Structure and Function of the Retina in Multiple Sclerosis

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Structure and Function of the Retina in Multiple Sclerosis

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A case control research project to investigate the function and structure of the complete visual pathway with routinely performed evoked potentials (pattern reversal ERGs and VEPs), additional flash electroretinograms (ERGs) and optical coherence tomography (OCT) in patients suspected of having multiple sclerosis (MS).

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'With the exception of any statements to the contrary, the contents presented in this thesis are the results of my own original efforts and have not previously been submitted in candidature for any other degree or diploma. In addition, no parts of this report have been copied from other sources. I understand that any evidence of plagiarism and/or the use of unacknowledged third-party contribution will be dealt with as a very serious matter.'

Nicola Broomfield (2023)

COVID-19 Statement:

This thesis was interrupted by the COVID-19 pandemic in a number of ways. Most significantly, the neurophysiology department was unable to perform any out-patient studies for a significant period and was only performing investigations for the acutely sick who were admitted to hospital. Additionally, staff, including myself, were retrained and redeployed to ward areas due to staff shortages. Academic modules that were part of the DClinSci programme that was outsourced to hospitals were also unable to go ahead as NHS organisations were unable to commit to training for a significant period of time. Collectively, there were many factors that had a negative impact on the DClinSci programme and my participation in it (2017–2023).

Background

I am submitting this research as a clinical scientist in clinical neurophysiology working within a district neuroscience and trauma centre for the NHS. It is the research aspect of the academic section of the Higher Specialist Scientist Training (HSST), which is supplemented by a professional portfolio gained over 5 years. It reflects my interest in a 'real world' clinical problem within my field that I have seen evolve during my career.

Abstract

Background: Multiple sclerosis (MS) is a complex heterogenous autoimmune inflammatory disease with a prolonged and variable time course. The visual system is frequently implicated, either as the presenting symptom, or, with advancement of the disease. This has been documented in the literature with changes in visual acuity (VA) that are accompanied by functional changes in the optic nerve, measured with the visual evoked potential (VEP) and possible retrograde degeneration involving the retinal ganglion cells in the retina, measured with the pattern reversal electroretinogram (PERG). However, inflammatory episodes may be clinical or subclinical in nature and may go unrecognised. Originating from the same embryological origins, the effect of inflammation in MS on the on the retina is less well known. The research hypothesis was that there is a measurable difference in the function of retinal cells in patients with newly diagnosed multiple sclerosis, suggestive of inflammatory retinopathy compared to healthy controls.

The overall aim was to investigate any differences in the electrophysiological function of the visual pathway of patients newly diagnosed with MS compared to healthy controls.

Methods: The visual system is explored with clinical (VA), electrophysiology (VEP and electroretinography (ERG – pattern and flash) and structural (OCT) measures, in patients presenting with symptoms suggestive of MS to a specialist service. This prospective case control study investigates the visual pathway at the earliest stage of the disease to look for differences in structure and function between patients and healthy volunteers that might serve as a biomarker in the future.

Results: There were a number of variables that were significantly different between the two groups, logistic regression analysis found that VA (p 0.038) and VEP P100 peak-time (p 0.014) from the right eye as significant. Dividing the participants by prolongation of the VEP P100 peak-time as defined in clinical practice, found a number of ERG amplitude variables as well as VA that were consistently different between the groups regardless of symptoms.

Conclusion: The study confirms optic nerve involvement in MS with VEP and VA abnormalities consistent with the literature in this cohort. Additionally, VA and some ERG amplitude variables were significantly reduced in participants with MS, when grouped according to VEP P100 peak-time, suggesting inner and outer retinal changes. Further work would be required to confirm these findings. No OCT structural changes were found in any of the analysis that included the macula thickness, ganglion cell layer or retinal nerve fibre layer.

Keywords: multiple sclerosis (MS), visual evoked potential (VEP), pattern electroretinogram (PERG), electroretinogram (ERG), optical coherence tomography (OCT)

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List of Abbreviations

AUC	Area under the curve
BBB	Blood brain barrier
CSF	Cerebrospinal fluid
CIS	Clinically isolated syndrome
CNS	Central nervous system
DA	Dark adapted
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease modifying treatment
EDRS	Early diabetic retinopathy screening
EDSS	Expanded Disability Status Scale
ERG	Electroretinogram
EP	Evoked potential
GCL	Ganglion cell layer
HV	Healthy volunteer
ILM	Inner limiting membrane
IQR	Interquartile range
Jx	Juxtacortical
LE	Left eye
LA	Light adapted
LogMAR	Logarithm of the minimum angle of resolution
LP	Lumbar puncture
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NfL	Neurofilament
ROC	Receiver operating characteristic
OCB	Oligoclonal band
OCT	Optical coherence tomography
ON	Optic neuritis
ONH	Optic nerve head
P	Participant
PERG	Pattern electroretinogram
PI	Principle investigator
PPMS	Primary progressive MS
PV	Periventricular
RAPD	Relative afferent pupillary defect
RE	Right eye
RRMS	Relapsing remitting multiple sclerosis
RGC	Retinal ganglion cell
RNFL	Retinal nerve fibre layer

SD	Standard deviation
VA	Visual acuity
VEP	Visual evoked potential
WML	White matter lesion

Summary chapter 1: Introduction

- Multiple sclerosis (MS) is explored with respect to incidence and prevalence at international, national and local levels.
- Aetiology and contributing factors illustrate the importance of exploring this multifactorial disease.

Chapter 1. Introduction

The eye and, more specifically, the retina have been implicated in a number of heterogenous neurological conditions with varying clinical implications. The ability of modern techniques to study the eye *in vivo* has led to an explosion of research into neurological conditions such as Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), prion disease and multiple sclerosis (MS) (London et al., 2013; International Federation, 2022). This thesis has evolved from the well-documented observation that the axons forming the optic nerves are commonly affected in MS. Patients often present with acute unilateral visual symptoms that include loss of vision, pain and altered colour vision that may resolve over several weeks. This can be attributed to inflammation of the optic nerve that may disrupt saltatory conduction, leading to secondary atrophy and axon loss.

The reported incidence of symptoms in the visual pathway in MS is high, termed optic neuritis (ON). This is the initial presenting symptom in up to 50% of cases and features as part of the disease course in up to 80% (Gundogan et al., 2007; Hamurcu et al., 2017). ON may be clinical or subclinical in nature. Where clinical attacks have occurred, there may be complete recovery of visual acuity (Hamurcu et al., 2017); however, conduction delays often remain and do not fully resolve with time (Halliday et al., 1973).

More recently, interest has turned to possible inflammatory effects on the distal visual pathway, the retina, in the hope that the eye can further explain the disease process and potentially be used as a biomarker to facilitate diagnosis, track progression and treatment (García-Portilla et al., 2019). Having originated from the same embryonic cells as the brain, but lacking in myelin, it is hoped that the retina will provide diagnostic evaluation of structure

and function of the central nervous system (CNS) in inflammatory disease (Martinez-Lapiscina et al., 2014; Janaky et al., 2017).

1.1 Multiple Sclerosis

MS is a multifactorial disease with genetic, environmental and lifestyle contributors. Worldwide there has been an increase in cases over recent decades, with a prevalence of 50–300 per 100,000, equating to 2.8 million globally (Figure 1); although this is likely to be an underestimate as data is lacking for some areas (International Federation, 2022). The incidence varies around the globe (Jobin et al., 2010); however, females are consistently diagnosed at higher rates than males and annual rates are increasing (International Federation, 2022).

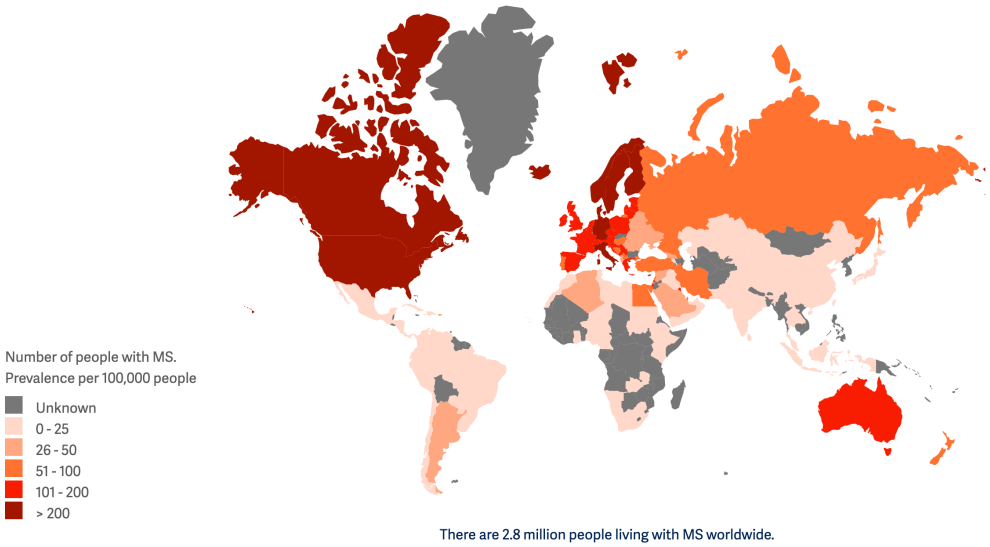

Figure 1. Prevalence of MS per 100,00 across the globe.
There is a clear higher incidence in regions further from the equator and paucity of data in some regions, (International Federation, 2022).

In the UK, MS is the most common neurological condition among young adults, with a prevalence of 196 per 100,000. There is thought to be a female to male ratio of 2.5:1, and an average age of diagnosis in the third decade (International Federation, 2022).

Figures from the south-west of England are in line with national data, showing a year-on-year increase (2016–2018) that was interrupted due to a combination of resource issues in 2018/9 and the COVID-19 pandemic. Since that time, services have struggled to recover due to a variety of operational and resourcing issues (Figure 2).

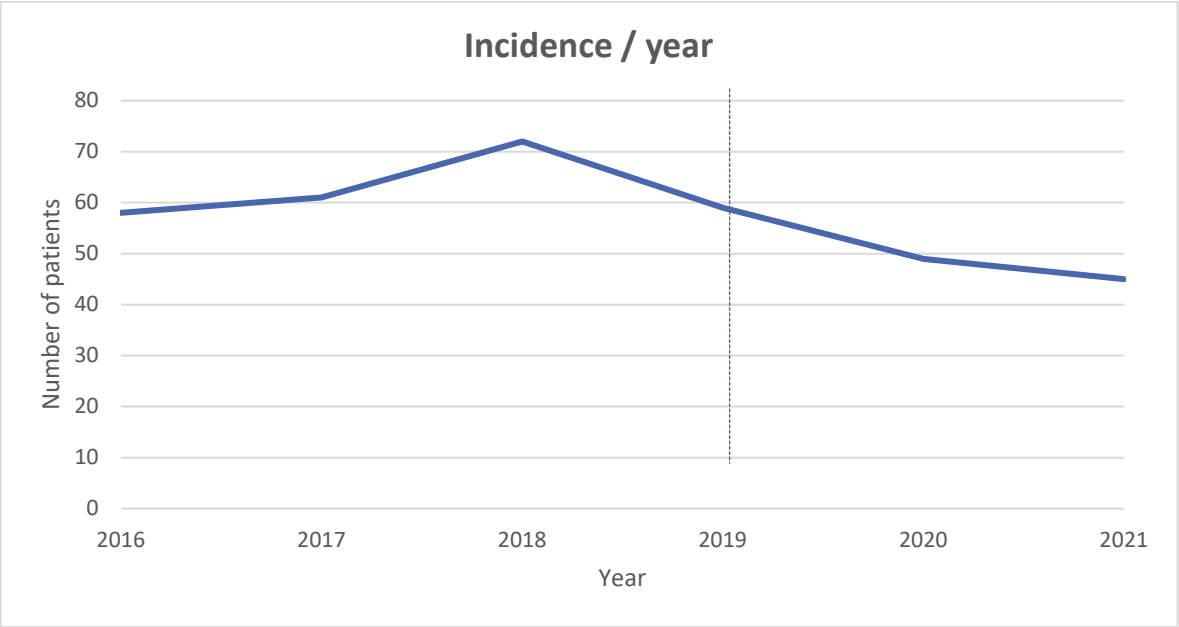

Figure 2. Incidence of MS from a single NHS site in the south-west of England.
The dashed line represents the approximate start of the COVID-19 pandemic in the UK, (Data prior to 2016 is unavailable).

1.2 Risk Factors

Although the determinants of MS are not entirely understood, there is repeated and consistent evidence that both genetic and external factors contribute to the expression of the disease.

Genetic determinants of MS suggest 'genetic burden' which combines all genetic and epigenetic factors that contribute to disease risk. Strong links with the major histocompatibility complex (MHC) alleles, in particular the genes in the human leukocyte antigen (HLA) region of chromosome 6, have long been associated with many human diseases, including MS. Carriers of the HLA-DRB1*1501 allele have been shown to be at three times higher risk of developing MS (Parnell and Booth, 2017), whereas those with HLA-A*02

have demonstrated a protective effect and are less likely to develop the disease (Nourbakhsh and Mowry, 2019). More recently, over 200 genetic variants have been identified through international efforts and whole genome sequencing, that have been associated with increased risk. These are thought to account for 20–30% of the overall hereditary risk, leaving large contributions from environmental and lifestyle factors (Hone et al., 2021). Some degree of inheritable risk has been shown with two interleukin receptor genes (IL2RA and IL7R) that have been identified with multiple polymorphisms. Additionally, a degree of racial clustering can be seen in some regions; however, the large number of genetic variants that are common in the general population make association difficult to prove (Thompson et al., 2018a).

Environmental factors are illustrated clearly with the global distribution generally increasing with distance from the equator, which is thought to be related to vitamin D levels. North America and Europe have the highest prevalence, while Eastern and Central Africa have the lowest (Wallin et al., 2019). This geographical variation, or 'latitudinal gradient', suggests that there are considerable environmental risks associated with the disease that favour more northern territories (Figure 1). Vitkova et al. (2022) recently performed a multi-centre review of patients with MS and place of residence, to further investigate the link between both sunlight exposure and disease severity. The results suggest a plateau above 40 degrees latitude. Additionally, migration studies have shown that region at birth (high risk or low risk) is largely associated with risk of developing the disease in both directions. However, the age at which migration occurs may be relevant with children that move prior to 15 years of age being thought to adopt the risk of the general population of the area that they move to (Gale and Martyn, 1995). Epidemiological studies in this area are difficult to perform and data is limited (Nourbakhsh and Mowry, 2019). Within the UK, there is a reported peak incidence in the Orkney Islands of Scotland, which is higher than anywhere in the world; here the incidence is one in every 170 women, for reasons that are not understood (Visser et al., 2012).

Smoking and obesity have both been shown to increase the risk of MS that can be combined with genetic factors to produce exponential risks of developing the disease. Similarly, prior exposure to the Epstein-Barr virus (EBV) measured in the serum, or by the presence of antibodies to the disease, show strong associations with subsequent MS across different races and ethnicities (Nourbakhsh and Mowry, 2019) (Figure 3).

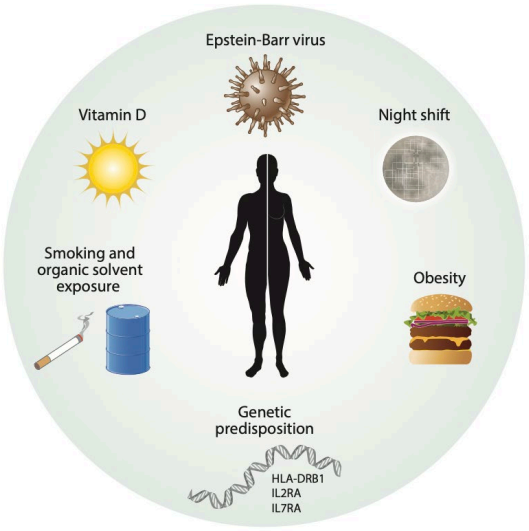

Figure 3. Genetic and environmental factors that contribute to MS.

(Nourbakhsh and Mowry, 2019).

The personal and public health implications are considerable, given the chronic and often protracted nature of the disease that is characterised by increasing disability over time. The financial burden includes direct and indirect costs. Attempts to quantify this in the literature have been hampered by lack of data and different methodologies (Trisolini et al., 2010). There is, however, general agreement that the worldwide burden is increasing due to better diagnostic facilities, disease modifying treatments (DMTs) and increased survival rates (Wallin et al., 2019; Trisolini et al., 2010). This highlights the need for sensitive and accurate diagnostic tests that can be utilised at the onset of the disease to facilitate early treatment choices.

Summary of chapter 1

- MS is a common, multifactorial disease that is seen around the world but appears more prevalent at greater latitudes.
- The incidence of MS is increasing nationally, which has significant individual and societal implications.
- Symptoms of MS are varied, but they frequently involve the optic nerves where conduction delays may remain after clinical recovery.

Summary chapter 2: Diagnostic criteria for Multiple Sclerosis

- The history of the disease to date is explored along with diagnostic criteria.
- Phenotypes and the disease time course are described to help contextualise this research project that was performed on patients at the start of their diagnostic journey.

Chapter 2. Diagnostic Criteria for Multiple Sclerosis

There is no current diagnostic test or pathognomonic sign that predicts the disease with certainty. Rather, clinical expertise and diagnostic criteria are combined to establish the diagnosis (Maggi et al., 2020). Symptoms are not limited to the visual system and any area of the CNS may be implicated, depending on the anatomical area of inflammation, resulting in a heterogenous set of symptoms that many of which also occur in a variety of other diseases (Repovic, 2019; Solomon, 2019). This has the potential to cause confusion for both the clinician and the patient that may prolonging time to the diagnosis, causing further anxiety for the patient and their family (Podbielska et al., 2021). Over the years there have been several diagnostic criteria devised by different groups (McAlpine et al., 1972; Fangerau et al., 2003). However, the most commonly used is the McDonald criteria which has undergone several revisions over the years (McDonald et al., 2001; Polman et al., 2011; Thompson et al., 2018b). These criteria are only applicable in patients presenting with symptoms suggestive of MS and are not appropriate for differentiating MS from other neurological diseases (Carroll, 2018; Thompson et al., 2018b).

Initial presentation is followed by thorough clinical assessment and careful documentation of the history. Neuro-ophthalmological assessment aims to document sensory and motor deficits that support the patient's symptoms. At a sensory level, signs may be subtle such as reduced touch or proprioception that goes unnoticed by the patient. Motor signs are consistent with an upper motor neuron dysfunction with weakness, increased tone or abnormal reflexes. At a brainstem level, there may be sensory loss of the face, nystagmus or vertigo. Additional signs such as Lhermitte's phenomenon and the 'MS Hug' may also provide important information.

