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MULTIFOCAL VISUAL EVOKED POTENTIALS IN DEMYELINATING DISEASES OF THE VISUAL PATHWAY

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A thesis submitted to The University of Sydney in fulfilment of the requirements for the degree of Doctor of Philosophy

Faculty of Medicine

THE UNIVERSITY OF SYDNEY

June 2015
Statement of originality

I declare that apart from the assistance that is acknowledged below, to the best of my knowledge, the research presented in this thesis is my original work and it has not been submitted toward the award for another degree in this university or other institutes.

Daniah A. Alshowaeir

15th June 2015
Acknowledgment

During the course of preparation of this thesis, I was privileged to know and learn from a talented group of people. The knowledge and help that I have received was vital in the completion of this work.

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I wish to dedicate this dissertation to my Dad, Abdulrahman, and Mum, Hessa, who taught me, at a tender age, that “knowledge is power”.
Thesis abstract

Multifocal visual evoked potentials (mfVEP) can offer vital information regarding the integrity of the visual system. In this thesis, the mfVEP changes in demyelinating diseases of the visual pathway were investigated. Four main areas were explored. First, the efficacy of the mfVEP technique compared to full-field pattern-reversal visual evoked potential (PVEP) was evaluated in six patients with known pathologies at various levels of the visual pathway. The case series demonstrated a potentially higher sensitivity of mfVEP compared to conventional PVEPs in detecting lesions affecting the peripheral visual fields and horizontal hemi-fields and lesions of the post-chiasmal pathway.

Second, mfVEP evolution in optic neuritis (ON) in the affected and fellow eyes during the first year after the attack was analysed. The mfVEP of 87 patients and 25 controls were analysed longitudinally and correlated with retinal nerve fibre layer (RNFL) thickness. Patients were classified into three groups: those diagnosed with multiple sclerosis (MS), and those at high risk (HR) or low risk (LR) for conversion to MS based on their magnetic resonance imaging (MRI). The results of this study demonstrated that recovery of amplitude and shortening of latency is fastest within the first three months. The largest amplitude reduction and longest latency delay of the affected eyes were recorded in the MS group. This was accompanied by statistically significant deterioration of both parameters in the fellow eyes. MfVEP remained stable in the fellow eyes of the LR group. Inter-eye asymmetry analysis was used to minimise the bilateral effect of any potential retro-chiasmal lesions and revealed a similar amount of amplitude reduction and latency delay in all three groups. RNFL thickness strongly correlated with mfVEP amplitude as early as three months after ON ($R^2 = 0.6$,
This study indicated that mfVEP amplitude can be used as an early predictor of post-ON axonal loss. In addition, the findings suggest that the apparently more severe involvement of ON eyes in the MS subgroup may be due to subclinical inflammation along the visual pathway.

The third aim was to analyse the mfVEP latency and waveform changes of the fellow eyes of patients with clinically isolated ON and MS-related ON in greater depth by evaluating mfVEP traces from individual segments, and to address the possible effect of cortical adaptation on latency change. The latency and waveform changes of mfVEP traces in fellow eyes of 15 ON patients were analysed and correlated with latency delay of the affected eyes. Eight age- and gender-matched controls were also included for comparison. The study showed that while there was a slight mfVEP latency change between three and 12 months post-attack in the fellow eyes of ON patients with low risk of MS that might support the hypothesis of cortical adaptation as the mechanism of its delay, the mfVEP latencies remained within the normal range. The significant mfVEP latency delay in the fellow eyes of MS patients and the change over time compared to clinically isolated ON patients and controls supports the assumption that the changes are due to subclinical demyelination in the visual pathway.

The fourth aim was to test the hypothesis that the latency delay of mfVEP in non-ON eyes of MS patients is related to retro-chiasmal demyelinating lesions. Fifty-seven MS patients with no history of ON at least in one eye and 25 age- and sex-matched controls were tested. Probabilistic tractography was used to reconstruct optic radiation (OR) fibres. MS lesion volume on MRI and diffusion tensor imaging (DTI) indices was measured. The relationship of the mfVEP latency with OR lesions and DTI indices was examined. A significant association was revealed between mfVEP latency delay and OR lesion load. There was also a significant correlation between mfVEP latency and
OR DTI. The findings of this study support our hypothesis that latency delay of the mfVEP in the eyes of MS patients without previous ON is related to retro-chiasmal demyelinating lesions.
Publications and presentations related to this thesis

Full article publications:


Abstracts at peer-reviewed conferences:

- Alshowaeir D, Yiannikas C, Garrick R, Van Der Walt A, Graham S, Fraser CL, Klistorner A. MfVEP assessment of optic neuritis evolution. The Young Investigators’ PhD seminar; Aug 2014; Sydney, Australia: The University of Sydney; 2014. [Oral presentation].
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<th>Definition</th>
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<td>AD</td>
<td>Axial diffusivity</td>
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<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
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<tr>
<td>BBB</td>
<td>The blood brain barrier</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor images</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion-weighted imaging</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
</tr>
<tr>
<td>FLAIR</td>
<td>Fluid-attenuated inversion recovery</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional MRI</td>
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<td>fVEP</td>
<td>Flash visual evoked potential</td>
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<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
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<td>HR</td>
<td>High risk for developing multiple sclerosis</td>
</tr>
<tr>
<td>HVF</td>
<td>Humphrey visual field</td>
</tr>
<tr>
<td>ISCEV</td>
<td>International Society of Clinical Electrophysiology of Vision</td>
</tr>
<tr>
<td>LGN</td>
<td>Lateral geniculate nucleus</td>
</tr>
<tr>
<td>LR</td>
<td>Low risk for developing multiple sclerosis</td>
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<td>M cells</td>
<td>Midget cells</td>
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<td>MD</td>
<td>Mean diffusivity</td>
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<tr>
<td>mfVEP</td>
<td>Multifocal visual evoked potential</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MS</td>
<td>Multiple sclerosis</td>
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<tr>
<td>ms</td>
<td>Millisecond</td>
</tr>
<tr>
<td>MTI</td>
<td>Magnetisation transfer imaging</td>
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<tr>
<td>NFL</td>
<td>Nerve fibre layer</td>
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<td>NON</td>
<td>Non-optic neuritis eyes</td>
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<td>OCT</td>
<td>Optical coherence tomography</td>
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<td>ON</td>
<td>Optic neuritis</td>
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<td>ONTT</td>
<td>Optic neuritis treatment trail</td>
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<td>PPMS</td>
<td>Primary progressive multiple sclerosis</td>
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<td>PVEP</td>
<td>Pattern visual evoked potential</td>
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<tr>
<td>RD</td>
<td>Radial diffusivity</td>
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<tr>
<td>RNFL</td>
<td>Retinal nerve fibres layer</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<td>RRMS</td>
<td>Relapsing remitting multiple sclerosis</td>
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<td>SNR</td>
<td>Signal to noise ratio</td>
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<td>SPMS</td>
<td>Secondary progressive multiple sclerosis</td>
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<td>V1</td>
<td>Primary visual cortex</td>
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<td>VEP</td>
<td>Visual evoked potentials</td>
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Chapter one: Thesis overview and background
1.0 Overview

This chapter is an introduction to the thesis and includes a brief background and review of relevant literature. The chapter is divided into three main sections. The first section briefly discusses the general aims and rational of this thesis. Section two describes the basic anatomy of the visual pathway whereas section three reviews visual evoked potentials including multifocal visual evoked potential (mfVEP) with a focus on cortical origin of generated signals and visual evoked potential changes reported in various visual pathway pathologies. The visual pathway is highly susceptible to multiple sclerosis (MS) lesions and therefore the last section briefly reviews MS and discusses the involvement of the visual pathway in MS.

1.1 Aims and rationale of this thesis

Visual evoked potentials (VEP) can offer vital information regarding the integrity of the visual system and provide a general objective measure of visual pathway function. Advances in recording techniques such as multifocal technology provide topographical measurements of visual function allowing localised damage to be identified.

This thesis has four main aims to address. The first aim is to assess the comparative efficacy of mfVEP and full-field pattern VEP techniques in patients with a known pathology at various levels of the visual pathway. The second aim is to evaluate the mfVEP evolution after acute optic neuritis (ON) and to examine the pattern of amplitude and latency changes in ON eyes and fellow eyes in a large cohort of patients. Additionally, the association between mfVEP parameters and long-term axonal loss will be examined.
The third aim of this thesis is to analyse latency and waveform changes of mfVEP traces in the fellow eyes of ON patients. Alteration of VEP in an absence of clinical symptoms has been reported in the fellow eye of patients with ON, which has been attributed to several factors including subclinical demyelination and cortical adaptation. MS patients and patients with normal magnetic resonance imaging (MRI) will be examined in an attempt to distinguish the effect of cortical adaptation from the effect of demyelinating lesions that may occur within the posterior visual pathway.

The last aim is to investigate the potential relationship of latency delay of mfVEP with optic radiation (OR) lesions in MS patients. The OR lesions will be identified using tractography and OR diffusion tensors indices (DTI) will be measured. Evaluating the correlation between structural and functional measures of visual pathway integrity will provide a better understanding of the nature of their relationship.

MfVEP is an evolving technology and is starting to find its place in the clinical setting. The need for more research to better understand the changes of mfVEP in visual pathway disorders is imperative. We believe that the studies included in this thesis will shed a light on some new aspects of this potentially important technique.
1.2 Background

1.2.1 The visual pathway

Visual perception occurs when light stimulus in the surrounding environment converts to nerve impulses at the level of photoreceptors, which then reach the brain to be processed. The light energy is converted to neuronal signals that are transmitted through several layers in the retina to reach the ganglion cells. The axons of the ganglion cells form the optic nerve. Signals are carried out from the optic nerve through the optic chiasm and optic tract, which is connected to the lateral geniculate body. From there, signals reach the visual cortex in the occipital lobe through the optic radiation (Fig 1.1).

Fig 1.1: The visual pathway anatomy.
Source: http://www.aao.org/theyeshaveit/anatomy/visual-pathway.cfm

The arrangement and distribution of visual pathway fibres have been extensively studied (1-3). Because damage in this pathway could be measured functionally and
structurally, understanding the optic fibres pathway will help to recognise lesion location based on the visual field defect and the associated functional disturbance.

This section discusses the anatomy of the visual pathway that is relevant to our research.

**Retina**

The retina, which is situated between the choroid and the vitreous, is the site where photons are converted into neuronal signals. Although the retina is around 0.4 mm in thickness, it has a well-organised structure consisting of ten layers, three of them nuclear layers (Fig 1.2) (2).

**Fig 1.2:** The anatomy of the retina. Source: Remington, L.A. Clinical anatomy of the visual system. Elsevier Health Sciences; 2011

The outermost layer is the retinal pigment epithelium, which is a single layer of pigmented cells between the neurosensory retina and the choroid. It has several functions including light absorption, controlled transport of substances, and
phagocytosis (4). The photoreceptor layer contains the outer and the inner segments of three types of cone and one type of rod photoreceptors. Cones are responsible for sharp, detailed, and colour vision while rods are responsible for vision under dim (scotopic) conditions. The highest distribution of cones is at the macula, which is at the central part of the retina. On the other hand, the rods have high distribution throughout the retina, which declines significantly at the macular area (2).

The external limiting membrane is between the photoreceptor layer and the outer nuclear layer. Histologically it is adherent junctions between photoreceptors and Müller cells which act as a metabolic barrier for some large molecules and therefore it is not considered a real membrane. The cell bodies of the photoreceptors form the outer nuclear layer. The outer plexiform layer consists of a network of neuronal synapses connecting the photoreceptors to bipolar cells and horizontal cells, while the inner nuclear layer consists of horizontal, bipolar and amacrine cells. The inner plexiform layer contains dendrites of retinal ganglion cells and processes of bipolar and amacrine cells (2).

The ganglion cell layer contains bodies of the ganglion cells, which receive their input from bipolar and amacrine cells and are the first cells to produce action potentials. There are three main types of ganglion cells (2, 5, 6). The first type is the midget cells, which receive direct input from a single bipolar cell and therefore have small receptive fields. Their axons form the parvocellular pathway. The second type is the parasol cells, which receive their input from diffuse bipolar cells and thus have larger receptive fields. They form the magnocellular pathway. The third type is the small bistratified cells, which have blue-ON receptive fields and project through the koniocellular pathway. Additionally, there are several other different ganglion cell types that have been
identified, for example, the melanopsin-containing ganglion cells which are involved in non-image forming visual functions and pupillary responses (7, 8).

The nerve fibre layer (NFL) is comprised of ganglion cell axons, which are generally unmyelinated in the retina. The innermost layer is an internal limiting membrane and it is formed by the footplates of Müller cells and a basement membrane (9). The outer retina receives blood supply from the choroid while the central retinal artery supplies the inner retinal layers.

**The optic nerve**

Ganglion cell axons turn 90° to enter the optic disc, where they form the optic nerve. The optic disc is supplied by a ring of branches from the short ciliary arteries called the circle of Zinn. Peripapillary arteries also contribute to the optic disc blood supply. The optic nerve consists of 2.2 million fibres with different sizes of diameter, ranging from 0.7 μm to 10 μm. Smaller fibres serve the central vision while larger ones come from the peripheral retina (10). The macular fibres are deep in the centre of the optic nerve while the fibres of the peripheral retina are more superficial.

The length of the optic nerve is around 6 cm and can be divided anatomically into four segments: intraocular (0.7 to 1 mm), intraorbital (30 mm), intracanalicular (6 to 10 mm), and intracranial (10 to 16 mm). The lamina cribrosa divides the intraocular part into prelaminar and laminar sections (2). It is important to note that this part of the nerve is not myelinated. Oligodendrocytes are responsible for the myelination of nerves and it is believed that the lamina cribrosa acts as a barrier preventing them from myelinating the intraocular section of the optic nerve (3).
Beyond the lamina cribrosa the optic nerve is myelinated and surrounded by a dural sheath and cerebrospinal fluid. The extraocular muscles surround the optic nerve in the orbit. The optic nerve sheet is adherent to the superior and medial rectus muscle, hence the pain with eye movement when the optic nerve is inflamed in cases such as ON. The ophthalmic artery is the first branch from the internal carotid artery and it forms the main blood supply for intraorbital and intracanalicular division of the optic nerve. The ophthalmic artery passes through the dural sheath of the optic nerve in the intracanalicular section. The intracranial optic nerve division is supplied by branches from the ophthalmic, interior cerebral, anterior communicating, and internal carotid arteries.

**Optic chiasm**

Ninety percent of the optic nerve fibres from both sides join in the optic chiasm while the remaining ten percent of fibres project to areas controlling pupillary responses (3). In the optic chiasm, the medial fibres partially cross (decussate) to terminate in the opposite side of the brain, representing about 53% of optic nerve fibres. Some nasal fibres may loop either into the end part of the opposite optic nerve (anterior knees of Wilbrand) or into the optic tract of the same side before crossing (posterior knees of Wilbrand). The temporal optic nerve fibres run directly through the chiasm to the optic tract on the same side. Nasal optic nerve fibres cross to the opposite side at the optic chiasm and terminate at the opposite side of the brain.

The optic chiasm has a rectangular shape, measuring 15 mm by 8 mm with a thickness of 4 mm. Similar to the optic nerve, the optic chiasm is surrounded by a dural sheath and cerebrospinal fluid (2).
Above the optic chiasm is the third ventricle and around 1 cm below it is the pituitary gland, resting in a small cavity called the sella turcica. The position of the optic chiasm above the sella turcica varies among individuals. Around 75% of the population has the chiasm directly above it, while 15% have the gland displaced posteriorly (prefixed) because of shorter optic nerves and 10% have it displaced anteriorly (postfixed) because the optic nerves are long and the gland lies just below the anterior part of the chiasm (2, 3). The optic chiasm lies within the circle of Willis from where it receives its blood supply via capillary beds from the anterior cerebral, anterior communicating, internal carotid, posterior cerebral, and posterior communicating arteries.

**The optic tract**

The optic tract originates from the posterior lateral side of the optic chiasm and ends at the lateral geniculate nucleus, where the fibres of the ganglion cells terminate. In the optic tract, fibres from the inferior peripheral retina run laterally while superior peripheral fibres run medially and the macular fibres in between. The optic tract measures around 5.1 mm in length and 3.5 mm in thickness (3). The main blood supply of the optic tract comes from the anterior choroidal artery, which originates from the internal carotid artery.

**Lateral geniculate nucleus**

The lateral geniculate nucleus (LGN) is located in the dorsolateral part of the thalamus. Retinal fibres end at the LGN and fibres from the LGN project to the visual cortex as the optic radiation. The LGN is composed of six layers where layers one and two receive information from the magnocellular pathway and layers three to six receive input from the parvocellular pathway. Those two pathways are anatomically and
functionally distinct (2). While the magnocellular pathway is responsible for processing motion and high flicker perception, the parvocellular pathway is mainly in charge of pattern and colour information analysis (11).

Because the LGN receives input from both cortex and subcortical centres, it has a very complex role in the visual process and acts as a selective regulator of visual information flow to the visual cortex (3). The LGN receives blood supply from branches of the posterior cerebral artery.

**Optic radiation (OR)**

The OR is composed of LGN fibres that run deep in the white matter of the cerebral hemispheres. Anatomically, the optic radiation is divided into three sections. The anterior section passes laterally to the inferior horn of the lateral ventricle. This section receives blood supply from the anterior choroidal artery and the middle cerebral artery. The second section is the middle one, through which OR fibres run laterally to the ventricles. It is supplied by the deep optic branch of the middle cerebral artery. The third section is the posterior part of the OR, which is supplied by branches of the posterior cerebral artery and the middle cerebral artery. Fibres from the inferior retina pass through the temporal lobe while fibres from the superior retina pass through the parietal lobe (12). Macular fibres are usually located in between the superior and inferior fibres (2). The OR fibres terminate at the primary visual cortex.

**Primary visual cortex**

The primary visual cortex (V1) extends from the posterior pole onto the medial surface of the occipital lobe. It is supplied mainly by the calcarine branch of the posterior cerebral artery and receives some contribution from the middle cerebral artery (2, 3).
The calcarine fissure divides the primary visual cortex into the upper division (cuneus gyrus) and lower division (lingual gyrus). The upper division receives fibres from the superior part of the optic radiation while the lower division receives fibres from the inferior part of the optic radiation. V1 has six main neuronal layers. The optic radiation axons terminate in the fourth layer, known also as the lamina granularis interna, which is further divided into 4A, 4B, and 4C. The magnocellular axons terminate in the upper half of 4C (also called 4Cα) while the parvocellular axons terminate in the lower half (4Cβ). V1 layers have rich interlayer connections and projections to other areas in the cortex and to subcortical areas.

Macular fibres project onto the most posterior part of V1 with the superior macular fibres projected onto the cuneus gyrus and the inferior macular fibres projected onto the lingual gyrus. Although the macular area at the retina is relatively small, macular fibres have a large representation at the primary visual cortex (Figure 1.3). Around 50-60% of V1 is devoted to the central 10° of the retina (3, 13).

![Fig. 1.3](image.png)  
**Fig. 1.3:** Left image: The medial side of the left occipital cortex showing the representation of the right visual field. The dotted lines represent eccentricity from the fovea in degrees. Right image: The layout map of the right visual hemi-field. The vertical lines indicate eccentricity in degrees. The dark spot corresponds to the right

In 1977, Hubel and Wiesel described a modular organisation of V1 (14). They proposed that the cortex is composed of modules with a set of orientation columns of cells crossed by ocular dominance columns and colour sensitivity blobs. Cortical cells are organised in specific horizontal and vertical patterns to analyse contour and depth perception accurately (3, 14, 15).

