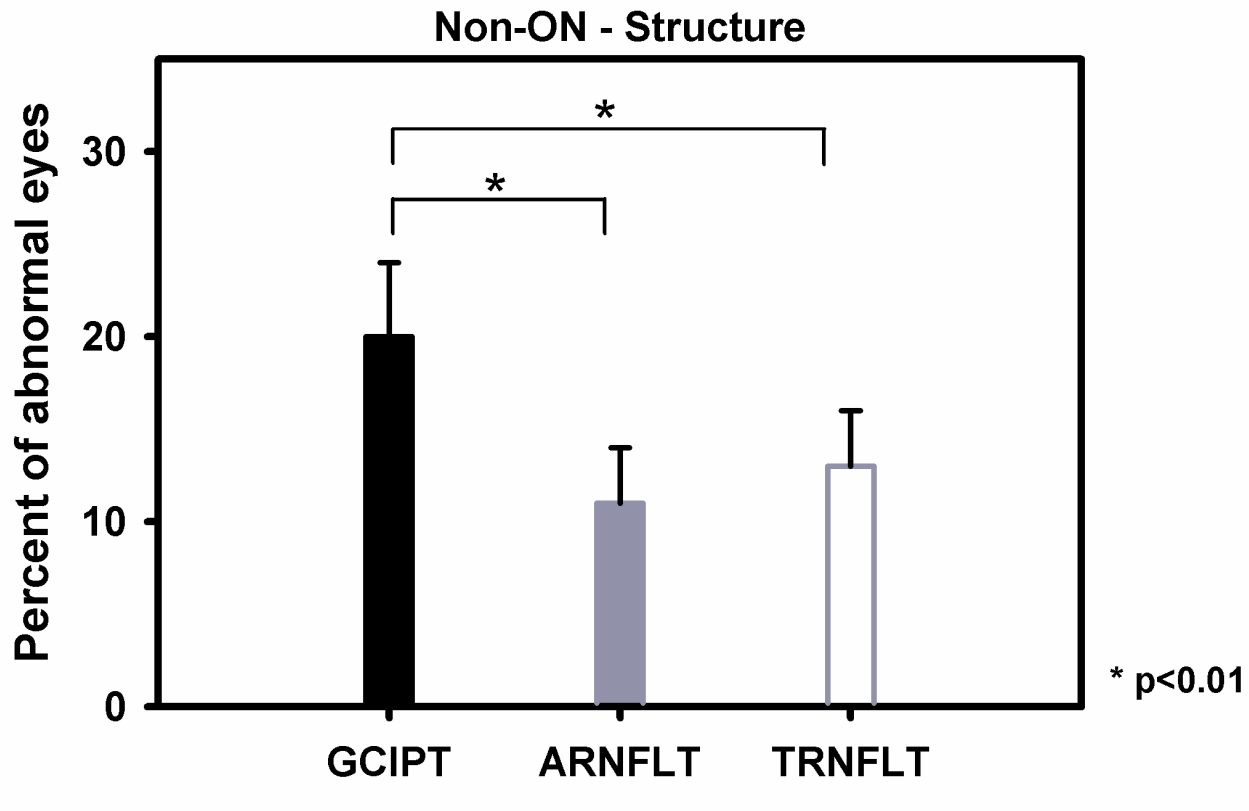

Figure 5-4: Percent of abnormal eyes detected by structural measures in ON eyes

The histogram shows the percentage of abnormal eyes detected by various structural measures in ON eyes. GCIPT detected significantly more abnormalities than ARNFLT and TRNFLT.

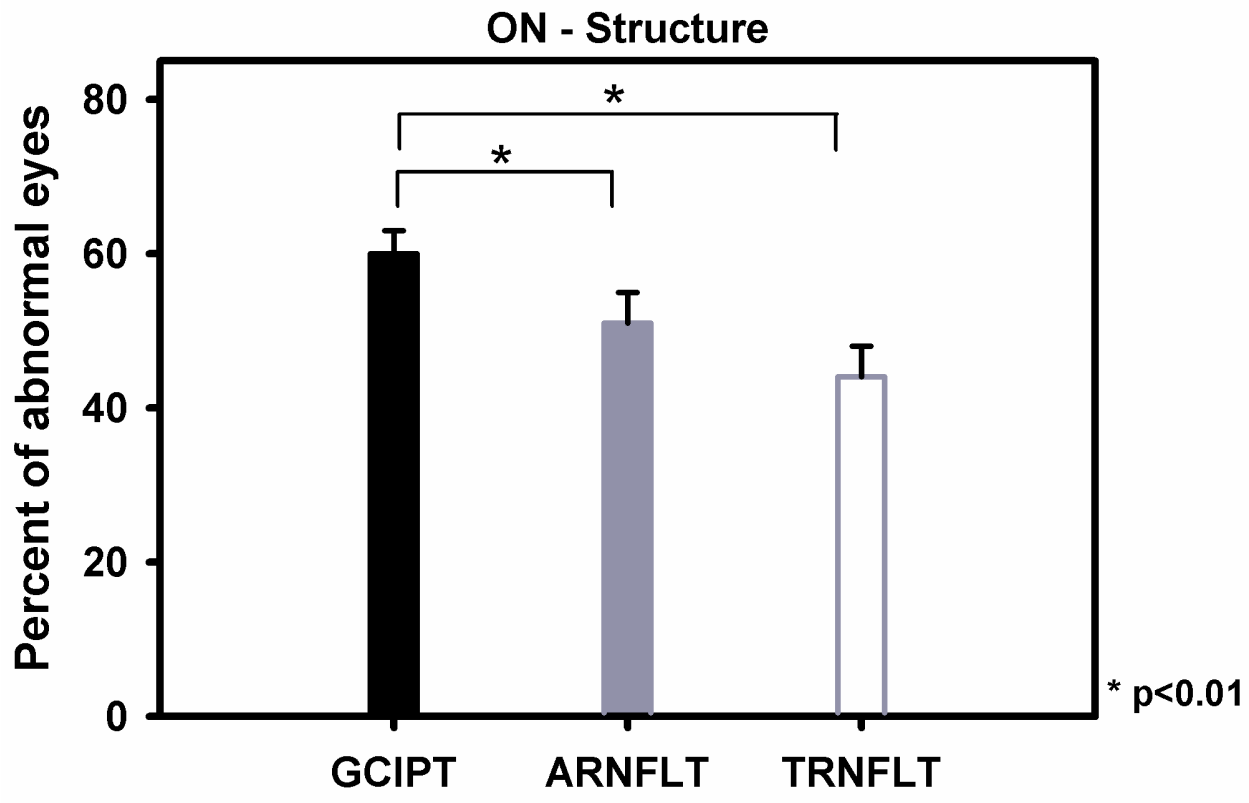

Figure 5-5: Functional tests vs GCIPT

Correlation between various functional tests and GCIPT in non-ON (circles) and ON eyes (triangles).The dashed line and solid lines are the fitted linear regression lines for non-ON and ON eyes respectively.