Clinical signs when patients present with ON may include loss of vision and pain on movement of the eye. On examination, there may be optic disc swelling, pallor, and on rare occasions evidence of ocular inflammation (Pane et al., 2018). A relative afferent pupillary defect (RAPD)

is regarded as a positive clinical sign for optic nerve disease (in the absence of retinal disease) and therefore supports optic nerve involvement in MS but does rely on unilateral pathology and careful comparison of the pupillary responses between the eyes.

2.1. Foundations of Diagnostic Criteria

The first accounts of MS date back to the 19th century when Sir Augustus d'Este described a case of what would be described today as a collection of classical MS symptoms and signs (London et al., 2013). Original attempts at standardising diagnostic criteria recognised the 'patterns of attack' but relied heavily on clinical interpretation of the patterns or 'risk' due to the lack of diagnostic tests (Schumacher et al., 1965). Five guiding principles were later identified by Schumacher and colleagues (1965) to facilitate the diagnosis: identification of a syndrome 'typical' of MS-related demyelination, objective evidence of CNS involvement, evidence of dissemination in space, demonstration of dissemination in time, and no better explanation other than MS. These remain apparent in current criteria, with paraclinical tests being incorporated to provide objective evidence to support (or rule out) the diagnosis (Solomon, 2019). New technologies have helped greatly in this respect; the widespread availability of magnetic resonance imaging (MRI) has been a major contributor, and it is now the basis for the widely adopted McDonald diagnostic criteria used in clinical practice (McDonald et al., 2001; Polman et al., 2011; Thompson et al., 2018a).

Under the current McDonald criteria, if at presentation there is a transient monophasic period of focal or multifocal neurological disturbance (>24 hours) that evolves over days or weeks, followed by a period of recovery that cannot be explained by any other cause (Thompson et al., 2018b), the term clinically isolated syndrome (CIS) is applied. Following diagnostic investigation, if the criteria are met, a diagnosis of MS can be made. If, however, the criteria are not fully met (a patient presenting with CIS but not meeting the criteria, i.e., no previous clinical episode in a separate area, negative imaging or a negative lumbar puncture (LP)), then the diagnosis is 'possible MS' and follow-up is required (Thompson et al., 2018b). Further attacks or relapses are also defined by the same criteria. The aim of all criteria has been to demonstrate the clinical (dissemination in space, DIS) and temporal (dissemination in time, DIT) characteristics of the disease, with the most recent revision of the McDonald criteria

aiming to facilitate early diagnosis. This has been achieved by including cerebrospinal fluid (CSF) analysis, which had not been included previously but had been included in other criteria Posner et al., 1983). Currently, the demonstration of two or more oligoclonal bands (OCBs), in the CSF (but not the blood serum), may now substitute for a second attack or dissemination in time (DIT). Significantly, this now means that MS may be diagnosed after a single clinical episode or attack rather than waiting for further clinical episodes, providing the criteria are met (Table 1.).

	Number of lesions with objective clinical evidence	Additional data needed for a diagnosis of multiple sclerosis
≥2 clinical attacks	≥2	None*
≥2 clinical attacks	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location†)	None*
≥2 clinical attacks	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI‡
1 clinical attack	≥2	Dissemination in time demonstrated by an additional clinical attack or by MRI§ OR demonstration of CSF-specific oligoclonal bands¶
1 clinical attack	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI‡ AND Dissemination in time demonstrated by an additional clinical attack or by MRI§ OR demonstration of CSF-specific oligoclonal bands¶

Table 1. The current McDonald criteria for MS.
**No additional tests are required to demonstrate dissemination in space and time. ¶The presence of CSF-specific oligoclonal bands does not demonstrate dissemination in time per se but can substitute for the requirement for demonstration of this measure. (Thompson et al., 2018b)*

ON is a common feature of the disease that may be an isolated event or progress with further inflammatory episodes. The risk of conversion of the diagnosis from CIS to MS is linked to the presence and type of lesions on MRI imaging at that point in time. A multi-centre 15-year follow-up study by the Optic Neuritis Study Group (2008) found that the risk is approximately 50% if using clinical criteria alone, that increased to 72% if the MRI also fit the diagnostic criteria at the time of presentation. In the same study, if there were no MRI lesions at the point of ON presentation, there was some (lesser) risk, which was greatest in the first 5 years, and reduced to almost zero by 10 years.

MRI is highly sensitive with regards to detecting white matter lesions that are typical of MS, and clear reporting guidelines have been developed that complement the McDonald criteria (Wattjes et al., 2021). The clinical interpretation of such lesions is often subjective and there

may be confounds depending on age and comorbidities that cause confusion; common differential diagnoses include migraine, vascular pathologies, or ischemic conditions (Solomon et al., 2016; Solomon, 2019). A recent multicentre study has found interrater agreement to be 'moderate to good' between experienced neuroradiologists in this context and reduced for less experienced radiologists (Hagens et al., 2019). White matter lesions may also feature as part of other diseases including the closely related neuroinflammatory spectrum disorders that can present with the same features of MS, including ON, but have different underlying mechanisms, treatments and outcomes (Omerhoca et al., 2018; Sa et al., 2020). It is also generally accepted that approximately 5% of patients with MS will not have MRI changes, and conversely a proportion of the general population will have asymptomatic white matter lesions (Palace, 2001).

Radiologically isolated syndrome (RIS) presents further diagnostic challenge for the clinician, whereby incidental MRI lesions suggestive of MS are found in asymptomatic patients. The current McDonald criteria (Thompson et al., 2018b) does not allow diagnosis based on imaging alone, and therefore, a clinical event is still required before the diagnosis can be made. However, the heterogeneous nature of the disease, with resolution of symptoms that may be disregarded as unimportant at the time, places a strong emphasis on clinical history taking and recognition of those symptoms and signs.

Paradoxically, the application of diagnostic criteria in this context can be challenging and has been reported as a major contributor to misdiagnosis and inappropriate treatment (Solomon et al., 2016; Siva, 2018). The problem lies in its inappropriate application, often in patients with atypical presentations, as the criteria have only been validated in populations presenting with clinical episodes typical of MS (Solomon, 2019). Clinicians are warned of so called 'red-flags' where symptoms or investigative findings do not fit their initial suspected diagnosis (Kelly et al., 2012; Maggi et al., 2020). Navigating the 'no better explanation' when clinical signs and diagnostic tests are non-specific and may feature in other diseases, suggests MS is entirely a clinical diagnosis (Kaschka et al., 2014; Omerhoca et al., 2018). For this reason, in the UK, the National Institute for Health and Care Excellence (NICE) (2022) guidance recommends that diagnosis be made by a neurologist using recommended and up-to-date diagnostic criteria such as the McDonald criteria (Thompson et al., 2018b).

There should also be consideration as to the populations in which the criteria were drawn from, which in the latest version of the McDonald criteria were predominantly white adults from European, American, and Canadian cohorts with a high likelihood of having MS. Therefore, the positive predictive value is likely to be higher than in the international population (Thompson et al., 2018b). How this translates to diverse populations and other ethnicities and younger age groups is uncertain (Solomon, 2019).

2.2. Phenotypes

Disease progression has always been the basis for describing MS phenotypes, with distinct patterns identified (Lublin and Reingold, 1996). More recent descriptions have since been developed that attempt to encompass both the evolution of the disease (active or inactive) and the pathophysiology to facilitate accurate prognosis and timely treatment choices (Lublin et al., 2014). The chronology is related to clinical and imaging findings such that with increased disability, lesion load increases and brain volume reduces (Figure 4).

Of the different phenotypes, relapsing remitting MS (RRMS) is the most common, accounting for approximately 80–85% of new cases, while the remaining 15–20% are the primary progressive forms of the disease (PPMS) (Thompson et al., 2018a). Of the RRMS phenotype, the majority will eventually convert to a progressive type (secondary progressive), although the time course is unpredictable (Kantarci, 2019). In clinical practice, patients with MS are monitored by their neurologist, often with periodic MRI imaging to establish whether there is evidence of active disease with symptomatic relapses or asymptomatic activity on MRI to differentiate periods of quiescence that indicate a slower or less aggressive progression that may influence treatment and life choices.

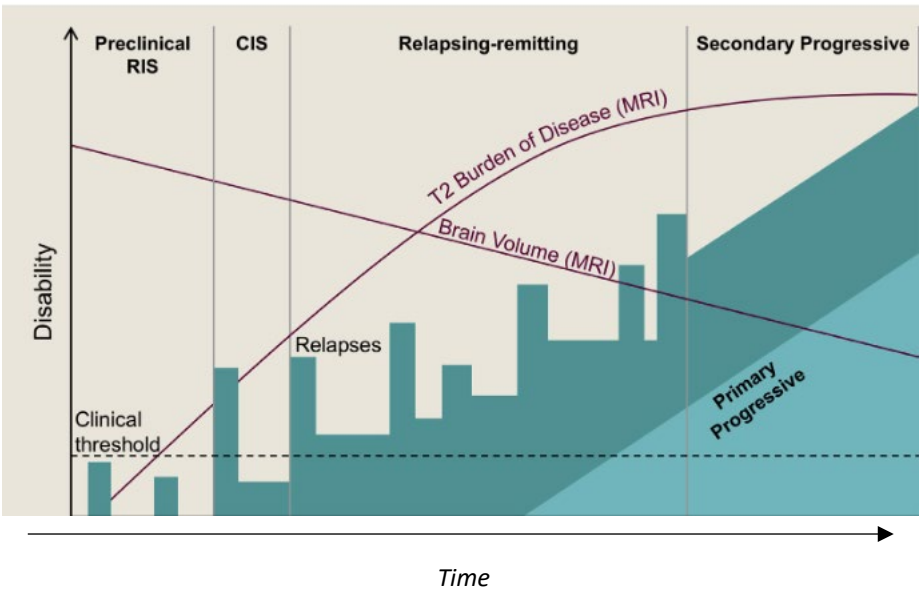

Figure 4. MS phenotypes relative to disability.
The MS phenotypes over time (x-axis) relative to disability (y-axis). Also included are typical MRI changes over time and the period prior to diagnosis termed preclinical and clinically isolated syndrome (at first presentation). Adapted from Baecher-Allan et al. (2018)

It is becoming more widely accepted that there is a prodromal phase that predates the diagnosis, which is supported by the observation that healthcare needs are increased in the years prior to diagnosis. Wijnands and colleagues (2017; 2019; 2019) have reviewed hospital records in a series of papers and found a significant increased number of hospital admissions and visits to doctors in at least 5 years prior to the diagnosis with a variety of symptoms. This has been consolidated with a 'Perspective Article' by Tremlett (2022), who reviewed the evidence to date, and suggesting an even longer prodromal period of up to 10 years. The authors acknowledge that all identified symptoms identified are non-specific in nature and are present in a large proportion of the general population. However, they argue that, combined, they could provide an opportunity for identifying those at greatest risk and could be combined with biomarkers to initiate earlier treatment (Tremlett, 2022). In practice, the disease onset may be difficult to identify with transient episodes over a prolonged period that go unreported. It is only when the attacks become more prolonged or disabling that medical advice may be sought.

There are no curative treatments for MS at present. Acute CIS and relapses may be treated with corticosteroids to reduce inflammation, provided they are administered by a specialist (Department of Health and Social Care, 2022). RRMS is the only phenotype for which there

are multiple approved treatments, in the form of disease-modifying treatments (DMTs) that aim to reduce inflammatory relapses and their severity but do not eliminate the disease. Research efforts are being made with stem cell treatments as a way of 'reformatting' the body's own immune system; at present, there is only one option, autologous hematopoietic stem cell transplantation (AHSCT), that is currently available when other treatments have failed for the RRMS phenotype (MS International Federation, 2022). In England, Ocrelizumab (Ocrevus) is the only NHS approved DMT for PPMS that can be effective early on in the disease and requires certain criteria to be met in order to qualify (MS Society, 2022). Similarly, Mayzent (Siponimod) has recently been licensed for use in active secondary progressive disease provided there is documented deterioration in EDSS while on DMT that is not related to relapse (Multiple Sclerosis Trust, 2022).

Summary of chapter 2: Diagnostic Criteria for Multiple Sclerosis

- Demonstration of dissemination in space and time is the aim of diagnostic criteria that have been revised periodically over the years.
- Clinical expertise is necessary to correctly apply the criteria and interpret diagnostic investigations to support the diagnosis.
- The literature suggests that the time course is prolonged and may include a prolonged prodromal phase that may provide an earlier treatment window.

Summary chapter 3: Paraclinical Assessment

The following routinely used investigations/assessments are recommended to provide additional evidence if required to support the diagnosis.

- Expanded Disability Severity Scale (EDSS)
- MRI
- Lumbar puncture (LP)
- Neurofilament
- Evoked Potentials

Chapter 3. Paraclinical Assessment

The importance of clinical history taking, and neurological examination are paramount in directing investigations appropriately to reach a diagnosis. However, the demographic of patients and the transitory nature of symptoms may mean that there may be minimal or no clinical findings by the time the patient presents in clinic.

Confirmation of a current clinical episode with objective evidence is the starting point of the diagnostic process, followed by the need to explore the possibility of a previous episode. If during that process evidence is found to support the DIT and DIS of CNS involvement (consistent with the current diagnostic criteria), then MS is the likely diagnosis. Equally, there is emphasis on 'red flags' or atypical signs that would be suggestive of an alternative differential diagnosis, of which there are many (Berger, 2022). Despite MRI currently being the 'gold standard', other paraclinical tests may be used to provide further diagnostic evidence and act as a baseline that can be useful in monitoring disease progression and response to treatment (Solomon, 2019). In clinical practice, a further separate second presentation with evidence of separate CNS involvement supported by imaging would ensure the most confident diagnosis.

3.1 Expanded Disability Status Scale (EDSS)

Although not a diagnostic test, the EDSS is an assessment tool used in clinical practice as an incremental measure of disability designed for MS patients that has been refined over the years (Kurtzke, 1955; Kurtzke, 1983). In its current iteration, it is 20-point scale in 0.5 increments (0 = no disability, 10 = death due to MS) that characterises disability across 8 different functional systems typically affected in MS, e.g., visual, sensory, pyramidal, etc. However, the scale is heavily weighted towards the ability to walk and does not necessarily separately score disability in different functional areas that could underrepresent disability. It is not able to differentiate loss of function due to comorbidities such as focal nerve entrapment or generalised neuropathy that may present in a similar fashion to MS, which further emphasises the need for careful history taking and clinical examination. Despite its drawbacks, EDSS is a frequently used tool in both clinical practice and research to assess and monitor disability.

3.2 Magnetic Resonance Imaging (MRI)

MRI became widely adopted in the 1980s, marking a change in diagnosis and treatment of MS, which has been further refined over the years. Unlike computer tomography (CT) or X-ray, MRI utilises a strong magnetic field to image internal structures with different physical properties. MRI of the brain in MS shows characteristic white matter lesions (WMLs) that are highly suggestive of MS that characterise clinical episodes, whereas, widespread brain atrophy is better correlated with long-term disability (Calvi et al., 2022). MRI can provide objective evidence of both DIT and DIS with WMLs, which is particularly useful at the first presentation when differentiation between CIS and MS is required. It also provides evidence of previous and ongoing inflammation that can be used to monitor and document disease progression (Kaunzner and Gauthier, 2017). Consequently, specific MRI protocols are now recommended for monitoring in clinical practice and as outcome measures in drug trials of DMTs (Wattjes et al., 2021). Additional radiological criteria have been devised that aim to ensure consistency and accuracy in clinical practice (Table 2).

	2017 McDonald criteria
Dissemination in space (DIS)	1 T2 lesion in at least two out of four areas of the CNS • Juxtacortical/ intracortical • Periventricular • Infratentorial • Spinal cord
Dissemination in time (DIT)	• A new T2 and/or gadolinium-enhancing lesion on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI **OR** • Simultaneous presence of symptomatic gadolinium-enhancing and non-enhancing lesions at any time

Table 2. The radiological criteria for MS required to demonstrate DIT and DIS (Barkhof and Smithuis, 2021)

Different MRI sequences are recommended to highlight different aspects of the disease process that rely on the breakdown of myelin (fatty insulation) surrounding the nerves which is essential for nerve action potentials communicated by saltatory conduction. The inflammatory process begins with the breakdown of the blood brain barrier (BBB), which allows lymphocytes to infiltrate the CNS leading to degeneration of oligodendrocytes that constitute myelin. With progression, there is also axonal degeneration that results in atrophy (Xue et al., 2021). T1-weighted MRI sequences are recommended to demonstrate the CNS anatomy and may show 'black-holes' or 'dark spots', where previous lesions have resulted in localised atrophy. In this way dissemination in time can be inferred. The use of gadolinium contrast agent with a T1 sequence may demonstrate blood brain barrier (BBB) breakdown (depending on the timing), with the contrast crossing the barrier which is normally impervious, preventing blood and pathogens entering the brain (Trip and Miller, 2005; Kimmy et al., 2009). For lesion identification and inspection, T2 and T2-FLAIR weighted sequences (Figure 5) are recommended that can indicate early active lesions (Calvi et al., 2022).

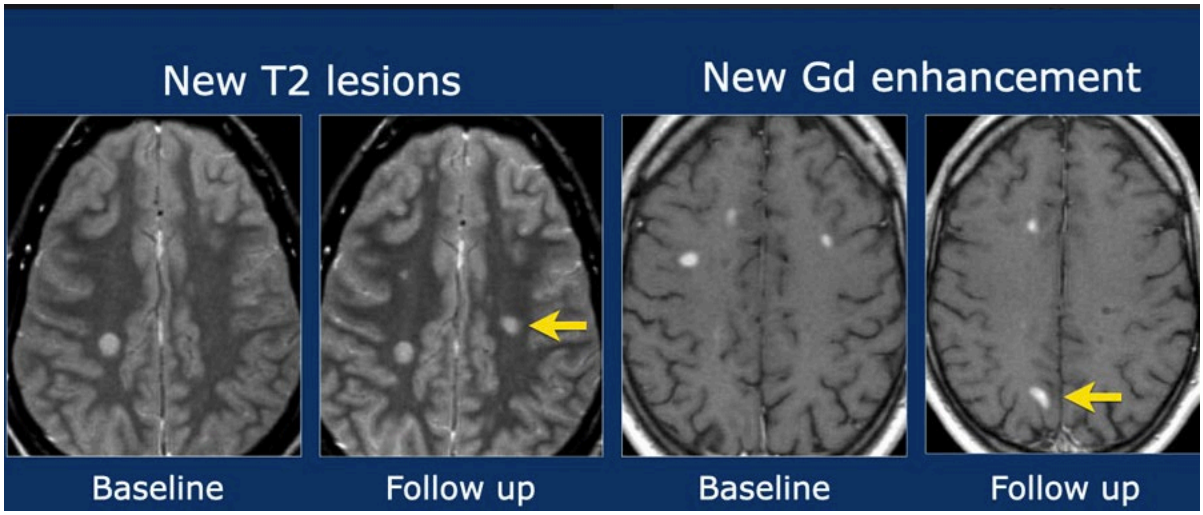

Figure 5. Baseline and interval MRI images in MS.
The images demonstrate new WMLs using T2 sequences (left) and T1 with enhancement (right). Lesions appear bright in both protocols (Barkhof and Smithuis, 2021).