One of the early attempts to study the retinotopic organisation in the visual cortex was by a Japanese ophthalmologist named Tatsuii Inouye in 1905 (16). Years later, Gordon Holmes proposed a map of the visual field representation in the human visual cortex based on the visual field loss resulting from gunshot wounds during World War I (17). Accordingly, it was demonstrated that the upper and lower visual fields are represented in the lower and the upper banks of the contralateral calcarine fissure respectively.

With the advances in MRI, Horton and Hoyt reassessed Holmes’ map and concluded that it provides an accurate estimate of the retinal projections to the visual cortex but that it underestimated the cortical magnification of the central vision, and proposed a revised map (Fig. 1.3) (13, 18).

The extra-striate cortex includes several functional areas within the visual cortex that are involved in processing of visual information. Brodmann area 18, also called V2, is adjacent to V1 and projects to other extra-striate areas. V3 receives input from both V1 and V2 and has a role in depth perception, motion and direction. V4 appears to receive input from the parvocellular pathway and has a role in colour perception. V5 receives input from V1 and directly from the magnocellular pathway and thus plays a
role in processing movement and direction of visual stimuli. V6 plays a role in facial recognition and saccadic movements (3). This brief anatomy review is clearly simplified but the complex details of the primary visual cortex function are beyond the scope of this thesis.
1.2.2 Visual evoked potentials (VEP) in the assessment of the visual pathway

VEP background

VEP is an electrical response recorded mainly from the visual cortex in response to light stimuli. It was first introduced in the 1930s and its role has evolved over the years (19). In 1961, Ciganek was the first to describe an electroencephalography (EEG) response to a flashlight stimulus in humans, followed by one of the earliest clinical studies of VEP reported by Halliday and colleagues on patients with optic neuritis (20, 21).

VEP provides an objective and reproducible measure of visual function and continues to have an imperative complementary role to other tests that provide information on the structure of the visual system such as MRI and optical coherence tomography (OCT).

The recording of VEP is performed using occipital mounted electrodes with, typically, monocular stimulation. Several forms of visual stimulus can be used to generate a VEP. The most common stimuli used are flash visual evoked potential (fVEP), pattern-onset VEP and reversing black and white checkerboard pattern (PVEP). Because of fVEP’s high inter-subject variability and low sensitivity, PVEP is preferred in most clinical setting. fVEP is frequently used in infants, uncooperative patients or if significant media opacity is present. The pattern-onset VEP is preferred in patients with fixation instability such as sytagms since the PVEP is severely reduced in those patients due to the effect of retinal image motion on the stimulus efficiency (22, 23).
The testing technique for these stimulus conditions has been standardised by the International Society of Clinical Electrophysiology of Vision (ISCEV) to reach a better consistency of results between different electrophysiology laboratories (24).

The PVEP waveform is triphasic with a prominent positive peak (P100) at around 100 ms, an earlier negative peak at around 75 ms, and a late negative peak at around 135 ms after stimulation (Fig 1.4). The amplitude of the P100 reflects the number of functional afferent axons reaching the cortex. The implicit time (latency) is believed to reflect the degree of demyelination. An abnormal VEP response indicates a functional disturbance in the afferent visual pathway and occasionally conventional VEP may provide some information on the location of the lesion (25). For example, based on the neuroanatomy of the visual system, a unilateral VEP abnormality implies an abnormality in the anterior optic pathway. Localisation is less likely when the delay is bilateral.

**Fig 1.4:** Normal waveform of a standard PVEP. Arrows showing first negative peak (N75), positive peak (P100) and a late negative peak (N135)
Cortical sources of VEP

The retinotopic organisation in V1 (discussed previously in 1.2.1) indicates that the upper visual hemi-field is mapped in lower banks while the lower visual hemi-field is mapped in the upper banks of the calcarine fissure. Therefore, visual stimulation of the upper hemi-field will generate evoked potentials that are opposite in polarity to that generated by the hemi-field stimulus. The polarity of the VEP would not change in response to changing stimulus position for VEPs generated from other visual areas. As a result, several studies have shown that the first major component of PVEP reverses in polarity when the stimulus position is changed between the upper and lower visual fields. Therefore, it arises primarily in V1. In contrast, the dominant later components seem to be generated in multiple extrastriate areas (25-28). In the multifocal recording technique, as discussed in the following chapters, the stimulation and recording conditions are different. This results in a different waveform generated mainly from V1 with less extrastriate contribution (29). Fortune and Hood suggested that the fast stimulation sequence used in the multifocal technique reduces the contribution of extrastriate areas and results in a waveform that is largely generated from V1 (30).

VEP in optic neuritis and multiple sclerosis

One of the earliest reports of VEP changes in patients with ON was in the early 1970s when Halliday and colleagues compared 19 cases of ON with healthy controls and reported a significant latency delay in the ON group, which persisted despite improvement in visual acuity (21). Since then, many studies have confirmed this observation (25, 31-33). During early stages of ON, PVEP shows reduced amplitudes and delayed latencies with amplitudes generally returning to normal or near normal after a few weeks. Severe inflammation in acute ON may result in reversible conduction
block with an extinguished VEP waveform. These changes in VEP are often accompanied by gadolinium enhancement of the optic nerve lesion on MRI.

Persistent amplitude reduction has been associated with reduced visual acuity, decreased retinal nerve fibre layer thickness on OCT, and optic nerve atrophy (34, 35). A more detailed background on ON changes is discussed in chapter three.

Although the rapid developments in MRI may seem to overshadow the role of VEP in the diagnosis and monitoring of MS patients, VEP studies still add a substantial insight into the pathophysiology of this complex disease. There is strong evidence that the majority of MS patients without visual involvement would have an abnormal VEP (36, 37).

There have been several attempts to assess whether MS affects the function of the magnocellular or parvocellular visual pathway. However, the results provided by many studies in this area were inconsistent. Some studies suggested that the magnocellular pathway is more commonly affected in MS, while others claimed that the parvocellular pathway is more sensitive to MS injury (25, 38, 39). There is no straightforward explanation for this contradiction. However, difference in disease subtype and duration could affect the results. For instance, it has been suggested that magnocellular involvement occurs early in the disease course while profound parvocellular pathway involvement may be evident in advanced cases of MS (25).

The presence of subclinical VEP abnormality in MS patients is in agreement with brain imaging and histopathological studies that provide evidence of disease activity even during the remission phase (40). In addition, VEP has been suggested as a surrogate marker to predict MS patients with high risk for long-term disability due to good
correlation between evoked potentials and disability scores observed in MS and has been used as an outcome in recent MS therapeutic trails (41, 42).

**Multifocal visual evoked potentials (mfVEP) as an objective measure of visual pathway function**

While VEP frequently detects optic nerve diseases, it is less sensitive in detecting or localising postchiasmal pathology (43). The full-field VEP response is dominated by the central vision due to cortical overrepresentation of the macular area. Furthermore, since the full-field VEP waveform is a vector sum of numerous differently oriented dipoles, it is prone to unpredictable changes, including cancellation, depending on the part of the nerve or visual field affected, which may affect the clinical usefulness of the full-field VEP. As a result, the need for an objective topographic test of optic nerve function with measurement of amplitudes and latencies from locally derived VEP responses in numerous small areas of the visual field has been recognised for more than two decades.

Attempts to record VEP from partial stimulation of the visual field, including the stimulation of hemi-fields, quadri-fields and segments, have been used in clinical practice particularly by neurologists. Although those techniques have improved the detection of peripheral visual field defects, they were often time-limited by serial stimulation and significant lower responses from the upper visual hemi-fields (44, 45).

In the early 1990s, Baseler and colleagues reported one of the earliest attempts to eliminate PVEP limitation by using a multi-input recording technique. They were able to record responses from multiple field locations simultaneously rather than a summed overall response (46). Recent technical refinements of mfVEP and electrode placement have significantly improved the quality of recordings and decreased inter-subject
variability, which has resulted in improved performance in cases of small or peripheral deficits (47, 48). It allows for a better assessment of the optic nerve function via stimulation of 58 different regions of the central 24° radius of the visual field. The checkerboard pattern used for stimulation is scaled based on cortical magnification. For instance, the foveal sectors are about 1° wide, while the peripheral sectors exceed 7°. This scaling results in stimulation of approximately equal cortical areas and therefore overcomes macular overrepresentation (47, 49).

**MfVEP visual field assessment and analysis**

**Amplitude assessment**

MfVEP also has an advantage over PVEP in providing an idea of the size and depth of a visual field defect. It should be noted, however, that the mfVEP arrays are not an objective version of visual field perimetry maps such as the Humphrey visual field (HVF) because the visual field is stimulated differently by the two techniques (29). For example, the stimuli used in the HVF test have the same size and are spaced equally while the mfVEP stimuli vary in size and spacing since they are cortically scaled (47). Several studies have shown a good agreement between the probability plots for the two tests (47, 50-53). To minimise the false positive defects on mfVEP plots, an amplitude cluster approach similar to the one used to define a defect on HVF was suggested (52, 54, 55). Goldberg and colleagues suggested that a local area of the visual field defect (scotoma) is present when there is a cluster of three adjacent zones with a p < 0.05 with at least one with a p < 0.02 (52). Hood and colleagues suggested slightly modified criteria with any two points with p < 0.01 or three points with p < 0.05 with at least one of them with p < 0.01 (54). Hemamalini Arvind and colleagues define a visual field defect on mfVEP as a cluster of three or more abnormal points, with at least two points
depressed by $p < 0.02$. Defects immediately above and below the blind spot were not considered part of the scotoma (55). These criteria ensure that results are valid and reliable (56).

**Latency delay assessment**

Measuring mfVEP latency has been challenging because of the small signal to noise ratio (SNR) and trace waveform variability. The latency of mfVEP sectors can be grouped and measured manually (57). This approach is subjective and time consuming. Moreover, sector grouping may reduce the spiral resolution of mfVEP.

Few automated methods have been suggested to calculate the latency of mfVEP traces (58-60). Hood and colleagues proposed a template method, which involved comparing the patient mfVEP traces to a template obtained by averaging the mfVEP response of 100 normal subjects using a cross-correlation (58). This method has some limitations such as dealing with traces with reverse polarity and the need for a large normal database. Thie and colleagues have suggested another method to measure the latency using cross-correlation with second order Gaussian wavelet kernels (60). The limitations of this method include dealing with the polarity of peaks and treating traces with “double humps” as it is uncertain whether one of the peaks in double humps traces is an artefact or a wide major peak that was pulled by an artefact (60). Advanced algorithms are under development to tackle some of these issues and improve the accuracy of latency measurement and progression (61). Another approach is to measure the latency of mfVEP by identifying waveform characteristics such as the start of response, which is the first response that crosses the 95th percentile of noise, and the first or second major peak (Fig 1.5). Sriram and colleagues have demonstrated less variability of the first and second major peaks compared to the start of response (62).
This indicates that the first and second major peaks are less dependent on noise levels and more reliable in measuring mfVEP latency.

![MfVEP waveform](image)

**Fig 1.5:** MfVEP waveform illustrating the three locations for potential latency measurement

A commonly used approach is described by Klistorner and colleagues which involves an algorithm to select the channel with the largest peak-to-peak amplitude for each segment with the second large peak used to measure the timing of latency (59, 63). This approach is used in the studies included in this thesis except for one study where latency was measured manually as described in the methodology section of chapter four.

A large bulk of mfVEP research has been done on the detection of glaucoma and monitoring its progression (29, 50, 55, 64). MfVEP showed promising results especially in patients with unreliable subjective visual fields. Blue-on-yellow mfVEP was suggested as a potential tool to detect pre-perimetric functional loss in patients with glaucomatous optic discs because of the stimulation of the blue-yellow pathway, which has a lower functional redundancy and a higher sensitivity to neuronal loss (55, 65).
However, it has been emphasised that the mfVEP test works as an adjunct for subjective visual field testing rather than replacing it.

The ability of mfVEP to provide valid measurements is essential in order to utilise its results. Both the sensitivity and specificity of mfVEP in detecting visual pathway defects are high (66-68). MfVEP demonstrated a higher performance in comparison to HVF and PVEP in a number of neuro-ophthalmological conditions. In ON, mfVEP was more sensitive than HVF and OCT, detecting up to 89% of cases in comparison to only 72% by HVF and 69% by OCT (66). Similar results were found when mfVEP was compared to full-field VEP in patients with typical ON. By using amplitude and latency asymmetry of mfVEP, defects were identified in 89% of cases in comparison to 73% using full-field VEP (67). MfVEP has a sensitivity of 95% and a specificity of 89% in detecting glaucomatous defects (50). Likewise, results from compressive optic neuropathies demonstrated high agreement between mfVEP and HVF (53).

Test-retest reliability is another important aspect in any newly introduced test. Several studies reported a good repeatability of mfVEP among normal individuals and glaucoma patients, which was comparable to and in some studies even better than HVF (56, 69-71).

As any other test, mfVEP has some limitations. One of the earliest limitations recognised was high inter-subject variability, which might affect the interpretation of test results (46). The main cause of variability in response between individuals is their cortical anatomy. Since the cortex is folded differently in every individual, the position of the primary visual area and its position in relation to the position of recording electrodes can result in noticeably different mfVEP responses (48, 72, 73). Additionally, differences in the conductivity of tissues such as skin thickness, the amount of
underlying fat, general brain activity, age and sex all have a role in increasing inter-subject variability (74). Recent developments, particularly EEG-based normalisation, have significantly improved the quality of recordings and decreased inter-subject variability allowing a gender- and sex-normative data base to be used by mfVEP software packages to create a grading scale with probability maps (47, 52, 74).

MfVEP responses from eyes of the same individual are usually very similar due to the fact that they project to the same cortical region and, hence, are not affected by cortical folding. Therefore, in cases of single eye involvement, an interocular comparison provides accurate and early detection of localised defects within the visual pathway (48). Technical limitations such as eyelid position, refractive errors and poor fixation may all influence the results of recordings and increase noise levels and should be taken into account and minimised if possible when recording mfVEP (49).

**MfVEP in demyelinating disorders**

MfVEP changes in demyelinating disorders have been evaluated in several studies (37, 63, 66-68, 75-78). The reduction of mfVEP amplitude during acute ON has been observed and was mainly attributed to acute inflammation and conduction block and, as in conventional VEP, mfVEP often demonstrated persistence of abnormal latency measurements in patients after visual acuity fully recovered (63). Hood and colleagues have demonstrated the ability of mfVEP to identify local defects following full recovery of visual acuity with amplitude reduction documented in areas of depressed visual sensitivity confirmed by HVF (79). Because of its ability to detect small and peripheral defects, mfVEP provided an evidence of heterogeneity of functional defects in patients with ON and allowed for better monitoring of functional recovery (59, 79). MfVEP amplitude has been shown to have a good topographic agreement with the retinal nerve
fibres layer (RNFL) thickness in corresponding areas, which provide an additional support for the role of mfVEP in disease monitoring (75). In addition, Klistorner and colleagues previously demonstrated that the degree of initial inflammatory demyelination of optic nerve is potentially a vital factor influencing the total long-term remyelination process (80). Further details on ON are discussed in chapter three. The following section provides a brief introduction to MS including its pathophysiology and the visual pathway involvement in this disorder.
1.2.3 Multiple sclerosis (MS)

Background

MS is a chronic autoimmune disease affecting the central nervous system (CNS) which is characterized by loss of myelin and nerve fibre degeneration (81). The early description of a case suggestive of MS dates back to the 14th century when partially recovered episodic neurological weakness and progressive neurological deterioration were described and documented (82). Jean-Martin Charcot was the first to correlate the clinical and the pathological features of this disorder in late 19th century (83).

MS is the commonest demyelinating disease causing disability in young adults with more than two million people diagnosed worldwide. It typically affects individuals from age 20 to 40, but occasionally it can present in children and late middle-aged adults. Females are more frequently affected than males (with a ratio of 2:1). Common clinical findings in MS are limb spasticity, sensory disturbance, ataxia, bladder dysfunction and ON.

According to the Australian Bureau of Statistics, the prevalence of MS in Australia is estimated to be 95.2 per 100,000 persons (84). However, the prevalence of this disorder varies from state to state. Several factors may influence this difference including latitude and demographic factors. In low latitude areas such as Queensland the prevalence of MS was estimated to be as low as 11 per 100,000 people while the prevalence increases in areas of higher latitude such as Tasmania (85). The explanation for this pattern of disease distribution is still not fully understood.
There are more than 23,000 people in Australia living with MS where about half of them have a severe disability (84). Because MS generally affects people in their mid-thirties, the long-term financial and social burden of the disease is substantial.

**MS diagnosis**

The early manifestation of MS usually involves neurological dysfunction (attack) lasting at least 24 hours. The dysfunction has to have a CNS origin and occurs in the absence of fever, infection or metabolic disturbance. MS is diagnosed based on clinical and para-clinical evidence of inflammatory demyelination of CNS after ruling out other possible conditions.

In 1983, Poser and colleagues proposed a scheme for MS diagnosis based on clinical, MRI, VEP, and cerebrospinal fluid (CSF) immunoglobulin abnormalities (86). Poser’s criteria divided patients into definite, probable, or possible MS. Since then, the role of MRI in the diagnosis of MS has been increasingly recognised. In 2001, the International Panel on the Diagnosis of Multiple Sclerosis proposed updated criteria for the diagnosis of MS (87). The aim was to have updated guidelines for the diagnosis of MS that could be useful to neurologists during their routine practice. These criteria are known as the “McDonald criteria” and named after the chair of that panel, Dr W. Ian McDonald. The latest revision of the McDonald criteria was released in 2011 (88). The revision included new evidence and refined some of the original definitions.

An essential criterion to diagnose MS is evidence of dissemination of lesions in both space and time, which means evidence of involvement of at least two areas of the CNS at least one month apart. The evidence is collected through a medical history, careful neurological examination and para-clinical findings such as MRI, CSF and VEP.
MS clinical course

The clinical course of the disease is quite variable. However, certain patterns have been recognised and MS generally can be divided into four main subtypes: relapsing remitting, primary progressive, secondary progressive, and progressive relapsing (89).

Relapsing remitting

Relapsing remitting MS (RRMS) is characterised by episodes of acute neurological attack such as optic neuritis, ataxia, or limb weakness followed by a variable amount of recovery with no disease progression between attacks. Up to 90% of patients will present with this form of the disease.

Primary progressive

Patients with this subtype will suffer from gradual deterioration of neurological signs and symptoms with minimal improvement but no well-defined relapses. Around 10% of patients will present with primary progressive MS (PPMS). This subtype is the most resistant to MS-modifying treatments.

Secondary progressive

Half the patients with RRMS will develop secondary progressive MS (SPMS) at a later stage with progressive deterioration and occasional relapses. Disease-modifying agents can delay this progression in many patients with RRMS if treatment is used early in the course of the disease.
Progressive relapsing

In this rare subgroup, the progression of MS is evident from onset and associated with distinct relapses with or without recovery. This pattern is evident in less than 5% of MS patients. Unfortunately there are still no biological or immunological markers to help in early recognition of MS subtypes to improve prognostic ability and allow for better use of various disease-modifying agents.