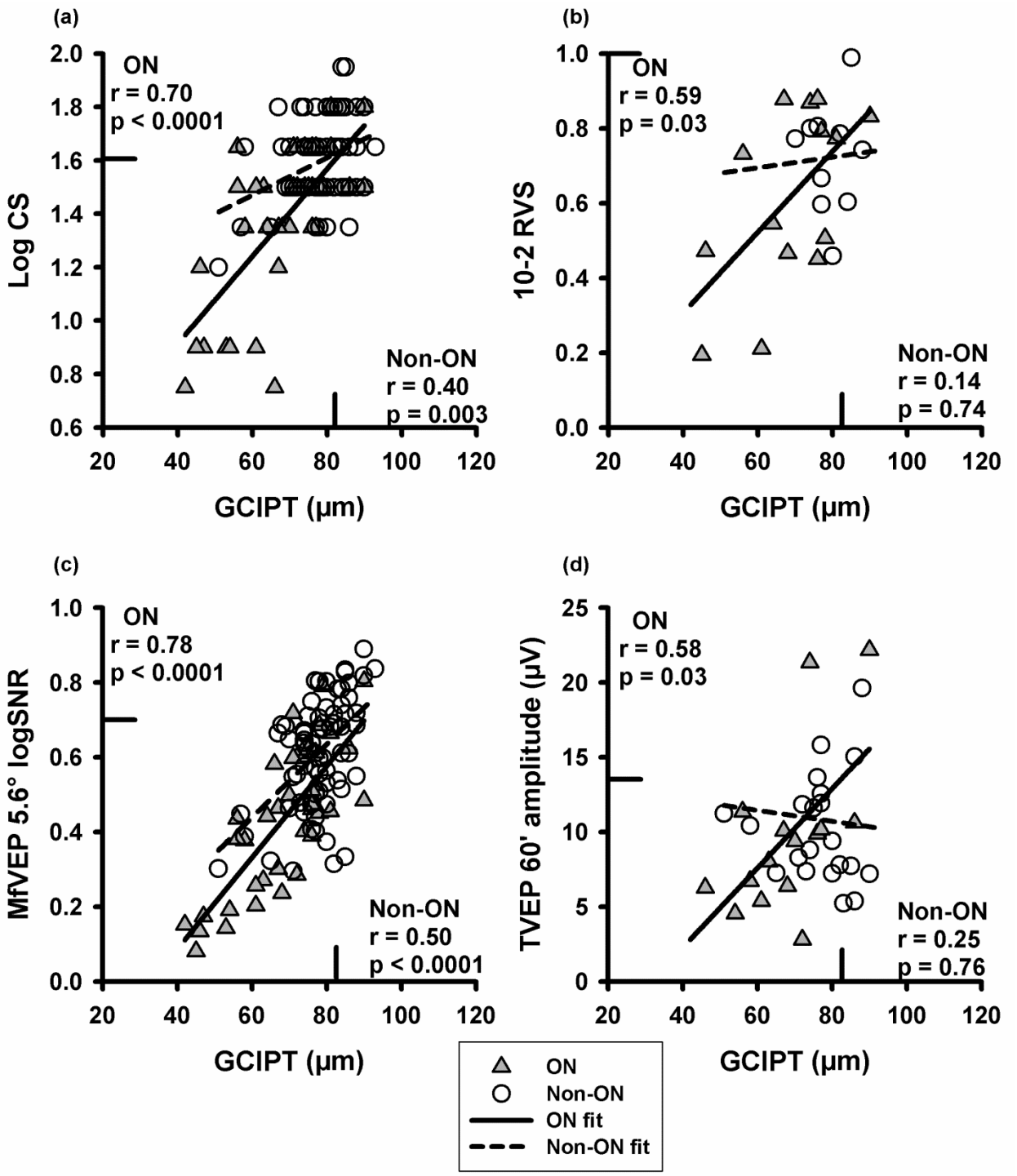
(a)
ON
r = 0.70
p < 0.0001
Non-ON
r = 0.40
p = 0.003
Log CS
GCIPT (μm)
(b)
ON
r = 0.59
p = 0.03
Non-ON
r = 0.14
p = 0.74
10-2 RVS
GCIPT (μm)
(c)
ON
r = 0.78
p < 0.0001
Non-ON
r = 0.50
p < 0.0001
MfVEP 5.6° logSNR
GCIPT (μm)
(d)
ON
r = 0.58
p = 0.03
Non-ON
r = 0.25
p = 0.76
TVEP 60' amplitude (μV)
GCIPT (μm)
ON
Non-ON
ON fit
Non-ON fit

Figure 5-6: Functional tests vs RNFLT

Correlation between various functional tests and RNFLT in non-ON (circles) and ON eyes (triangles).The dashed line and solid lines are the fitted linear regression lines for non-ON and ON eyes respectively.

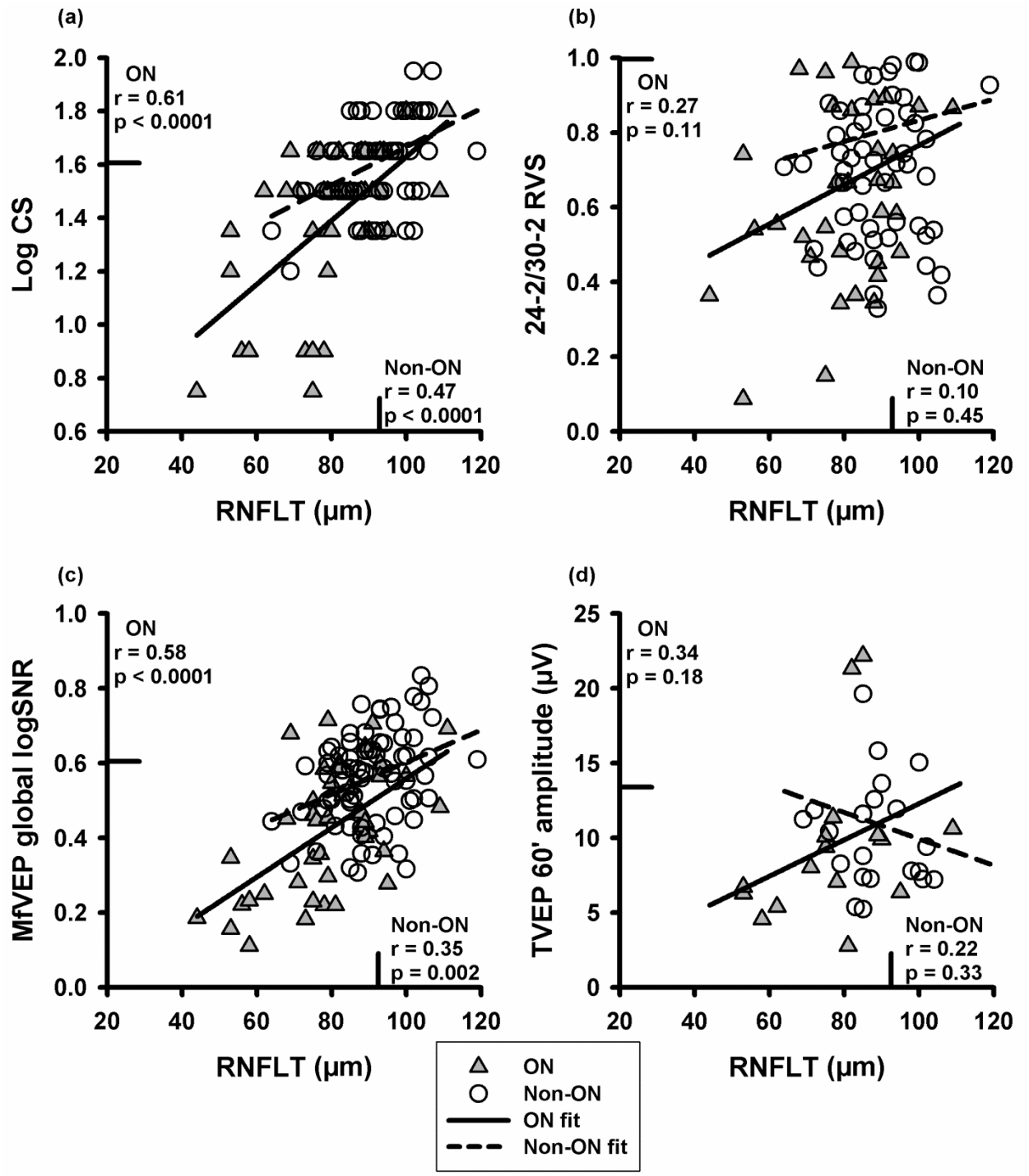
(a)
ON
r = 0.61
p < 0.0001
Non-ON
r = 0.47
p < 0.0001
Log CS
RNFLT (μm)
(b)
ON
r = 0.27
p = 0.11
Non-ON
r = 0.10
p = 0.45
24-2/30-2 RVS
RNFLT (μm)
(c)
ON
r = 0.58
p < 0.0001
Non-ON
r = 0.35
p = 0.002
MfVEP global logSNR
RNFLT (μm)
(d)
ON
r = 0.34
p = 0.18
Non-ON
r = 0.22
p = 0.33
TVEP 60' amplitude (μV)
RNFLT (μm)
ON
Non-ON
ON fit
Non-ON fit

Figure 5-7: MfVEP/tVEP latency vs GCIPT/RNFLT

Correlation between mfVEP/tVEP latency and GCIPT/RNFLT in non-ON (circles) and ON eyes (triangles).The dashed line and solid lines are the fitted linear regression lines for non-ON and ON eyes respectively.