To meet the criteria for dissemination in space, at least 1 lesion must be demonstrated in either the juxtacortical/intracortical, periventricular, or infratentorial regions within the brain, or the spinal cord, demonstrated below with yellow arrows (Figure 6) (Thompson et al., 2018b; Barkhof and Smithuis, 2021). Imaging of the spinal cord is not essential in every case depending on the presentation and history (Thompson et al., 2018b).

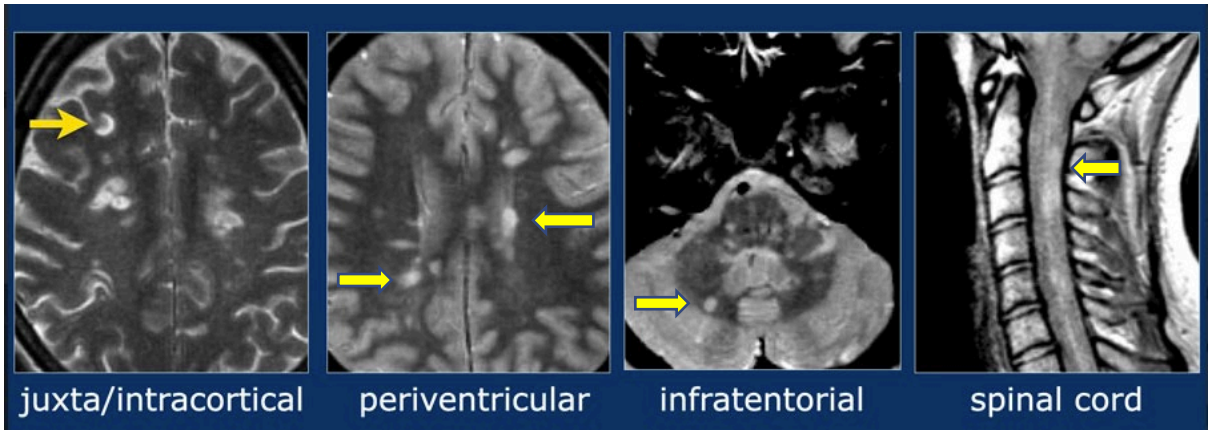

Figure 6. MRI images typical of MS in the locations defined by the McDonald criteria.
From left to right, juxta/intracortical axial view, periventricular axial view, infratentorial axial view, spinal cord sagittal view (arrows denote lesions). (Barkhof and Smithuis, 2021)

The Magnetic Resonance Imaging in Multiple Sclerosis network (MAGNIMS) have produced international consensus practice recommendations to facilitate the application of the clinical

criteria and standardise practice (Wattjes et al., 2021) that provides further detail as to the type of lesion, shape and size that facilitates radiological interpretation.

The use of MRI is not without its drawbacks; MRI is sensitive but not very specific (Hemond and Bakshi, 2018), and in clinical practice, patients may have comorbidities and non-specific abnormalities (red flags) that may provide diagnostic doubt. Some of these confounds are common in the general population and may be considered a consequence of aging. As a result, additional MRI lesions are suggested (but not mandatory) in individuals over 50 years old (Thompson et al., 2018b). There is also the concept of a 'clinical-radiological paradox', whereby clinical signs are discordant with imaging (Mollison et al., 2017). The emphasis given to such findings is open to interpretation by the neurologist and may lead to both false positives and false negatives.

The practicalities of performing MRI are also an important consideration that may make this type of imaging unsuitable. Claustrophobia, implanted metal devices, high body mass index and concerns over the use of gadolinium contrast may make patients unsuitable for this type of imaging. Technical considerations can make images suboptimal due to artefact such as movement, as the images take some time to acquire, requiring the patient to lie still for a considerable number of minutes. Additionally, different scanners have different technical specifications that may make comparisons difficult, which is particularly important when looking for progression or interval change. The COVID-19 pandemic has increased waits for most diagnostic tests, that has subsequently been highlighted by a recent government report that attempts to address the issue with new care models and the creation of diagnostic centres (Richardson, 2020).

3.3 Cerebrospinal Fluid (CSF) Analysis

Lumbar puncture is typically performed as a 'day-case' procedure using local anaesthesia to withdraw CSF from the spinal canal to identify antibodies in the form of OCBs, (Figure 7). The presence of OCBs in the CSF and not the blood serum (or higher in the CSF than the serum) is suggestive of a chronic immune response within the CNS (Dobson et al., 2013; Deisenhammer et al., 2019) and intrathecal synthesis of immunoglobulins (Rodriguez M et al., 2022). There

are a small number of different OCB antibodies, termed isotope IgA, IgD, IgE, IgG and IgM, of which IgG is regarded as the most sensitive in MS. The cascade of events in the immune response involves many cells and molecules that culminate in neuronal damage that characterise the disease, e.g., demyelination, axonal degeneration, and atrophy.

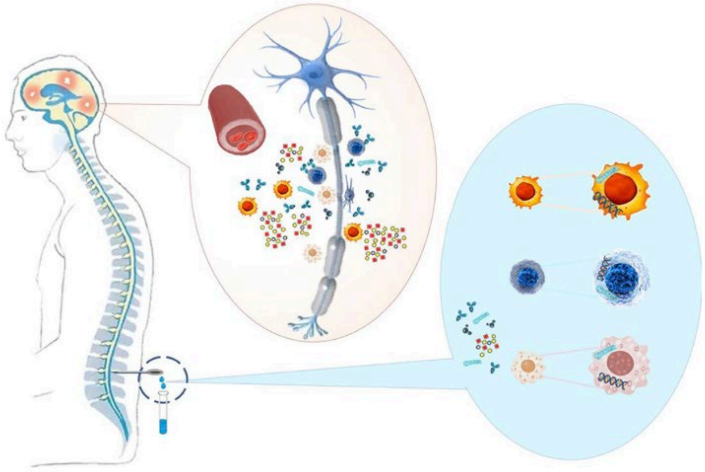

Figure 7. LP Procedure.

CSF is drawn from the spinal cord for analysis. It will contain immune cells, antibodies, cytokines and other inflammatory markers that result in neuronal damage. (Deisenhammer et al., 2019).

OCBs in the CSF are not unique to MS, and OCBs can be a consequence of several inflammatory conditions including systemic lupus erythematosus (SLE), brain tumour and paraneoplastic syndromes. However, the absence of OCBs in cases of suspected MS carries a high negative predictive value and could be considered a 'red flag' in this context (Tintore et al., 2001).

Several methodologies for measuring abnormalities in CSF analysis have been described both quantitatively with the IgG index, that is the ratio of IgG in CSF to serum (in relation to albumin), or qualitatively with agarose gel electrophoresis with isoelectric fixing and immune blotting. Currently, the agarose gel method is recommended (Thompson et al., 2018b), which relies upon the different electrical charge of different molecules to separate them into the 'bands' that can be visualised by staining. This preferred method must demonstrate at least 2 OCBs in order to substitute for DIT with the most recent diagnostic criteria (Thompson et al., 2018b). CSF analysis may include other measures such as red and white blood cell count, glucose, protein, and lactate that are compared to reference values and may suggest an

alternative diagnosis. Quantitative interpretation of the results may be hampered by laboratory OCB reference values that are based on historical data and are unvalidated or not available at all (McCudden et al., 2017; Deisenhammer et al., 2019). Similarly, qualitative results are open to subjective interpretation (Lo Sasso et al., 2019).

The process of obtaining the sample requires medical expertise and carries with it some risk of side effects including transient infection, headache, bruising or swelling for the patient. The analysis is more specialised and not available onsite in every hospital, requiring a significant amount of time, making the procedure quite resource intense and collectively makes LP something that is not practical to repeat unless necessary. This makes CSF analysis unsuitable for monitoring purposes, despite the cells and markers of inflammatory response being a target for therapeutic monitoring (Rodriguez M et al., 2022).

Similar to other investigations, CSF analysis is not without its weaknesses as approximately 8% of MS patients will not have positive OCBs, and OCBs can be seen in a variety of other neurological diseases (acute and chronic), as well as in a proportion of the general population. This represents a weakness in the current diagnostic criteria, which added OCBs in the CSF since the previous revision to expedite early treatment, but it may do so at the expense of specificity.

3.4 Neurofilament Light Chain (NfL)

Neurofilament light chain (NfL) is a protein marker that can be measured in both the blood serum and CSF that is released when neurons are damaged, which is regarded as a biomarker for MS (Thebault et al., 2020). A recent review and meta-analysis by Ning and Wang (2022) found that levels are correlated with disease progression which has not been described with any other biochemical markers. Additionally, NfL levels respond to DMT and may even normalise following treatment, making it a potential candidate to measure drug efficacy (Ferreira-Atuesta et al., 2021; Ning and Wang, 2022).

Although levels of NfL are lower in the blood serum compared to the CSF, new technology and techniques make serum levels preferable. A drawback of NfL in this context is its lack of

specificity as raised levels can be seen in a multitude of neurodegenerative disease and traumatic brain disease. Additionally, normative data are not available that take into account other variables such as age, sex and body mass index, all of which have been reported to affect NfL levels (Manouchehrinia et al., 2020).

3.5 Evoked Potentials (EPs)

In addition to assessment of the visual pathway with visual evoked potential (VEP), other evoked potentials (EPs) can be used to provide additional functional information of other nerve pathways. This may include the auditory and somatosensory pathways from the upper and lower limbs of the ascending sensory system and the descending motor pathways (Walsh et al., 2005). The nerve being tested will determine the method of stimulation. For example, the auditory nerve is stimulated with a sound delivered through headphones while the somatosensory pathways require cutaneous electrical stimulation. Motor stimulation requires transcutaneous magnetic stimulation with specialised equipment. The tests are non-invasive, rapidly acquired, painless and well tolerated (Walsh et al., 2005; Canham et al., 2015). Service provision may vary around the country and not all modalities may be available in all departments (Fuller, 2021).

Irrespective of the pathway being tested, the aim is to elicit a time-locked cerebral response that represents conduction time in that pathway. This is achieved by presenting a high number of repeated stimuli (up to thousands) to extract the latency and amplitude of the averaged responses that represent the underlying neurophysiological processes. This can provide electrophysiological evidence of conduction delays (latency) and axonal integrity (amplitude) that support the diagnosis, or provide evidence of deterioration or response to treatment (Canham et al., 2015; Hardmeiser et al., 2017).

Multimodality evoked potentials are non-specific, and delays in conduction and latency delays may be caused by a variety of pathological processes including trauma, ischemia and inflammatory causes. Reference data should be acquired locally and may need correcting for height (somatosensory). By testing different nerve pathways, irrespective of symptoms, subclinical or ‘silent’ neuronal dysfunction may be demonstrated that further supports the

diagnosis. Multimodal evoked potentials have been used as biomarkers and predictors of future disease (Canham et al., 2015; Hardmeier et al., 2017).

Summary of Chapter 3

- Diagnostic criteria are largely determined by clinical and MRI findings, which is regarded as highly sensitive but lacks specificity.
- There are several other paraclinical tests available to support diagnosis, however, none are highly specific.
- The tests measure physiological, biochemical and structural changes that may change over the course of the disease.
- Service provision varies between organisations and not all investigations may be available.

Summary Chapter 4: CNS and Ocular Inflammation

- There are many common structural and adaptive similarities between the retina and CNS.
- Additionally, there are genetic and pathological links between MS and ocular inflammation.
- Other demyelinating diseases may present similarly to MS but have distinct pathophysiology.

Chapter 4. CNS and Ocular Inflammation

Also originating from the neuroectoderm of the diencephalon, the eye is considered part of the CNS, and it shares many similar structures and characteristics. It would therefore be logical to assume that diseases that affect the brain and spinal cord may also affect the eye, and there may be some cross-over between ocular and neurological disease (London et al., 2013). Research into neurological disease has focussed on this close association to look for biomarkers and has found retinal changes (typically retinal thinning as measured via OCT), in a number of disorders, including Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis and Susac syndrome (Yap et al., 2019; Marchesi et al., 2021). Conversely, inflammatory disorders including uveitis and periphlebitis have been linked to MS, although the precise relationship is unknown (Ortiz-Perez et al., 2013; Abraham et al., 2021). The incidence of MS and uveitis is rare, ranging between 0.52–1.3% (Kaya et al., 2014), which is approximately 10 times higher than the general population (Kaya et al., 2014; Casselman et al., 2021), although study designs and criteria vary. In the other direction, a study by Jakob et al. (2009) found that uveitis (intermediate) was a strong risk factor for MS (>13%) in women with bilateral visual symptoms. This raises the possibility that in MS, antibodies other than those that target myelin may play a part in the disease that may result in ocular inflammation. Alternatively, inflammation of the eye maybe a separate autoimmune process in some cases and conditions.

Both the eye and the CNS (as well as other organs) exhibit 'immune privilege' (Medawar, 1947), that acts as a protective mechanism against immune mediated damage. This protection limits the body's own immune response to prevent damage from foreign pathogens and resultant inflammation. Other immune privileged structures include the

placenta and sperm which, like the brain and eye, have limited ability for regeneration but are essential for survival, which is thought to be an evolutionary advantage.

Both ocular inflammation and MS are diseases that begin with peripheral activation of the immune system that results in changes within the CNS, involving both the adaptive and innate immune systems.

4.1 Common Features of the CNS and Eye

There are some prominent structural similarities between the eye and brain. Both possess protective barriers to protect them from inflammation in the form of the blood-brain-barrier (BBB) and the blood-retinal-barrier (BRB), respectively. These robust barriers comprise of multiple layers of specialised cells that provide structural and homeostatic support preventing unwanted cells and organisms from entering the parenchyma. In the brain, the dura and arachnoid layers of the meninges enclose the circulating CSF, whereas the cornea, sclera and uveal tract provide a barrier to the aqueous humour in the anterior chamber in the eye. The circulating fluids in both structures serve similar functions, offering support and protection while providing a transport system for nutrients, immunoglobulins and removing waste products (Sen et al., 1977; Forrester et al., 2018). In the eye, aqueous humour is produced by filtration of blood from the capillaries in the ciliary processes which then passes into the posterior chamber. After circulating, it drains from the anterior chamber through the trabecular meshwork and Schlemm's canal. This process that takes approximately 90 minutes (Tortora and Derrickson, 2011). Movement of substances across the vascular endothelium in the eye is enabled by way of water channels termed aquaporins that form channels in the membrane. In the brain, CSF is produced by the choroid plexus within the walls of the ventricles where ependymal cells (ciliated epithelial cells) filter blood and secrete CSF, which is separated by the subarachnoid space and layers of the meninges. CSF circulates through the system to be reabsorbed by the arachnoid villi, found in the venous sinuses, at a similar rate to its production, which helps ensure a constant intracranial pressure (ICP) and CSF volume (Figure 8).

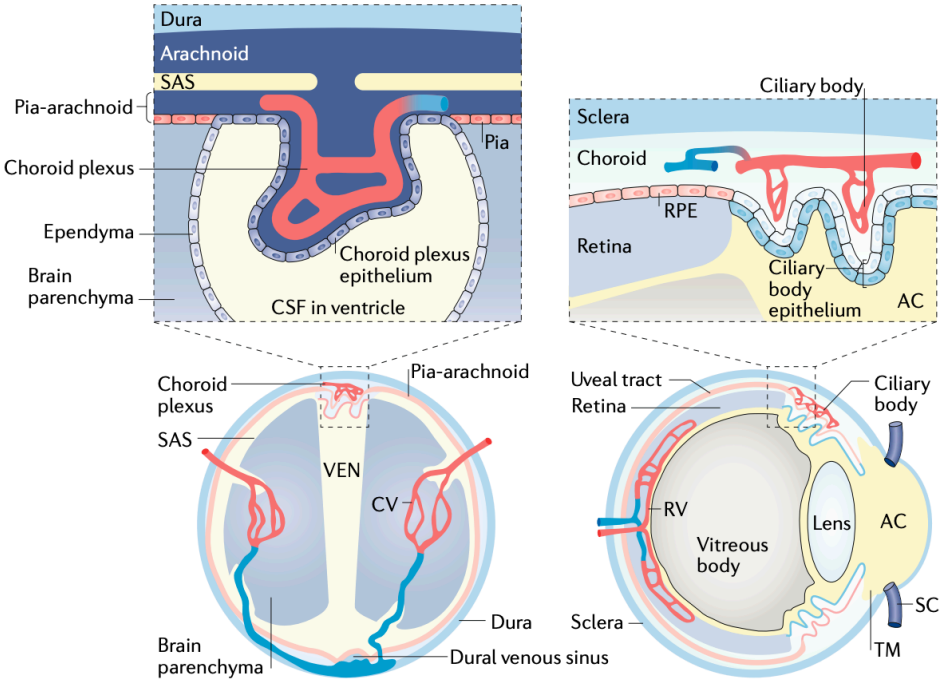

Figure 8. Comparison of the meninges and choroid plexus with the retina.
Comparison of the meninges and choroid plexus (left) with the retina (right) to show structure and fluid transfer. SAS – sub-arachnoid space, CV – cerebral vasculature, RV – retinal vasculature, AC – anterior chamber, TM – trabecular network, SC – Schlemm's canal, RPE – Retinal pigment epithelial cells (Adapted from Forrester, 2018).

Tight junctions between the ependymal cells help prevent foreign bodies entering the CNS. However, the barrier is not entirely secure as host cells, which as white blood cells, are required to cross the barrier to respond to infection and inflammation. Three methods of transfer of systemic cells into the CNS have been identified (Dando et al., 2014). The first is termed transcellular penetration, by movement through the ependymal cells, either by absorption or by way of ligand-receptors interactions that provide high specificity. Secondly, paracellular penetration, whereby the tight junctions between cells and other structures are disrupted allowing passage between. Thirdly, infected phagocytes may carry pathogens into the CNS, termed the 'Trojan Horse' method (Figure 9). These tightly controlled and regulatory entry mechanisms also make treatment efforts difficult as therapeutic drugs must be able to cross the BBB to exert their effects.

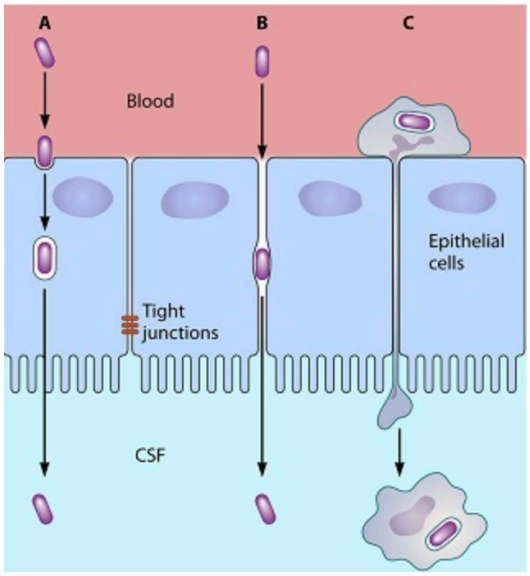

Figure 9. Mechanisms of blood-CSF penetration by pathogens.