MS pathophysiology

The basic pathology of MS was described in the late 19th century as a chronic inflammatory demyelinating disorder characterised by the presence of demyelinating plaques (83). It has become apparent over the past decade that MS pathogenesis is far more complicated than predicted previously with much more widespread damage of the brain and spinal cord, including white and grey matter, particularly at late stages of the disease. Although demyelination is still considered a distinctive histopathological feature, in recent years the contribution of axonal damage to functional disability has been increasingly recognised (90, 91). The loss of white matter integrity remains a key area of MS pathophysiology.

Axons in CNS are insulated by a material known as myelin, which assists in the fast conduction of action potentials along nerve axons. Oligodendrocytes are the cells responsible for synthesising and maintaining myelin integrity. These cells and other neural cells in CNS are generally protected from inflammatory cells circulating in the blood by the blood brain barrier (BBB). The BBB is composed of endothelial cells, which line the walls of blood vessels in the central nervous system. Compared to normal endothelial cells, the cells lining the BBB are connected by occludin and
claudin, which form tight junctions in order to create a barrier to keep out larger molecules such as proteins. In order to pass through, molecules must be taken in by transport proteins or an alteration in the BBB’s permeability must occur. Therefore, normally most immune cells are effectively separated from the CNS by this barrier. Immune cells are essential for detecting and eliminating abnormal cells, whether infected or neoplastic, and under normal circumstances they would not attack normal tissues.

It is believed that in MS this harmony is disturbed and T lymphocytes, B lymphocytes and plasma cells infiltrate the white matter in the brain and spinal cord, producing inflammation, demyelination and axonal damage (92).

**Cell-mediated myelin damage and oligodendrocyte death**

**T lymphocytes**

An important immune cell in the pathogenesis of MS is the T lymphocyte. T helper cells, also known as CD4+ T cells because they express the CD4 glycoprotein on their surface, and cytotoxic T cells, known as CD8+ T cells since they express the CD8 glycoprotein, reach the CNS either due to the corrupted BBB or due to the fact that activated T cells are allowed to patrol the brain parenchyma. Both mechanisms may also occur simultaneously. It is likely that the T lymphocytes are first activated in the periphery and then migrate into the CNS, where they become reactivated against target antigens, resulting in cytotoxic damage (93).

It is believed that myelin components act as an antigen for T lymphocytes resulting in myelin sheath damage and impaired nerve conduction (94).
The exact role and the extent of the damage related to CD8+ and CD4+ T cells are not yet well defined. It is recognised that CD4+ T cells play a major role in this disease (95). This is supported by the fact that certain human leukocyte antigen (HLA) class II molecules, that have a role as antigen-presenting molecules to pathogenic CD4+ T cells, are a strong genetic risk factor for MS.

Furthermore, there is mounting evidence on CD8+ T cells involvement in the pathogenesis of MS. CD8+ T cells were present at the lesion edge as well as perivascular regions in animal models of MS (94). Cabarrocas and colleagues have shown that activated CD8+ T cells travel freely into the CNS and induce inflammation and tissue damage without the need of CD4+ T cell help (96). Therefore, it is likely that CD4+ T cells and CD8+ T cells are equally important in MS pathogenesis.

**Humoral-immune-mediated myelin damage and oligodendrocyte death**

**B lymphocytes**

While the involvement of antibodies in the pathogenesis of multiple sclerosis has been long suspected, the role of B cell lymphocytes has been increasingly recognised. There is evidence for B cell clonal expansion in the CSF of the majority of clinically isolated syndrome patients and it was suggested that antibody to myelin proteins may contribute to loss of myelin (97, 98). However, the understanding of the precise function of B cells in this disease is still evolving.

**Neuro-degeneration**

Traditionally, it was believed that the prominent early manifestation of the disease is mainly caused by demyelination due to inflammatory processes and that neurodegeneration is just a consequence of that, appearing in the later stages of the
disease. There is cumulative evidence from studies on histopathology as well as brain imaging challenging this view and demonstrating that neurodegenerative changes are an early pathological feature in MS (99).

An additional issue of debate is whether demyelination is a vital requirement for axonal loss or both conditions appear independently. Axon loss might be acute, caused by the effects of inflammation, or chronic, caused by insidious inflammation or lack of trophic support from myelin. Both immunologically mediated and non-immunological processes have been suggested as mechanisms of neurodegeneration in MS including diffuse microglial activation, glutamate excitotoxicity, calcium influx, mitochondrial collapse and damage from acute cytokine release (99).

Post-mortem evidence suggests that axonal degeneration does not only take place in the context of acute lesions, but continues to occur during the later stages of the disease when acute inflammatory episodes are generally absent (100). One potential explanation for ongoing axonal degeneration in the absence of obvious inflammation is that the absence of myelin sheaths leads to a lack of axonal support that ultimately results in axonal degeneration (101). Persisting diffuse inflammation may also contribute to axonal loss.

**Possible antigens involved in MS**

There has been substantial research to identify relevant antigens in MS with the greatest focus on myelin proteins (102). Non-myelin antigens such as the α-B crystallin protein have also been suggested as potential antigens (103). However, it is possible that other antigens which have not yet been identified, could contribute to the autoimmune process in MS (97).
Genetic predisposition and environmental factors

Predisposition to MS involves a complex interaction between genetic and environmental factors. The genetic influence in MS was suggested due to familial clustering of cases and the high incidence in some ethnic populations, such as groups of northern European origin (104). MS nowadays is considered a polygenic disorder even though greater numbers of MS patients do not have an affected family member.

Genetic associations with major histocompatibility complex class I alleles have been recognised in several studies. Furthermore, patients with HLA alleles such as HLA-A3 and HLA-DR2 may have an increased risk of developing MS. Some studies have suggested that HLA-DR2 is an independent risk factor for MS, proposing a relative risk of 4 (105, 106).

Several environmental factors, including place of residence, infections (such as Epstein-Barr virus), lower vitamin D levels and lack of sun exposure, have long been assumed to potentially play a role in and increase the risk of MS development (107).

Visual pathway involvement in MS

The visual pathway is highly susceptible to inflammatory demyelination injury with more than a third of MS patients having some kind of visual impairment (108). Involvement of the optic nerve is common in MS and ON occurs in 50-70% of patients during the course of their disease. 15-20% of patients present with ON as their first manifestation of MS (109, 110). MS patients without ON often demonstrate lower visual function including low contrast, visual acuity and colour sensitivity testing when compared with controls (111). In addition, reduced RNFL thickness has been documented in MS patients without a clinical history of ON. The optic chiasm and post-
chiasmal involvement is common as well. Evidence of inflammatory demyelinating lesions in the posterior visual pathway has been reported in 70-80% of MS patients although the majority of them are clinically asymptomatic (36, 112). The visual pathway involvement in MS is discussed further in chapter three, four and five.

The following chapter elaborates further on the rationale of choosing mfVEP over full-field PVEP by evaluating both techniques in several cases with different visual pathway disorders.
Chapter two: Assessment of mfVEP and PVEP changes in patients with visual pathway disorders: a case series

(The finding from this chapter have been submitted to Neuro-Ophthalmology)
2.0 Overview

In this chapter both PVEP and mfVEP are used to assess comparative efficacy of the techniques in detecting visual field defects in patients with a known visual pathway pathology where other tests such as MRI were used to establish diagnosis. This chapter consists of four sections. The first section is an introduction and a review of comparative studies on mfVEP and PVEP. The second section includes aims and methodology used in the current study. Section three describes the participants’ clinical and para-clinical findings and reports the results of mfVEP and PVEP. Section four includes a discussion on the relative utility of mfVEP in clinical setting compared to the conventional pattern reversal technique.

2.1 Introduction

Visual pathway disorders can be diagnosed by clinical evaluation and imaging. However, if subjective tests such as visual acuity, visual field analysis and color vision assessment are inconclusive or not explained by clinical findings, objective investigations such as visual evoked potentials may be of use.

The VEP has been used in the diagnosis of various neuro-ophthalmological diseases for many years. As has been discussed in the previous chapter, it is known that the upper retina (lower visual field) projects to the upper bank of the sulcus calcarinus (cuneus gyrus), while the lower retina (upper visual field) projects to lower bank of the sulcus calcarinus (lingual gyrus). Since both banks are facing each other, the polarity of the cortical dipoles from the lower and upper hemi-fields is almost opposite. Since the full-field PVEP is a vector sum of numerous differently oriented dipoles, the waveform of the full-field PVEP is prone to cancellation and distortion (47, 113). Furthermore,
because of cortical over-representation of the central macula region, the full-field VEP response is greatly dependent on the function of that area (114, 115). Up to 65% of the VEP response is produced by stimulation of the central two degrees of the visual field (116) and, as a result, a lesion localised in the periphery of the visual field could easily be missed.

MfVEP, on the other hand, enables simultaneous recording from multiple regions of the visual field, allowing assessment of a much larger cross-sectional area of the optic nerve and, therefore, more accurate functional evaluation of the visual pathway (47). Such an objective visual field topographic map may have useful applications in clinical practice.

Currently the mfVEP has been predominantly used in the assessment of patients with glaucoma and optic neuritis and has been shown to have a sensitivity of more than 92% and specificity above 90% in detecting visual field defects (68, 117). However, relatively few studies have targeted other visual pathway disorders (53, 118-120). Furthermore, although there have been some studies comparing mfVEP with conventional VEP predominantly in the setting of optic neuritis and glaucoma (67, 68, 121). Grippo and colleagues studied the effect of glaucoma on latency delay measured by both PVEP and mfVEP. Both tests agreed in results and showed only modest latency delay. Klistorner and colleagues have compared mfVEP and full field PVEP in the setting of ON and reported a good agreement in amplitude and latency between the two tests. In addition, they have shown that mfVEP allowed independent assessment of multiple areas simultaneously by including information from peripheral visual fields (67). Grover and colleagues have also compared mfVEP and PVEP in ON confirming the superiority of mfVEP in detecting local damage from optic neuritis (68).
We could not find studies comparing the utility of the techniques in the setting of confirmed chiasmal and retro-chiasmal pathology, particularly using selective half and central field in addition to full field stimulation. In this study we assess the relative utility of mfVEP compared to PVEP in several cases with pathology at various levels of the visual pathway confirmed by other objective techniques.
2.2 Aims and methodology

Subjects

Six patients with different visual pathway pathology diagnosed by a neurology consultant were selected from a large neurology/neuro-ophthalmology service. The selected cases included vascular ischaemic events, compressive optic neuropathy and inflammatory demyelinating event. The tenets of the Declaration of Helsinki were followed and informed consent was obtained from all patients.

MfVEP recording

Stimulus display

MfVEP testing was performed using Accumap (ObjectiVision Pty. Ltd., Sydney, Australia). The stimulus consisted of a cortically scaled dartboard pattern of 58 segments (eccentricity up to 24°). Each segment contained a 4 × 4 grid of black (1.1 cd/m²)-and-white (146 cd/m²) checks (Michelson contrast, 99%), which reversed patterns according to a binary pseudorandom sequence (Fig 2.1). On average, eight runs (each has a 54-second duration) were recorded to reach good SNR. The visual stimulus was generated on a 21-in CRT display. Participants were best refracted for near vision and were seated 30 cm away from the screen. All recordings were performed monocularly.
Fig 2.1:  a) Cortically scaled reversed pattern visual stimuli with central fixating target that changes randomly. b) Normal mfVEP traces of the right eye

**Electrode placement**

Four gold-cup electrodes (Grass Telefactor, West Warwick, RI) were used for bipolar recording, with two electrodes placed 4 cm on either side of the inion, one electrode 2.5 cm above and one 4.5 cm below the inion in the midline. Another electrode is attached to the patient’s right ear lobe as the ground electrode.

**MfVEP recordings**

Signals were recorded along four channels around the inion to maximise signal detection. The horizontal channel is between the superior and inferior electrodes, the vertical channel is between the left and right electrodes, and the oblique channels are between the horizontal and inferior electrodes (Fig 2.2). Visual evoked responses were amplified $1 \times 10^5$ times and band-pass filtered 1 to 20 Hz.
Fig 2.2: An illustration for electrode placement used for mfVEP recordings. The four channels used to record signals are shown as CH followed by the channel number.

**MfVEP amplitude and latency calculation**

OPERAs software (Accumap; ObjectiVision Pty. Ltd., Sydney, Australia) was used to correlate the pattern-reversal binary sequence with the electrical signals recorded. The largest peak-to-trough amplitude within the interval of 70 to 210 ms was selected for each channel. The software automatically chose the wave of maximum amplitude among the four channels, to create a combined topographic map which then was compared to normal built-in database to create amplitude and latency probability plots (50). A visual defect for mfVEPs was defined as a cluster of at least 3 abnormal points on the amplitude deviation plot with 2 segments $p < 0.02$ and at least 1 segment $p < 0.01$ or a cluster of 3 or more abnormal segments on inter-eye asymmetry deviation plot with $p < 0.01$ or 2 or more zones with $p < 0.005$ (117). For latency analysis, the second peak of the largest wave for each segment was automatically determined for latency measurement by a specially designed algorithm.
**OCT recording and analysis**

OCT was performed using a Spectralis scanner (Heidelberg Engineering, Heidelberg, Germany). A peri-papillary circular scan (12° peripapillary ring, axonal protocol, high resolution) was used to obtain measurement of RNFL. The pupils were not dilated. Scan quality was considered acceptable if the quality scores were more than 25 decibels and the scan was well centred on the optic nerve.

**Visual field recording**

Monocular visual fields were tested using HVF analyser (Carl Zeiss Meditec, Inc., Dublin, CA). SITA standard 24-2 protocol was used.

**PVEP recording and analysis**

Full-field, right and left half-field and central field VEPs were tested using a Medelec Synergy Version 15.0. Pattern reversal stimulation was performed using a Dell CRT monitor with a 20” screen, alternating black and white checkerboard stimulation (32 min checks) reversed at a rate of 2/s. A fixation point was located in the centre of the screen positioned at the corner of four checks. The contrast between black and white checks was greater than 80%. The mean luminance of the stimulus was 50 cd/m² and there was no change in mean luminance during the reversal of the pattern. The luminance of the screen was uniform and varies less than 10% between the centre and periphery of the visual field. Lighting in the laboratory room was homogenous with an average of room luminance equal to the average stimulus luminance. Monocular testing was performed with the non-tested eye covered by an eye patch. Gold cup disc electrodes (10 mm in diameter) were used. In accordance with the international 10/20 system (24), the active electrode placed on the scalp over the visual cortex at Oz with
the reference electrode was placed at Fz and the ground electrode placed on the forehead. The distance between the patient and the stimulus was 70 cm. Sweep duration was 300 ms post stimulus and the electrode impedance was measured prior to each recording and was 5Kohms or less. The amplifier band-pass was 3 to 200 Hz.

VEPs were recorded in two trials for each eye, averaging at least 128 responses. Cut-offs for normal values were < 112 ms for P100-peak latencies, ≥ 2 µV for amplitudes, the inter-eye right to left half field latency asymmetry was 7 ms and the inter-eye left to right half field amplitude ratio was 3:1. These cut-off limits were previously established laboratory normal measurements and represent values beyond two standard deviations from the mean.

**Brain MRI testing**

Brain MRI was performed using 3.0 Tesla GE MR750 scanners (GE Healthcare, Little Chalfont, UK).

**2.3 Results**

**Case 1 (branch retinal artery occlusion)**

An 83-year-old woman presented with a five week history of painless right eye visual impairment. There were no other visual or systemic symptoms. She had a past history of polymyalgia rheumatica and cataract surgery in her left eye.

On examination, Snellen visual acuity was 6/12 in the right eye and 6/6 in the left. Fundus examination of the right eye showed an ischaemic pale retina inferiorly with a cholesterol embolus in the inferior retinal artery (Fig. 2.3). Inferotemporal branch retinal artery occlusion was diagnosed. Full-field VEP and central VEP amplitude and
latency were normal (Fig.2.4). However, mfVEP of the right eye showed significant reduction in amplitude in the upper field (Fig.2.5). This correlated well with the reduction of RNFL thickness of the right eye inferiorly (Fig.2.6).

**Fig. 2.3:** Fundus photography of the right eye showing an embolic inferotemporal branch retinal artery occlusion

**Fig. 2.4:** Full-field PVEP of the right and left eyes showing normal amplitude and latency. The right eye PVEP has higher amplitude and better-defined waveform
**Fig. 2.5:** MfVEP showing reduction of amplitude in the upper field of the right eye

**Fig. 2.6:** OCT showing an inferior RNFL thickness reduction in the right eye

**Comments: case 1**

In this case, full-field PVEPs in the affected eye were normal. Moreover, the waveform had higher amplitude and was better defined than in the unaffected eye. This contradiction is likely caused by the anatomy of the visual cortex. As discussed previously, the polarity of the cortical dipoles from the lower and upper hemi-fields is
almost opposite. This results in a cancellation effect of amplitude in the non-affected eye. In the affected eye, however, since the response from the upper hemi-field is almost extinguished, there is no cancellation and the average full-field signal looks larger, although it is mostly generated by the lower hemi-field. The findings suggest a greater sensitivity of mfVEP compared to full-field PVEP in the setting of pathology selectively affecting one horizontal hemi-field.

**PVEP and mfVEP changes in compressive optic neuropathy**

**Case 2 (pituitary adenoma)**

A 75-year-old woman presented with a history of progressive visual loss of her left eye during the previous six months. She had a history of pituitary adenoma that had been operated on in 1998. Visual acuity in her left eye was hand motion with an afferent pupillary defect. Visual acuity in her right eye was 6/9. MRI scans showed residual and possibly recurrent pituitary macro-adenoma affecting the left optic nerve and chiasm (Fig. 2.7). Visual field perimetry showed almost total visual field loss in the left eye and an upper temporal scotoma in the right eye (Fig. 2.8).

Full field VEP showed no consistent response from the left eye. The right eye full field latency and amplitude were normal. The right half-field amplitude of the right eye was small (1.2 µV), whereas the response from the left half-field demonstrated a normal amplitude (2.3 µV) (Fig. 2.9). PVEP of both half fields of the right eye showed P100 latency within normal limits. MfVEP showed a clear right eye temporal hemianopia with normal amplitude of the left hemi-field (Fig. 2.10). This example highlights the importance of mfVEP in accurate detection of vertical hemi-field pathology.
**Fig. 2.7:** Axial T1-weighted MRI with contrast shows a large mass lesion (white arrow) occupying the pituitary fossa and extending into the suprasellar cistern and right cavernous sinus consistent with a pituitary tumour

**Fig. 2.8:** Humphrey 24-2 visual fields showing total visual field loss in the left eye and upper temporal scotoma in the right eye
Fig. 2.9: Full-field PVEP of both eyes showing no consistent response in the left eye and normal amplitude and latency in the right eye. Half-field PVEP of the right eye shows smaller amplitude in the right half with normal latency in both half-fields.

Fig. 2.10: MfVEP showing total reduction of amplitude in the left eye and temporal hemianopia in the right eye
Case 3 (pituitary tumor in a multiple sclerosis patient)

A 51 year-old woman presented with deterioration of vision in her right eye. She described it as an area of obscuration in the temporal field. There was no associated pain or change in the colour vision. The patient had a long history of MS.