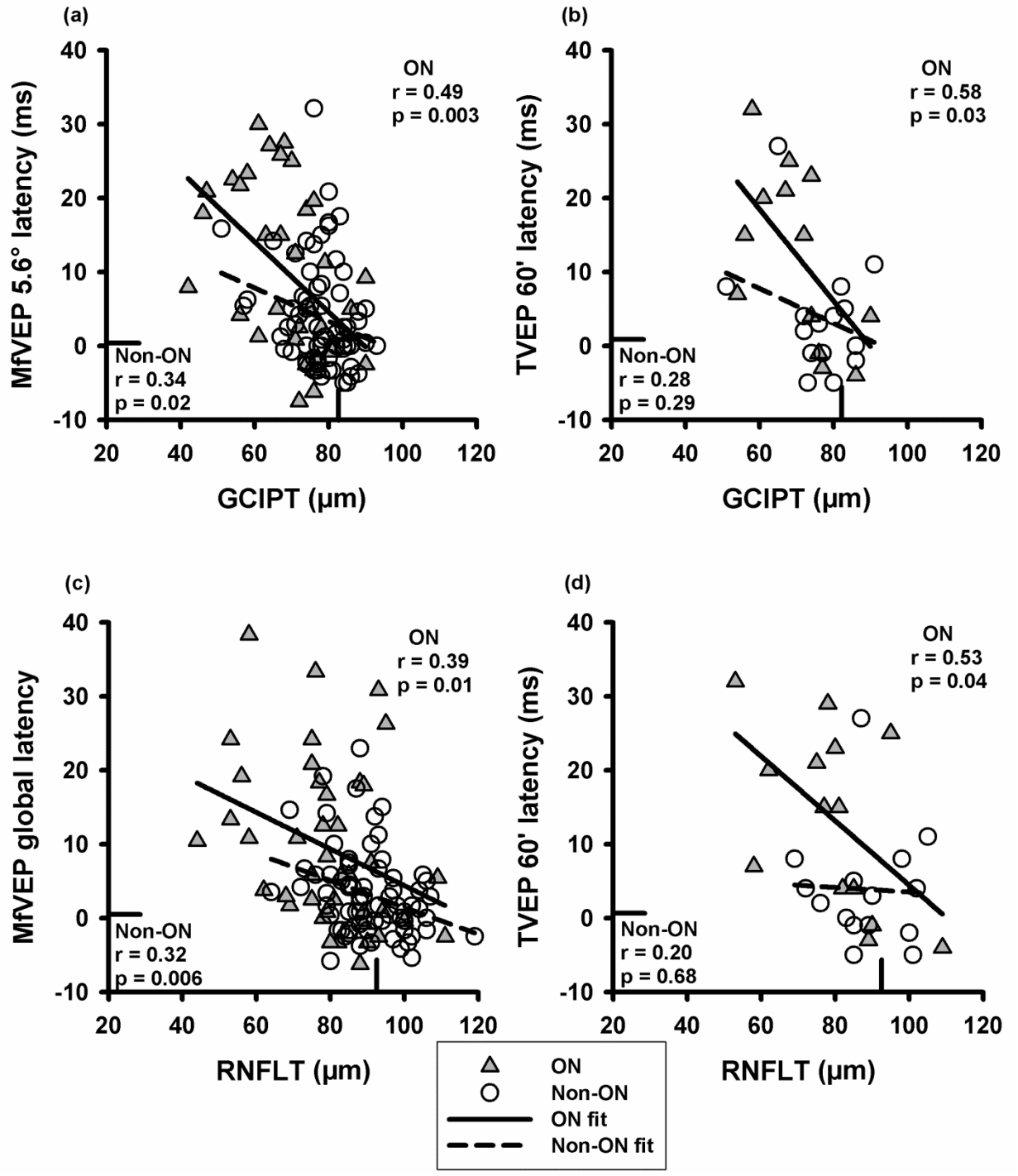
(a)
MfVEP 5.6° latency (ms)
ON
r = 0.49
p = 0.003
Non-ON
r = 0.34
p = 0.02
GCIPT (μm)
(b)
TVEP 60' latency (ms)
ON
r = 0.58
p = 0.03
Non-ON
r = 0.28
p = 0.29
GCIPT (μm)
(c)
MfVEP global latency
ON
r = 0.39
p = 0.01
Non-ON
r = 0.32
p = 0.006
RNFLT (μm)
(d)
TVEP 60' latency (ms)
ON
r = 0.53
p = 0.04
Non-ON
r = 0.20
p = 0.68
RNFLT (μm)
ON
Non-ON
ON fit
Non-ON fit

Table 5-1: Agreement between mfVEP AMP and GCIPT in non-ON and ON eyes

(a) Non-ON

	GCIPT Abnormal	GCIPT Normal
MfVEP AMP Abnormal	5 %	17 %
MfVEP AMP Normal	15 %	63 %

Agreement = 68 %, AC1 = 0.67

(b) ON

	GCIPT Abnormal	GCIPT Normal
MfVEP AMP Abnormal	50 %	13 %
MfVEP AMP Normal	10 %	27 %

Agreement = 77 %, AC1 = 0.65

Table 5-2: Agreement between mfVEP LAT and GCIPT in non-ON and ON eyes

(a) Non-ON

	GCIPT Abnormal	GCIPT Normal
MfVEP LAT Abnormal	4 %	21 %
MfVEP LAT Normal	16 %	59 %

Agreement = 63 %, AC1 = 0.43

(b) ON

	GCIPT Abnormal	GCIPT Normal
MfVEP LAT Abnormal	45 %	15 %
MfVEP LAT Normal	15 %	25 %

Agreement = 70 %, AC1 = 0.40

Table 5-3: Pearson correlation between GCIPT, RNFLT and subjective functional tests

	ON eyes		Non-ON eyes	
	GCIPT	RNFLT[c]	GCIPT	RNFLT[c]
Pelli-Robson CS	0.70(<0.0001)	0.61(<0.0001)	0.40(0.003)	0.47(<0.0001)
10-2 RVS	0.59 (0.03)	0.20 (0.44)	0.14(0.74)	0.18 (0.62)
30-2/24-2 RVS	0.45 (0.006)	0.27 (0.11)	0.14 (0.52)	0.10 (0.45)

[c]Temporal RNFLT used for correlation with 10-2 RVS, average RNFLT used for others.