Bacteria (purple) may invade the CNS via (A) transcellular penetration involving either absorption or receptor-mediated mechanisms; (B) by paracellular entry following the disruption of junctions between choroidal epithelial cells or adjacent structures; (C) by the 'Trojan horse' mechanism, where microbes may transmigrate with infected phagocytes (Dando et al., 2014).

It is not clear whether an endogenous or exogenous stimuli trigger the autoimmune response in MS or uveitis (Casselman et al., 2021). Animal studies have suggested a link between stress, gut microbiome and immune dysregulation leading to altered autoimmune responses (Werbner et al., 2019; Merchak et al., 2023). This has been observed in humans, although the precise mechanisms are still unknown and a complex relationship between the neuroendocrine system is thought to contribute (Calcagni, 2006; Porcelli et al., 2016).

A predilection for inflammation to occur around the vascular structures, in the form of periphlebitis in the eye, and the medullary veins of the brain (i.e., 'Dawson's fingers' sign) in MS is noted in the literature (Kaya et al., 2014; Flanagan, 2019; Abraham et al., 2021). This has been explored by Sepulcre et al. (2007), who found a correlation between retinal nerve fibre layer (RNFL) thinning and MS relapse rates in a cohort of established MS disease patients over a 2-year period which was independent of previous symptomatic optic nerve involvement. They also found a moderate decrease in both grey and white matter volume in the MS cohort leading them to postulate that retinal measures might serve as a biomarker for MS. They acknowledge that the relationship between retinal measures and brain volume is unclear and that secondary processes might contribute to their findings. They did not attempt to measure relapse or progression clinically and only measured brain volume with MRI and retinal layers with OCT.

In MS, it seems that the majority of the associated genes discovered so far are involved in the regulation of the adaptive and innate immune response, and differ from other neurodegenerative diseases (Baecher-Allan et al., 2018). Common genes have been found in both MS and uveitis, with the HLA-DR15 allele suggesting a common predisposition (Forooghian et al., 2003; Kaya et al., 2014). A more recent review (1980–2019) by Casselman and colleagues (2021) found some consistent features between uveitis and MS in immunological processes and genetics. However, longitudinal studies are lacking. The authors found some evidence to suggest that uveitis onset is at a younger age than MS. They further described that retinal vasculitis could be linked to MS relapses.

The adaptive immune system has been implicated with CD4+ and CD8+ cells associated in MS and uveitis, initiating and driving the response (Bando, 2020). Additionally, B cells have been shown to be implicated in both diseases that also exerts a more direct effect (Figure 10) (Smith et al., 2016; Huang et al., 2017).

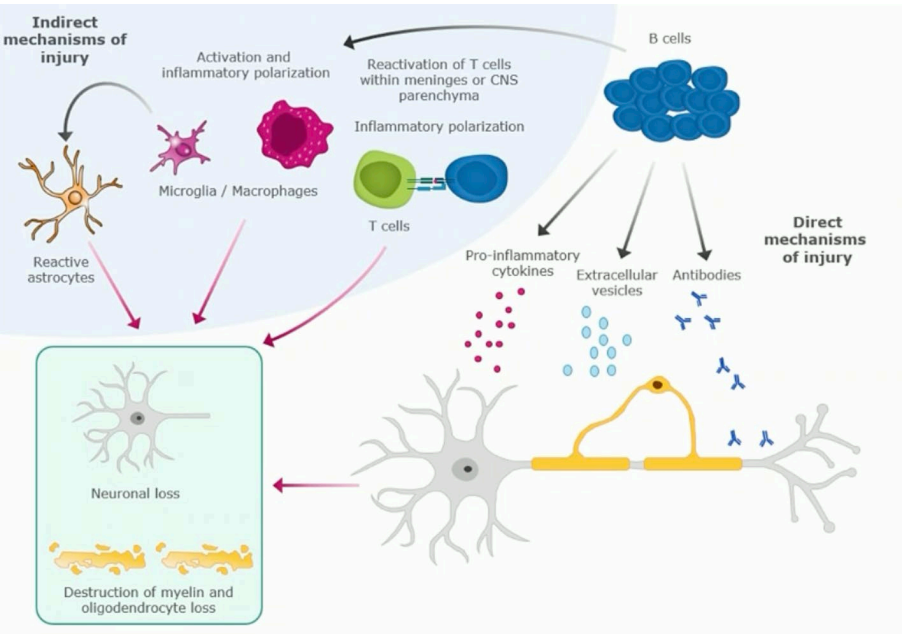

Figure 10. The neuronal damage in MS.
The neuronal damage caused in MS is the result of the complex interaction between T cells and B-cells (Smolders, 2022).

Circulating antibodies other than those targeting myelin antigens have been found and are thought to independently contribute to inflammation in the eye (Saidha et al., 2011).

Animal studies have been used by some authors to explore the relationship between myelin antigens (myelin basic protein [MBP] and myelin oligodendrocyte glycoprotein [MOG]) in mice (London et al., 2013; Forrester et al., 2018). The rodent induced equivalent of MS, termed experimental autoimmune encephalomyelitis (EAE), has been found to be associated with anterior uveitis, although the mechanism is unclear given the lack of myelin in the eye. One theory proposed by Casselman and colleagues (2021) is that there is some immunological 'cross-reactivity', which has been observed in humans with other pathogens (Tejada-Simon et al., 2003) in a process similar to allergies and hypersensitivity.

4.2 Inflammation in MS

The exact pathogenesis of MS is not clear, and different mechanisms have been suggested (van Langelaar et al., 2020; Rodriguez M et al., 2022). Historically, MS has been termed a demyelinating disease of the white matter leading to destruction of the oligodendrocytes that surround nerve axons, resulting in loss of function. The damage caused results in gliotic scarring and axonal loss that forming the characteristic lesions or 'plaques' seen on MRI imaging. This has been reported in the literature to frequently involve the optic nerves, resulting in conduction slowing and block. This limits the anatomical site of damage to being proximal to the lamina cribrosa. When damage occurs, the response includes the release of inhibitory factors that limit further damage that may also affect axons spared of the original insult (Figure 11). Subsequently, there has been a well-documented link with inflammation of the optic nerves and retrograde degeneration of the retinal ganglion cells (RGCs), which may not be demonstrable for a number of weeks after the acute phase of ON (Holder, 1997). This can be demonstrated with reduction in the pattern electroretinogram (PERG) N95, which is generated by spiking of the retinal ganglion cells (RGCs) and, in severe cases, shortening of the preceding P50, which is thought to have a contribution from the RGCs (Holder, 1991; Marmoy and Viswanathan, 2021).

The pathology of the disease is complex, involving a sequence of events beginning with the initial inflammatory event followed by axonal degeneration, microglial activation, mitochondrial injury, oxidative stress and glutamatergic excitotoxicity (Mahad et al., 2015; Thompson et al., 2018a). There is considerable grey matter CNS involvement in MS with

lesions being a prominent feature of the disease. This important for patients as it may indicate time course and progression but also explain symptoms (Calabrese et al., 2013).

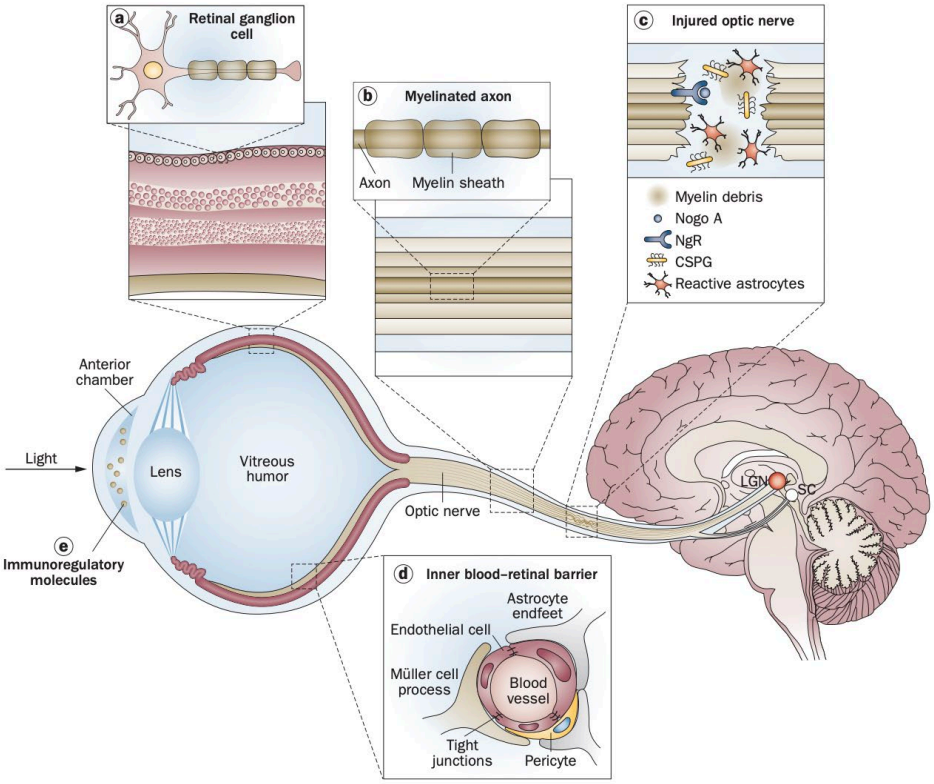

Figure 11. The visual pathway.
(a) RGCs in the retina that exit the eye where their axons (b) become myelinated. In MS (c) the inflammation results in injury to the nerve that triggers the release of signalling molecules that limit damage. The arrangement and properties of the tight junctions, blood vessels and other cells (d) help regulate movement of material in and out of the cell. (London et al., 2013).

The time course of RRMS is characterised by alternating periods of relative quiescence or partial recovery with periods of relapse. This suggests further signalling events within the immune system that represent suppression and activation of the pro- inflammatory response that is characteristic of the disease (Figure 12A). This may be associated with immune modulation that initiates repair and remyelination. More recently, the concept of 'burn-out' in MS has been proposed in response to the observation that the disease often appears to run its course and no observable progression is seen (Figure 12B). This has given rise to the term 'no evidence of disease activity' (NEDA), which refers to no evidence of disease progression on imaging, no increase in EDSS, or clinical relapse (Baecher-Allan et al., 2018).

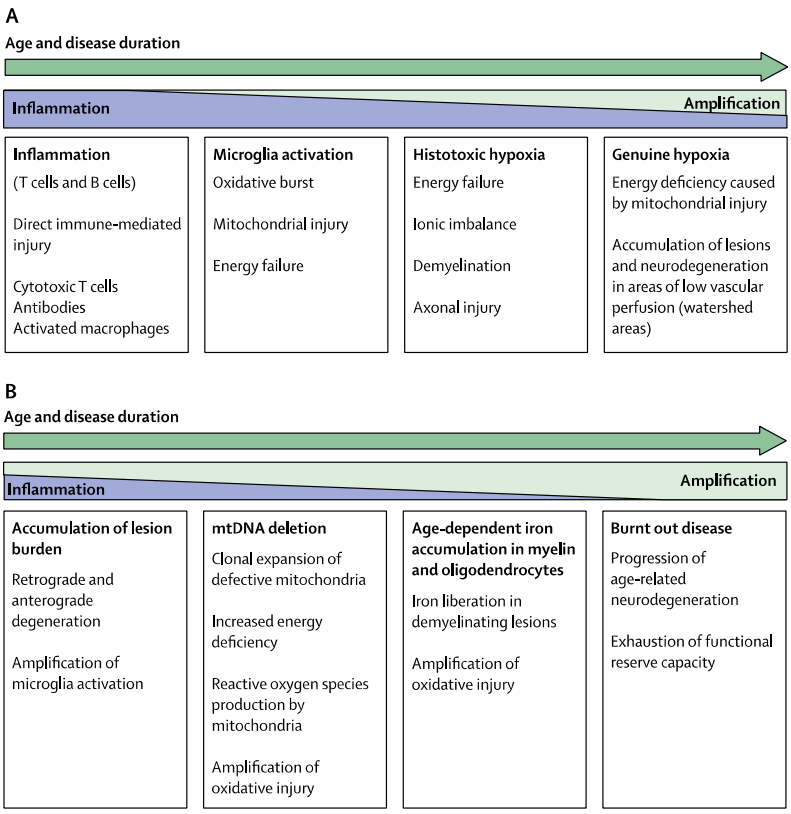

Figure 12. Disease duration and disease activity in RRMS MS.
Inflammation dominates early in the time course (A) and amplification dominate in the progressive phase (B), (Mahad et al., 2015).

The concept of brain reserve has been recently postulated by Vollmer et al. (2021) who argue that MS is a continuum rather than a disease with distinct subtypes based on the lack of definitive markers. Rather, the concept of neurological reserve is used to describe the compensatory ability of an individual's CNS which declines with age. The idea shares similarities to the 'functional reserve capacity' described by Mahad et al. (2015). Vollmer et al. (2021), however, suggest a more fixed capacity for reserve rather than amplification which could be expediential in nature.

Pathologically, retinal atrophy has been demonstrated post mortem in all MS. Vascular changes have been confined to RRMS and secondary progressive cases (Green et al., 2010). However, the number of post-mortem studies in MS is limited.

4.3 Inflammation in Uveitis

There are two subdivisions of uveitis depending on the cause. Infectious uveitis occurs as a result of known infection, such as the herpes viruses or as part of a systemic inflammatory disease such as rheumatoid arthritis or inflammatory bowel disease. The site of inflammation within the eye is also used to categorise the disease. This can involve the whole uveal tract (panuveitis), the anterior vitreous, ciliary body and the retina (intermediate uveitis), or the iris (anterior uveitis) (Figure 13). Typical symptoms include reduced acuity, floaters, eye pain and light sensitivity affecting both eyes (National Eye Institute, 2021). This contrasts with ON typically associated with MS, where symptoms include an acute loss of vision, colour desaturation, and pain associated with eye movements in one eye. It is for this reason that the two conditions are often not considered linked in the out-patient clinic (Forooghian, 2017; Casselman et al., 2021).

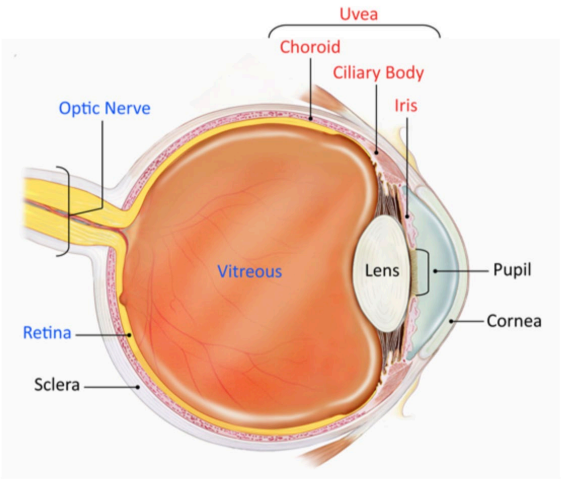

Figure 13. Cross-section of the eye. Uveitis may affect the iris (anterior), the anterior vitreous, ciliary body and retina (intermediate) or the whole tract (panuveitis)(The National Eye Institute, 2021).

4.4 Other Demyelinating CNS Diseases

Another group of other demyelinating diseases that share some common clinical features with MS have relatively recently been described under the heading of neuromyelitis optica spectrum disorders (NMOSD). These include myelin oligodendrocyte glycoproteins (MOG) and Aquaporin 4 (AQP4) antibody disease. These conditions may be seropositive or seronegative (Narayan et al., 2018; Flanagan, 2019). Although these disorders have been described in the literature for a long time under varying names including Devic's disease (Jarius and Wildemann, 2013), it was the discovery of serum antibodies that has enabled the

identification of these distinct entities that are diseases of the oligodendrocytes and the astrocytes, respectively (Figure 14). Clinically, these distinct diseases can present very similarly to MS and have some common pathological features. These include demyelination of the optic nerve(s) and longer sensory and motor tracts. However, the underlying mechanisms and evolution are quite distinct with clearly identified antibodies. The NMOSD diseases also contain some clinical features that might be regarded as 'red flags' that might suggest an alternative to MS if a careful clinical history is taken. The collective prevalence of NMOSD is uncertain as data is lacking. Estimates vary across the globe; in Europe it is thought to be 4.4 per 100,000 with a slightly higher rate in Asia (Flanagan, 2019).

There are also a large number of diseases that may 'mimic' MS which are not primary demyelinating. These that may be genetic, neoplastic, vascular, structural or inflammatory in nature. This underlines the need for careful history taking and reliable biomarkers.

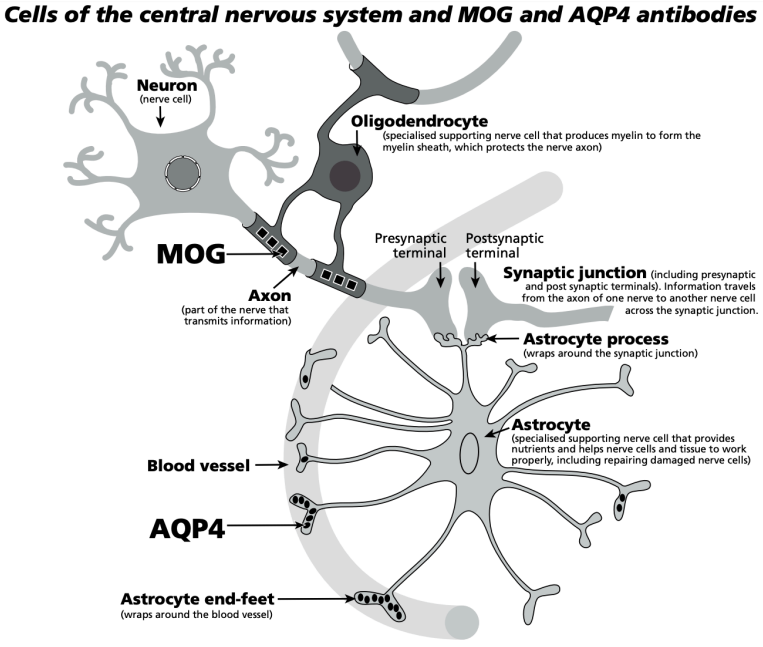

Figure 14. The CNS sites of AQP4 and MOD antibody diseases.
AQP4 is a disease of the astrocytes, whereas MOG is a disease of the oligodendrocytes, (Palace and Everett, 2019).

Summary of chapter: CNS and Ocular Inflammation

- It is likely that some individuals may have an underlying genetic predisposition to both ocular and/ or CNS inflammation.
- Some demyelinating conditions clinically similar to MS have identifiable sites of dysfunction and identifiable antibodies.
- Some conditions are MS 'mimics' that present with signs and symptoms which present a challenge for accurate diagnosis.
- Disease endpoint may be influenced by pre-existing brain health and the individual's capacity to biologically adapt.