On examination her visual acuity was 6/18 in the right eye and 6/6 in the left eye. The optic disc appeared normal. Full-field PVEP of right eye showed delayed P100. The amplitude was smaller than the left eye, but still within normal limits (5.5 µv). Half-field PVEP waves in the right eye were poorly formed on both sides. Left eye full-field and half-field VEPs were within normal limits (Fig 2.11). In view of her MS history optic neuritis was considered. MfVEP, however, demonstrated a dramatic loss of amplitude in the right eye and significant involvement of the left eye, predominantly on the temporal side (Fig 2.12).

An MRI scan of the brain confirmed extensive demyelination in keeping with her MS but in addition, a suprasellar mass extending toward the right optic nerve (Fig 2.13).
Fig. 2.11: Full-field PVEP of both eyes showing normal amplitude and latency. Half-fields PVEP of the right eye showing poorly formed waves with reduction of amplitude and latency delay. Both half-fields of the left eye were within normal limits.

Fig. 2.12: MfVEP showing severe reduction of amplitude in the right eye, with apparent left eye involvement.
**Fig 2.13:** T1-wheighted axial MRI showing suprasellar mass extending toward the right optic nerve consistent with pituitary adenoma

**Comments: cases 2 and 3**

Patients with a pituitary adenoma can present to the neuro-ophthalmology clinic with visual symptoms secondary to the tumour. Up to 80% of non-functional pituitary adenomas and around 20% of patients with growth hormone or adrenocorticotropic hormone-secreting tumours will present with visual disturbance as their primary complaint (122). The classic visual field defect is a bitemporal hemianopia due to the compression of the crossed nasal fibres in the chiasm. Many other variants of the typical visual field defect have been reported in the literature (122). Around 16% of patients will present to the neuro-ophthalmologist as in case 2 with one eye blind and a temporal visual field loss in the other eye. Conventional VEP abnormalities in this setting are well described (123) however in case 2 the full-field VEP was normal in the right eye and normal in both eyes in case 3. The extent of the defect was more obvious in the mfVEP compared with half-field PVEP, which showed only a mild asymmetry in case 2. Previous studies that have compared mfVEP with HVF in patients with compressive optic neuropathies showed a higher sensitivity of mfVEP (53, 124) but there are no
studies comparing VEP techniques. The findings in these cases support the usefulness of the mfVEP in this pathological condition.

**PVEP and mfVEP changes in central visual pathway lesions**

**Case 4 (anterior choroidal artery infarction)**

A 42-year-old woman presented with a history of acute onset headache, nausea and vomiting. This was associated with visual disturbance as well as facial paraesthesia and heaviness in the left arm and leg. The symptoms improved over the next 24 hours except for the visual disturbance. Her MRI scan showed features consistent with an anterior choroidal artery infarction involving the right hippocampus, medial temporal lobe and posterior thalamus (Fig 2.14). Visual field testing revealed left relative hemianopia (Fig 2.15). Full-field VEP for both eyes was within normal limits. Left field VEP showed lower amplitude and prolonged latency for both eyes, worse in the left eye (Fig 2.16). MfVEP confirmed the presence of left superior homonymous quadrantanopia (Fig 2.17).

**Fig 2.14:** Humphrey 24-2 visual fields showing left congruous relative hemianopia
Fig 2.15: Full-field PVEP of both eyes showing normal amplitude and latency and half-field PVEP shows lower amplitude and prolonged latency of the left field in both eyes, worse in the left eye
**Fig 2.16:** Flair and T2-weighted images showing signs of infarction (white arrow) involving the right hippocampus, medial temporal lobe and posterior thalamus, including a region of the lateral geniculate body

**Fig 2.17:** MfVEP showing left superior homonymous quadrantanopia
Case 5 (sub-acute infarcts due to septic emboli)

A 73-year-old woman presented with a one-week history of fever after a trip to Europe and Hong Kong. She was diagnosed with endocarditis and required mitral valve replacement. Her condition was complicated by septic emboli with sub-acute infarcts in the right occipital, left cerebellar, right parietal and right frontal lobes (Fig 2.18).

She was left with a residual field defect, but otherwise her neurological function returned to normal. The P100 latency and amplitude of full-field PVEP of both eyes were within normal limits. Although the half-field PVEPs of the right eye were within normal limits, the amplitude was asymmetrical (right half-field 3.1µV, left half-field 2µV). Similar findings were seen in the left eye. The latency was within normal limits for both sides (Fig 2.19). While full-field PVEP of the left eye amplitude was relatively reduced compared to the right eye, half-field PVEP was inconclusive. MfVEP, on the other hand, clearly demonstrated an incongruous left homonymous hemianopia, which corresponded to the lesion seen on the MRI (Fig 2.20).

**Fig 2.18:** a) Fast field echo MRI images and b) Diffusion-weighted images (DWI) showing sub-acute infarcts in the right occipital lobe
Fig 2.19: Fullfield PVEP of both eyes showing amplitude asymmetry not exceeding the cut-off for normal values. Half-field PVEP of both eyes is within normal limits.

Fig 2.20: MfVEP showing an incongruous left homonymous hemianopia
Comments: cases 4 and 5

Several studies have reported the ability of conventional VEP to detect field loss due to lesions involving the visual pathway and higher visual centres (125-129). Although Bradman and colleagues (125) reported that PVEP quadrantic-field testing has a sensitivity and specificity in detecting visual field defects, others disagree. Maitland and associates (129) concluded that it was possible to lateralize the brain lesion, but not to predict the site of the lesion within the hemisphere and therefore, concluded that PVEP analysis is of limited value in assessing patients with homonymous or bitemporal hemianopias.

MfVEP, as demonstrated by case 4 and 5, has the capacity to detect abnormalities in the posterior visual pathways that may be missed by conventional pattern reversal studies. Klistorner and colleagues have previously reported a good correlation between HVF defects and mfVEP in patients with retro-chiasmatic visual pathway lesions (118). However, it should be noted that in the setting of lesions in higher visual centres (e.g. V2/V3) the mfVEP may be normal as it arises mostly from V1 (119).

Case 6 (ischaemic optic neuropathy)

A 50-year-old man presented with a history of painless visual loss in his left eye. On examination his visual acuity was 6/6 in both eyes but the left optic disc was swollen.

An MRI scan of his brain and optic nerves was normal. Lumbar puncture was also unremarkable. Full-field and half-field PVEP were normal in both eyes (Fig 2.21). HVF testing and mfVEP, however, demonstrated a peripheral visual field defect in his left eye, which was accompanied by a corresponding reduction in RNFL thickness (Fig
2.22). This could only be revealed on full-field PVEP through annular stimulation after macular masking. The patient was treated for an anterior ischaemic optic neuropathy.

**Fig 2.21:** Full-field PVEP of both eyes and half-field PVEP of the left eye were within normal limits
Fig 2.22: (a) Humphrey 24-2 visual fields showing left eye peripheral visual field defect. (b) MfVEP traces and amplitude asymmetry maps of the left eye showing peripheral reduction of amplitude. (c) The area of amplitude reduction on mfVEP correlates well with RNFL thinning on OCT
Comments: case 6

Full-field and half-field PVEP were normal in this patient because the majority of PVEP is driven by the central 4° of the visual field, which was intact. Hence, despite his extensive peripheral visual field defects, he had a normal full-field PVEP and his central visual acuity was unaffected. MfVEP, on the other hand, was able to detect pathology affecting peripheral fibres with relatively preserved central vision.

2.4 Discussion

There is limited number of studies comparing conventional VEP and mfVEP changes in cases of confirmed visual pathway pathology (67, 121). This case series demonstrates that mfVEP, as an objective test for visual field, is potentially more sensitive than conventional PVEP in detecting focal visual pathway pathology. The findings in our cases of a normal PVEP response when the central vision was preserved even if there was a significant peripheral visual field defect, as in case 1 and 6, can be explained by the fact that a large proportion of the VEP is generated by macular fibres. Since the macula is responsible for sharp detailed vision, it has a higher density of cones and ganglion cells in comparison to the peripheral retina. At the same time, larger numbers of cortical neurons are involved in the processing of visual stimuli from the central visual field in comparison to the peripheral visual field. It has been estimated that around 50-60% of the visual cortex is devoted to macular representation (130). In addition, studies have confirmed that the ideal check size to obtain best response differs based on receptive fields’ location in the retina. Small check sizes are optimal for fovea while larger check sizes are better for peripheral visual fields (131, 132). The check size used routinely for PVEP stimulation is selected to obtain an optimal response from
central and para-central visual fields (133-136) and, as a consequence, is sub-optimal for the peripheral retina.

MfVEP, on the other hand, uses a dartboard pattern of 58 segments that contains 4x4 checks cortically scaled to increase in size from the centre to the periphery in order to optimise the response from different parts of the visual field (137). It is therefore capable of greater resolution of visual pathway function including fibres from the peripheral visual field. Moreover, mfVEP techniques allow independent assessment of fibres subserving different regions of the visual field, minimising the susceptibility to phase cancellation and distortion, which may be evident in PVEP as a result of potential summation as in case 1 and 2.

It is important to note that a normal mfVEP does not always mean a normal visual pathway. Considerable caution should be exercised if the visual field defects on subjective perimetry have the characteristic higher visual centre field defects i.e. quadrinopic field defect respecting horizontal midline.

In summary, mfVEP may provide a more easily demonstrated topographic representation of the visual pathway when compared with conventional VEP. The independent assessment of different areas in the visual field improves the detection and localisation of lesions and provides an objective topographical map that can be used in clinical practice. Although the observational nature of the study limits the application of the findings to a larger population of patients, the findings support the need for larger studies to evaluate the relative utility of mfVEP in this clinical setting compared to the conventional pattern reversal technique.
Chapter three: MfVEP assessment of acute optic neuritis evolution

(The results of this chapter have been published in *Clinical Neurophysiology*)
3.0 Overview

In this chapter, the assessment of ON evolution during the first 12 months using mfVEP is explored. The chapter has four main sections. The first section briefly reviews literature on ON. The second section discusses objectives and methodologies used to evaluate ON changes in the current study. The third section includes study results and explores a possible relationship between functional and structural measurements used and the last section discusses the importance of those results and places them in context with previous research in this field.

3.1 Background

Optic neuritis clinical presentation

Typical ON is characterised by inflammation of the optic nerve associated with loss of vision. The visual loss usually progresses over a few days to two weeks. Mild orbital pain exacerbated by eye movement is often present, although no pain is reported in 10% of patients (138). The amount of visual loss and speed of recovery vary among patients but continued visual deterioration after the first two to three weeks may point to a different diagnosis. Visual field loss is nonspecific with almost all types of visual fields having been previously reported (139). Central field scotoma seems to be more characteristic of ON (140). Colour vision and contrast sensitivity are typically reduced and a relative afferent pupillary defect is present in the majority of unilateral cases. Anterior ON is present when the ON lesion is close to the optic nerve head, resulting in optic disc swelling, whereas retro-bulbar ON typically spares the optic disc (138). Retinal examination is
usually unremarkable. Atypical manifestations such as no light perception, vitritis, and severe retinal haemorrhages and exudates may suggest a different diagnosis.

**Differential diagnosis of ON:**

Extensive discussion of the differential diagnosis of ON is beyond the scope of this review. However, many disorders can present with a clinical picture similar to ON. Thus, meticulous examination and exclusion of those disorders is crucial. Inflammatory conditions (such as systemic lupus erythematosus, sarcoidosis, or Behçet’s disease), infectious diseases, vascular lesions or space occupying lesions could present with similar manifestations but require different types of management and should be ruled out through clinical and para-clinical tests (138).

**Epidemiology**

Typical ON patients are young adults (aged 20–40 years), yet, presentation at younger or older ages is possible. Females are more frequently affected than males in a ratio of 2.5:1. The incidence increases in populations living at high latitudes and decreases in areas close to the equator. Whites are affected more than blacks but visual outcome tends to be worse in blacks. The reported annual incidence of ON ranges from 1 to 5 per 100,000 (141, 142).

ON can present as an isolated condition or as a demyelinating attack in patients known to have MS.
**Pathophysiology**

Many aspects of ON pathogenesis are similar to the pathogeneses of MS discussed in chapter one.

**Recovery process**

The process of damage and recovery in ON are not totally understood and the time course of demyelination and remyelination in lesions is also still indeterminate. However, we know that clinical improvement starts early during the recovery process. This could be explained by several mechanisms including remyelination, increased expression of sodium channels along the demyelinated segment to improve conduction, and cortical plasticity (143).

Myelin regeneration is mainly mediated by oligodendrocyte precursor cells. These cells are frequently observed in demyelinating lesions during the early, active stages of ON episodes (144). The first signs of remyelination can be observed few days after the acute onset of demyelination (145). Although the precise role of inflammation in promoting remyelination is still unclear, it is believed that the acute inflammation provides the required environment for many of the regulators needed for remyelination, which are absent in more chronic settings. For example, macrophages, which initially appear at the border of demyelinated lesions, play an important role in the phagocytosis of myelin-associated inhibitors aiding remyelination in the early stages (146).

Furthermore, the recovery of function requires secure action potential conduction. Several studies have demonstrated an increase in the expression of sodium channels as a mechanism to restore impulse conduction after a demyelinating attack (147).
The third mechanism that could contribute to rapid recovery after acute ON is cortical adaptation. Functional MRI (fMRI) studies of optic neuritis patients have shown that there is an increased activation in higher visual centres in response to binocular stimulation but that these changes have subsided after a few months, suggesting that temporary adaptive changes happen during recovery (143, 148). Rocca and colleagues evaluated the extent to which the fMRI changes correlated with the total axonal injury in clinically isolated syndromes, including ON, brain stem injury, and spinal cord syndromes. They showed that acute axonal injury could elicit adaptive cortical reorganisation in order to limit the functional effects of irreversible axonal damage (149).

**ON prognosis and MS risk**

Recovery from ON is generally good but many patients are at a greater risk of recurrence and/or progression to MS. A great amount of our knowledge on the natural history of ON is drawn from the Optic Neuritis Treatment Trial (ONTT), where 457 acute ON patients were enrolled in to a randomised control study (150). These patients were followed up for visual outcome, recurrence rate and risk of MS development after 15 years’ time (151).

Pain associated with ON generally lasts for only a few days. After around two weeks, spontaneous improvement of vision takes place. Initial improvement is rapid which is followed by slower recovery of vision for up to a year after the initial episode. Ninety percent of patients in the ONTT had a visual acuity better than 6/12 by the end of first year (151). However, the amount of visual loss at presentation seems to influence the final visual outcome. The proportion of patients with 6/12 vision decreased to 64% in patients with visual acuity of light perception on presentation (138).
Persistent residual deficits are frequent including reduced contrast sensitivity and colour saturation deficits. Patients also may complain of vision fluctuation with increased body temperature, known as *Uhthoff’s phenomenon* (152).

The risk of having recurrent ON after five years of follow-up in either eye was 28% in the ONTT. Recurrence was more frequent in MS patients and in patients who received oral prednisolone (153). The risk of MS after ON becomes greater as the length of the follow-up period increases. In a cohort study of 156 ON patients, the 10-year risk of multiple sclerosis was 39% but increased to 60% by 40 years (141). The most important risk factor for the development of MS is the presence of white-matter lesions on MRI of the brain. In the ONTT, the 5-year risk of MS was 16% in patients with no MRI lesions in comparison to 51% in patients with more than two brain lesions (154). Another study evaluated the risk of MS in patients with different clinically isolated syndromes including ON, spinal cord and brainstem sundroms and showed that the 10-year risk for MS in patients with normal MRI was 11% while the risk increased to 83% in patients with one or more lesions consistent with demyelination (155). Other risk factors that have been related to developing MS are being female, HLA-DR-positive and having oligoclonal bands in the CSF (156, 157).

**Assessment of ON damage**

Damage from ON can be evaluated by several available tests. Different tests are aimed to capture different aspects of function and structure changes. Subjective visual acuity using Snellen chart or logMAR scoring is commonly used in clinical practice and research. Low contrast acuity charts were found to be more sensitive in detecting visual dysfunction in mild ON cases and after visual acuity recovery (158). Colour testing can be done with Ishihara plates, which are more readily available in the clinical setting or
with the Farnsworth-Munsell 100-hue test, which is more comprehensive and preferred in research practice (159). The visual field can be tested with several tools but most commonly either with a static target using Humphrey perimetry or with a kinetic target using Goldmann perimetry. The choice of which to select is usually a balance between the advantages and disadvantages of those tests.

Imaging the optic nerves can be challenging because of several factors such as their small size, movement artifacts, and the fact that they are surrounded by fat, CSF, and air sinuses makes it difficult to identify them accurately. However, rapid advances in imaging techniques have improved the quality of images and allowed quantitative data to be collected. It has been reported that an abnormal contrast enhancement of the optic nerve is evident in more than 90% of acute ON cases. However, the location and length of the enhanced segment was not correlated to visual recovery (160).

Lesions to the optic nerve cause physical and/or functional transection of axons and subsequent retrograde degeneration resulting in changes in RNFL thickness. Early evidence supporting such hypotheses came from animal studies with ON models where optic nerve transection and retrograde RGC degeneration were evident after such events (161, 162).

Peripapillary RNFL thickness is of particular interest in optic neuropathies. RNFL thickness can be measured reliably using OCT to quantify axonal loss through creating a high resolution cross sectional image of the retinal layers by measuring the backscattered infrared light generated from low-coherence interferometry. Parisi and colleagues were the first to report a reduction in RNFL thickness following ON using OCT (163). Numerous studies have confirmed this finding compared to fellow unaffected eyes and to controls (63, 164-167).
Functional assessment of ON damage using PVEP and mfVEP has been discussed in chapter one. Several studies have demonstrated a correlation between VEP parameters and other functional and structural visual tests including visual acuity, MS disability scales, RNFL thickness, and optic nerve atrophy (41, 168-170). Having an agreement between functional and structural tests in general is useful clinically as it helps to overcome some of the limitations of each individual test and captures various stages of injury and repair. A good correlation between amplitude and RNFL thickness measured by OCT was reported previously in ON after the resolution of optic disc swelling (63, 75, 77). In a cross-sectional study of 32 patients with ON, Klistorner and colleagues demonstrated a good correlation between mfVEP parameters and RNFL thickness (63). MfVEP amplitude had a better correlation ($r = 0.9$) with RNFL thickness compared to the latency ($r = -0.6$). Similar findings were reported in another cross-sectional study which demonstrated a topographic association between the amplitude and reduction of RNFL thickness in ON eyes (75). Furthermore, mfVEP changes have been observed in the fellow eyes after ON, although these changes are usually subtle and asymptomatic (63, 171).

Previous cross-sectional studies have noted a difference in mfVEP parameters in ON eyes when patients were stratified on the bases of their risk for developing MS, where MS-related ON eyes showed sectorial latency delay on the mfVEP map (76, 172). Another cross-sectional study reported mfVEP changes in fellow eyes in MS-related ON, suggesting a possible insidious inflammatory demyelination process (171).
3.2 Aims and methodology

Purpose of the study

1. To evaluate mfVEP evolution in both affected and fellow eyes during the first 12 months after a unilateral acute ON
2. To investigate whether mfVEP measurements differ based on participants’ MRI findings and their future conversion to MS
3. To study the relationship between functional and structural changes by measuring RNFL thickness at the twelfth month and correlating it with mfVEP amplitude changes during different follow-up time points

Rationale of the study

Most of the studies that have addressed the mfVEP changes after ON were cross-sectional, had relatively small sample sizes or did not take MRI findings into consideration (76, 78, 79, 171, 172). Therefore, there is a need for a longitudinal analysis examining a larger sample size of patients with ON and assessing changes in both affected and fellow eyes simultaneously to provide a better understanding of mfVEP changes and the time frame of their occurrence. Finding a significant mfVEP difference between different patient groups may shed a light on subtle pathological changes and suggest a potential role for mfVEP in identifying early pathological dysfunction.
Methodology

Subjects

Patients with clinically diagnosed typical acute unilateral ON were recruited from Sydney Eye Hospital and The Royal Victorian Eye and Ear Hospital.