Table 5-4: Pearson correlation between GCIPT, RNFLT and objective functional tests

Objective functional tests	Structural tests r (p value)			
	ON eyes		Non-ON eyes	
	GCIPT	RNFLT[c]	GCIPT	RNFLT[c]
Amplitude				
MfVEP 5.6°	0.78 (<0.0001)	0.43 (0.003)	0.50 (<0.0001)	0.36 (0.001)
MfVEP global	0.76 (<0.0001)	0.58 (<0.002)	0.55 (<0.0001)	0.35 (0.002)
TVEP 15’	0.48 (0.09)	0.26 (0.37)	0.14 (0.48)	0.08 (0.72)
TVEP 60’	0.58 (0.03)	0.34 (0.18)	0.25 (0.76)	0.22 (0.33)
TVEP 120’	0.61 (0.01)	0.41 (0.10)	0.23 (0.29)	0.30 (0.17)
Relative latency				
MfVEP 5.6°	0.49 (0.003)	0.38 (0.03)	0.34 (0.02)	0.32 (0.004)
MfVEP global	0.40 (0.02)	0.39 (0.01)	0.39 (0.01)	0.32 (0.006)
TVEP 15’	0.60 (0.03)	0.56 (0.03)	0.36 (0.18)	0.31 (0.38)
TVEP 60’	0.58 (0.03)	0.53 (0.04)	0.28 (0.29)	0.20 (0.68)
TVEP 120’	0.51 (0.07)	0.39 (0.16)	0.15 (0.65)	0.20 (0.46)

[c] Temporal RNFLT used for correlation with mfVEP 5.6°, average RNFLT used for others

References

Amato MP, Ponziani G. 2000. A prospective study on the prognosis of multiple sclerosis. Neurol Sci 21(4 Suppl 2): S831-838.

Ames A, 3rd. 2000. CNS energy metabolism as related to function. Brain Res Brain Res Rev 34(1-2): 42-68.

Arditi A. 2005. Improving the design of the letter contrast sensitivity test. Invest Ophthalmol Vis Sci 46(6): 2225-2229.

Balachandran C, Graham SL, Klistorner A, Goldberg I. 2006. Comparison of objective diagnostic tests in glaucoma: Heidelberg retinal tomography and multifocal visual evoked potentials. J Glaucoma 15(2): 110-116.

Balcer LJ, Baier ML, Pelak VS, Fox RJ, Shuwairi S, Galetta SL, et al. 2000. New low-contrast vision charts: reliability and test characteristics in patients with multiple sclerosis. Mult Scler 6(3): 163-171.

Balk LJ, Petzold A. 2014. Current and future potential of retinal optical coherence tomography in multiple sclerosis with and without optic neuritis. Neurodegener Dis Manag 4(2): 165-176.

Barkhof F, Calabresi PA, Miller DH, Reingold SC. 2009. Imaging outcomes for neuroprotection and repair in multiple sclerosis trials. Nat Rev Neurol 5(5): 256-266.

Baseler HA, Sutter EE, Klein SA, Carney T. 1994. The topography of visual evoked response properties across the visual field. Electroencephalogr Clin Neurophysiol 90(1): 65-81.

Bechtold DA, Miller SJ, Dawson AC, Sun Y, Kapoor R, Berry D, et al. 2006. Axonal protection achieved in a model of multiple sclerosis using lamotrigine. Journal of neurology 253(12): 1542-1551.

Beck RW, Cleary PA. 1993. Optic neuritis treatment trial. One-year follow-up results. Arch Ophthalmol 111(6): 773-775.

Bjartmar C, Trapp BD. 2003. Axonal degeneration and progressive neurologic disability in multiple sclerosis. Neurotox Res 5(1-2): 157-164.

Bjerre A, Grigg JR, Parry NR, Henson DB. 2004. Test-retest variability of multifocal visual evoked potential and SITA standard perimetry in glaucoma. Invest Ophthalmol Vis Sci 45(11): 4035-4040.

Bland JM, Altman DG. 1996. Measurement error. BMJ 313(7059): 744.

Bone II. 2000. McAlpine's multiple sclerosis. Third edition. J Neurol Neurosurg Psychiatry 68(5): 682A.

Bermel RA, Balcer LJ. 2013. Optic neuritis and the evaluation of visual impairment in multiple sclerosis. Continuum (Minneap Minn) 19(4 Multiple Sclerosis): 1074-1086.

Brusa A, Jones SJ, Kapoor R, Miller DH, Plant GT. 1999. Long-term recovery and fellow eye deterioration after optic neuritis, determined by serial visual evoked potentials. Journal of neurology 246(9): 776-782.

Brusa A, Jones SJ, Plant GT. 2001. Long-term remyelination after optic neuritis: A 2-year visual evoked potential and psychophysical serial study. Brain 124(Pt 3): 468-479.

Bruce CC, Zhao C, Franklin RJ. 2010. Remyelination - An effective means of neuroprotection. Horm Behav 57(1): 56-62.

Budenz DL, Fredette MJ, Feuer WJ, Anderson DR. 2008. Reproducibility of peripapillary retinal nerve fiber thickness measurements with stratus OCT in glaucomatous eyes. Ophthalmology 115(4): 661-666 e664.

Castro-Borrero W, Graves D, Frohman TC, Flores AB, Hardeman P, Logan D, et al. 2012. Current and emerging therapies in multiple sclerosis: a systematic review. Ther Adv Neurol Disord 5(4): 205-220.

Cadavid D, Butzkueven H, Yaou L. 2013. Effect of LINGO-1 blockade on optic nerve axonal injury in MOG-EAE rodent models. Neurology 80: (Meeting Abstracts 1): P05.186.

Cohen JA, Reingold SC, Polman CH, Wolinsky JS. 2012. Disability outcome measures in multiple sclerosis clinical trials: current status and future prospects. Lancet Neurol 11(5): 467-476.

Celebi AR, Mirza GE. 2013. Age-Related Change in Retinal Nerve Fiber Layer Thickness Measured with Spectral Domain Optical Coherence Tomography. Invest Ophthalmol Vis Sci.

Chang A, Tourtellotte WW, Rudick R, Trapp BD. 2002. Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 346(3): 165-173.

Chauhan BC, Johnson CA. 1999. Test-retest variability of frequency-doubling perimetry and conventional perimetry in glaucoma patients and normal subjects. Invest Ophthalmol Vis Sci 40(3): 648-656.

Chen CS, Hood DC, Zhang X, Karam EZ, Liebmann JM, Ritch R, et al. 2003. Repeat reliability of the multifocal visual evoked potential in normal and glaucomatous eyes. J Glaucoma 12(5): 399-408.

Cheng H, Laron M, Schiffman JS, Tang RA, Frishman LJ. 2007. The relationship between visual field and retinal nerve fiber layer measurements in patients with multiple sclerosis. Investigative ophthalmology & visual science 48(12): 5798-5805.

Coman I, Aigrot MS, Seilhean D, Reynolds R, Girault JA, Zalc B, et al. 2006. Nodal, paranodal and juxtaparanodal axonal proteins during demyelination and remyelination in multiple sclerosis. Brain 129(Pt 12): 3186-3195.

Compston A, Coles A. 2008. Multiple sclerosis. Lancet 372(9648): 1502-1517.

Confavreux C, Vukusic S, Adeleine P. 2003. Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. Brain 126(Pt 4): 770-782.

Confavreux C, Vukusic S. 2006. Natural history of multiple sclerosis: a unifying concept. Brain 129(Pt 3): 606-616.

Costello F, Hodge W, Pan YI, Eggenberger E, Coupland S, Kardon RH. 2008. Tracking retinal nerve fiber layer loss after optic neuritis: a prospective study using optical coherence tomography. Mult Scler 14(7): 893-905.

Costello F. 2013. The afferent visual pathway: designing a structural-functional paradigm of multiple sclerosis. ISRN Neurol: 134858.

Costello K. 2013. Multiple sclerosis research: diagnostics, disease-modifying treatments, and emerging therapies. J Neurosci Nurs 45(6 Suppl 1): S14-23.

Craner MJ, Newcombe J, Black JA, Hartle C, Cuzner ML, Waxman SG. 2004. Molecular changes in neurons in multiple sclerosis: altered axonal expression of Nav1.2 and Nav1.6 sodium channels and Na+/Ca2+ exchanger. Proc Natl Acad Sci U S A 101(21): 8168-8173.