Summary: Assessment of the visual pathway

- Visual acuity is a subjective measurement of visual function that is often impaired due to pathological or non-organic disorders.
- Visual electrophysiology can be used as quantitative measures of visual pathway function, from retina to cortex.
- OCT is a measure of retinal structure that produces quantitative and qualitative measures of the retinal layers and macula.

Chapter 5. Assessment of the Visual System

The eye is regarded as an extension of the brain due to the common embryological origins. Research in animals (Talla et al., 2013; Teixeira et al., 2016) and humans has suggested that retinal cells are implicated in MS, along with other neurological diseases, although the exact mechanisms are unknown (Barboni et al., 2019; Vujosevic et al., 2023). Given that the optic nerves are a common site of inflammation in MS, it was postulated that the disease also affects other parts of the visual pathway such as retinal cells. Visual processing begins with the first order neurons, the photoreceptors of the retina. These synapse onto the bipolar cells (second order neurons) which in turn synapse onto the RGCs (third order neurons) such that the cell bodies form the unmyelinated RGC layer (unmyelinated) within the retina, whose axons converge to form the optic nerve where they become myelinated. Clinical assessment is a necessity for any patient presenting with sensory or motor symptoms suggestive of MS and should include a full neurological examination, including the cranial nerves. The optic nerve, the second cranial nerve (CN II), is assessed for acuity, accommodation, visual fields, light and pupillary reflex, RAPD, and colour perception, as well as with fundoscopy on both sides.

5.1 Visual Acuity

Visual acuity measures are the mainstay of clinical practice that encompasses the whole of the visual pathway including higher levels of visual processing. Assessment was made using a full contrast early treatment diabetic retinopathy study (ETDRS) chart with Logarithm of the

Minimum Angle of Resolution (LogMAR) notation (Figure 15). The chart consists of 5 letters on each line with a graduated scale with each letter subtending 5 minutes of arc equating to a score of 0.02. Thus, the lower the score, the better the visual acuity. This method was preferred over the traditional Snellen chart because it provides an equal number of letters on each line with proportional spacing and sizing of each letter, providing a more sensitive assessment and analysis (Lay et al., 2009). Visual acuity is an overall assessment of the visual function and is unable to localise areas of dysfunction. It is therefore potentially sensitive to disease but not very specific in nature. Supplementary examinations and investigations are needed to add further information.

Figure 15. Example of ETDRS chart.

(Bailey and Lovie, 1976).

The LogMAR method of measuring acuity has been shown to have a test-retest variability of 0.2 LogMAR, that is, 2 lines of letters or greater has been shown to reliably distinguish a change in VA while maintaining sensitivity and specificity (>95%) (Rosser et al., 2003).

Additional methods of measuring visual performance include contrast sensitivity, of which there are different versions, that attempt to measure the minimum contrast that can be perceived. This typically uses a letter chart also with deceasing contrast which is read under standard conditions e.g., distance and room illumination. This measure is thought to be closely related to the stimulation of particular RGCs and has been implicated in MS (Sisto et al., 2005).

5.2 Electrophysiology

Electrophysiological methods are used as an objective measure of visual pathway function from the retina to the visual cortex by utilising different stimulation techniques to evoke the

visual evoked potential (VEP), pattern electroretinogram (PERG) and flash electroretinogram (ERG) (Figure 16). The international society for electrophysiology of vision (ISCEV) has developed standards and guidelines for recording all modalities (VEP, PERG and ERG) to facilitate comparison and standardise practice (Bach et al., 2013; McCulloch et al., 2015; Odom et al., 2016). Convention may vary between neurophysiology and ophthalmological specialities where waveforms are depicted with positivity downwards in the former and upwards in the latter. Irrespective of this configuration, responses from each test are labelled with standard nomenclature, with the polarity (positive or negative) followed by the expected peak-time (in milliseconds) or amplitude (in microvolts). For example, the VEP from the cortex evokes an initial negative deflection N75 (75ms), followed by a major positivity P100 (100ms), and a second negativity N135 (135ms). The size of the individuals' responses ranges from several microvolts for the pattern ERG to a few hundred microvolts for the full field flash ERG. Consequently, signal averaging techniques are required along with adequate amplification, with low signal to noise ratios, to ensure the signals can be separated from ongoing brain activity or noise (both physiological and artefactual).

It is recommended that all tests (VEP, PERG and ERG), are checked for reproducibility with a minimum number of two trials and that individual laboratories collect their own normative data (Robson et al., 2018).

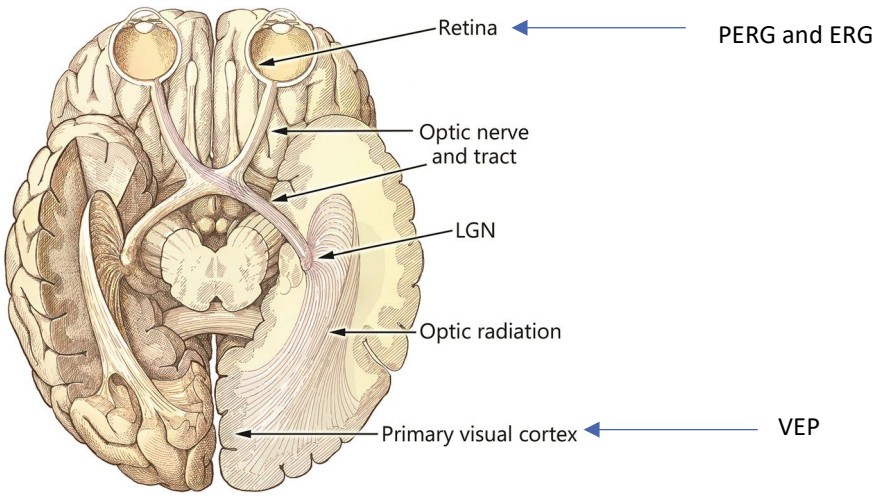

Figure 16. Visual pathway with electrophysiological generators.
Adapted from Mirochnik & Pezaris (2019)

5.2.1 Visual Evoked Potential (VEP) – Cortical Function

The VEP can be evoked by a pattern or diffuse flash stimulus and recorded from the primary visual cortex, close to Brodmann's area 17 (Figure 17). It represents the overall ability of the visual system to convert light into an electrical impulse that is conducted along the pathway to the primary visual cortex.

The retinotopic representation of the primary visual cortex can be utilised to elicit a broad, more variable response from the striate cortex by using a diffuse flash of light or preferentially from the central 8–10 degrees of the visual field by using a pattern stimulus. Until the widespread adoption of MRI, the VEP was an integral part of the diagnostic criteria for MS (Posner et al., 1983) that was used to demonstrate conduction delays in the visual pathway. The VEP is not included in current diagnostic criteria for MS but is recommended as a paraclinical test that provides additional supporting evidence (Thompson et al., 2018b).

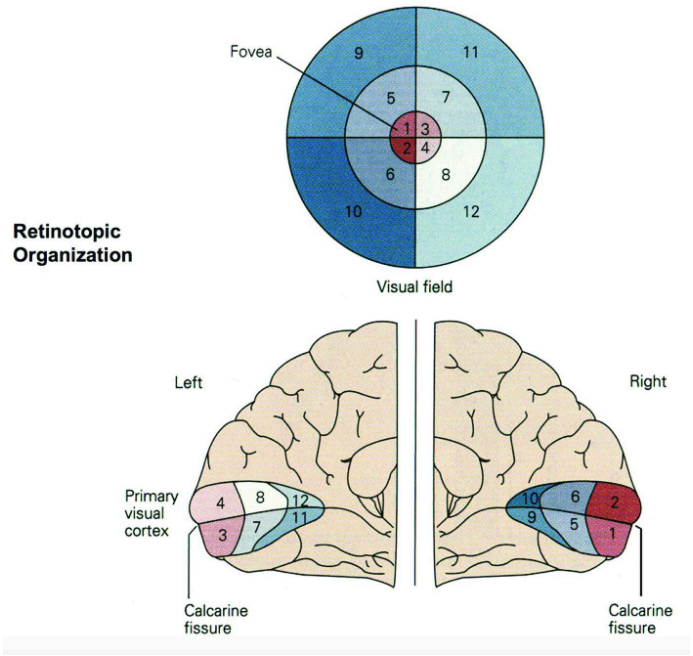

Figure 17. Retinotopic organisation of the primary visual cortex.

Up to 50% of the primary visual cortex processes foveal signals (cortical magnification). Signals from the top left of the visual field are mapped to the bottom right of the visual cortex (retinotopy) (Wurtz and Kandel, 1990)

The pattern reversal VEP is elicited using a reversing checkerboard (Figure18 left), producing a robust response from the visual cortex (Figure 18 right), with low intrasubject variability due to the focal nature of the response (Odom et al., 2016). The evoked P100 waveform is a measure of voltage over time with nomenclature denoting the peak-time and polarity of the

response (Figure 19). Bipolar recordings are made with an active electrode on the scalp over the calcarine fissure with a frontal reference electrode. The use of additional electrodes placed 5 cm lateral of the mid occipital region and one placed 3 cm above the Oz mid occipital region, named the 'Queen Square' placement system, additionally facilitates the detection of hemispheric and chiasmal abnormalities. Each eye is tested separately to facilitate localisation of any conduction defects that can be further examined with half-field testing if necessary.

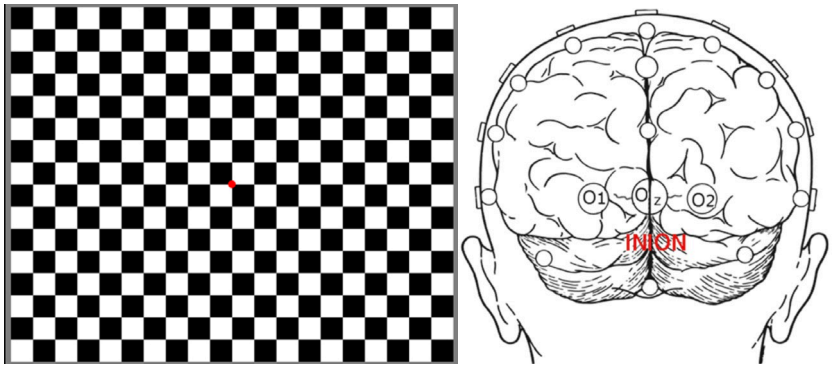

Figure 18. VEP stimulation and recording methods.
Checkerboard stimulus (left).) Electrode placement with midline (Oz) and lateral electrodes (O1 left occipital and O2 right occipital) (right) (Creel, 2015).

The test conditions are critically important and are set out in the ISCEV guidelines (Robson et al., 2022). Of note is that the stimulus should be high contrast with an equal number of black and white squares and there should not be any overall change in luminance which could introduce artefacts. The patient should be positioned as to ensure both a constant field size and check size and in such a way that compliance can be monitored. The method of display is also of important as liquid crystal displays (LCDs) and organic light emitting diodes (OLEDs) stimulators can introduce delays due to refresh rates and frames rates being slower than cathode ray tubes (CRTs).

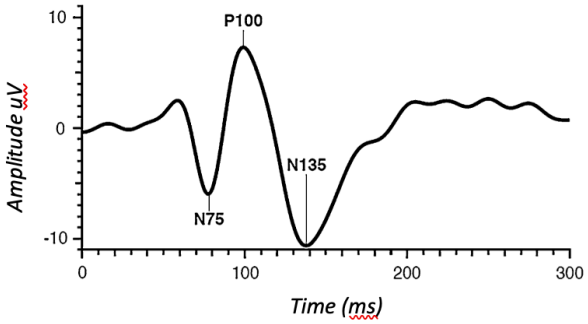

Figure 19. Pattern reversal VEP waveform.

Positivity upwards (Odom et al., 2016).

The cortical generators of the VEP components are not definitively defined but modelling work and functional imaging suggests that the dorsal extrastriate cortex of the middle occipital gyrus contributes to the early component (N75), and the major positivity (P100) has been localised to ventral extrastriate cortex of the fusiform gyrus. While the later negative component (N135) is thought to have contributions from multiple regions including the parietal lobe (Di Russo et al., 2002).

5.2.2 Pattern Electroretinogram (PERG) – Macular Function

The PERG can be used to help differentiate diseases of the macular and optic nerve when used in conjunction with the VEP. PERGs have been shown to be affected in conditions such as glaucoma and optic neuritis (Holder, 1991; Holder et al., 2009). In routine clinical practice a transient PERG is recorded using a checkerboard reversal rate of approximately 3 Hz (6 reversals per second). Higher rates of reversal (>8 Hz) produce a 'steady state' response that produces a waveform that is continuous and more difficult to measure (Asanad and Karanjia, 2022).

There is still some debate in the literature as to the generators of the individual components of the PERG, but it is generally accepted that the P50 is derived jointly from the photoreceptors with a contribution from the RGCs. In contrast, the N95 is thought to be solely derived from the RGCs that can also be affected in optic nerve disease and compression (Parmar et al., 2000) (Figure 20). Both the P50 and N95 are of low amplitude, typically between 2–8 μV (Figure 21 right), making them technically challenging to record, which is made possible by differential amplifiers and signal averaging techniques. Some authors have

found that measurement of the P50 to N95 ratio a more sensitive measure of optic nerve function due to the selective reduction in the N95 component in optic nerve disease (Holder, 1989; Atilla et al., 2006).

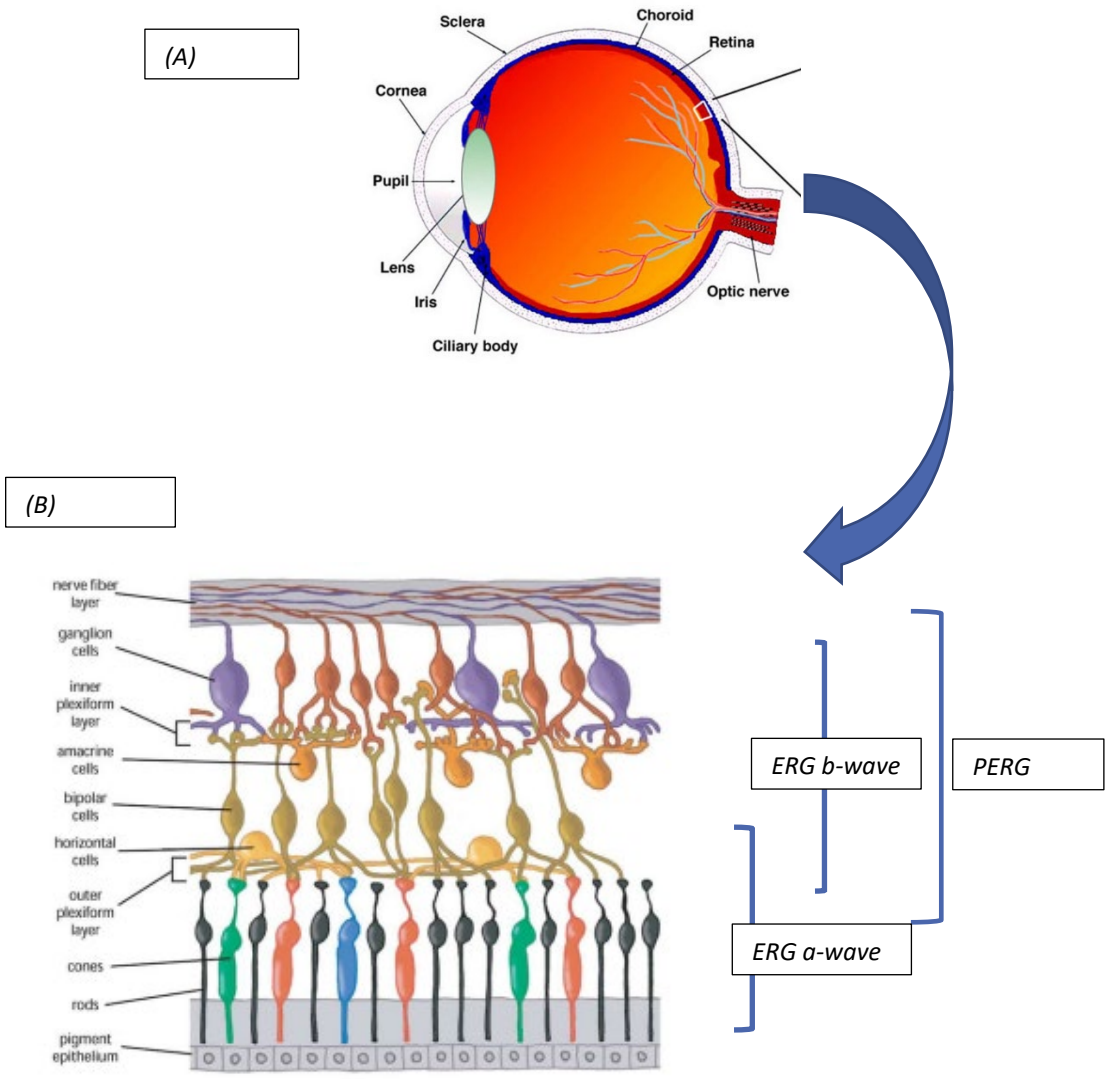

Figure 20. Anatomy of the eye and retina
(A) Retinal structure (B) Cellular generators of the individual ERG responses (Kolb).

Recording electrodes should ideally be placed with the active electrode on the cornea and a reference placed on the ipsilateral outer canthus, and not placed anywhere that may be

contaminated by EEG activity (Bach et al., 2013). There are various corneal electrodes available (mostly monopolar) that are made from different materials. In practice, the most frequently favoured is the Dawson-Trick-Litzkow (DTL) thread (Figure 21 right). This type of electrode does not require a lid speculum or contact lens, which is more comfortable for the patient and increases compliance. It is also single use, reducing infection control risks.

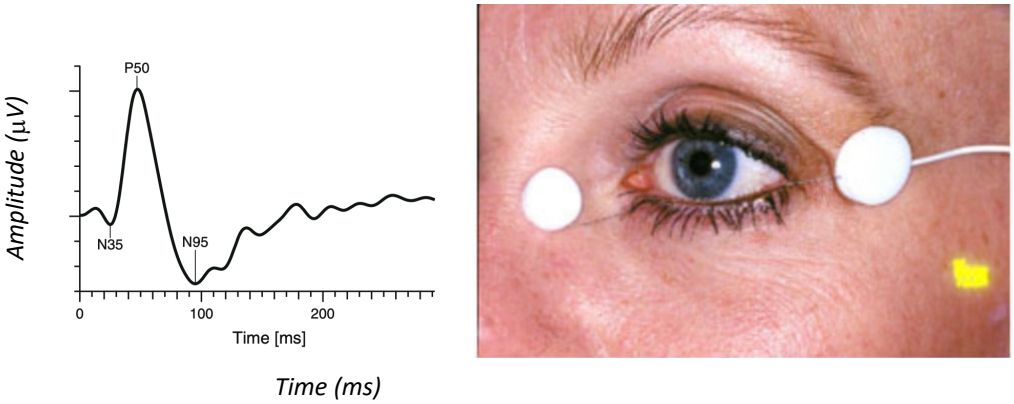

Figure 21. Normal pattern electroretinogram and DTL placement
(left) Normal pattern electroretinogram (PERG) (positivity upwards) (Bach et al., 2013), DTL electrode placement (right) (Creel, 2015).