The inclusion criteria were:

- Adult (>16 years old) presenting with ON with no previous history of other inflammatory demyelinating attacks
- Signs and symptoms of typical acute ON including visual acuity and colour vision reduction, afferent pupillary defect and pain on eye movement (138)

The exclusion criteria were:

- History of previous demyelinating events
- Atypical presentation (138)
- Involvement of the other eye
- Presence of other ophthalmic conditions that could affect the mfVEP or OCT measurements (for example, optic neuropathies such as glaucoma, dense cataract, retinal detachment or amblyopia)
- Mental or physical disabilities, which require continuous and special care, as this may interfere with performing reliable tests
- Inability to fixate at a point from 30 cm distance due to poor vision (usually if best corrected vision is less than 6/60)

Potential participants undertook a full ophthalmic examination and patients matching the inclusion criteria were offered to be part of the study. Twenty-five age-and-gender
matched healthy subjects were recruited from the general community as controls. All procedures adhered to the tenets of the Declaration of Helsinki. Test procedures and other related concerns were discussed and informed consent was obtained from all participants. Ethical approval was obtained from the Human Research Ethics Committee of the University of Sydney (protocol no. 2013/106).

Patients had an MRI within two weeks of the ON attack and at least one follow-up MRI within the next 12 months. MfVEP recordings were performed at 1, 3, 6 and 12 months from the onset of ON. OCT was performed at 12 months post-attack.

Controls underwent visual acuity testing, ophthalmic evaluation, and were tested once using mfVEP and OCT.

As a standard of care, all ON patients were followed up in the neurology/neuro-ophthalmology clinic with routine clinical and MRI assessment. Within the following four years, participants were retrospectively stratified to three groups:

- **Group 1**: multiple sclerosis (MS) – ON patients who converted to MS (diagnosis was made based on revised McDonald Criteria for multiple sclerosis) (173)
- **Group 2**: high risk group (HR) – ON patients with MRI lesions who did not fulfil the McDonald Criteria for MS
- **Group 3**: low risk group (LR) – ON patients with normal MRI
- **Group 4**: (control) – age-and-gender-matched controls
Study procedures

MfVEP recording and analysis

MfVEP was recorded as described in methodology section of chapter two. In order to accurately follow the evolution of the latency after the ON attack, only eyes that had at least 44/58 (75%) of traces with sufficient amplitude at one month post-attack were included. To determine sufficient amplitudes, the software automatically calculated the SNR at each segment of the stimulated visual field. The signal was considered non-recordable in segments where the amplitude of the response was less than 1.96 times that of the noise level (determined as standard deviation of the trace within the interval 400-1000 ms) (80). The amplitude of multiple segments added after cross-correlation is performed and individual traces are defined, avoiding, therefore, cancellation of the dipoles, which often occurs in full-field VEP. Mean values of both amplitude and latency, which were used in the final analysis, were calculated by averaging the amplitude and latency of the individual sectors (see mfVEP example in Fig 3.1).

Fig 3.1: MfVEP of a patient with left eye optic neuritis showing localised amplitude reduction centrally with latency delay
**OCT recording and analysis**

OCT was performed by a trained operator using Stratus OCT-3 scanner (Stratus; Carl Zeiss Meditec Inc, Dublin, California, USA). When the study began the Stratus OCT machine was the standard machine in the clinics. The Fast RNFL protocol, consisting of three circular scans with diameters of 3.4 mm centred on the optic disc was used. The pupils were not dilated. The OCT scan was considered acceptable if the signal strength score was 7 or more and the scan was well centred on the optic nerve. The mean total RNFL thickness was assessed.

**Inter-eye asymmetry analysis**

Inter-eye asymmetry has been used earlier in studies of both mfVEP and RNFL thickness and has proved to be more sensitive in detection of abnormality as well as revealing relationships between various measures compared to absolute values (48, 164, 168). Therefore, the inter-eye asymmetry of both amplitude and latency of the mfVEP and inter-eye asymmetry of RNFL thickness were calculated and analysed in this study. The inter-eye asymmetry was calculated as a difference between fellow and affected eyes and was expressed in nanovolts, milliseconds and micrometers for mFVEP amplitude, latency and RNFL thickness respectively.

In addition, the correlation between mfVEP amplitude asymmetry at different time points of follow-up and RNFL thickness at 12 months was evaluated. The 12-month cutoff point was selected because RNFL thickness is known to be affected by oedema during the acute period of inflammation and it takes time for retrograde degeneration to reach the retina after the transection of optic nerve fibres during the acute attack.
Latency Z-scores were calculated for each patient using following formula:

\[
\frac{\text{Patient’s latency} - \text{mean normal latency}}{\text{Normal latency standard deviation}}.
\]

A Z-score greater than 1.96 was classified as latency delay.

**Statistical analysis**

Statistical analysis was performed using SPSS 21.0 software. To evaluate the pattern of amplitude and latency change over time, we tested the trend of change with mixed model repeated measure analysis using the follow-up visits as a continuous variable. One-way ANOVA was used to assess difference between groups. As the examined variables followed a normal distribution, a Spearman rank correlation and linear regression analysis were used to determine correlations between the mfVEP values and OCT values. A p value of 0.05 or less was considered statistically significant.
3.3 Results

A total of 98 patients with typical acute ON were enrolled. Subsequently, 11 ON patients were excluded from analysis: three participants due to a second episode of ON in the same eye during the 12-month study period, six due to an ON attack in the other eye during the study period; one patient due to noisy traces; and another one due to the development of a maculopathy that interfered with reliable measurements. Therefore, the total number of enrolled participants was 87 patients and 25 controls.

Demographic and clinical characteristics of participants

At presentation, 27 of the 87 patients without other demyelinating MRI lesions were classified as low risk (LR) for developing MS. Sixty of the 87 patients had brain and/or spinal cord demyelinating lesions on MRI, but did not meet the criteria for a diagnosis of MS. This group was considered to be at high-risk (HR) for developing MS (174).

During the follow-up period (range 1-4 years) after the attack, 38 patients converted to MS with 36 from the HR group 60 (60%) and 2 from the LR group of 27 (7%).

The demographic and clinical characteristics of the study participants are summarised in Table 3.1. There was no significant difference between groups with respect to age (p = 0.6, one-way ANOVA). The LR group displayed a higher male: female ratio compared to patients who converted to MS and the HR group.
Table 3.1: Demographic and clinical characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients converted to MS</th>
<th>Patients remained at HR</th>
<th>Patients remained at LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>25</td>
<td>38</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Mean age</td>
<td>35.9±11.2</td>
<td>34.4±10.5</td>
<td>33.9±8.3</td>
<td>36.5±11.3</td>
</tr>
</tbody>
</table>

Abbreviations: MS, multiple sclerosis; HR, high risk; LR low risk.

Amplitude of mfVEP in ON eyes

Entire study cohort

There was a significant reduction of amplitude at one month in ON eyes compared to normal controls and fellow eyes (p < 0.0001, one-way ANOVA; p < 0.0001, post hoc Tukey test). During the 12-month follow-up period, amplitude recovered significantly (p < 0.01, mixed model repeated measure analysis). The largest improvement in amplitude was seen within the first three months (Fig 3.2a). Although the amplitude continued to improve after three months, the change was not significant (p = 0.47, mixed model analysis repeated measure analysis).

At the 12-month time-point, amplitude remained significantly reduced compared to both controls and fellow eyes (p < 0.0001, one-way ANOVA; p < 0.0001, post hoc Tukey test).
Group analysis

Separate analysis of LR, HR and MS patients revealed a similar trend for all three groups with a significant difference between the ON eyes versus fellow eyes and controls (p < 0.01) (Fig 3.2a).

Fig 3.2: (a) Amplitude changes during the study period in the ON eyes in patient groups. (b) Latency recovery during study period in ON eyes. Bars show standard error of the mean (entire study cohort is shown as a cross, LR as triangles, HR as squares, and MS as diamonds)

The mean amplitude of the MS group was less than in the HR and LR groups throughout the study period, but this difference did not reach statistical significance at any point in time.

The largest improvement in amplitude, in all three groups, was seen within the first three months (p = 0.03, 0.05, 0.02, for MS, HR, and LR respectively) with modest, but not significant, improvement afterward (p= 0.5, 0.3, 0.6 mixed model repeated measure analysis for MS, HR, and LR respectively).
Latency of mfVEP in ON eyes

Entire study cohort

As discussed in the methods section of this chapter, only eyes that had at least 75% of traces with sufficient amplitude at one month for latency to be reliably measured were included, leaving 57 eyes (MS – 24; HR – 18; LR – 15) suitable for analysis.

ON eyes had a substantial mfVEP latency delay at one month when compared to controls and fellow eyes (p < 0.0001, one-way ANOVA; p < 0.0001, post hoc Tukey test). A significant trend of latency recovery was observed during the entire follow-up period (p < 0.001, mixed model repeated measure analysis) (Fig 3.2b). The average rate of latency recovery was 1.33 ms/month between one and three months, which declined between three and six months to 0.76 ms/month, and slowed to 0.33 ms/month between six and twelve months.

There was still a significant residual latency delay at 12 months in comparison to controls and fellow eyes (157.9 ±8.3, 145.6±6.7, 141±5.1 ms, for ON, fellow eyes and controls respectively; p < 0.0001, one way-ANOVA-post hoc test).

Group analysis

Affected eyes in all groups showed significant latency delay in comparison to controls and fellow eyes throughout the study period (p ≤ 0.003).

All three groups followed a similar trend of latency recovery (p < 0.001, mixed model repeated measure analysis for each group) (Fig 3.2b).

The LR group had less latency delay throughout the study period. There was a significant difference in latency delay between the LR group and the MS and HR
groups at three months (p = 0.01, one-way ANOVA; p =0.01 for MS and p=0.05 for HR, Tukey post-hoc analysis) and six months (p = 0.01, one-way ANOVA; p=0.01 for MS and p=0.05 for HR, Tukey post-hoc analysis). The latency difference between groups decreased at 12 months (p = 0.057, one-way ANOVA).

Analysis of the latency Z-scores also revealed that a smaller proportion of ON eyes in the LR group were abnormal as compared to the MS and HR groups (Table 3.2).

**Table 3.2: Z-scores* of latency for ON eyes and fellow eyes according to patients subgroups**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>ON eye</th>
<th>Fellow eye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z-score ≥ 2</td>
<td>Z-score &gt; 2</td>
</tr>
<tr>
<td></td>
<td>at 1 month</td>
<td>at 12 months</td>
</tr>
<tr>
<td>MS</td>
<td>21/24 (88%)</td>
<td>17/24</td>
</tr>
<tr>
<td></td>
<td>(71%)</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>17/18 (94%)</td>
<td>13/18</td>
</tr>
<tr>
<td></td>
<td>(72%)</td>
<td></td>
</tr>
<tr>
<td>LR</td>
<td>9/15 (62%)</td>
<td>5/15</td>
</tr>
<tr>
<td></td>
<td>(36%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47/57 (82%)</td>
<td>35/57</td>
</tr>
<tr>
<td></td>
<td>(61%)</td>
<td></td>
</tr>
</tbody>
</table>

*Z-score less than 1.96 classified as normal latency
Abbreviations: ON, optic neuritis; MS, multiple sclerosis; HR, high risk; LR low risk
Retinal nerve fibre thickness analysis

At the 12-month test, the RNFL thickness of the entire cohort was significantly reduced in ON eyes in comparison to fellow eyes (84 ± 16 vs. 103 ± 11 µm, p = 0.0001). This reduction was similar between the three studied groups (p = 0.46, one-way ANOVA).

To assess the predictive power of mfVEP amplitude on long-term axonal loss, the mfVEP amplitude at every time point was compared with final inter-eye asymmetry of the RNFL thickness. A high degree of correlation between the mfVEP amplitude and RNFL thickness was seen as early as three months after the attack, and this increased marginally with time (Table 3.3). Separate analysis for each group was also performed. A similar trend was observed for all groups. The correlation by three months was already strong, but continued to improve with time (Table 3.3).

Table 3.3: Linear correlation between relative asymmetry of mfVEP amplitude and retinal nerve fibre thickness.

<table>
<thead>
<tr>
<th>Patients Group</th>
<th>1month</th>
<th>3months</th>
<th>6months</th>
<th>12months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.28</td>
<td>0.59</td>
<td>0.61</td>
<td>0.71</td>
</tr>
<tr>
<td>MS</td>
<td>0.27</td>
<td>0.63</td>
<td>0.6</td>
<td>0.73</td>
</tr>
<tr>
<td>HR</td>
<td>0.29</td>
<td>0.59</td>
<td>0.71</td>
<td>0.75</td>
</tr>
<tr>
<td>LR</td>
<td>0.29</td>
<td>0.49</td>
<td>0.65</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Abbreviations: RNFL, retinal nerve fibre layer; MS, multiple sclerosis; HR, high risk; LR, low risk
Amplitude of mfVEP in fellow eyes

Entire study cohort

The fellow eyes demonstrated lower amplitude in comparison to controls (p < 0.001, one-way ANOVA) at all time points. There was no significant change in the amplitude throughout the study period (p = 0.5, mixed model repeated measure analysis) (Fig 3.3a).

Group analysis

The amplitude in the fellow eye was significantly lower in all three groups, however the LR group demonstrated less amplitude reduction compared to the HR and MS groups (p < 0.01 for all).

The amplitude of the fellow eye in the LR group remained stable during the follow-up period (Fig 3.3a), while progressive amplitude reduction in the fellow eye was seen in the MS group with the difference between the MS and LR groups reaching significance at six months (p = 0.01, one-way ANOVA; p = 0.02, post-hoc) and at 12 months (p = 0.02, one-way ANOVA; p = 0.03, post-hoc).

Latency of mfVEP in fellow eyes

Entire study cohort

Latency of the fellow eye was not significantly delayed at the first visit in comparison to the controls (p = 0.17). However, a slow but consistent increase in latency was seen during the study period. By three months this latency delay reached significance (p = 0.03), which continued to increase during subsequent visits (p = 0.02, and 0.018 in the six and 12-month visits) (Fig 3.3b).
Fig 3.3: (a) Amplitude changes during study period in fellow eyes. (b) Latency changes during study period in fellow eyes. Bars show standard error of the mean (entire study cohort is shown as a cross, LR as triangles, HR as squares, and MS as diamonds)

**Group analysis**

There was no significant difference between the latency of each individual group and normal controls at one month (p = 0.2, one-way ANOVA). By three months, the MS group displayed significant latency delay compared to controls (p = 0.024, one-way ANOVA; p = 0.023, Tukey test post-hoc). This trend continued at six and 12-months visits (p = 0.018, one-way ANOVA; p = 0.013, Tukey test post-hoc, and p = 0.01, one-way ANOVA; p = 0.01, Tukey test post-hoc respectively) (Fig. 3.3b).

While latency delay of the fellow eye in HR group increased over time, it was not statistically significant. The latency of the LR group remained stable.

Z-score analysis of the fellow eyes revealed abnormal latency in more than third of patients (38%) who converted to MS, but none of the patients from the LR group (Table
3.2). The HR group occupied an intermediate position with 22% of fellow eyes displaying abnormally long latency.

**Asymmetry analysis.**

As noted above, the amplitude of ON and fellow eyes in the MS group was generally lower compared to the other two groups. To examine the possible effect of posterior visual pathway damage on these results, an inter-eye asymmetry analysis of the amplitude was performed. It demonstrated no significant difference between groups at any time point (p = 0.6, 0.3, 0.3, 0.4 for one, three, six and 12 months respectively, one-way ANOVA) (Fig 3.4a), suggesting that the difference in amplitude between the groups is likely due to retro-chiasmal damage.

In addition, all three groups demonstrated very similar trends in latency recovery. There was less latency asymmetry in the LR group compared to the MS and HR groups, which, however, did not reach statistical significance at any time points (p = 0.2, 0.07, 0.18, 0.29 for one, three, six, 12 months respectively, one way-ANOVA) (Fig.3.4b).

**Fig 3.4:** (a) Amplitude inter-eye asymmetry changes during study period. (b) Latency delay inter-eye asymmetry during study period in patient groups. Bars show standard error of the mean (MS group is shown as diamonds, HR as squares, and LR as triangles)
3.4 Discussion

This report provides prospectively acquired, longitudinal mfVEP data over 12 months of patients presenting with ON as clinically isolated syndrome. During the following four years 58% of patients who had lesions on initial MRI scans converted to MS, while only 7% of patients without initial MRI lesions developed the disease. While the conversion rate is lower compared to other reports (155, 175), the follow-up period in this study is considerably shorter. Therefore, more patients, particularly from the HR group, are expected to develop MS. The study demonstrated that both the amplitude and latency of the mfVEP are grossly abnormal at the early stages of ON. While amplitude of the mfVEP improves considerably during the first year after acute ON, the majority of the recovery occurred within the first three months. These results are similar to those reported for full-field VEPs (176). This pattern of amplitude recovery is also in line with visual acuity, which demonstrates rapid improvement of vision within the early post-acute period (33, 79).

Similar to amplitude, the speed of latency recovery was fastest during the first three months. It is believed that latency shortening after ON is mostly due to the process of remyelination. Remyelination occurs most efficiently during the early post-acute stage, where cells engaging in the formation of new myelin sheaths are frequently observed (144). The environment for successful remyelination may be severely altered afterward (177). Thus, the significant latency recovery during the first few months after the attack supports the concept of a “window of opportunity” as being fundamentally important for the success of remyelination (177, 178).

Spontaneous remyelination, which is an early and frequent phenomenon in MS, is often incomplete (144, 179). Our results confirm this, as we showed significant residual
latency delay even 12 months after acute ON. This chronically persisting latency delay has been demonstrated in multiple studies and remains the major hallmark of previous ON (180).

In this study, patients were divided into groups of those who converted to MS and those who remained at high risk or low risk of conversion to the disease. Overall, the group analysis demonstrated smaller amplitude and longer latency in the ON eyes of MS patients compared to LR patients. However, the progressive deterioration of both amplitude and latency in the fellow eyes of the MS group, and to a lesser extent the HR group, suggests that the apparent more severe involvement of ON eyes in these subgroups is due to superimposed burden of subclinical inflammatory demyelinating activity along the posterior visual pathway. Hence, the severe involvement of ON in the MS group is likely to be an indirect reflection of lesion load and disease burden at presentation and not related to the difference in remyelination pattern between the MS and LR groups.

The latency of the fellow eye in the LR group did not differ from the latency of normal controls and was stable throughout the study period. However, there was a clearly visible trend of latency increase in the fellow eye of the MS group. The difference between MS patients and controls reached statistical significance by three months and continued to increase thereafter. The latency of the fellow eye in the HR group also demonstrated a tendency to increase, although not to the same extent as patients in the MS group. Delayed latency in the fellow eyes of ON patients has been suggested as being part of central adaptive mechanism at the cortical level to compensate for delayed cortical visual input from ON eyes (181). The fact that the fellow eyes of the LR subgroup in this study did not show significant latency delay argues against this suggestion. Since both ON and fellow eyes appear to be involved, it is reasonable to
assume that this pathological process is occurring in a post-chiasmal location. The optic tract and OR are two potential sites of such retro-chiasmal lesions. Lesions of the optic tract, however, are rare in MS (43, 182). Furthermore, it is unlikely for the acute inflammation of the optic tract to be missed clinically due to its small diameter and supposedly preferential damage of small central fibres (183), which would result in an acute binocular visual deficit.