Cree BA. 2014. 2014 multiple sclerosis therapeutic update. Neurohospitalist 4(2): 63-65.

Curcio CA, Allen KA. 1990. Topography of ganglion cells in human retina. J Comp Neurol 300(1): 5-25.

Davies MB, Williams R, Haq N, Pelosi L, Hawkins CP. 1998. MRI of optic nerve and postchiasmal visual pathways and visual evoked potentials in secondary progressive multiple sclerosis. Neuroradiology 40(12): 765-770.

De Stefano N, Giorgio A, Battaglini M, Rovaris M, Sormani MP, Barkhof F, et al. 2010. Assessing brain atrophy rates in a large population of untreated multiple sclerosis subtypes. Neurology 74(23): 1868-1876.

Di Maggio G, Santangelo R, Guerrieri S, Bianco M, Ferrari L, Medaglini S, et al. 2014. Optical coherence tomography and visual evoked potentials: which is more sensitive in multiple sclerosis? Mult Scler.

Drasdo N, Millican CL, Katholi CR, Curcio CA. 2007. The length of Henle fibers in the human retina and a model of ganglion receptive field density in the visual field. Vision Res 47(22): 2901-2911.

Duncan ID, Brower A, Kondo Y, Curlee JF, Jr., Schultz RD. 2009. Extensive remyelination of the CNS leads to functional recovery. Proc Natl Acad Sci U S A 106(16): 6832-6836.

Dutta R, Trapp BD. 2011. Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. Prog Neurobiol 93(1): 1-12.

Ebers GC, Koopman WJ, Hader W, Sadovnick AD, Kremenchutzky M, Mandalfino P, et al. 2000. The natural history of multiple sclerosis: a geographically based study: 8: familial multiple sclerosis. Brain 123 Pt 3: 641-649.

Fairless R, Williams SK, Hoffmann DB, Stojic A, Hochmeister S, Schmitz F, et al. 2012. Preclinical retinal neurodegeneration in a model of multiple sclerosis. J Neurosci 32(16): 5585-5597.

Ford C, Goodman AD, Johnson K, Kachuck N, Lindsey JW, Lisak R, et al. 2010. Continuous long-term immunomodulatory therapy in relapsing multiple sclerosis: results from the 15-year analysis of the US prospective open-label study of glatiramer acetate. Mult Scler 16(3): 342-350.

Fortune B, Hood DC. 2003. Conventional pattern-reversal VEPs are not equivalent to summed multifocal VEPs. Invest Ophthalmol Vis Sci 44(3):1364-1375.

Fortune B, Zhang X, Hood DC, Demirel S, Johnson CA. 2004. Normative ranges and specificity of the multifocal VEP. Documenta ophthalmologica 109(1): 87-100.

Fortune B, Demirel S, Zhang X, Hood DC, Johnson CA. 2006. Repeatability of normal multifocal VEP: implications for detecting progression. J Glaucoma 15(2): 131-141.

Fox EJ. 2004. Mechanism of action of mitoxantrone. Neurology 63(12 Suppl 6): S15-18.

Franklin RJ, ffrench-Constant C, Edgar JM, Smith KJ. 2012. Neuroprotection and repair in multiple sclerosis. Nat Rev Neurol 8(11): 624-634.

Fraser CL, Klistorner A, Graham SL, Garrick R, Billson FA, Grigg JR. 2006. Multifocal visual evoked potential analysis of inflammatory or demyelinating optic neuritis. Ophthalmology 113(2): 323 e321-323 e322.

Frohman EM, Costello F, Stuve O, Calabresi P, Miller DH, Hickman SJ, et al. 2008a. Modeling axonal degeneration within the anterior visual system: implications for demonstrating neuroprotection in multiple sclerosis. Arch Neurol 65(1): 26-35.

Frohman EM, Fujimoto JG, Frohman TC, Calabresi PA, Cutter G, Balcer LJ. 2008b. Optical coherence tomography: a window into the mechanisms of multiple sclerosis. Nat Clin Pract Neurol 4(12): 664-675.

Garcia-Martin E, Pueyo V, Almarcegui C, Martin J, Ara JR, Sancho E, et al. 2011. Risk factors for progressive axonal degeneration of the retinal nerve fibre layer in multiple sclerosis patients. Br J Ophthalmol 95(11): 1577-1582.

Galetta KM, Calabresi PA, Frohman EM, Balcer LJ. 2011. Optical coherence tomography (OCT): imaging the visual pathway as a model for neurodegeneration. Neurotherapeutics 8(1): 117-132.

Givogri MI, Costa RM, Schonmann V, Silva AJ, Campagnoni AT, Bongarzone ER. 2002. Central nervous system myelination in mice with deficient expression of Notch1 receptor. J Neurosci Res 67(3): 309-320.

Gordon-Lipkin E, Chodkowski B, Reich DS, Smith SA, Pulicken M, Balcer LJ, et al.2007.Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology 69(16): 1603-1609.

Grazioli E, Zivadinov R, Weinstock-Guttman B, Lincoff N, Baier M, Wong JR, et al.2008. Retinal nerve fiber layer thickness is associated with brain MRI outcomes in multiple sclerosis. Journal of the neurological sciences 268(1-2): 12-17.

Green AJ, McQuaid S, Hauser SL, Allen IV, Lyness R. 2010. Ocular pathology in multiple sclerosis: retinal atrophy and inflammation irrespective of disease duration. Brain 133(Pt 6): 1591-1601.

Grippo TM, Hood DC, Kanadani FN, Ezon I, Greenstein VC, Liebmann JM, et al. 2006. A comparison between multifocal and conventional VEP latency changes secondary to glaucomatous damage. Invest Ophthalmol Vis Sci 47(12): 5331-5336.

Grover LK, Hood DC, Ghadiali Q, Grippo TM, Wenick AS, Greenstein VC, et al. 2008. A comparison of multifocal and conventional visual evoked potential techniques in patients with optic neuritis/multiple sclerosis. Documenta ophthalmologica 117(2): 121-128.

Gwet K. 2002. Inter-rater reliability: dependency on trait prevalence and marginal homogeneity. Statistical Methods for Inter-Rater Reliability Assessment No.2

Hammond SR, Yiannikas C. 1986. Contribution of pattern reversal foveal and half-field stimulation to analysis of VEP abnormalities in multiple sclerosis. Electroencephalogr Clin Neurophysiol 64(2): 101-118.

Hammond SR, MacCallum S, Yiannikas C, Walsh JC, McLeod JG. 1987. Variability on serial testing of pattern reversal visual evoked potential latencies from full-field, half-field and foveal stimulation in control subjects. Electroencephalogr Clin Neurophysiol 66(4): 401-408.

Halliday AM, McDonald WI, Mushin J. 1972. Delayed visual evoked response in optic neuritis. Lancet 1(7758): 982-985.

Hawker K. 2011. Progressive multiple sclerosis: characteristics and management. Neurol Clin 29(2): 423-434.

Henderson AP, Barnett MH, Parratt JD, Prineas JW. 2009. Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. Ann Neurol 66(6): 739-753.

Henderson AP, Altmann DR, Trip AS, Kallis C, Jones SJ, Schlottmann PG, et al. 2010a. A serial study of retinal changes following optic neuritis with sample size estimates for acute neuroprotection trials. Brain 133(9): 2592-2602.

Henderson AP, Trip SA, Schlottmann PG, Altmann DR, Garway-Heath DF, Plant GT, et al. 2010b. A preliminary longitudinal study of the retinal nerve fiber layer in progressive multiple sclerosis. Journal of neurology 257(7): 1083-1091.