The PERG is performed with corrected vision where appropriate, at a fixed distance from the screen and usually with simultaneous recording from both eyes to maximise fixation. If there is significant strabismus, it can be more helpful to stimulate each eye individually (Asanad and Karanjia, 2022). As with VEP protocols, it is critical that the overall luminance of the stimulus remains constant, and the contrast is high.

5.2.3 Flash Electroretinogram – Diffuse Retinal Function

In contrast to the PERG, the flash ERG is a mass response from the entire retina that can be used to elicit responses from the photoreceptor pathways using a diffuse flash of light, ideally from a Ganzfeld stimulator (Figure 22). Responses are recorded under dark adapted (DA), and

light adapted (LA) conditions to elicit responses from the rods, cones, and inner retinal layers (Figure 23).

The standard nomenclature defines the stimulus parameters, state of adaptation (LA or DA), and the flash strength measured in photic units, phot (photo candelas/m2, $cd{\cdot}s{\cdot}m^{-2}$). It is recommended that the LA stimuli are presented on top of a background luminance of $30cd{\cdot}s{\cdot}m^{-2}$ after at least 10 minutes of light adaptation (McCulloch et al., 2015).

Figure 22. Ganzfeld stimulator for ERG testing.

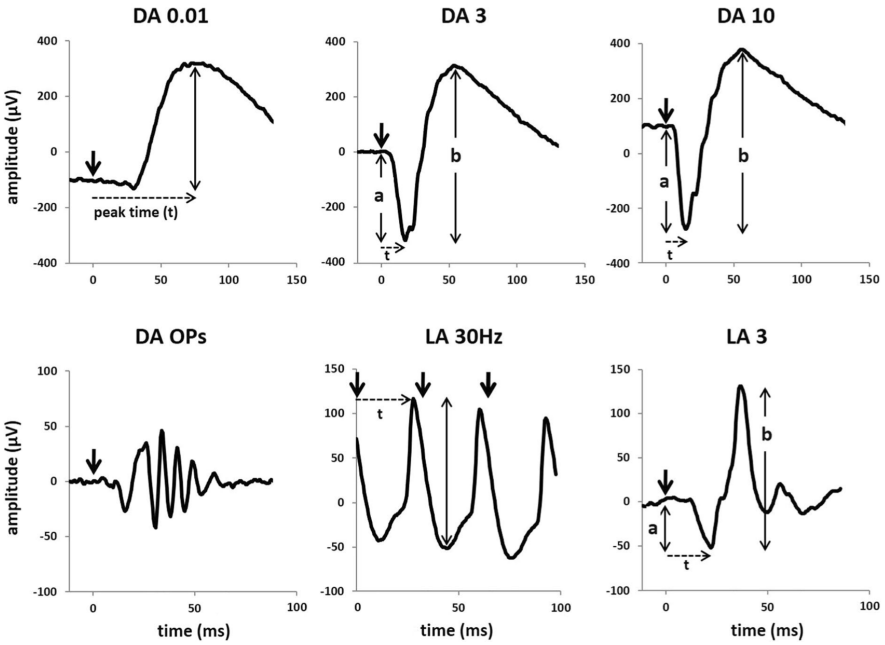

Figure 23. Standard ISCEV flash ERG responses and their measurement.
DA dark adapted, LA light adapted, t: time a: a wave amplitude (baseline to trough), b: b-wave amplitude (trough to peak) Flash strength (photo candelas/m2, $cd{\cdot}s{\cdot}m^{-2}$). *(Robson et al., 2022)*

The loss of cells due to atrophy or apoptosis produces a reduction in the ERG amplitude whereas cell 'stress' or dysfunction is expressed as a peak-time delay (Creel, 2018).

5.3 Optical Coherence Tomography (OCT)

OCT imaging is a non-invasive technique that uses infra-red light (approximately 840nm), to image the optic nerve head and macula regions, producing high-resolution cross-sectional images similar to ultrasound imaging techniques. Spectral domain OCT (SD-OCT) utilises fast Fourier transformation to analyse the inference spectrum of two broad spectrum light waves, allowing for simultaneous measurement of different depths. This allows for the fast acquisition of high-resolution images. Thus, OCT is a mathematical reconstruction of the retina (transposed into an image) rather than a direct image, unlike a photograph that is dependent on the reflective properties of the different layers and their position in relation to the light source. For instance, structures that are more parallel to the light source do not reflect as much light as those more perpendicular, e.g., the retinal nerve fibre layer (RNFL). Within each layer, other cells are present such as interstitial fluid and supporting cells which may remain when pathology exists such that, a layer may never be absent or unrecordable even when significant pathology exists. Figure 24 shows a normal OCT scan (left) with retinal layers labelled, whereas a fundus photograph of the macula is shown with further detail of the fovea (right). The scans utilised in this study rely on protocols that automatically identify the fovea, which has a reduced area of reflection. If the equipment is unable to do this, there is a warning and a manual facility to find the fovea.

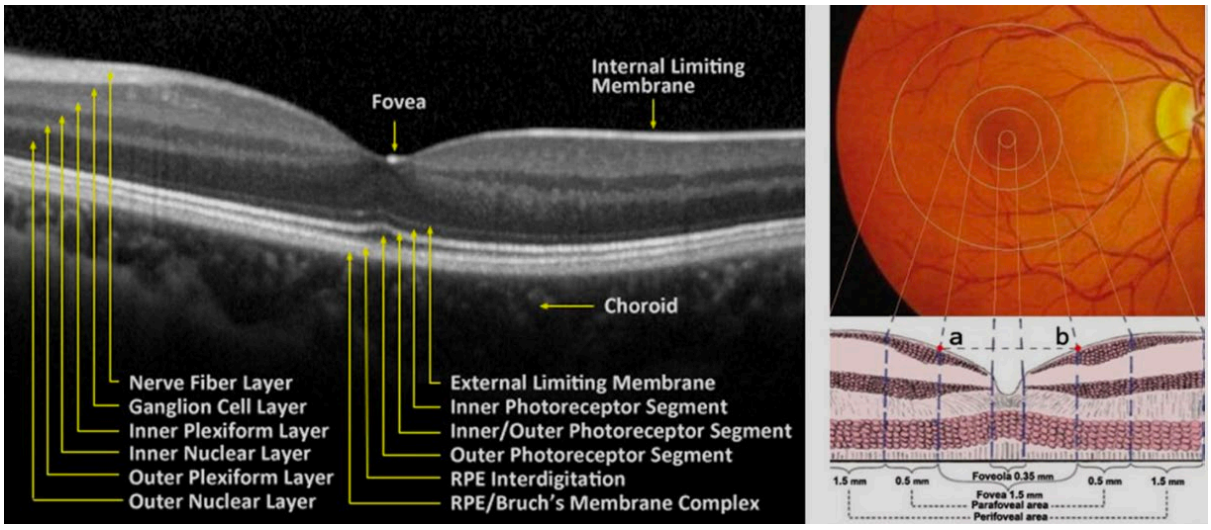

Figure 24. Normal OCT image with retinal layers.

Normal OCT image (left). Fundus photograph (right) with approximate macula measures (Fu et al., 2016).

Different scan protocols can be used to highlight different aspects of the retina. The macula scan captures a predefined area of 6 mm x 6 mm centred on the fovea to produce a cross sectional image (Figure 24 left) or map. The scans are recorded with 128 horizontal scans each composed of 512 A-scans and a central horizontal HD B-scan. This provides greater resolution in the horizontal plane than the 200 x 200 protocol, but at the expense of the vertical resolution (Zeiss, 2015). The software includes inbuilt age-matched normative data that is used for comparison in the reports that show quantitative and qualitative displays of the data for each eye. Quality of the scan is measured by 'signal strength' which the manufacturer recommends should be greater than 6/10.

5.3.1 Retinal Nerve Fibre Layer (RNFL)

The RNFL consists mainly of the unmyelinated axons of the RGCs that converge to form the optic nerve that exits at the back of the eye. These axons are arranged in a distinct pattern, with nasal fibres going directly to the optic nerve head while the temporal fibres project superiorly and inferiorly around the central region that maintains a clear horizontal division about the midline (Figure 25). In. normal healthy individuals the RNFL is thickest superior and inferior to the optic nerve head, and varies with distance from the centre of the disc (Lamirel et al., 2010).

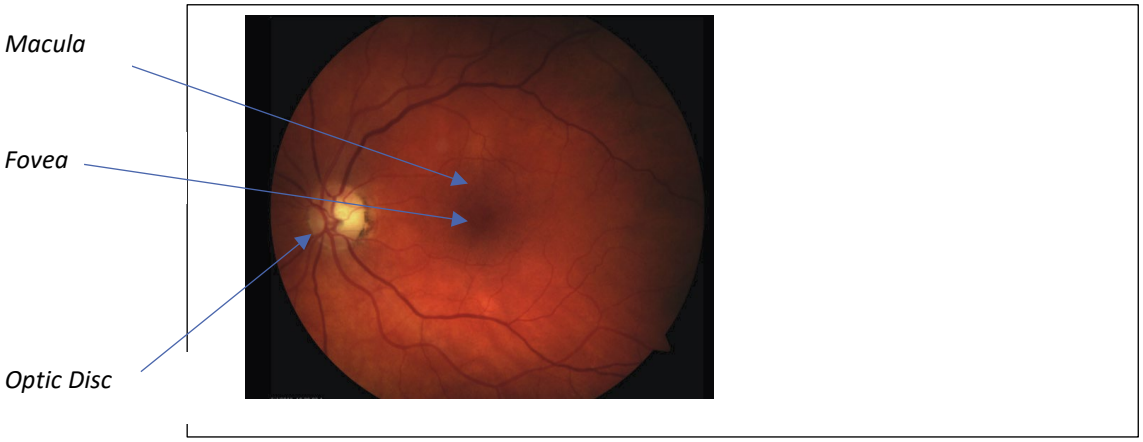

Figure 25. Retinal photograph.
Photograph showing optic disc, macula and fovea (right) drawing showing the topography of the RGC axon arrangement (left) Left eye Adapted from Costello (2013).

OCT is able to produce colour coded maps of the RNFL that differentiates thickness as well as quantitative data that can be divided into discrete regions – typically divided into quadrants or a 'clock face' (Figure 26).

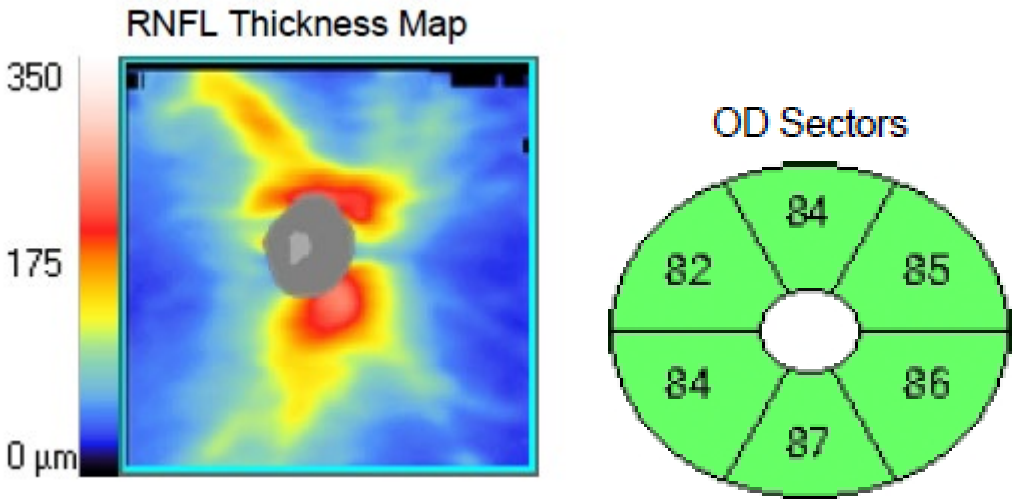

Figure 26. RNFL maps.
A colour coded map of the RNFL, demonstrating the greater thickness over superior and inferior regions (left). The quantitative data displayed as segments (right); measurements are in microns (µm) (RE HV 55).

5.4 Ganglion Cell Layer (GCL)

The ganglion cell layer is made up of the cell bodies of the RGCs that synapse with interneurons that connect the photoreceptors to the RGCs. The lack of myelin in both the RGC and RNFL layers is analogous to the grey matter in the brain and supports the use of the eye as a site that may provide information about other areas of the CNS.

OCT analysis of RGC layer also uses a 6 mm x 6 mm cube containing an elliptical annulus over the fovea to create a contour map using colours to indicate thickness (Figure 27).The fovea is represented by a darker colour representing the depression or 'pit' whereas thicker areas are represented by lighter colours. The software also calculates the inner boundary of the ganglion cell layer (that also corresponds to the outer boundary of the RNFL), and the outer boundary of the inner plexiform layer to illustrate the cross-section (shown as purple and yellow lines, respectively (Figure 28). The data detailed in the reports for the ganglion cell layer is a combination of the GCL and IPL layer with averages and minimum and maximum values if required.

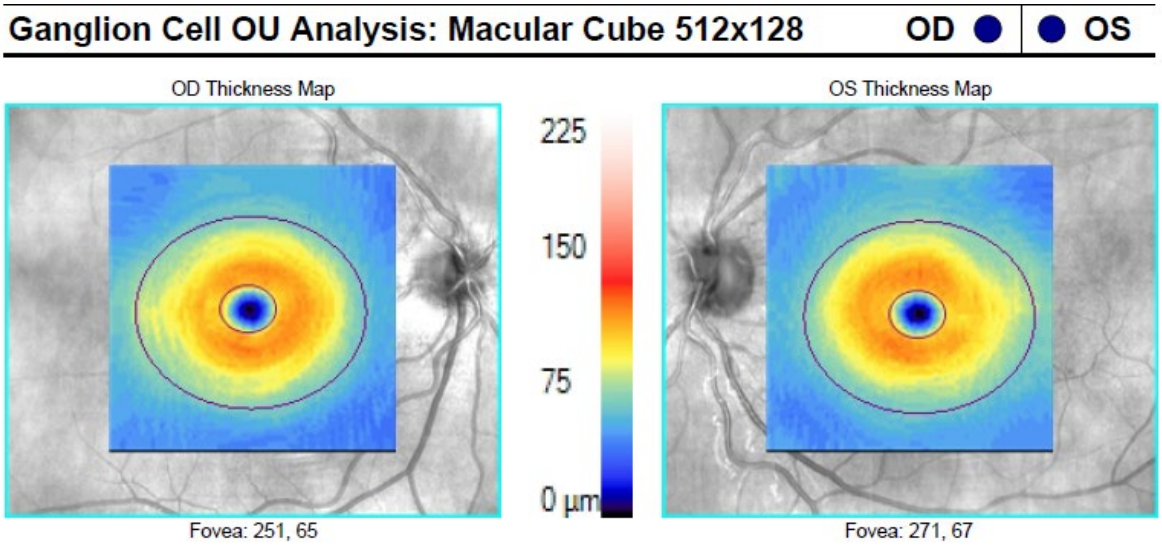

Figure 27. Ganglion cell thickness maps centred on fovea
(HV 55)

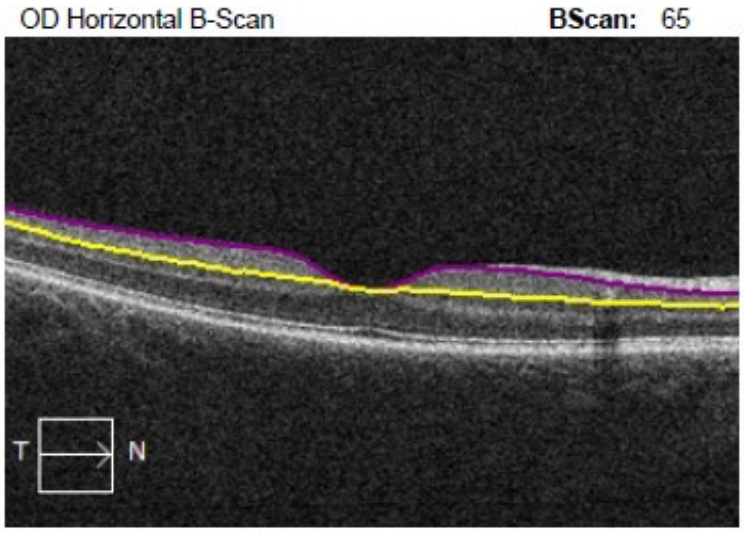

Figure 28. OCT of Ganglion cell and Inner plexiform layer
(HV 55).

The majority of RGCs are concerned with vision but a small proportion (0.4–1.5%) of them have a different function, termed intrinsically photosensitive retinal ganglion cells (ipRGCs); these cells contribute to circadian rhythms, mood, alertness and the pupillary light reflex (PLR) (Mure, 2021).

5.3.2. Macula Thickness and Optic Nerve Head

The equipment defaults to the 512 x 128 scan due to the increased resolution, measuring between the inner limiting membrane (ILM) and the retinal pigment epithelium (RPE). The quantitative data is displayed as a graphical representation of the fundus (Figure 29), as well as a cross sectional image (Figure 30).

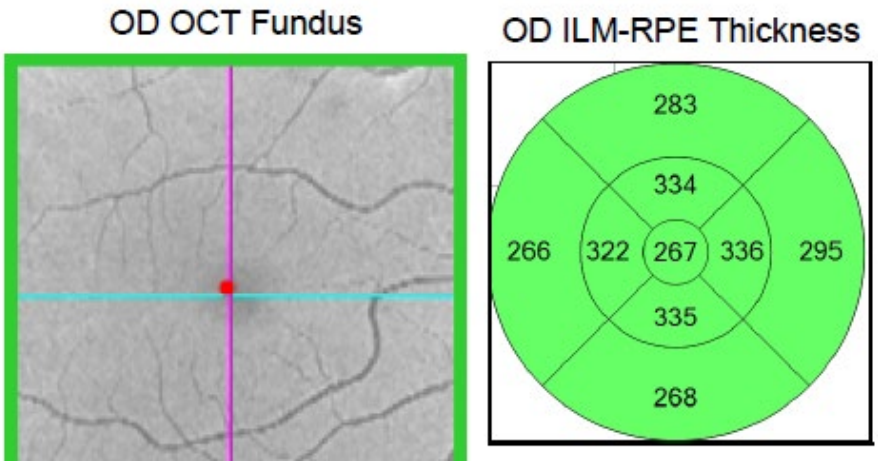

Figure 29. Fundus image and macula thickness map.
Fundus image (left) and average macula thickness divided into sections (right) (RE HV 55).