On the other hand, OR lesions are very common in MS (184) and are often clinically silent. This apparent clinical “invisibility” is due to a wide spread of OR fibres, and non-preferential distribution of the lesions, which causes rather small and more peripherally located visual field defects, easily missed by patients. However, since mfVEP covers a significant part of the visual field (48°), demyelinating lesions of the OR are likely to cause amplitude reduction and latency delay for both ON and fellow eyes. Consistent with this, as will be discussed in chapter five, we reported a correlation between the volume of OR lesions and latency delay in the fellow eyes of MS patients (185). The findings of this study also concur with previous reports demonstrating that latency prolongation and amplitude decline 12 months after acute ON were proportional to the risk of MS (171). Likewise, full-field VEP studies have also suggested that the cause of the observed asymptomatic deterioration of VEP latency in unaffected eyes of MS patients is a demyelinating process in the posterior visual pathway (176).

Therefore, to analyse the effects of acute ON on the amplitude and latency of the mfVEP, the potential effect of retro-chiasmal damage has to be eliminated. Assuming that retro-chiasmal damage influences both eyes similarly, its effect can be removed by subtracting values of the fellow eye from the ON eye. Consequently, inter-eye asymmetry analysis showed no difference in amplitude reduction, recovery or residual deficit between patients who converted to MS and patients who remained at high risk or
low risk of conversion to MS, indicating a similar degree of inflammation caused by acute ON for all three groups. This is also supported by high (and very similar) correlation between the residual amplitude of the mfVEP and RNFL thickness in all three groups and by the similar inter-eye asymmetry of the RNFL thickness between the groups.

Results of the inter-eye latency analysis also revealed a similar picture, suggesting that the demyelinating effect of the acute ON is independent of MS.

While several studies have reported a shorter latency in eyes with acute ON as part of mono-symptomatic disease (analogous to the LR group) as compared to MS-related ON (186-188), none of them assessed the potential effect of retro-chiasmal pathology by analysing inter-eye asymmetry. Our data, however, indicates that retro-chiasmal demyelination is the major factor contributing to differences in amplitude and latency between MS and non-MS patients.

Another important aspect of this study is a demonstration of an early predictive power of mfVEP amplitude in post-ON axonal loss. The amplitude reduction predicted a significant portion of the final axonal loss as early as three months. The early association of mfVEP amplitude with the degree of post-inflammatory neuronal loss may suggest a possible role of the electrophysiological measure as a potential functional surrogate marker in neuro-protective trials.

The limitation of this study is its retrospective design in regards to conversion to MS. Therefore, a significant number of patients in the HR group and some patients in the LR group are in reality MS patients, who, due to limited follow-up time, have not yet demonstrated evidence of conversion to MS. Another limitation is the use of time-domain OCT. Since there is a low incidence of ON in Australia, the enrolment process
took several years and started at the time when spectral-domain OCT machines were not yet available. However, we believe that for the purpose of the current study the resolution of time-domain OCT was adequate.

In conclusion, in this longitudinal analysis of mfVEP evolution of ON we demonstrated significant recovery of the amplitude and shortening of the latency during the 12-month follow-up period, which was fastest within the first three months after an acute episode. We also showed a high predictive value of mfVEP amplitude in subsequent axonal loss as early as three months after acute ON. Our result suggested the presence of the progressive retro-chiasmal inflammatory demyelination in patients with MS-related disease.
Chapter four: Mechanism of delayed conduction of fellow eyes in patients with clinically isolated optic neuritis

(The results of this chapter have been submitted to *clinical electrophysiology*)
4.0 Overview

The delayed conduction of fellow eyes in patients with clinically isolated ON and MS-related ON is analysed in more depth in this chapter by evaluating latency and waveform of mfVEP traces from individual segments. Four sections are included in this chapter. The first section briefly reviews the VEP changes in fellow eyes after ON. The second section discusses the aims and methodology used in the current study. Results are described in section three and the findings are discussed and placed in the relevant context in the last section of this chapter.

4.1 Background

ON can be a single demyelinating episode of unknown aetiology or a manifestation of MS. Patients with ON, presenting as clinically isolated syndrome, have a 40% risk for developing MS within 10 years’ time (138, 141). Chapters one and three included a detailed discussion on VEP amplitude and latency changes in ON eyes. In brief, the amplitude of the VEP reflects the number of functional afferent fibres reaching the striate cortex, which is determined by a combination of the severity of the inflammation (acute or chronic) along the visual pathway, axonal degeneration and the degree of synaptic activity in V1 (176). Therefore, diminished amplitude indicates either inflammatory conduction block or axonal atrophy, or both.

Delayed conduction of the VEP in the affected eye has been found in the majority of patients with ON and is thought to reflect demyelination of the optic nerve fibres (21, 189) while a subsequent shortening of latency is thought to represent the process of remyelination (59, 176). Significant latency delay is also found in a large proportion of MS patients with no history of ON (37, 180, 190, 191).
An alteration of the VEP has been reported in the fellow eyes of patients with ON in an absence of clinical symptoms (32, 171, 192-194). In a small group of ON patients (n = 12), Brusa and colleagues reported a significant subclinical VEP latency deterioration in the fellow eyes during the first three years after an ON attack (32). Beck and colleagues found that ON patients with fellow eye abnormalities are more likely to have clinical or MRI evidence of MS than ON patients with normal fellow eyes (193). Similar findings were reported in a cross-sectional study using mfVEP (171) and were in agreement with our longitudinal study included in the previous chapter of this thesis, which reported a significant deterioration of amplitude and latency of fellow eyes in patients with MS-related ON. The reported VEP changes in the fellow eyes have been attributed to various factors including sub-clinical ON, inflammation spillover from the affected eye at the chiasm or retro-chiasmal inflammatory demyelination, which is frequent in ON patients with MS (171, 192-194).

Recently, however, adaptive cortical plasticity has been suggested as an important factor that may contribute to prolongation of the latency in the fellow eye. It has been argued that temporal reorganisation at the cortical level causes latency delay in fellow eyes to compensate for delayed transmission of visual information, which serves to improve binocular vision (181).

In the current study, we tested this hypothesis by analysing the latency and waveform changes of mfVEP traces in fellow eyes of ON patients during the first 12 months after acute ON. MfVEP provides a unique opportunity to evaluate locally induced responses, therefore increasing the technique’s sensitivity and improving its spatial resolution by minimising waveform cancellation effect and including peripheral visual field segments (47).


4.2 Aims and methodology

**Aims of the study**

1. To assess the latency delay in fellow eyes of ON patients by evaluating mfVEP traces at early (three months) and late (12 months) periods after ON through measurements of the first and second peaks of mfVEP traces and assessment of their waveform changes in comparison to age-and-gender matched controls

2. To evaluate the correlation between the latency delay of ON eyes and latency change of both peaks of fellow eyes. The assumption is that patients with more latency delay in the affected eyes would experience more delay in the fellow eyes if the original hypothesis was true

3. In order to isolate the effect of cortical plasticity from potential pathological changes that may occur in the visual system in disseminated disease, patients with ON and a normal MRI are examined separately from those with a diagnosis of MS.

**Methodology**

**Subjects**

Patients presenting with unilateral typical acute ON, as determined by a neuro-ophthalmologist or neurologist, with no previous history of inflammatory demyelinating episodes were enrolled.

Patients with atypical presentation, clinical involvement of the other eye or other ophthalmic conditions that could affect the mfVEP measurements were excluded.
Patients had brain and spine MRI within two weeks of the ON attack and at least one follow up MRI scan within the next 12 months. A diagnosis of MS was made by a neurology consultant based on the revised McDonald Criteria for multiple sclerosis (88).

ON patients were analysed as one group and then divided retrospectively on the basis of MS diagnosis into two subgroups:

1. ON with low risk of developing MS (LR) – patients with normal MRI (no inflammatory demyelinating lesions in brain or spine) for the first 12 months after the attack

2. MS group – ON patients with brain or spine inflammatory demyelinating lesions on MRI who were later diagnosed with MS

3. Eight age-and-gender-matched controls were enrolled for comparison

All participants underwent visual acuity testing and ophthalmic evaluation. ON patients were tested with mfVEP at three and 12 months after the attack and controls were tested once.

The University of Sydney Ethics Committee approved the study (protocol no. 2013/106) and all procedures adhered to the tenets of the Declaration of Helsinki with informed consent obtained from all participants.
MfVEP recording and analysis

MfVEP testing was performed as described in the methodology section in chapter two. Raw data were exported into Microsoft Excel format for analysis. Good SNR was calculated by dividing amplitude of signal by noise. Noise level was calculated as the standard deviation of amplitude between 400 and 800 ms. Only traces with SNR > 2 in both eyes at both sessions were included. Traces of 5/32 segments from three inner rings (eccentricity from 2 to 10 deg) and 5/24 segments from two outer rings (eccentricity between 10 and 24 deg) were randomly selected and analysed at three months and 12 months after the attack in both affected and fellow eyes. Five inner segments and five outer segments in one randomly selected eye were analysed for controls (Fig 4.1). The first and second major peaks between 70 and 200 ms were recorded and analysed (Fig 4.2). Since the first and second peak latency of mfVEP is more reproducible than the onset of response (62), waveform width was evaluated by subtracting the latency of first peak from the second peak.

Fig 4.1: mfVEP traces of the right eye. Five traces from three inner rigs and five traces from outer two rings were selected for analysis
**Fig 4.2:** waveform of mfVEP recording illustrating first peak, second peak and waveform width used for analysis

**Statistical analysis**

Statistical analysis was performed using SPSS 21.0 software. A paired student t-test was used to evaluate latency change between the tests at three months and twelve months. One-way ANOVA with post-hoc Bonferroni correction was used to assess difference between groups. Spearman rank correlation and linear regression analysis were used to determine correlations between mfVEP values. A p-value of 0.05 or less was considered statistically significant.
4.3 Results

Demographic and clinical characteristics of participants

15 acute ON patients were included. Seven patients had normal brain and spine MRI and eight patients had brain lesions and were given a diagnosis of MS at a later stage. There was no significant difference in age or gender between groups (Table 4.1).

A total of 380 traces were analysed (15 patients x 10 segments x 2 tests and 8 controls x 10 segments). Vertical channel recording constituted 57% of traces followed by horizontal channel with 35%. Right and left oblique channels made up less than 10% of total traces. The vertical channel usually has the best recording because the majority of visual fibres project to the upper and the lower banks of the sulcus calcarinus (195). There was no significant difference between groups in regard to selected channels ($p = 0.66$).

Table 4.1: Demographic and clinical characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Number of subjects</th>
<th>Mean age (years)</th>
<th>Male: female ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>8</td>
<td>36±10</td>
<td>3:8</td>
</tr>
<tr>
<td>Total ON patients</td>
<td>15</td>
<td>36±7</td>
<td>1:3</td>
</tr>
<tr>
<td>MS</td>
<td>8</td>
<td>33±2</td>
<td>3:8</td>
</tr>
<tr>
<td>LR</td>
<td>7</td>
<td>38±9</td>
<td>2:7</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.9</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Abbreviations: ON, optic neuritis; MS, multiple sclerosis; HR, high risk; LR low risk
**Fellow eye latency in optic neuritis patients**

At three months, the mean latency of the first and second peaks was significantly delayed in fellow eyes compared to controls (p = 0.002 and 0.004 for first and second peak respectively) (Table 4.2). The latency delay of both peaks increased significantly at 12 months with the second peak shifting more than the first peak (1.4ms for first peak and 2.4ms for second peak, paired t-test p < 0.001 for both peaks).

**Subgroup analysis**

When analysing patients’ data based on their diagnosis of either MS or LR, mfVEP traces of LR group showed a small increase of latency of both peaks from three months to 12 months (1.6±1.7 ms and 1.7±3 ms). The shift of the first and second peak was similar (p = 0.54). However, latency values still remained within the normal range and there was no difference in both peaks compared to controls at both time points (Table 4.2).
Table 4.2: latency delay of first and second peaks in fellow eyes compared to controls*

<table>
<thead>
<tr>
<th>Fellow eye</th>
<th>1st peak</th>
<th>2nd peak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 months</td>
<td>12 months</td>
</tr>
<tr>
<td></td>
<td>p-value#</td>
<td>p-value#</td>
</tr>
<tr>
<td>Total</td>
<td>99.8±11.6</td>
<td>101.2±11</td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>LR</td>
<td>97±8.6</td>
<td>98.6±8.5</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>MS</td>
<td>102.2±13.3</td>
<td>103.3±13</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Controls had a first peak latency of 95±10 ms and a second peak latency of 140±14 ms.

# p-value (one-way ANOVA post-Bonferroni correction) compared to controls

Abbreviations: MS, multiple sclerosis; LR low risk

While the shift of first peak between three months and 12 months was similar in the MS and LR groups (p = 0.62), the second peak demonstrated significantly a larger increase in latency (3.2±3 ms, p = 0.016).

Furthermore, in the MS group both peaks were significantly delayed, compared to controls and the LR group (p < 0.02 for both peaks) even at three months after ON, which increased further at 12 months (p ≤ 0.01).
Correlation between latency delay of ON eyes and latency change of both peaks of fellow eyes

If latency shift is due to cortical adaptation, patients with more latency delay in the ON eye will experience more shift delay in the fellow eye. However, correlation between the latency delay of ON eyes and latency change of both peaks of fellow eyes was poor (Fig 4.3).

![Graphs showing correlation between latency delay of ON eyes and latency change of fellow eyes peaks](image)

**Fig. 4.3:** left side shows linear regression plot between latency delay of the first peak in ON eyes and change in latency delay of the first peak in fellow eyes. The right plot shows the latency delay of the second peak in ON eyes and change in latency delay of the second peak in fellow eyes. R-squared values and p-values are included
MfVEP waveform width in fellow eyes

There was no significant difference between the waveform width of fellow eyes of ON patients and controls at three months or 12 months (Table 4.3).

Table 4.3: waveform width of mfVEP at 3 and 12 month in fellow eyes

<table>
<thead>
<tr>
<th></th>
<th>Waveform width at 3 months</th>
<th>Waveform width at 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (44.9±8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR (44.4±9)</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>MS (47±7)</td>
<td>0.2</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Abbreviations: MS, multiple sclerosis; LR low risk

Group analysis, however, revealed that while waveform width in the isolated ON group continued to be comparable to controls, the MS group displayed a significant increase in waveform width which was mainly due to a larger shift of the second peak.
4.4 Discussion

In the current study, we evaluated changes in latency of the mfVEP in the fellow eye of ON patients between three and 12 months after an acute ON event and confirmed earlier reports by demonstrating a significantly longer latency in the fellow eyes of ON patients as compared to normal controls (32, 171, 192).

In the previous chapter we demonstrated a progressive deterioration in latency and amplitude of fellow eyes in patients with a high risk of developing MS and in MS-diagnosed patients, but not in patients with a low risk of developing MS. Moreover, in chapter five, as discussed in the next chapter, we show that latency delay is related to OR lesions in MS patients, who never experienced ON, with OR lesions evident in the majority of MS patients even at earlier stages of the disease. We also demonstrate that there is a significant correlation between latency delay and OR diffusivity indices, which provide further evidence linking latency delay with retro-chiasmal inflammatory demyelination (185).

Raz and colleagues suggested that “delayed latencies in the fellow eyes may reflect adaptive mechanisms at the cortical level” (181). The authors hypothesised that the temporal reorganisation of cortical processing, which is manifested as latency delay of the fellow eye, may compensate for the delayed transmission of visual information from the ON eye to the cortex. The basis of their theory came from a cross-sectional study of ON patients which showed that PVEP of fellow eyes had a wider waveform morphology rather than a delay in time-to-start response with a negligible effect of retro-chiasmal lesions. However, PVEP can be impaired by waveform cancelation/distortion and macular over-representation, due to large field stimulation, which means the effect of small peripheral visual pathway defects could be easily
missed. Additionally, MS lesions in the OR area are often orientated to venules rather than to OR fibres. This decreases the proportion of fibres damaged by a lesion and further reduces the likelihood of PVEP detection.

We hypothesised that the cortical adaptive mechanisms are similar in MS and isolated ON patients and will affect the latency of the fellow eye similarly in both groups. In addition, the magnitude of the adaptive effect and, therefore, the magnitude of fellow eye latency delay should be proportional to the latency disparity between ON and fellow eyes.

Our results, however, demonstrate considerable differences in latency values and in the magnitude of its alteration in the fellow eye of MS patients compared to LR subgroup during the follow-up period. While the fellow eyes showed significant delays in comparison to controls early after the ON attack, which increased even more by 12 months, this change was driven by the MS group. Thus, latency delay in fellow eyes of the LR group at both three months and the follow-up visit was not statistically different to latency observed in controls for both peaks. There was, however, a significant delay of the first and second peaks in MS patients in comparison to both controls and LR patients.

Therefore, our data suggests that pre-existing demyelinating activity may be responsible to a significant degree for the mfVEP latency delay in the fellow eye of the ON patients. This is also supported by the fact that the magnitude of the latency change in the fellow eye observed during the follow-up period did not correlate with the severity of latency delay in the affected eye.

Furthermore, while a similar increase of both latency peaks between three and 12 months was observed in LR group, MS patients demonstrated a significantly larger
prolongation of the second peak during the follow-up period, resulting in waveform widening. The mfVEPs are largely but not entirely generated from striate cortex with some extrastriate contribution (29). Since this widening of the waveform was only seen in the MS group, it may indicate the evolving character of demyelination in the primary visual cortex itself or in higher visual centres, which is related to the nature of the disseminated disease, rather than cortical plasticity.

In conclusion, while there was a slight mfVEP latency change between three and 12 months in the fellow eyes of ON patients with a low risk of MS which might support the hypothesis of cortical adaptation as the mechanism of its delay, the mfVEP latencies remained within the normal range. The significant mfVEP latency delay in the fellow eyes of MS patients and the change over time compared to the LR patients and controls supports the assumption that the changes are due to subclinical demyelination in the visual pathway outside of the affected optic nerve and is reflective of the burden of disease in MS patients rather than adaptation.
Chapter five: The relationship of mfVEP latency delay with optic radiation inflammatory demyelinating lesions and diffusion tensor indices changes in non-optic neuritis eyes of MS patients

(The results of this chapter have been published in Investigative Ophthalmology & Visual Science 55.6 (2014): 3758-3764)
5.0 Overview

As discussed in chapters three and four, significant latency delay was observed in the fellow eyes of ON patients. Since latency delay was mainly driven by the patients who later converted to MS, we have suggested that this latency delay may be a result of MS-related subclinical retro-chiasmal inflammatory demyelination. The focus of this chapter is to assess the relationship between latency changes and markers of retro-chiasmal damage in order to evaluate the potential role of mfVEP latency as a measure of retro-chiasmal lesions in MS patients. The first section of this chapter includes a brief literature review on VEP changes in non-optic neuritis eyes of MS patients and an overview of MRI and diffusion tensor indices (DTI) acquisition and available analysis methods. A brief description of DTI changes in MS patients will also be included. The second section describes the objectives and methodology used in the current study. The third section reports results of data analysis and explores potential correlation between the structural and functional parameters included. In the last section, the main results of the study are discussed and their implications on the potential role of mfVEP latency as a marker for retro-chiasmal demyelination are considered.
5.1 Introduction

**VEP assessment of non-optic neuritis eyes (NON) in MS**

In the current study the focus is shifted to mfVEP changes in MS patients with no history of ON. The visual pathway is frequently affected in patients with MS. Although the optic nerve is most commonly involved either early at presentation or during the disease course, significant VEP latency delay has also been reported in a large proportion of MS patients with no history of optic neuritis (37, 180, 190, 191).