Herrero R, Garcia-Martin E, Almarcegui C, Ara JR, Rodriguez-Mena D, Martin J, et al. 2012. Progressive degeneration of the retinal nerve fiber layer in

patients with multiple sclerosis. Invest Ophthalmol Vis Sci 53(13): 8344-8349.

Hickman SJ, Brierley CM, Brex PA, MacManus DG, Scolding NJ, Compston DA, et al. 2002. Continuing optic nerve atrophy following optic neuritis: a serial MRI study. Mult Scler 8(4): 339-342.

Hickman SJ, Toosy AT, Jones SJ, Altmann DR, Miszkiel KA, MacManus DG, et al. 2004. A serial MRI study following optic nerve mean area in acute optic neuritis. Brain 127(Pt 11): 2498-2505.

Hood DC, Odel JG, Zhang X. 2000. Tracking the recovery of local optic nerve function after optic neuritis: a multifocal VEP study. Invest Ophthalmol Vis Sci 41(12): 4032-4038.

Hood DC, Greenstein VC. 2003. Multifocal VEP and ganglion cell damage: applications and limitations for the study of glaucoma. Progress in retinal and eye research 22(2): 201-251.

Hood DC, Ohri N, Yang EB, Rodarte C, Zhang X, Fortune B, et al. 2004. Determining abnormal latencies of multifocal visual evoked potentials: a monocular analysis. Documenta ophthalmologica 109(2): 189-199.

Hood DC, Raza AS, de Moraes CG, Liebmann JM, Ritch R. 2013. Glaucomatous damage of the macula. Progress in retinal and eye research 32: 1-21.

Howe CL, Bieber AJ, Warrington AE, Pease LR, Rodriguez M. 2004. Antiapoptotic signaling by a remyelination-promoting human antimyelin antibody. Neurobiol Dis 15(1): 120-131.

Howell OW, Palser A, Polito A, Melrose S, Zonta B, Scheiermann C, et al. 2006. Disruption of neurofascin localization reveals early changes preceding demyelination and remyelination in multiple sclerosis. Brain 129(Pt 12): 3173-3185.

Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, et al. 1991. Optical coherence tomography. Science 254(5035): 1178-1181.

Irvine KA, Blakemore WF. 2008. Remyelination protects axons from demyelination-associated axon degeneration. Brain 131(Pt 6): 1464-1477.

Ikuta F, Zimmerman HM. 1976. Distribution of plaques in seventy autopsy cases of multiple sclerosis in the United States. Neurology 26(6 PT 2): 26-28.

Jindahra P, Petrie A, Plant GT. 2012. The time course of retrograde trans-synaptic degeneration following occipital lobe damage in humans. Brain 135(Pt 2): 534-541.

Jolanda Munzel E, Williams A. 2013. Promoting remyelination in multiple sclerosis-recent advances. Drugs 73(18): 2017-2029.

Jones SJ, Brusa A. 2003. Neurophysiological evidence for long-term repair of MS lesions: implications for axon protection. Journal of the neurological sciences 206(2): 193-198.

Keltner JL, Johnson CA, Spurr JO, Beck RW. 1993. Baseline visual field profile of optic neuritis. The experience of the optic neuritis treatment trial. Optic Neuritis Study Group. Arch Ophthalmol 111(2): 231-234.

Keltner JL, Johnson CA, Spurr JO, Beck RW. 1994. Visual field profile of optic neuritis. One-year follow-up in the Optic Neuritis Treatment Trial. Arch Ophthalmol 112(7): 946-953.

Keltner JL, Johnson CA, Cello KE, Dontchev M, Gal RL, Beck RW. 2010. Visual field profile of optic neuritis: a final follow-up report from the optic neuritis treatment trial from baseline through 15 years. Arch Ophthalmol 128(3): 330-337.

Klistorner A, Graham S, Fraser C, Garrick R, Nguyen T, Paine M, et al. 2007. Electrophysiological evidence for heterogeneity of lesions in optic neuritis. Invest Ophthalmol Vis Sci 48(10): 4549-4556.

Klistorner A, Fraser C, Garrick R, Graham S, Arvind H. 2008. Correlation between full-field and multifocal VEPs in optic neuritis. Documenta ophthalmologica 116(1): 19-27.

Klistorner A, Arvind H, Garrick R, Graham SL, Paine M, Yiannikas C. 2010a. Interrelationship of optical coherence tomography and multifocal visual-evoked potentials after optic neuritis. Invest Ophthalmol Vis Sci 51(5): 2770-2777.

Klistorner A, Arvind H, Garrick R, Yiannikas C, Paine M, Graham SL. 2010b. Remyelination of optic nerve lesions: spatial and temporal factors. Mult Scler 16(7): 786-795.

Klistorner A, Sriram P, Vootakuru N, Wang C, Barnett MH, Garrick R, et al. 2014. Axonal loss of retinal neurons in multiple sclerosis associated with optic radiation lesions. Neurology 82(24): 2165-2172.

Knight OJ, Girkin CA, Budenz DL, Durbin MK, Feuer WJ. 2012. Effect of race, age, and axial length on optic nerve head parameters and retinal nerve fiber layer thickness measured by Cirrus HD-OCT. Arch Ophthalmol 130(3): 312-318.

Koch-Henriksen N, Sorensen PS. 2010. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol 9(5): 520-532.

Kotter MR, Li WW, Zhao C, Franklin RJ. 2006. Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. J Neurosci 26(1): 328-332.

Kremenchutzky M, Cottrell D, Rice G, Hader W, Baskerville J, Koopman W, et al. 1999. The natural history of multiple sclerosis: a geographically based study. 7. Progressive-relapsing and relapsing-progressive multiple sclerosis: a re-evaluation. Brain 122 (Pt 10): 1941-1950.

Kremenchutzky M, Rice GP, Baskerville J, Wingerchuk DM, Ebers GC. 2006. The natural history of multiple sclerosis: a geographically based study 9:

observations on the progressive phase of the disease. Brain 129(Pt 3): 584-594.

Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Bruck W. 2002. Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain 125(Pt 10): 2202-2212.

Kurtzke JF, Beebe GW, Nagler B, Kurland LT, Auth TL. 1977. Studies on the natural history of multiple sclerosis--8. Early prognostic features of the later course of the illness. J Chronic Dis 30(12): 819-830.

Kurtzke JF. 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33(11): 1444-1452.

Laron M, Cheng H, Zhang B, Schiffman JS, Tang RA, Frishman LJ. 2009. Assessing visual pathway function in multiple sclerosis patients with multifocal visual evoked potentials. Mult Scler 15(12): 1431-1441.

Laron M, Cheng H, Zhang B, Schiffman JS, Tang RA, Frishman LJ. 2010. Comparison of multifocal visual evoked potential, standard automated perimetry and optical coherence tomography in assessing visual pathway in multiple sclerosis patients. Mult Scler 16(4): 412-426.

Lassmann H. 2010. Axonal and neuronal pathology in multiple sclerosis: what have we learnt from animal models. Exp Neurol 225(1): 2-8.

Lee J, Koh D, Ong CN. 1989. Statistical evaluation of agreement between two methods for measuring a quantitative variable. Comput Biol Med 19(1): 61-70.

Leung CK, Yu M, Weinreb RN, Lai G, Xu G, Lam DS. 2012a. Retinal nerve fiber layer imaging with spectral-domain optical coherence tomography: patterns of retinal nerve fiber layer progression.Ophthalmology 119(9): 1858-1866.

Leung CK, Yu M, Weinreb RN, Ye C, Liu S, Lai G, et al. 2012b. Retinal nerve fiber layer imaging with spectral-domain optical coherence tomography: a prospective analysis of age-related loss. Ophthalmology 119(4): 731-737.

Lopez-Diego RS, Weiner HL. 2008. Novel therapeutic strategies for multiple sclerosis--a multifaceted adversary. Nat Rev Drug Discov 7(11): 909-925.