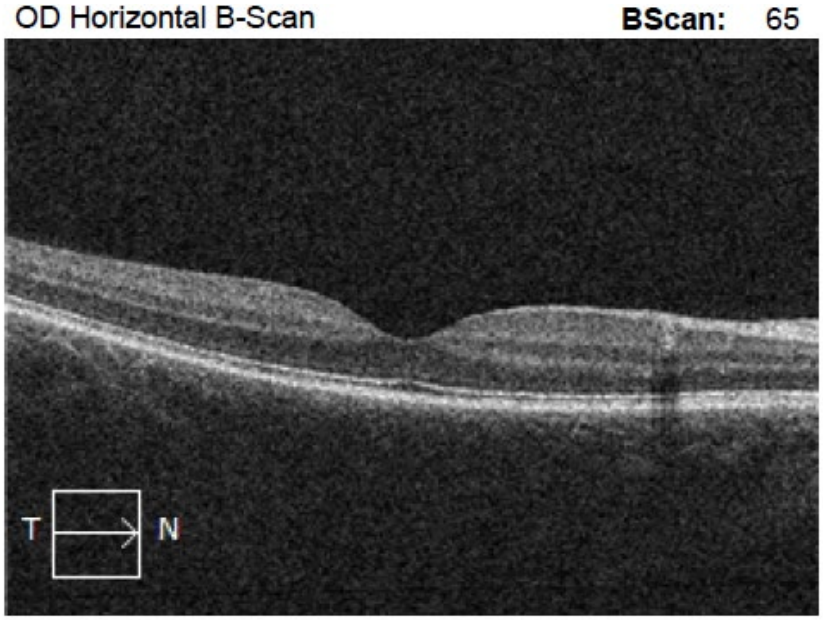

Figure 30. OCT Cross sectional view through the macula
(RE HV 55).

Summary: Investigations of the visual pathway

- Combining function and structural measures can localise areas of dysfunction in the visual pathway.
- Standardisation with established protocols helps ensure consistency and comparability.

Summary: Literature Review

- Early accounts of electrophysiology in visual disturbance attempted to localise the area of dysfunction within the visual pathway.
- Technology and standardisation have facilitated work in this area.
- Later studies in MS have looked to differentiate eyes with previous clinical episodes from those without, to further delineate the disease process.
- The use of these paraclinical measures may serve as a way of differentiating patients with MS and those without.

Excerpts from this review have been submitted previously as part of the C1 literature review for the DClin.Sci Broomfield, N. (2020) C1:6ACP8024: Doctoral Research and Innovation in Clinical Science Literature Review Manchester Metropolitan University

Chapter 6. Literature Review

6.1 Methodology

An in-depth literature review was undertaken using PubMed, Medline and CINHAL databases on 29 October 2019 (Appendix 1a) updated on 1 February 2022 (Appendix 1b). Search terms included: “multiple sclerosis”, “MS”, “clinically isolated syndrome”, “demyelinating disease”, “chronic”, “progressive”, “relapsing remitting”, “electroretino”, “electroretinogram”, “pattern” and “flash”. The search terms were exploded using the thesaurus function where available. Limitations were placed to confine the results to those written in English and those studies involving adults. References from individual papers were also screened and expert opinion was also sought from Professor Hobart and Dr Almasari to guide the search. Studies relating to stimulation protocols that were not used in mainstream clinical practice, e.g., steady state PERG, have not been included. Studies where the focus of the electrophysiology was the relatively new ISCEV photopic negative response (PhNr), the multifocal VEP (mfVEP) and the multifocal ERG (mfERG) protocols were excluded. Abstracts were screened from 994 results (Figure 31), which were reduced to 33 titles for full review after removal of duplicates and excluded content (Table 4).

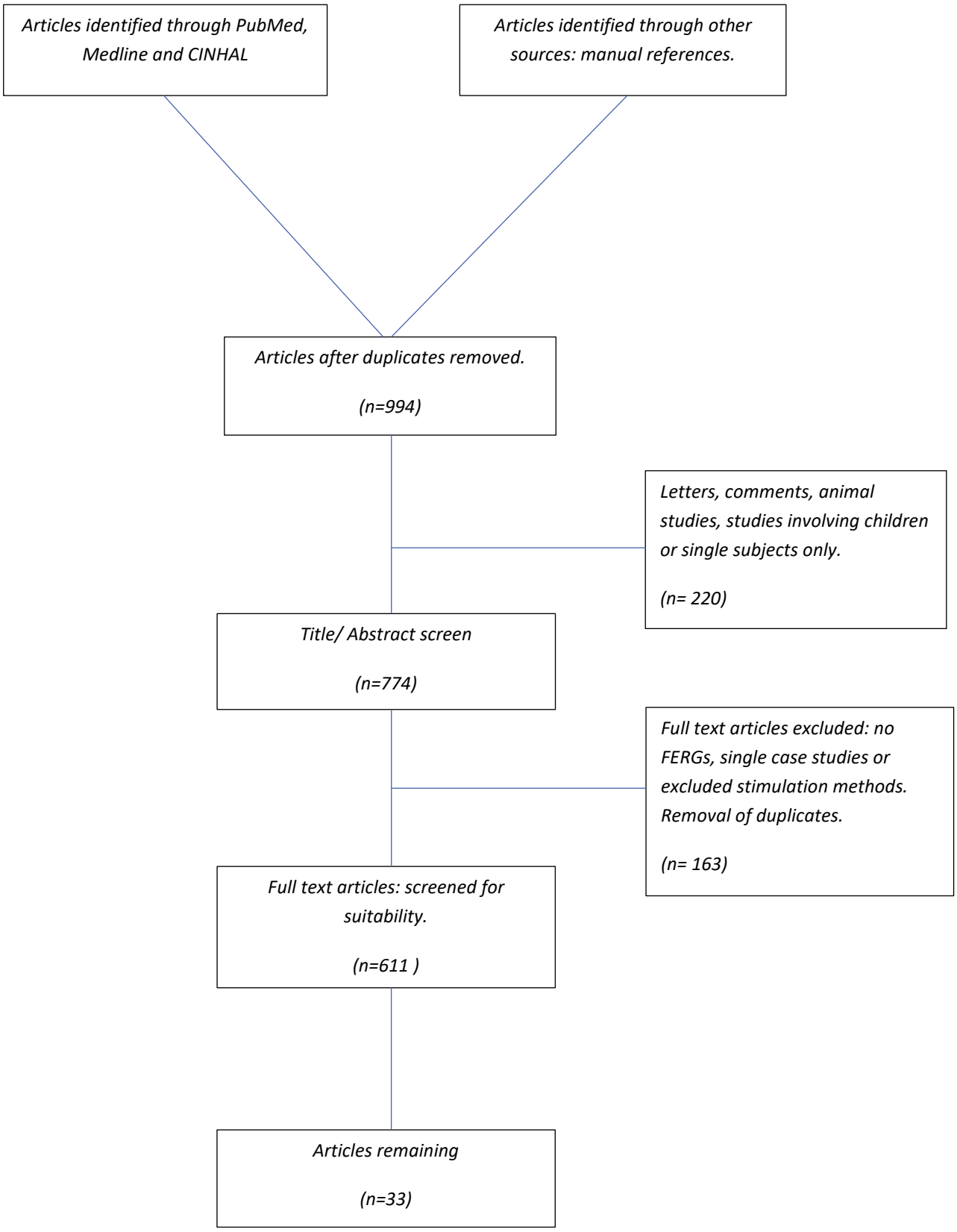

Figure 31. Literature review process map

The review aimed to assess the available evidence to support the theory that there are measurable physiological differences in the retinas of patients with MS that are recordable with ERGs. The evidence is clear with regards to the high incidence of visual impairment in MS and that the optic nerves are frequently a target for inflammation and demyelination. However, less is known about the distal pathway and individual retinal layers. There are conflicting reports in the literature regarding which cells or pathways may be affected, and if this data could be used as a reliable biomarker. This work is inevitably related to other electrophysiological measures (VEP and PERG) as they are the only methods of quantitatively localising dysfunction in the visual pathway (or ruling it out). The close relationship between structure and function is demonstrated by the number of studies that also added OCT measures to the electrophysiology.

The importance of the visual system in MS is demonstrated by the link between ON and axonal damage, which in turn has been shown to be related to disability (Garcia-Martin et al., 2011), making it an attractive measure of monitoring and possibly predicting outcomes. Longer term studies have also shown good correlation with brain atrophy (Saidha et al., 2015).

Apart from one, all the studies found in the literature search were single centre observational studies, some of which contained a small number of patients. There were three longitudinal studies performed over up to 3 years in patients with established MS. Different diagnostic criteria for MS were used across the individual studies, although the McDonald criteria was the most frequently used, particularly in more recent studies.

A common methodological approach was to compare patients with a history of MS with or without previous ON, or alternatively, target patients with CIS involving inflammation of the optic nerves. In some studies, these groups were compared to controls; in others, the 'good eye' was used as a control, which assumes no subclinical involvement. Other groups chose to compare MS to other conditions, e.g., MNOSDs, either with or without ophthalmological involvement. It has been shown that although one eye may be affected in ON or MS, there may be changes in both eyes with or without symptoms of ON (Parisi et al., 1999; Sriram et al., 2014).

The confound of intrasubject correlation is often neglected in many of the studies, and it was often unclear how sample sizes were decided or how eyes were chosen (one or both) within groups. In some studies, a single measure was reported without reference as to which eye it was recorded from or if the measures from both eyes were combined. Some authors have dealt with this by using the generalised estimating equation (GEE) which takes into account the correlation between eyes within subjects, which is highlighted as a problem in studies involving both eyes (Armstrong, 2013).

Table 3. Literature review.

Year	Author	Single/Multi centre	Sample size - patients (n)	Type of study	Group studied	MS classification	Electrophysiology	Other paraclinical measures
1966	Gills	Single centre	27	Case control	MS (+/- ON)	NS	FERG	Histology
1971	Feinsod et al.	Single centre	69	Mixed cases, adults and children	Mixed optic nerve pathologies including MS	NS	FVEP, FERG	
1973	Feinsod et al.	Single centre	35	Case control	MS (+/- ON)	McAlpine, Lumsden & Acheson	FVEP, PERG, FERG	
1977	Zeese	Single centre	30	Case control	MS v mixed neurological disorders v healthy volunteers	McAlpine, Lumsden & Acheson	VEP	
1982	Coupland & Kirkham	Single centre	105	Case control	MS (+/- ON)	McDonald	FVEP, FERG	
1983	Kirkham & Coupland	Single centre	28	Case control	MS +ON	McDonald	PERG, VEP	
1984	Serra et al.	Single centre	20	Case control	MS (+/-ON)	McDonald	VEP, PERG, FERG	
1984	Person & Wanger	Single centre	15	Case control	MS (+/- ON)	NS	VEP, PERG, FERG	
1985	Pierelli et al.	Single centre	15	Case control	MS	McDonald	VEP, ERG	
1986	Celesia et al.	Single centre	35	Case Control	MS	McAlpine	VEP, PERG	
1987	Veagan & Billson	Single centre	15	Case Control	Mixed optic nerve pathologies	NS	PERG	mfERG
1989	Holder	Single centre	67	Observational	Mixed optic nerve pathologies and retinopathies	NS	PERG, VEP	
1989	Papakostopoulos et al.	Two centres	25	Case Control	MS +ON	McAlpine	VEP, ERG	

1991	*Holder*	*Single centre*	*141*	*Case Control*	*MS*	*McDonald*	*PERG*	
1991	*Stefano et al.*	*Single centre*	*18*	*Case Control*	*MS*	*NS*	*PERG, VEP*	
1999	*Parisi et al.*	*Single centre*	*14*	*Case Control*	*MS (+/-ON)*	*Poser*	*PERG*	*OCT*
2006	*Forooghian et al.*	*Single centre*	*34*	*Case control*	*MS*	*NS*	*FERG*	*Antiretinal antibodies*
2007	*Forooghian et al.*	*Single centre*	*34*	*Case control*	*MS*	*NS*	*FERG*	*Enolase autoantibodies*
2007	*Gundogan*	*Single centre*	*39*	*Case control*	*MS (-ON)*	*NS*	*VEP, ERG*	*OCT mfERG*
2010	*Almarcegui*	*Single centre*	*19*	*Case control*	*MS*	*Poser*	*PERG, VEP*	*OCT, VF*
2011	*Fraser*	*Single centre*	*46*	*Case control*	*ON*	*NA*	*ERG, PERG, VEP*	
2011	*Garcia-Martin*	*Single centre*	*34*	*Case control/ Longitudinal (2yr FU)*	*MS +ON*	*NS*	*VEP, PERG*	*OCT, laser polarimetry*
2013	*Hokazono*	*Single centre*	*38*	*Case control*	*MS (+/-ON MNOD)*	*McDonald*	*PERG,*	*OCT, VF*
2013	*Rodriguez-Mena et al.*	*Single centre*	*114*	*Case control*	*MS*	*McDonald*	*VEP, PERG*	*OCT*
2014	*Sriram et al.*	*Single centre*	*62*	*Case control*	*MS (-ON)*	*NS*	*FERG*	*OCT mfVEP*
2017	*Hamurcu et al.*	*Single centre*	*51*	*Case control*	*MS (+/- ON)*	*NS*	*VEP, ERG*	*OCT*
2017	*Janaky*	*Single centre*	*85*	*Case Control*	*MS +/-ON*	*NS*	*VEP, PERG*	-
2017	*Behbehani*	*Single centre*	*50*	*Case control*	*MS*	*McDonald*	*VEP*	*OCT*
2017	*You*	*Single centre*	*77*	*Longitudinal (3yr)*	*MS -ON*	*NS*	*ERG*	*OCT*
2018	*Hanson et al.*	*Single centre*	*32*	*Case control*	*MS and CIS*	*McDonald*	*ERG*	*OCT, mfERG*

2019	*Pisa*	*Single centre*	*107*	*Case control (comparative + longnitudinal)*	*MS , NMOSDs*	*McDonald*	*VEP*	*OCT*
2021	*Hanson*	*Single centre*	*23*	*Longitudinal (3yr FU)*	*MS*	*McDonald*	*ERG*	*OCT*
2021	*Nowacka*	*Single centre*	*32*	*Case control*	*MS +ON (Treated v not treated)*	*McDonald*	*PERG, VEP*	*OCT*

Key:
CIS Clinically isolated syndrome
ERG Electroretinogram (flash)
FU Follow Up
FVEP Flash VEP
MS (+/- ON) confirmed MS with and without clinical optic nerve involvement.
MS (+ON) confirmed MS with a pervious history of optic neuritis.
MS (-ON) confirmed MS without previous clinical history of optic neuritis.
NMOD Neuromyelitis optica spectrum disorder
NS Not specified.
NA Not applicable
ON unilateral optic neuritis history
PERG Pattern electroretinogram
VEP Pattern reversal VEP

6.2 Early Studies

Early efforts in the literature focussed on the optic nerves and the VEP, followed by investigation of the macula driven PERG that is now known to be generated (in the most part) by the RGCs. The narrative has shifted to the distal pathway more recently, looking for evidence of wider inflammation affecting the structure and function of the retina measured with OCTs and ERGs, respectively. Many of the early studies were limited by the technology at the time. Methodologies and nomenclature often varied between researchers, with no standardisation in recording or stimulation parameters, until the introduction of the various ISCEV guidelines (Marmor et al., 1989).

The initial discovery by Caton (1875) that responses from the human cortex could produce localised 'negative' waves in response to sensory, visual or auditory stimulation reportedly predates Berger's recording of the electroencephalogram (EEG), by almost 100 years (Serra and Serra, 1990). It was many years later, in the 1940s, that technology enabled the reliable recording of these electrical potentials, enabling non-invasive assessment of the function of the CNS. As a result, clinical practice evolved accordingly, which is evident in the literature with different groups formulating different diagnostic criteria that utilise these techniques to varying degrees over the years to select their patient cohorts. This makes comparisons between studies difficult.

Although researchers had previously been investigating the electrophysiology of the retina in optic atrophy, it was Gills (1966) who looked specifically at patients with a long history of MS. His early studies in patients with long standing disease revealed a reduction in many of the individual ERG b-wave amplitudes generated in the inner retina from the rod and cone pathways along with reduced cellularity of the inner nuclear layer on histological review. This unique study is the only one to combine structure and function in this way and has not been duplicated since. The degree of these abnormalities reportedly paralleled the severity and duration of the disease, with the earliest changes noted in the cone driven flicker and red flash responses based on disease duration. In his discussion, he contrasts this with reports that elevated ERG responses have been found in surgical section studies of the optic nerves, leading him to postulate that [in MS] a different mechanism must be responsible and 'that there may be other factors affecting the retina than the lesions of the optic nerve' (Gills, 1966).

These findings were partially contradicted by others who subsequently found a mixture of enhanced and reduced b-wave abnormalities with concomitant optic nerve involvement. One group consistently found these variable findings, initially, in a cohort of mixed neurological conditions and in a follow-up study containing MS patients only (Feinsod et al., 1971; Feinsod et al., 1973). They looked to animal studies to explain the unexpected and seemingly counterintuitive increased b-wave findings and postulated that the loss of 'centrifugal fibres', that had previously been demonstrated in birds and cats, may cause a reduced inhibitory effect at the bipolar cell level. This group were the first to divide MS participants into those with a previous history of ON and those without, and for the first time described subclinical involvement of both the optic disc and retina that was reportedly not related to disease severity (although this was not quantified statistically). The increase in ERG amplitude was only partially replicated by one another group who recorded an amplitude increase in the b-wave using a red flash; however, recording methods including stimulation and recording parameters varied greatly (Pierelli et al., 1985). They also postulated that centrifugal fibres but could not explain the isolated increase in amplitude of the cone driven red flash that was not seen with other flash stimuli.

The technical limitations in these early studies meant that flash ERGs were evoked with stroboscopes and recordings were typically made with Polaroid photographs of oscilloscope traces. There was considerable variation in the environmental and technical conditions that the authors acknowledge made comparisons between studies difficult. They did, however, generate interest, paving the way for future work.

Almost one hundred years after Caton's initial observations, a variety of different visual stimulators were developed with varying success to 'drive' to elicit the cortical and retinal responses. The stroboscope was later superseded by the development of ganzfeld and pattern stimulators that led to an explosion of research using pattern or steady state stimulation that produced reproducible, robust VEP responses with low intrasubject variability (Odom et al., 2016).

Halliday et al. (1973), are credited with the discovery that the pattern reversal VEP is reliably prolonged in optic neuritis and MS, and that the characteristic delays may be subclinical in nature in a high proportion of patients (>90%), even in the presence of normal examination.