The source of this latency delay is still debatable. It has been suggested that VEP delay in NON eyes of MS patients may be caused by subclinical optic nerve involvement (191), chiasmal spread of inflammation from the optic neuritis side (196), subclinical retro-chiasmal inflammatory demyelination, and/or cortical adaptation (176, 181).

Latency delay of the mfVEP in MS patients without a history of ON in either eye was recently reported (197). A possible retro-chiasmal origin was suggested due to the binocular nature of the delay and the fact that VEP response is generated at the level of the primary visual cortex and, therefore, possibly influenced by demyelinating lesions along the entire visual pathway.

MS is known to affect the posterior visual pathway. Lesions of the optic tract are rare and tend to be clinically apparent (182, 198). On the other hand, OR lesions are very common (184, 199, 200). Autopsy studies as well as MRI studies have reported OR lesions in up to 70-80% of ON and MS patients (184, 201). The orientation of MS lesions is often directed to venules rather than to OR fibres, which decreases the proportion of fibres damaged by a lesion and reduces the likelihood of symptomatic...
visual field defect. Less than 9% of participants in ONTT had an evidence of retrochiasmal visual field defects (139, 202).

Previous studies have failed to find a relationship between OR lesions and VEP latency (43, 184). Two main factors may have contributed to this lack of correlation: the limitation of conventional VEP to detect small and peripheral field defects, which has been discussed in chapter two, and the degree of accuracy of MRI methods of lesion identification and optic radiation localisation.

Until recently studies of OR lesions in vivo were hampered by the observation that optic radiation fibres are undistinguishable from the surrounding white matter. However, new technological advances in MRI have improved the ability to distinguish the optic tract and OR from the surrounding white matter more accurately.

A brief background on MRI techniques and findings in MS are discussed next.

**MRI background**

In the mid-1940s, Felix Bloch, from Stanford University, and Edward Purcell, from Harvard University, revealed that magnetic fields and radio waves cause atoms to emit tiny radio signals (203). Both were awarded a Nobel Prize for their work. Years later, Paul Lauterbur, a Professor of Chemistry at the State University of New York moved science from the single dimension of nuclear magnetic resonance images to two dimensional spatial orientation which is considered the foundation of MRI (203). Peter Mansfield demonstrated how the signals could be mathematically analysed, which made it possible to develop a useful imaging technique (204). During the 1970s, Raymond Damadian, a physician and scientist, developed the basis for using magnetic resonance
imaging in medical diagnosis (203). Since then, the medical use of the technology has advanced rapidly.

At present, MRI is the method of choice when evaluating CNS tissue pathology. It provides a superior image quality compared to computed tomography (CT) due to the lack of bony structure artefacts and the absence of ionising radiation.

Different image contrast is achieved by changing pulse sequences and image parameters. The main two basic pulse sequences in MRI are T1 and T2. Their signal intensity depends on specific tissue characteristics and brain pathology.

As with any other tests, MRI has some limitations including longer scanning time and motion artefacts. In addition, MRI is contraindicated in patients with cardiac pacemakers, cochlear implants, ferromagnetic aneurysm clips, and metallic foreign bodies in their eyes.

**MRI and MS**

MS is characterised by the presence of pathological inflammatory demyelinating lesions called plaques found in brain tissue and the spinal cord. Lesions are typically distributed along the corpus callosum, juxtacortical, and temporal white matter. They affect white as well as grey matter and are usually located perpendicular to the ventricles.

**T1-weighted images**

MS lesions may appear hypointense on T1 (Fig 5.1a). Chronic hypointensities, also known as “black holes”, have been suggested as signs of irreversible axonal loss and tissue destruction (205) and they have been linked to worsening Expanded Disability Status Scores (206, 207). In addition, gadolinium can be used to enhance lesions on T1
images and to evaluate BBB disruption during active inflammation. The number of enhanced lesions was found to have a moderate ability to predict the relapse rate in MS (208).

**T2 fluid-attenuated inversion recovery (FLAIR)**

MS lesions appear as hyper-intense foci on T2-weighted FLAIR images (Fig 5.1b). T2-FLAIR is widely used in MS imaging. In this technique CSF signalling is suppressed to differentiate bright lesions commonly found in the periventricular area from bright CSF signals, which results in improvement in lesion detection and follow-up (209).

![Fig 5.1](image)

**Fig 5.1:** (a) T1-weighted image of an MS patient with a hypointense lesion (black hole) shown with a white arrow. (b) FLAIR T2-weighted image of an MS patients with multiple hyperintense foci (one of them shown with a white arrow). Both images are from patients that have participated in research that is part of this thesis

Clinical disability was found to correlate better with lesion load on T1 images compared to lesion load on T2 images. This is possibly because lesions on T2 images represent temporary demyelination, inflammation or oedema rather than permanent axonal loss.
This disproportion between T2 findings and clinical disability was referred to as clinic-radiological paradox and it was therefore, recommended to consider several outcome measures such as brain atrophy, spinal cord involvement and masking effect of cortical adaptation in evaluating patients to overcome this inconsistency (210).

While brain atrophy is evident in the late stages of MS, subtle atrophy is increasingly recognised early in the disease process especially with improved image quality and advanced image processing (211, 212). Furthermore, studies have shown that general brain atrophy rate and atrophy in particular areas such as the corpus callosum are significant predictors of subsequent disability (213, 214).

**Principles of diffusion tensor images**

One of the recent MRI developments is diffusion-weighted imaging (DWI), which is created using the thermal agitation of random movements of water molecules, known as Brownian motion. Molecular diffusion in tissues occurs inside, outside and around cellular structures and interacts with the cellular environment including many microscopic structures such as fibres and membranes.

One of the early attempts to create and utilise DWI in humans was by Le Bihan, Basser and colleagues in the early 1990s (215, 216). They used pulsed magnetic field gradients included in a standard spin echo sequence to measure an apparent diffusion coefficient (ADC) in normal and pathological brain diffusion images. Diffusion MRI has rapidly advanced since then and has been applied to investigate a variety of diseases including stroke, epilepsy and MS.

Diffusion tensor imaging (DTI) is an extension of DWI, which measures the signal attenuation from water diffusion to create a three-dimensional map to assess the
structural integrity of tissues (217). In contrast to DWI, which uses only the average of diffusivity, three diffusion directions can be measured in DTI based on their orientation and are referred to as eigenvectors (Fig 5.2).

Fig 5.2: An illustration of diffusion eigenvalues represented with diffusion ellipsoid. $\lambda_1$ represents the main eigenvector. $\lambda_2$ and $\lambda_3$ are two eigenvectors perpendicular to $\lambda_1$.

The largest eigenvalue ($\lambda_1$) reflects the diffusion parallel to the white matter fibre bundle known as axial diffusivity (AD), the average of two eigenvectors perpendicular to the axonal fibres ($\lambda_2$ and $\lambda_3$) known as radial diffusivity (RD). The average of all three eigenvalues, so-called mean diffusivity (MD), is a measure of diffusion that is independent of the orientation of structures (218). Therefore, greater MD values imply more isotropic diffusion. The fourth parameter measured in DTI is fractional anisotropy (FA), which is calculated based on the equation:
FA has a value between 0 and 1. Theoretically, an FA value of 0 means that the molecular diffusion is equal in all directions (isotropic). On the other hand, a value of 1 means that the diffusion occurs only in one axis or direction. Since the white matter is composed of well-structured nerve fibres surrounded by myelin, diffusion is relatively restricted perpendicular to axons and greater along the fibre tract.

It is believed that diffusion indices, and RD in particular, represent a measure of MS damage since they are severely abnormal within the MS lesions (219). AD has associated with axonal injury in animal models as well as in humans, while fractional anisotropy (FA) is considered a measure of fibre coherence (220, 221).

Animal studies have shown that myelin is considered one of the main barriers for water diffusion in central and peripheral nerve fibres (222-226). A model of Wallerian degeneration in the sciatic nerve of a frog showed an increase in RD and decrease in AD due to myelin and axonal degeneration (222). Optic nerve diffusion measurements in a mouse model of retinal ischaemia showed an early decrease of AD due to axonal injury followed by an increase in RD in later stages in correspondence with myelin degradation (223). In another study with experimental demyelination of the corpus callosum in a mouse brain, the extent of RD reflected the severity of demyelination with evidence of RD reduction during the process of remyelination (224). Similar DTI changes have been reported in humans with an increase in radial diffusivity and a reduction in axial diffusivity in degenerated white matter tracts (227).
Thus, the integrity of white matter and the microscopic changes during pathological processes can be assessed *in vivo* using DTI. However, the acquisition of DTI and interpretation are complex and should be performed with caution (228).

**Methods for DTI analysis**

Several methods have been introduced to evaluate the effect of certain disorders on diffusion properties of the CNS. The most common approaches are discussed below.

**Region of interest (ROI) based approach**

In this approach, a geometric shape such as a square or a circle is placed manually within the anatomical structure of interest. The DTI indices are then measured from the voxels that are included within this area. As the ROI is delineated manually, this method is prone to low reproducibility and bias. The datasets of patients can be transformed to a certain atlas space so that a single ROI can be used to delineate the structure in all participants (229, 230).

**Tractography approach**

The tractography approach provides the ability to identify white matter bundles and gives the opportunity to selectively analyse their diffusion properties.

It is assumed that the orientation of the largest eigenvector runs parallel to white matter tracks in a DTI coloured map. Therefore, a white matter tract can be estimated by starting at a specific location, also known as an ROI, then assessing the direction of the major eigenvector until reaching another ROI. Tractography algorithms can be divided into two main groups: deterministic and probabilistic (231). Deterministic tractography was introduced in the late 1990s (232). The algorithm is based on line propagation of
streamline techniques, where the tracking of fibres starts at a pixel or a ROI and continues as long as the main diffusion vectors are aligned. Tracing is terminated if vector orientation becomes random or has an isotropic diffusion with low FA (231). Accordingly, the algorithm only creates one reconstructed trajectory per ROI without consideration of branching fibres and may result in premature termination of neural tracts.

The probabilistic tractography algorithm, on the other hand, addresses this limitation by considering multiple pathways along the reconstructed trajectories (233). Therefore, instead of a distinct trajectory, the probabilistic approach generates potential pathways. However, anatomical knowledge is essential in evaluating such pathways as the connectivity map may involve unexpected regions of the brain.

In spite of the promising results in tractography, several limitations of this method should be noted and considered during DTI acquisition and analysis. These include image artefacts and thermal and physiological fluctuation, which can affect the greatest eigenvector estimation and its direction (228). Partial volume averaging between tissues in large voxels may result in signal mixing of grey matter, white matter and CSF resulting in less accurate measurements (234). Lastly, algorithms based on the major eigenvector are still incapable of estimating white matter tracts in regions with crossing white matter pathways (235). While DTI technology is still in its infancy, attempts are underway on several fronts, including improved data acquisition approaches and analysis methodology, to overcome these limitations (236).
DTI in MS

Several studies have highlighted the ability of DTI to detect abnormality in white matter affected by lesions and in normal appearing white matter of MS patients (237-244).

DTI and demyelination

Myelin contributes significantly to the anisotropic nature of the diffusion in white matter fibres as it limits diffusion of water within the axons. It has been suggested that myelin destruction therefore disturbs the structural barrier and increases the diffusion perpendicular to white matter fibres, which as a result increases RD and MD measurements and reduces FA (158, 224, 238, 242, 245). Klawiter and colleagues showed a significant correlation between RD and demyelination severity in acute MS lesions of the spinal cord using autopsy material (246). Fox and colleagues reported a reduction of RD in active MS lesions after the use of natalizumab, a neuro-protective agent, and inferred that the observed change after treatment may signify a process of remyelination (247).

DTI and inflammation

There is limited work on the specific effect of inflammation on DTI measurements, but a few studies have suggested that AD reduction may be attributed to axonal damage (225, 240). Tievsky and colleagues examined the difference of ADC and FA measurements in acute and chronic MS lesions and showed that there is increased ADC and decreased FA in acute lesions (244). Acute inflammatory changes can result in increased endothelial permeability, inflammatory cell migration and oedema, which result in a reduction of tissue anisotropy and an increase in mean diffusivity. Werring and colleagues compared MD in acute and chronic MS lesions. They reported a higher
MD in acute lesions compared to chronic ones, which reflects the acute inflammatory status and oedema, while chronic lesions demonstrated a large range of MD, reflecting possible pathologic heterogeneity including gliosis, axonal loss and persistent demyelination. A lower FA was also observed in T1 hypointense lesions compared with T1 isointense lesions and was related to presumed axonal loss (240).

**DTI and clinical disability in MS**

Whether DTI measurements differ based on MS subtypes is still uncertain. Scanderbeg and colleagues reported higher diffusivity in SPMS as compared to RRMS (248). Their findings were in agreement with another study that reported higher abnormal DTI values in whole brain MD in SPMS compared with RRMS (249). A significant correlation between DTI measurements and lesion load was observed in patients with RRMS and SPMS, but not PPMS (250). This may be explained by the atypical MRI features in PPMS and the more severe clinical disability in this subgroup.

Several studies reported a moderate to strong correlation between different DTI indices and clinical disability in MS patients (242, 248, 251). Ciccarelli and colleagues reported a stronger correlation between DTI measurements and clinical disability in SPMS compared to RRMS (251). FA has a negative correlation with Expanded Disability Status Scale, which was also more severe in advanced stages (242). A correlation between DTI of the optic nerves and several visual parameters including visual acuity, VEP and RNFL thickness has been recently reported (252, 253).

Therefore, the evidence from the literature suggests that DTI is a useful tool in assessing the microstructural integrity of white matter tracts and was therefore included in the current study to explore the relationship between mfVEP latency and retrochiasmal damage of the visual pathway in MS patients.
5. 2 Aims and methodology

The purpose of the study

4. To evaluate the mfVEP latency in MS patients with no previous history of ON and to compare it with the mfVEP latency of age-and-gender matched controls.
5. To examine the relationship between mfVEP latency delay and optic radiation lesions identified on T1 and FLAIR T2-weighted MRI.
6. To assess the correlation between mfVEP latency delay and optic radiation DTI indices.

Rational of the study

Significant latency delay has been reported in MS patients with no history of optic neuritis (37, 180, 191). It has been suggested that VEP delay in those eyes is caused by subclinical optic nerve involvement (191) or chiasmal spread of inflammation from the optic neuritis side, if one eye has been affected (193). However, based on the binocular nature of the delay and the fact that VEP response is generated at the level of the primary visual cortex, a possible retro-chiasmal origin was suggested (197, 254). The current study was conducted to test this hypothesis by evaluating the relationship between mfVEP latency delay, optic radiation inflammatory demyelinating lesions and DTI changes in NON eyes of MS patients.
Methodology

Subjects

Consecutive patients with RRMS with no history of clinical ON, at least in one eye were enrolled from MS clinic at Brain and mind institute, Royal North Shore Hospital and St. Vincent’s Hospital together with 25 age- and sex-matched healthy controls.

Pre-enrolment assessment

All participants were asked about previous signs and symptoms of ON and underwent visual acuity testing, full ophthalmic evaluation including slit lamp examination as well as optic disc evaluation. Patients matching the study inclusion criteria were offered to be part of the study.

Patients with other systemic or ocular diseases that could confound results such as retinal or optic nerve diseases were excluded.

All procedures adhered to the tenets of the Declaration of Helsinki. Ethical approval was obtained from the University of Sydney Ethics Committee (protocol no. 2013/106). Written Informed consent was obtained from all participants after the study Procedures, time involved and potential risks were discussed.

The inclusion criteria were:

- Adult (>16 years old)
- RRMS diagnosed by a neurology consultant
- No history of ON in at least one eye
The exclusion criteria:

- Presence of eye disease that could interfere with the mfVEP measurements such as optic neuropathies, glaucoma, dense cataract, retinal detachment or amblyopia.
- Mental or physical disabilities that may interfere with performing reliable tests.
- Inability to fixate at a point from a distance of 30 cm due to poor vision (usually if best corrected vision is less than 6/60)

All participants were tested using brain and spine MRI and mfVEP once.

Study procedures

MfVEP recording and analysis

MfVEP testing was performed with Accumap (ObjectiVision Pty. Ltd., Sydney, Australia) as described in the methodology section of chapter two. One eye was randomly selected for MS patients with no previous ON, while the NON eye was tested in patients with a documented previous ON. Averaged latency values from all segments of the visual field were used for latency analysis.

MRI recording and analysis

MRI protocol

MRI data was collected using a 3.0 Tesla GE MR750 scanner (GE Healthcare, Little Chalfont, UK). Three sequences were implemented: Sagittal 3D T1 (GE BRAVO sequence, FOV 256mm, slice thickness 1 mm, Discovery MR750, TE 2.7 ms, TR 7.2 ms, Flip angle 12°, pixel spacing 1mm); FLAIR CUBE (GE CUBE T2 FLAIR sequence, FOV 240mm, slice thickness 1.2mm, acquisition matrix (Freq. x Phase)
256×244, TE 163ms, TR 8000ms, Flip angle 90°, Pixel spacing 0.47 mm); and DTI pulse sequence (Spin Echo, 64 directions, FOV 256 mm, acquisition matrix (Freq.×Phase) 128 ×128, slice thickness 2 mm, TE 83ms, TR 8325 ms).

**Tractography**

Probabilistic tractography using the ConTrack algorithm, as described by Sherbondy and colleagues (255), was used to reconstruct the optic tract and OR fibers. The ConTrack algorithm was chosen because it uses the probabilistic tractography technique and therefore has the ability to overcome some of the limitations of deterministic algorithms in detecting white matter pathways (as discussed in the previous section of this chapter). Additionally, the ConTrack algorithm appears to be superior to other probabilistic algorithms in detecting Meyer’s loop as well as direct optic radiation pathways (255, 256).

DTI and FLAIR T2 images were co-registered to a high resolution T1 structural image. Prior to the reconstruction of OR, two ROIs were determined. The first was the LGN on both sides. To identify the LGN, the optic chiasm was located from the FA map using deterministic tractography. A 10 mm ROI was placed on the chiasm and was used for seeding of the deterministic algorithm. The optic tract was then followed to the LGN area (around 4 mm tract). The position of the LGN was determined and a ROI (diameter 7 mm) was placed at the end of the tract on the posterior lateral area of the thalamus on both sides. The ROI size was chosen to ensure that the whole LGN in included as the size of LGN varies among individuals (257).

The second ROI was the calcarine sulcus, which was segmented manually in each hemisphere using ITK-SNAP software (258). Probabilistic tractography software (ConTrack) was run between the first ROI (LGN) and the second ROI (calcarine sulcus)
to reconstruct the optic radiation. 70,000 fibres were collected initially and the 30,000 best fibres were selected by scoring algorithm. Optic radiation fibres were then manually cleaned using Quench software.