Luessi F, Siffrin V, Zipp F. 2012. Neurodegeneration in multiple sclerosis: novel treatment strategies. Expert Rev Neurother 12(9): 1061-1076; quiz 1077.

Maghzi AH, Minagar A, Waubant E. 2013. Neuroprotection in multiple sclerosis: a therapeutic approach. CNS Drugs 27(10): 799-815.

Mancardi G, Saccardi R. 2008. Autologous haematopoietic stem-cell transplantation in multiple sclerosis. Lancet Neurol 7(7): 626-636.

Matthews WB, Small DG, Small M, Pountney E. 1977. Pattern reversal evoked visual potential in the diagnosis of multiple sclerosis. J Neurol Neurosurg Psychiatry 40(10): 1009-1014.

McAlinden C, Khadka J, Pesudovs K. 2011. Statistical methods for conducting agreement (comparison of clinical tests) and precision (repeatability or reproducibility) studies in optometry and ophthalmology. Ophthalmic Physiol Opt 31(4): 330-338.

Menge T, Weber MS, Hemmer B, Kieseier BC, von Budingen HC, Warnke C, et al. 2008. Disease-modifying agents for multiple sclerosis: recent advances and future prospects. Drugs 68(17): 2445-2468.

Metz I, Lucchinetti CF, Openshaw H, Garcia-Merino A, Lassmann H, Freedman MS, et al. 2007. Autologous haematopoietic stem cell transplantation fails to stop demyelination and neurodegeneration in multiple sclerosis. Brain 130(Pt 5): 1254-1262.

Meyer-Moock S, Feng YS, Maeurer M, Dippel FW, Kohlmann T. 2014. Systematic literature review and validity evaluation of the Expanded Composite (MSFC) in patients with multiple sclerosis. BMC Neurol 14:58.

Mi S, Miller RH, Lee X, Scott ML, Shulag-Morskaya S, Shao Z, et al. 2005. LINGO-1 negatively regulates myelination by oligodendrocytes. Nat Neurosci 8(6): 745-751.

Mi S, Hu B, Hahm K, Luo Y, Kam Hui ES, Yuan Q, et al. 2007. LINGO-1 antagonist promotes spinal cord remyelination and axonal integrity in MOG-induced experimental autoimmune encephalomyelitis. Nat Med 13(10): 1228-1233.

Mi S, Miller RH, Tang W, Lee X, Hu B, Wu W, et al. 2009. Promotion of central nervous system remyelination by induced differentiation of oligodendrocyte precursor cells. Ann Neurol 65(3): 304-315.

Mitsunaga Y, Ciric B, Van Keulen V, Warrington AE, Paz Soldan M, Bieber AJ, et al. 2002. Direct evidence that a human antibody derived from patient serum can promote myelin repair in a mouse model of chronic-progressive demyelinating disease. FASEB J 16(10): 1325-1327.

Munzel EJ, Williams A. 2013. Promoting remyelination in multiple sclerosis-recent advances. Drugs 73(18): 2017-2029.

Murray PD, McGavern DB, Sathornsumetee S, Rodriguez M. 2001. Spontaneous remyelination following extensive demyelination is associated with improved neurological function in a viral model of multiple sclerosis. Brain 124(Pt 7): 1403-1416.

Mwanza JC, Chang RT, Budenz DL, Durbin MK, Gendy MG, Shi W, et al. 2010. Reproducibility of peripapillary retinal nerve fiber layer thickness and optic nerve head parameters measured with cirrus HD-OCT in glaucomatous eyes. Invest Ophthalmol Vis Sci 51(11): 5724-5730.

Mwanza JC, Durbin MK, Budenz DL, Girkin CA, Leung CK, Liebmann JM, et al. 2011a. Profile and predictors of normal ganglion cell-inner plexiform layer thickness measured with frequency-domain optical coherence tomography. Invest Ophthalmol Vis Sci 52(11): 7872-7879.

Mwanza JC, Oakley JD, Budenz DL, Chang RT, Knight OJ, Feuer WJ. 2011b. Macular ganglion cell-inner plexiform layer: automated detection and thickness reproducibility with spectral domain-optical coherence tomography in glaucoma. Invest Ophthalmol Vis Sci 52(11): 8323-8329.

Narayanan D, Cheng H, Bonem KN, Saenz R, Tang RA, Frishman LJ. 2014. Tracking changes over time in retinal nerve fiber layer and ganglion cell-inner plexiform layer thickness in multiple sclerosis. Mult Scler.

Neuhaus O, Kieseier BC, Hartung HP. 2004. Mechanisms of mitoxantrone in multiple sclerosis--what is known? Journal of the neurological sciences 223(1): 25-27

Nikic I, Merkler D, Sorbara C, Brinkoetter M, Kreutzfeldt M, Bareyre FM, et al. 2011. A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. Nat Med 17(4): 495-499.

Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. 2000. Multiple sclerosis. N Engl J Med 343(13): 938-952.

Odom JV, Bach M, Brigell M, Holder GE, McCulloch DL, Tormene AP, et al. 2009. ISCEV standard for clinical visual evoked potentials (2009 update). Documenta ophthalmologica 120(1): 111-119.

Optic Neuritis Study Group. 1991. The clinical profile of optic neuritis. Experience of the Optic Neuritis Treatment Trial. Arch Ophthalmol 109(12): 1673-1678.

Optic Neuritis Study Group. 2008. Multiple sclerosis risk after optic neuritis: final optic neuritis treatment trial follow-up. Arch Neurol 65(6): 727-732.

Ouardouz M, Malek S, Coderre E, Stys PK. 2006. Complex interplay between glutamate receptors and intracellular Ca2+ stores during ischaemia in rat spinal cord white matter. J Physiol 577(Pt 1): 191-204.

Patani R, Balaratnam M, Vora A, Reynolds R. 2007. Remyelination can be extensive in multiple sclerosis despite a long disease course. Neuropathol Appl Neurobiol 33(3): 277-287.

Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H, et al. 2006. Remyelination is extensive in a subset of multiple sclerosis patients. Brain 129(Pt 12): 3165-3172.

Petzold A, de Boer JF, Schippling S, Vermersch P, Kardon R, Green A, et al. 2010. Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. Lancet Neurol 9(9): 921-932.

Pirko I, Ciric B, Gamez J, Bieber AJ, Warrington AE, Johnson AJ, et al. 2004. A human antibody that promotes remyelination enters the CNS and decreases lesion load as detected by T2-weighted spinal cord MRI in a virus-induced murine model of MS. FASEB J 18(13): 1577-1579.

Polman CH, Wolinsky JS, Reingold SC. 2005a. Multiple sclerosis diagnostic criteria: three years later. Mult Scler 11(1): 5-12.

Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, et al. 2005b. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 58(6): 840-846.

Prineas JW, Kwon EE, Cho ES, Sharer LR. 1984. Continual breakdown and regeneration of myelin in progressive multiple sclerosis plaques. Ann N Y Acad Sci 436: 11-32.

PRISMS Study Group.1998. Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis.Lancet 352(9139): 1498-1504.

Quelly A, Cheng H, Laron M, Schiffman JS, Tang RA. 2010. Comparison of optical coherence tomography and scanning laser polarimetry measurements in patients with multiple sclerosis. Optom Vis Sci 87(8): 576-584.

Ramagopalan SV, Dobson R, Meier UC, Giovannoni G. 2010. Multiple sclerosis: risk factors, prodromes, and potential causal pathways. Lancet Neurol 9(7): 727-739.

Ratchford JN, Saidha S, Sotirchos ES, Oh JA, Seigo MA, Eckstein C, et al. 2013. Active MS is associated with accelerated retinal ganglion cell/inner plexiform layer thinning. Neurology 80(1): 47-54.

Rodriguez M, Scheithauer B. 1994. Ultrastructure of multiple sclerosis. Ultrastruct Pathol 18(1-2): 3-13.

Rodriguez M, Warrington AE, Pease LR. 2009. Invited article: human natural autoantibodies in the treatment of neurologic disease. Neurology 72(14): 1269- 1276.

Rovaris M, Confavreux C, Furlan R, Kappos L, Comi G, Filippi M. 2006. Secondary progressive multiple sclerosis: current knowledge and future challenges. Lancet Neurol 5(4): 343-354.

Rudick RA, Cutter GR, Baier M, Weinstock-Guttman B, Mass MK, Fisher E, et al. 2005. Estimating long-term effects of disease-modifying drug therapy in multiple sclerosis patients. Mult Scler 11(6): 626-634.

Saidha S, Syc SB, Durbin MK, Eckstein C, Oakley JD, Meyer SA, et al. 2011. Visual dysfunction in multiple sclerosis correlates better with optical coherence tomography derived estimates of macular ganglion cell layer thickness than peripapillary retinal nerve fiber layer thickness. Mult Scler 17(12): 1449-1463.

Scalfari A, Neuhaus A, Degenhardt A, Rice GP, Muraro PA, Daumer M, et al. 2010. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. Brain 133(Pt 7): 1914-1929.

Seewann A, Vrenken H, van der Valk P, Blezer EL, Knol DL, Castelijns JA, et al. 2009. Diffusely abnormal white matter in chronic multiple sclerosis: imaging and histopathologic analysis. Arch Neurol 66(5): 601-609.

Serbecic N, Aboul-Enein F, Beutelspacher SC, Vass C, Kristoferitsch W, Lassmann H, et al. 2011. High resolution spectral domain optical coherence tomography (SD-OCT) in multiple sclerosis: the first follow up study over two years. PLoS One 6(5): e19843.

Shahrokhi F, Chiappa KH, Young RR. 1978. Pattern shift visual evoked responses. Two hundred patients with optic neuritis and/or multiple sclerosis. Arch Neurol 35(2): 65-71.

Smith KJ, Blakemore WF, McDonald WI. 1979. Central remyelination restores secure conduction. Nature 280(5721): 395-396.

Sokol S. 1976. Visually evoked potentials: theory, techniques and clinical applications. Surv Ophthalmol 21(1): 18-44.

SPECTRIMS study group. 2001. Randomized controlled trial of interferon- beta-1a in secondary progressive MS: Clinical results. Secondary Progressive Efficacy Clinical Trial of Recombinant interferon-Beta-a1 in MS. Neurology 56(11): 1496-1504

Sriram P, Klistorner A, Arvind H, Graham SL. 2012. Reproducibility of multifocal VEP latency using different stimulus presentations. Documenta ophthalmologica 125(1): 43-49.

Stys PK, Zamponi GW, van Minnen J, Geurts JJ. 2012. Will the real multiple sclerosis please stand up? Nat Rev Neurosci 13(7): 507-514.

Stys PK. 2013. Pathoetiology of multiple sclerosis: are we barking up the wrong tree? F1000Prime Rep 5: 20.

Syc SB, Saidha S, Newsome SD, Ratchford JN, Levy M, Ford E, et al. 2012. Optical coherence tomography segmentation reveals ganglion cell layer pathology after optic neuritis. Brain 135(Pt 2): 521-533.

Talman LS, Bisker ER, Sackel DJ, Long DA, Jr., Galetta KM, Ratchford JN, et al. 2010. Longitudinal study of vision and retinal nerve fiber layer thickness in multiple sclerosis. Ann Neurol 67(6): 749-760.

Thomae E, Niklas A, Sebraoui H, Baum P, Wagner A, Then Bergh F. 2010. Improving test-retest variability of visual-evoked responses in multiple sclerosis: implications for trial design. J Clin Neurophysiol 27(4): 270-273.

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(5): 278-285.

Trapp BD, Ransohoff R, Rudick R. 1999. Axonal pathology in multiple sclerosis: relationship to neurologic disability. Curr Opin Neurol 12(3): 295-302.

Trapp BD, Nave KA. 2008. Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci 31: 247-269.

Trapp BD, Stys PK. 2009. Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet Neurol 8(3): 280-291.

Tremlett H, Yinshan Z, Devonshire V. 2008. Natural history of secondary-progressive multiple sclerosis. Mult Scler 14(3): 314-324.

Tremlett H, Zhao Y, Rieckmann P, Hutchinson M. 2010. New perspectives in the natural history of multiple sclerosis. Neurology 74(24): 2004-2015.

Toussaint D, Perier O, Verstappen A, Bervoets S. 1983. Clinicopathological study of the visual pathways, eyes, and cerebral hemispheres in 32 cases of disseminated sclerosis. J Clin Neuroophthalmol 3(3): 211-220.

Tullman MJ. 2013. Overview of the epidemiology, diagnosis, and disease progression associated with multiple sclerosis. Am J Manag Care 19(2 Suppl): S15-20.

Vukusic S, Confavreux C. 2003. Primary and secondary progressive multiple sclerosis. J Neurol Sci 206(2): 153-155.

Wall M, Johnson CA, Kutzko KE, Nguyen R, Brito C, Keltner JL. 1998. Long- and short-term variability of automated perimetry results in patients with optic neuritis and healthy subjects. Arch Ophthalmol 116(1): 53-61.

Wang J, Cheng H, Hu YS, Tang RA, Frishman LJ. 2012. The photopic negative response of the flash electroretinogram in multiple sclerosis. Invest Ophthalmol Vis Sci 53(3): 1315-1323.

Wangsupadilok B, Greenstein VC, Kanadani FN, Grippo TM, Liebmann JM, Ritch R, et al. 2009. A method to detect progression of glaucoma using the multifocal visual evoked potential technique. Documenta ophthalmologica 118(2): 139-150.

Walker R. 2000. McAlpine's multiple sclerosis. Third edition. J Neurol Neurosurg Psychiatry 68(6): 801A.

Warrington AE, Bieber AJ, Ciric B, Pease LR, Van Keulen V, Rodriguez M. 2007. A recombinant human IgM promotes myelin repair after a single, very low dose. J Neurosci Res 85(5): 967-976.

Warrington AE, Asakura K, Bieber AJ, Ciric B, Van Keulen V, Kaveri SV, et al. 2000. Human monoclonal antibodies reactive to oligodendrocytes promote remyelination in a model of multiple sclerosis. Proc Natl Acad Sci U S A 97(12): 6820-6825.

Waxman SG, Black JA, Stys PK, Ransom BR. 1992. Ultrastructural concomitants of anoxic injury and early post-anoxic recovery in rat optic nerve. Brain Res 574(1-2): 105-119.

Weinshenker BG, Bass B, Rice GP, Noseworthy J, Carriere W, Baskerville J, et al. 1989. The natural history of multiple sclerosis: a geographically based study. 2. Predictive value of the early clinical course. Brain 112 (Pt 6): 1419-1428.

Weinshenker BG, Rice GP, Noseworthy JH, Carriere W, Baskerville J, Ebers GC. 1991. The natural history of multiple sclerosis: a geographically based

study. 4. Applications to planning and interpretation of clinical therapeutic trials. Brain 114 (Pt 2): 1057-1067.

Wingerchuk DM, Carter JL. 2014. Multiple sclerosis: current and emerging disease-modifying therapies and treatment strategies. Mayo Clin Proc 89(2): 225-240.

Yang EB, Hood DC, Rodarte C, Zhang X, Odel JG, Behrens MM. 2007. Improvement in conduction velocity after optic neuritis measured with the multifocal VEP. Invest Ophthalmol Vis Sci 48(2): 692-698.

Zambonin JL, Zhao C, Ohno N, Campbell GR, Engeham S, Ziabreva I, et al. 2011. Increased mitochondrial content in remyelinated axons: implications for multiple sclerosis. Brain 134(Pt 7): 1901-1913.