**Identification of MS lesions**

MS lesions were identified on co-registered FLAIR T2 and T1 images and segmented semi-automatically using ITK-SNAP software. Lesions were then intersected with optic radiation fibres to calculate their volume within and outside of the optic radiation (Fig 5.3). An averaged (between the left and right side) lesion volume was used for analysis. DTI indices (FA, MD, AD, and RD) were calculated along the OR (between ROI 1 and ROI 2).

**Fig 5.3:** (a) 3-D tractographic image demonstrates optic tract fibres in green and optic radiation in yellow. (b) Lesions (in red) were intersected with visual pathway fibres
**Statistical analysis**

Statistical analysis was performed using SPSS 21.0 (SPSS, Chicago, IL, USA). The examined variables followed a normal distribution, Spearman rank correlation and linear regression analysis with adjustment for confounding factors were used to determine correlations between variables. The entire data were analysed as one group. Then, since the fellow eyes of ON patients may potentially be affected by chiasmal spill-over of inflammation from the ON eye, participants were separated to two groups: a group of fellow eyes of optic neuritis patients (“ON group”) and a group of study eyes of patients who never experienced ON in either eye (“NON group”).

Paired or unpaired student t-test or ANOVA were used when suitable and a p-value of 0.05 or less was considered statistically significant.
5.3 Results

Demographic and clinical characteristics of participants

Fifty-nine MS patients were enrolled. Two participants with chiasmal involvement were excluded. Twenty-seven patients had a previous history of optic neuritis in one eye while 30 patients had no previous history of optic neuritis. One eye was randomly selected for patients without a history of ON, while the non-optic neuritis eye was used in patients with a history of previous ON. These two MS subgroups were analysed together and then individually for correlations. Demographics are presented in Table 5.1.

Table 5.1: Demographic and clinical characteristics of participants:

<table>
<thead>
<tr>
<th></th>
<th>Number of subjects</th>
<th>Age (years)</th>
<th>Female: male ratio</th>
<th>Disease duration (years)</th>
<th>Latency of mfVEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>25</td>
<td>39.9±10.2</td>
<td>19:6 (76%)</td>
<td></td>
<td>149.3±5.1</td>
</tr>
<tr>
<td>MS patients</td>
<td>57</td>
<td>40.8±11.9</td>
<td>42:15 (74%)</td>
<td>4.68±3</td>
<td>161.2±9</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.7</td>
<td>0.8</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: MS, multiple sclerosis

The average mfVEP latency of non-optic neuritis eyes in the entire MS cohort was significantly delayed compared to healthy controls (p < 0.0001, Student’s t-test, Table 5.1).

No lesions were identified in the optic tract in any of the patients; however, 77% (44/57) of patients had OR lesions demonstrated on T2 FLAIR images. The lesion
volume varied between 24 mm$^3$ and 4512 mm$^3$ and constituted about 10% of total brain lesion volume. T1 OR lesions were detected in 72% (41/57) of patients with lesion volume varying between 20 mm$^3$ and 3060 mm$^3$. T1 lesion volume also represented about 10% of total T1 brain lesion volume. T1 lesion volume was significantly lower compared to T2 FLAIR both within and outside of the OR (p = 0.001 and < 0.0001 respectively, Table 5.2).

**Table 5.2:** lesion volume/mm$^3$ on T2 FLAIR and T1-weighted images

<table>
<thead>
<tr>
<th></th>
<th>T2 FLAIR</th>
<th>T1-weighted</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR lesion volume (mm$^3$)</td>
<td>745±144</td>
<td>525±100</td>
<td>0.001</td>
</tr>
<tr>
<td>Lesion volume outside OR (mm$^3$)</td>
<td>7727±1064</td>
<td>5194±744</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

*p-value is the statistical difference between T2 FLAIR and T1 lesion volume/mm$^3$.

Abbreviations: OR, optic radiation

**Correlation between mfVEP latency and lesion volume**

**Entire study cohort**

OR lesion volume was strongly associated with total brain lesion volume for both T2 FLAIR and T1 images (r = 0.85, p < 0.001 and r = 0.89, p < 0.001 respectively). Therefore, in order to examine tract-specific relationships between mfVEP latency delay and OR lesion volume, correlation between the two was corrected for lesion volume outside of OR. In addition, correlation was also adjusted for age, gender and disease duration. Partial correlation demonstrated a significant positive association between mfVEP latency and optic radiation T2 FLAIR lesion load (Table 5.3). An
example is presented in Fig 5.4. There was also a significant (albeit on a lesser scale) correlation between mfVEP latency and OR T1 lesion load.

**Table 5.3:** mfVEP latency correlation with diffusion indices and OR lesion volume after adjusting for age, gender, disease duration, and brain lesion outside OR

<table>
<thead>
<tr>
<th></th>
<th>Entire cohort</th>
<th>Fellow eye of ON patients</th>
<th>Study eye in patients without previous history of ON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation</td>
<td>p-value</td>
<td>Correlation</td>
</tr>
<tr>
<td>T2 OR lesion volume</td>
<td>0.50</td>
<td>&lt; 0.001</td>
<td>0.30</td>
</tr>
<tr>
<td>T1 OR lesion volume</td>
<td>0.31</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>FA</td>
<td>-0.34</td>
<td>0.016</td>
<td>-0.3</td>
</tr>
<tr>
<td>MD</td>
<td>0.5</td>
<td>&lt; 0.001</td>
<td>0.38</td>
</tr>
<tr>
<td>AD</td>
<td>0.42</td>
<td>0.003</td>
<td>0.20</td>
</tr>
<tr>
<td>RD</td>
<td>0.48</td>
<td>0.001</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Abbreviations: OR, optic radiation; FA, fractional anisotropy; MD, mean diffusivity; AD, axial diffusivity; RD, radial diffusivity.
Fig 5.4: MS patient with no history of optic neuritis showing (a) mfVEP latency delay (darker zones) and (b) left optic radiation lesions (in red, with tract fibres in yellow) with corresponding increase of radial diffusivity.

Fig 5.5: partial regression plots for the entire cohort of study participants and sub-group analysis according to the presence or absence of previous ON in fellow eyes.
**Group analysis**

Participants were separated into two groups, an ON group and NON group to assess the potential effect of ON on the observed correlation. The groups demonstrated similar ages, female-to-male ratio and disease duration. Latency of the mfVEP in both groups was significantly delayed, but there was no difference between the groups ($p = 0.8$, Student’s t-test). The total lesion volumes and optic radiation lesion volumes for both T1 and T2 FLAIR were also similar between the two groups (Table 5.4). Correlations between mfVEP latency and OR lesion volume were performed for each group separately. While in the ON group the correlation between mfVEP latency of the fellow eyes and OR T2 FLAIR lesions volume lost significance, the correlation for the study eye in the NON group was increased considerably compared to the entire cohort. A similar result was observed for T1 lesions: there was a loss of significance for the fellow eyes in the ON group but there was an increased correlation for the study eyes in the NON group (Table 5.3). Noticeably, even for the study eyes of NON-patients, the correlation of T1 lesion volume with mfVEP latency was less strong compared to T2 FLAIR lesion volume.
Table 5.4: Demographic and clinical characteristics of MS participants with and without history of ON in fellow eye

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Age (years)</th>
<th>Female: male ratio</th>
<th>Disease duration (years)</th>
<th>Latency of mfVEP</th>
<th>T2 OR lesion volume (mm³)</th>
<th>T2 total lesion volume (mm³)</th>
<th>T1 OR lesion volume (mm³)</th>
<th>T1 total lesion volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fellow eye of ON patients</td>
<td>27</td>
<td>40.1±11.3</td>
<td>21: 6 (76%)</td>
<td>4.71±3.1</td>
<td>161.6±8.2</td>
<td>874</td>
<td>9327</td>
<td>603</td>
<td>6508</td>
</tr>
<tr>
<td>Study eye of NON patients</td>
<td>30</td>
<td>41.3±12.4</td>
<td>21: 9 (72%)</td>
<td>4.66±3.0</td>
<td>160.0±9.7</td>
<td>653</td>
<td>7988</td>
<td>417</td>
<td>4853</td>
</tr>
<tr>
<td>p-value</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>0.5</td>
<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MS, multiple sclerosis; ON, optic neuritis; NON, non-optic neuritis
Correlation between mfVEP latency and OR DTI metrics

Entire study cohort

There was a significant and very similar increase of axial and radial diffusivity in the entire patient cohort compared to normal controls. As a result, MD (i.e. diffusion in all directions) also increased, while FA (relative directional diffusivity) remained stable (Table 5.5).

Table 5.5: Optic radiation DTI characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>MS patients</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>0.49±0.04</td>
<td>0.50±0.02</td>
<td>0.16</td>
</tr>
<tr>
<td>MD</td>
<td>0.88±0.07</td>
<td>0.82±0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AD</td>
<td>1.4±0.07</td>
<td>1.32±0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RD</td>
<td>0.62±0.07</td>
<td>0.56±0.04</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: DTI, Diffusion tensor images; FA, fractional anisotropy; MD, mean diffusivity; AD, axial diffusivity; RD, radial diffusivity.

All four indices were significantly correlated with mfVEP latency. RD and MD demonstrated strongest association followed by AD. FA displayed the weakest correlation with latency (Table 5.3).

Group analysis

Group analysis of the relationship between the DTI indices and mfVEP latency demonstrated a behaviour similar to the one described above for the lesion volume: correlation increased for the NON group, but was insignificant for the fellow eyes of
ON patients (Table 5.3 and Fig 5.5).

Except for FA, all DTI indices in both groups remained significantly different from normal controls (ANOVA, Tukey post-hoc test, p < 0.0001). However, diffusivity changes in the fellow eyes of ON patients displayed larger deviation from normal controls compared to the study eyes of NON patients, particularly in RD, for which the difference between the two groups demonstrated a tendency for significance (0.64±0.01 vs. 0.6±0.01 for the fellow eyes of ON and NON patients respectively, ANOVA, Tukey post-hoc test, p = 0.06).
5.4 Discussion

In this study we analysed the relationship between latency of the mfVEP and structural markers of primary MS-related damage in the posterior visual pathway. We hypothesised that the latency delay of mfVEP in the eyes of MS patients without previous ON may be caused by retro-chiasmal demyelinating lesions. This assumption was based on the binocular nature of the mfVEP latency delay in MS patients without ON in either eye, which has been reported previously (197).

The findings of the current study support this hypothesis. Thus, in addition to confirming extensive latency delays in NON eyes compared to normal controls, we found a significant association between latency of the mfVEP and lesion volume of the optic radiation as determined by both T1 and T2 FLAIR MRI. Since lesion volume within the OR correlates highly with volume of the lesions in the rest of the brain, correction for non-optic radiation white matter lesion volume was necessary to determine a tract-specific relationship. This correction, as well as adjustment for age, sex and disease duration had minimal effect on the revealed association.

Lesion volume determined on T2 FLAIR sequence was generally larger and its volume within the OR demonstrated a higher degree of correlation with the mfVEP latency delay than T1 lesion volume. This is possibly related to the fact that, while both T1 and T2 lesions have low histopathological specificity, chronic T1-weighted lesions (“black holes”) are associated with severe tissue destruction and axonal loss, while T2 lesions are more linked to demyelination (205, 259, 260).

Another novel finding of this study is the demonstration of a significant correlation between the latency of the mfVEP and OR diffusivity indices. Changes of DTI indices
in the OR of MS patents have been reported previously (261, 262). Reich and colleagues demonstrated significant abnormality in all DTI indices in the OR of MS patients (lower FA and higher MD, AD and RD values) (262). Similar alterations of OR DTI indices in MS patients were recently confirmed by Rocca and colleagues (261). More importantly, both studies also demonstrated a strong association between DTI changes, particularly RD, with the presence of OR lesions. Therefore, correlation between latency delay of the mfVEP and DTI indices provides further evidence linking the mfVEP delay with retro-geniculate inflammatory demyelination.

An important observation is also related to the sub-analysis based on the history of ON: patients without previous ON in either eye demonstrated considerably stronger (and more significant) correlation between mfVEP latency and OR lesion load compared to the entire cohort. On the other hand, when only the fellow eyes of ON patients were analysed, the correlation between mfVEP latency and OR lesions became insignificant. This trend was similar for all lesional measures including T2 and T1 lesions and DTI indices. Since both groups have similar demographics and disease burden, it is likely that the presence of ON may mask the relationship between mfVEP latency and OR lesions in the fellow eye.

The effect of ON on the OR is still unclear with a limited number of studies that addressed the impact of optic neuritis on the posterior visual pathway (183, 262, 263). Evangelou and colleagues suggested a process of retrograde, anterograde or trans-synaptic degeneration as a mechanism of neuronal atrophy in the visual pathway. Ciccarelli and colleagues studied the effect of ON on the OR using fast marching tractography and DTI and reported subtle anatomical and functional changes in the OR. The effect of ON on the posterior visual pathway, which may result in significant
alteration of OR diffusivity, could potentially affect the correlation between mfVEP latency and DTI metrics (262, 263).

In addition, a potential chiasmal spill-over of the inflammatory demyelination from the ON eye, even in a few cases, may dramatically change mfVEP latency, but would not have any effect on OR lesions. It has also been suggested that adaptive cortical mechanism may contribute to the VEP latency delay in the fellow eyes of ON patients (particularly to the late VEP waves, which are measured in this study). It was proposed that the delay in cortical processing of visual information from the unaffected eye may compensate for the slowdown of visual input from the affected eye caused by demyelination of the optic nerve (181).

There are several limitations in this study. Firstly, magnetization transfer imaging (MTI) has not been used. However, while MTI may potentially help in lesion identification, we believe that combination of T2 FLAIR and DTI-reconstructed optic radiation allowed us to detect and measure the majority of the lesions in the posterior visual pathway.

Secondly, cortical damage, which may potentially affect the OR, has not been analysed. However, current detectability of cortical lesions (even using most sensitive double inversion recovery sequence) is very poor. In addition, DTI has not been shown to identify cortical damage of the occipital brain.

Thirdly, a potential effect of cortical plasticity and sub-clinical ON on the correlation between the latency of the mfVEP and lesions of the posterior visual pathway has not been investigated. In addition, severe damage to axons in some lesions may result in axonal loss and a decline in mfVEP amplitude, rather than latency delay, which may also have an impact on correlation.
In conclusion, the current study evaluated the relationship between mfVEP latency and structural changes of the OR on MRI in MS patients. The result of this study supports our hypothesis that optic radiation lesions are responsible for latency prolongation in NON eyes of MS patients. Previous ON, however, may have a significant contribution to latency delay even in the fellow eye. Care should therefore be taken to adjust for this factor if mfVEP latency is to be used as a marker of demyelination in the posterior visual pathway.
Chapter six: conclusions and future directions
6.0 Overview

This chapter summarises the main findings and conclusions of this thesis. The contribution of mfVEP in the assessment of damage and recovery of the visual pathway is highlighted and directions for future research are identified.

6.1 General discussion and conclusions

MfVEP provides an objective functional measure of the integrity of the visual pathway. The results of this thesis shed a new light on changes in the mfVEP caused by visual pathway lesions. In chapter two, full-field PVEP limitation in detecting focal visual field defects was demonstrated in a group of patients with different visual pathway disorders. Our findings showed that mfVEP offers a greater resolution of visual pathway function including peripheral visual field fibres and may therefore provide a more accurate assessment of visual defects when compared with conventional full-field PVEP.

Chapter three evaluated the evolution of mfVEP following acute inflammation of the optic nerve through a longitudinal analysis of mfVEP parameters of affected and fellow eyes in a large cohort of patients during the first 12 months after acute ON. The findings confirm previous reports of grossly abnormal amplitude and latency of affected eyes at early stages of ON (21, 176). Both amplitude and latency of the mfVEP improve considerably during the first year after acute ON with the majority of the recovery occurring within the first three months. This pattern of recovery is also in line with previous studies, which have demonstrated a rapid improvement of vision within the early post-acute period (33, 79, 176). Our results also demonstrated significant residual latency delay even 12 months after acute ON. This chronically persisting latency delay
remains the major hallmark of previous ON. We argued that more severe involvement of ON eyes in MS patients (and to a lesser extent in patients at high risk of developing MS) is probably due to a retro-chiasmal inflammatory demyelination, which occurs frequently in MS and is not related to the difference in remyelination pattern between MS and LR groups. This is in line with the observed progressive deterioration of both amplitude and latency in the fellow eyes of MS and HR groups and was supported by inter-eye asymmetry analysis (which was used to minimise retro-chasmal lesion effect).

Another key finding presented in this chapter was a demonstration of an early predictive power of mfVEP amplitude in post-ON axonal loss suggesting a possible role of this measurement as a potential functional surrogate marker in neuro-protective trials.

In chapter four we took our investigation in regard to the mechanism of latency delay in fellow eyes in ON patients a step further by testing the recently proposed hypothesis that prolongation of the latency in fellow eyes after ON is an adaptive cortical plasticity to compensate for delayed transmission of visual information (181). We evaluated mfVEP latency and waveform changes over a one-year period in individual traces from mfVEP segments in patients with isolated ON and MS-related ON. Our results, which demonstrated significant mfVEP latency delay in the fellow eyes of MS patients early after attack and a change in waveform over time compared to the LR patients and controls, support the assumption that the observed changes are due to subclinical demyelination in the visual pathway outside of the affected optic nerve and a reflection of the burden of disease in MS patients. Moreover, while there was slight mfVEP latency change between 3 and 12 months in the fellow eyes of ON patients with a low risk of MS (this might support the hypothesis of cortical adaptation mechanism), the mfVEP latencies remained within normal range. The poor correlation between latency delay of ON eyes and latency change of both peaks of fellow eyes also argues against
the assumption that latency delay in fellow eyes is a compensation for the delayed transmission of the visual information from ON eyes.

In the last chapter we focused on an evaluation of the relationship between mfVEP latency and posterior visual pathway lesions in MS patients. We demonstrated a significant association between latency of the mfVEP and lesion volume within the posterior visual pathway on both T1 and T2 FLAIR MRI. A potential masking effect of previous ON on the correlation between latency delay in the fellow eyes and OR lesions was discussed and we suggested that care should be taken to adjust for this factor if mfVEP latency is to be used as a marker of demyelination in the posterior visual pathway. A novel finding of this chapter is the demonstration of a significant correlation between the latency of the mfVEP and OR diffusivity indices, which provides evidence linking the mfVEP delay with retro-geniculate inflammatory demyelination.

6.2 Directions for future research

MfVEP is a relatively new and evolving technique. Future advances in various aspects of mfVEP recording and analysis techniques will certainly improve the reliability and the clinical utility of this technique. Recently, promising new software programs have been proposed to analyse signal progression in mfVEP with higher accuracy using cross-correlation between signals (61, 264). Further studies are needed to verify and assess their utility in the clinical setting.

Combining different tests to evaluate structural and functional integrity of the visual pathway in longitudinal studies is an interesting prospect allowing more comprehensive assessment of the visual pathway pathology. The strong correlation between mfVEP amplitude and long term axonal loss measured by OCT which was demonstrated in chapter three suggests a role for mfVEP amplitude as a functional biological marker for
axonal loss. This is an area that merits further research. The relationship between latency delay and DTI of the optic radiation has not been previously investigated and it would be interesting to assess this in a longitudinal study with a larger sample size.
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