This discovery, for the first time, provided reliable functional information about the visual pathway before MRI imaging techniques were widely available, marking a major milestone that was later incorporated into the Posner's diagnostic criteria for MS (Posner et al., 1983). This has been omitted in subsequent alternative versions, but it remains a recommended paraclinical test to identify previous inflammatory episodes (Thompson et al., 2018a; Solomon, 2019).

During the 1980s, studies by rival groups on either side of the Atlantic worked on delineating the generators of the individual retinal responses, reasoning that different retinal cells would respond differently to different stimuli, similar to the cortical generators of the VEP. Coupland and Kirkham (1982b; 1982a; 1983), in Canada, published several consecutive papers exploring the effect of manipulating the stimulus on the VEP, PERG and ERG in MS patients. They were able to demonstrate that orientation specific pattern VEPs increased the yield of VEP abnormalities in a small number (11%) of MS patients compared to the established reversal stimulus, and that a large proportion of the whole cohort had subclinical delays. In a separate paper, acknowledging Gills (1966), they used flash stimulation to evoke VEPs and ERGs that showed delays in both the VEP as expected but also in the b-wave implicit time of the flicker ERG (using skin electrodes). Their study recorded a binocular flash VEP which has no localising value when looking for unilateral optic nerve pathology due to desiccation at the chiasm, which was a curious choice as their cohort was divided into groups that included right, left, and bilateral ON. The choice to only use a flicker stimulus for the ERG confines the results to the cone driven responses due to their temporal resolution that was combined with 3 minutes of dark adaptation prior to testing. The small sample sizes of the right and left ON groups were combined in their analysis, which in the case of the ERGs, were abnormal in all of the patients, with more patients showing abnormalities in the bilateral ON group. The latency variability was described as greater in the patient group compared to controls, although this was not quantified. The flash VEP was abnormal in 75–80% of patients. This group were the first to analyse interocular latency differences and describe temporal dispersion of the ERG responses (although this was not statistically analysed). Although acknowledging previous work that had reported reduced and increased ERG b-wave amplitudes, the group confined their analysis to latency measures only in this paper. They concluded that there were demonstrable abnormalities in the distal unmyelinated structures of the retina in patients

with MS that, in this study, were independent of previous ON. They postulated that either transsynaptic degeneration to the inner nuclear layer or unrecognised 'humoral factors' may be responsible for the retinal delays they observed. On reflection, this may have been what Gills (1966) had been alluding to when he suggested that something else other than optic nerve pathology may be contributing to the abnormalities seen with optic nerve pathology in MS. The ERG studies to date, therefore, suggested pathological processes occurring in the eyes of MS patients that were secondary to demyelination. Or that separate mechanisms within the disease were responsible.

At about the same time, Arden et al. (1982) had shown, in children, the differing properties of full field ERGs and focal ERGs with manipulation of the luminance and contrast. This was made possible by the use of computer averaging techniques and improved amplifiers that enabled the separation of the two ERG components (now accepted as the P50 and N95, respectively) due to the innate small size of the responses.

In their final paper, Kirkham and Coupland (1983) used pattern reversal stimulation to simultaneously record the VEP and PERG in response to different check sizes. They use novel nomenclature to describe the baseline, P50 and N95 components of the PERG. On this occasion, they did include amplitude measurements, however, only of the initial component which they designated as 'Q', and they chose not to analyse the entire response neglecting the later component which has now become correlated with RGC function, representing an opportunity missed. PERG changes with optic nerve pathology have subsequently shown that that the initial component ('Q' or P50) can have a reduced peak-time (not amplitude) and that the proceeding component ('R' or N95) can be reduced (Holder, 2004). Not surprisingly, their analysis did not show any difference in the PERG response as they defined it between normal and MS patients.

The studies that followed frequently combined retinal recording in form of the PERG and the VEP to further localise areas of dysfunction within the visual pathway. Animal studies of the PERG had shown that complete dissection of the optic nerve resulted in progressive PERG deterioration and the development of optic atrophy, but the flash ERG persisted unaffected (Maffei and Fiorentini, 1982). This led to the theory that the PERG must be generated in the RGC layer in the proximal retina, and the mass response of the ERG must be distal to the RGCs.

Holder (1989; 1991; 1997; 2009) has contributed much to exploring the diagnostic value of VEP and PERGs, initially in identifying optic nerve dysfunction and using electrophysiology to differentiate it from retinal (macula) disease, which has since been widely adopted in clinical practice.

The introduction of the ISCEV standards for visual electrophysiology (1989), has facilitated greatly in ensuring methods for recording, measuring and comparing results are standardised, both in research and clinical practice. The majority of studies from this date use these the individual guidelines for VEP, PERG and ERG as a minimum.

6.3 Introduction of Structural Measures

The first study to include structural measures with the use of OCT was by Parisi et al. (1999), who described a difference in the RNFL thickness that correlated with changes in the P50 peak-time in a cohort of patients with a previous history of MS. Their sample size was small but was the first to show a difference in the P50 component thought to be partly derived from outer retinal cells (photoreceptors) and GCL. Since then, many studies have included the PERG as a physiological measure but found, in contrast, that a proceeding N95 reduction correlated well with OCT thinning (Almarcegui et al., 2010; Rodriguez-Mena et al., 2013). Additionally, the N95 correlated well with RNFL thinning that was independent of ON which remained constant over two years (Garcia-Martin et al., 2011). The discrepancy is difficult to explain given the different generators of the P50 and N95 components and has been attributed to retrograde degeneration of the optic nerve and subsequent degeneration of the RGCs. It may have been that Parisi et al. (1999) had selected participants at an early stage of the disease and therefore P50 peak-time delays representing cell dysfunction rather than atrophy were recorded. However, this is discrepant with the OCT findings.

The widespread adoption of spectral domain OCT (SD-OCT), from approximately 2006, enabled the rapid acquisition of higher resolution images than with the previous time domain machines (Fujimoto and Swanson, 2016). The literature reflects the ease and accessibility of these measures with all of the later studies including these measures. It is now generally accepted that thinning of the GCL and RNFL is a feature of MS and may contribute to

documenting the disease but has not yet been incorporated into diagnostic criteria (Petzold et al., 2010; Saidha et al., 2011; Saidha et al., 2015). What is still not clear, is the temporal relationship and how this relates to the different phenotypes. Several authors revisited the relationship between VEP and OCT with SD-OCT and found that VEP remained correlated with RNFL thinning (Hamurcu et al., 2017) even when disease duration was relatively short (Behbehani et al., 2017).

Forooghian et al.(2006; 2007) were the only group to explore the relationship between ERGs and circulating antibodies in MS, in a search for an autoimmune marker to further explore retinal changes that may involve more distal layers. Their first study demonstrated higher antibodies in a subset of MS patients that also had delayed DA b-wave peak-times and reduced oscillatory potentials compared to controls. In their second paper, they specifically looked for alpha-enolase antibodies (in the same cohort), which are associated with auto-immune retinopathy mediated by T-cells, causing apoptosis. The levels of these antibodies were found to be higher in a proportion of the MS patients (38%) than controls; however, there was no correlation with the ERG peak-time delays. This reportedly contradicts findings from a separate group who found decreased ERG amplitudes that correlated with alpha-enolase antibodies (Gorczyca et al., 2004).

6.4 Correlating Structure and Function

Importantly, Green et al. (2010) performed pathological studies in a cohort of mixed phenotypes in MS and were able to confirm inflammatory changes in the RNFL and GCL that were independent of disease duration. They also found atrophic changes in the inner nuclear layer (bipolar and horizontal cells), but not the outer nuclear layer, that appeared to be related to the changes in the RGC and RNFL, but they were not able to quantify this. This study provided definitive anatomical evidence that provided some support to the structural and physiological measures that had been made up to this date.

Saidha et al. (2011) followed this by describing a distinct subset of MS patients that were found to have macular and INL thinning in the absence of ON or the typical RNFL and GCL clinical signs associated with ON, and in contrast, they tended to report photophobia and

positive signs such as photopsia and glare. This new finding or subtype has not been reported elsewhere but is important as the authors suggest that this group experienced an accelerated deterioration compared to both a group of MS patients with normal macula thickness and a group with thinning of the RNFL and GCL. Their study reinforces the findings of Green (2010) and adds further weight to a separate retinal process in some patients with MS. In practice, the diagnosis of ON is largely based on clinical assessment, although diagnostic criteria have been proposed (Petzold et al., 2022). Similar to the MS criteria, the ON criteria are based on a combination of clinical and paraclinical evidence that provides a framework for diagnosis that also categorises the many diseases that may feature ON. The use of electrophysiology is not a recommended paraclinical test in the criteria, but it is mentioned as a useful measure when VEPs are used along with PERGs (Holder, 2004) to help assess complex cases and functional visual loss. The omission of electrophysiology in the acute stage is entirely reasonable given that acuity is often reduced and would limit the patient's ability to perform the test.

Hanson et al. (2018) were the first group to attempt to specifically address the question as to how the outer retina is affected in MS with a longitudinal study over 3 years. Their first study provided baseline evidence of outer retinal dysfunction without any observable changes in OCT measures. Their cohort were recruited from a group with established disease both with and without previous ON. They found differences between four of the seven ERG peak-times measures (DA and LA) and mild evidence of a single amplitude measure (DA 10 b-wave) in healthy volunteers. There were no differences in those participants with or without previous ON. They did report the expected GCL and IPL layer changes that were significant in those with previous ON. Thus, their electrophysiological findings suggested dysfunction to the photoreceptor and inner retinal layers in the absence of the corresponding OCT changes. The suggestion that retrograde degeneration of the optic nerves may be responsible seems unlikely given that there were no OCT differences between the groups; instead, the findings suggest a primary retinal process. The authors acknowledge the absence of myelin in the retina and therefore antigens against it are unlikely, but they do not suggest that an alternative inflammatory process may be responsible. Instead, they suggest that the changes may be related to faulty neurotransmission, specifically regarding glutamate, the most prominent excitatory neurotransmitter within the brain which is related to T-cell function in

MS (Levite, 2017). In their follow-up study (2021), they did not find any significant progression in the previously noted ERG abnormalities, reporting that they appeared stable and within the measurement accuracy limits of the equipment. This group were the only group to measure ERGs using a 'normalised' method by using the a:b wave ratio rather than absolute values, which departs from the ISCEV standard. OCT variables also remained stable despite some EDSS deterioration between studies. There was some loss of participants in their follow-up study, which the authors also acknowledge.

Another longitudinal study performed by You et al. (2018b) compared an established MS group without previous ON with controls, although VEPs were not recorded. This group recorded ERGs peak-time delays in two ERG stimuli (DA 3.0 a-wave and LA 3.0 b-wave) involving the rod and cone pathways. The authors were able to correlate the ERG differences at baseline with reductions in GCL-IPL and average RNFL as well as GCL-IPL layers, respectively. No ERG amplitude measures were found to be significant when compared to controls at baseline. Additionally, they were able to correlate the observed ERG changes with other parameters including disability, lesion load and disease duration. After 3 years, there was a reduction in a number of the ERG DA and LA a and b wave amplitudes. The most significant of which was an 11% reduction in the rod mediated (DA .001), involving the rod bipolar cells. No ERG peak-time measures were found to have changed at follow-up. OCT measures showed a reduction in RNFL and GCL-IPL measures over the 3 years. At the end time-point, correlation remained between the ERG variables and disease duration and disability but not with lesion load, which may reflect grey matter changes and neurodegeneration rather than WMLs associated with relapses. This group also experienced some loss of participants over the course of the study. This provides further evidence to support outer retinal dysfunction in MS. However, the findings differed from that of Hanson et al. (2018) where peak-time delays were the most prominent finding and no significant progression was seen over a similar period (Hanson et al., 2021). The changes, it was postulated, could be due to subclinical inflammation similar to that seen in other inflammatory retinopathies where ERGs are commonly used to facilitate diagnosis and monitor progression. Or there may be localised inflammation related to infiltrates and blood vessels. A limitation of this study is that no VEPs were recorded, which does not exclude clinically silent optic ON and may explain the progressive deterioration, although the literature is unclear as to whether this is relevant. The lack of correlation with

lesion load may reflect the disease course, which appears to include less relapses (associated with WMLs) with time.

Several authors have compared OCT and ERG measures in MS with NMODs to explore any differences between the conditions that have many over lapping clinical features but differ in their treatment. You and colleagues (2019) found amplitude reductions confined to the dark-adapted b wave along with thinning of specific retinal layers in aquaporin+4-IgG MNOSD compared to those without the IgG marker, MS patients and controls. However, this is not a demyelinating disease, and the authors were looking at methods to differentiate the disease. They used a novel measurement system outside of the ISCEV guidelines to separate the cells that contribute to the b wave (bipolar and Muller glial cells) to further localise the area of dysfunction which they suggest is the Muller cells in this rare condition. An earlier study by Hokazono and colleagues (2013) found good correlation between OCT and PERGs amplitude measures when detecting RNFL thinning.

There is only one study concerned with treatments, and that is with steroid treatment of ON. Nowacka and Lubinski (2021) retrospectively compared participants with a history of ON that had been treated with intravenous steroids with those who have not, evaluating them at an average of 5 years later. They performed OCT, VEP and PERGs, along with a VA, physical examination and visual field testing, none of which were significantly different. The group studied had an established diagnosis with an average duration of 5 years, but the authors did not report if they had received any DMTs, which may be a potential confound. The use of OCT has been used in some DMT trials as an outcome measure, with the INL being shown to dynamically respond to both inflammation with thickening and normalisation with DMT (Knier et al., 2016; Hanson et al., 2018). This further suggests that other mechanisms other than those affecting the RNFL and GCL are a feature of the disease and supports work of others (Saidha et al., 2011).

6.5 Summary

There is a clear narrative in the literature that sees investigation of the visual pathway evolve over time, moving from assessment of the entire pathway with the VEP, moving more distally to the retina. This has paralleled the understanding of the disease process, which was originally thought to be a CNS disease of the white matter, whereby antibodies target myelin, to now being accepted as equally being a disease affecting the grey matter. This has been concomitantly illustrated with technological advances in MRI and OCT imaging and electrophysiological techniques that have revealed additional aspects of the disease. Heterogeneity in study design adds additional perspectives but makes comparisons difficult.

Pathological and electrophysiological changes have been demonstrated in the outer retinal layers in a small number of studies with ERGs that may be a means of measuring damage before structural changes occur. The literature would suggest that these changes are independent of previous ON episodes and are likely, therefore, to be subclinical in nature. Whether this occurs independent of structural damage and could be used in clinical practice or research is also uncertain.

Summary -Literature Review

- Early studies were limited by the technologies at the time.
- The lack of myelin in the retina provides opportunity for exploring MS from a different perspective adding to the pathophysiology of the disease.
- Studies to date have been limited and have found variable ERG changes.

Summary: Concept and Feasibility

- The study aimed to quantify the number of patients that get referred to a specialist MS team that present with visual symptoms.
- Structural and functional measures of the visual system were made in patients at the beginning of their diagnostic journey and compared to healthy controls.
- To look for evidence of retinal involvement in this inflammatory condition with or without optic nerve involvement.
- A power calculation was performed to determine how many participants would be required to make the study meaningful.

Chapter 7. Concept and Feasibility

The lack of definitive diagnostic tests and the high propensity for visual pathway involvement in MS motivated this study into the structure and function of the retina, by adding to the routine standard of care investigations.

The aim of the research was to ascertain if there are differences in the electrophysiological function of the visual pathway of patients newly diagnosed with MS compared to healthy controls. This generated the following hypothesis:

There is a measurable difference in the function of retinal cells in patients with newly diagnosed multiple sclerosis compared to healthy controls.

Additionally, the following secondary aims were to be investigated:

- To assess retinal structure with OCT in patients and compare to healthy volunteers.
- To assess ERGs with optic nerve VEP measures to assess for clinical and subclinical ON.
- To correlate any differences between structure and function between the groups.
- To quantify any previous clinical episodes that precede the diagnosis.
- To use post hoc analysis to look for grouping after the final diagnosis has been given, which will include participants not diagnosed with MS.

7.1 Participant and Patient Involvement

Four focused telephone interviews were arranged through the specialist MS team to inform the recruitment process. Common themes from the interviews suggested that patients would like direct contact, in person or over the telephone, to explain the study which would be supplemented by information sheets. Most indicated that they would be happy to participate on the day of their routine appointment, although logistical issues such as childcare would have to be considered, along with not being able to drive home afterwards. The wide geographical catchment area of referrals was highlighted as a potential problem for some patients but was not deemed preventative. This information was used to help compile information sheets and inform the study protocol.

7.2 Preliminary Data

Prior to recruitment, there was a review of all patients referred by the MS team to the neurophysiology department over a three-week period for routine standard of care investigation. Referrals and patients were reviewed with regards to the number of patients seen and the number presenting with visual symptoms in one or both eyes, and abnormalities were categorised (Table 3). This included one patient that was referred for additional ERG testing on clinical grounds. Based on this information, the study would recruit enough patients with a mixture of symptoms within approximately 16 weeks subject to consent.

Patient	Visual symptoms	VEP	PERG	ERG
1	Y	n	ab	
2	N	n	n	
3	Y	ab	n	
4	N	n	n	
5	N	n	n	
6	Y	ab	ab	ab
7	N	ab	n	

Table 4. Review of patients and referrals to the neurophysiology department. Y-Yes, N-No, n-normal, ab-abnormal.

7.3 Statistical Analysis

A power calculation was performed based on previous studies using Minitab 18 software. Using a two-tailed t-test, with a significance level a=0.05 and an assumed standard deviation of 1.0 (Hanson et al., 2018), two groups each of n=35 will give a power of at least 0.90 (90%) for finding a difference of 0.79 between the group means.

Participants were given time to consider their participation and any additional requirements that may be needed. Any additional costs such as travel, and parking would be reimbursed with production of receipts.

Summary: Concept and Feasibility

- The patient interviews suggested that patients were willing to contribute to the study if consideration to their personal circumstances were made, e.g., travel time, caring responsibilities.
- Based on current referral rates the study would recruit enough patients within the timeframe.
- The number of participants required to power the study was calculated to be 35 for each group, which was thought to be achievable within the time constraints.

Summary: Method

- Patients who were thought to have MS at the start of their diagnostic journey were prospectively recruited from the regional specialist team.
- Additional recordings were made to the routine standard of care tests, to assess retinal function and structure.
- Clinical outcomes were recorded at least 6 months after recordings were made to check the eventual diagnosis.

Chapter 8. Method

This is a prospective cross-sectional study that recruited newly referred patients to the specialist MS service for the south-west region. Written and verbal consent was obtained from both the patients and healthy volunteers. All subjects underwent OCT and ERG testing as well as the routine standard of care investigations (VEP and PERG) where appropriate. OCTs were recorded by the lead optometrist, for the NHS Trust, who also prescribed mydriasis for ERG testing. Electrophysiology was performed by a single trained clinical scientist, the principal investigator.

Patients were fully informed prior to attending and given the option to withdraw their consent at any time. No patient has subsequently requested to be removed from the study and most patients have requested to be updated by email with a summary, once the study is complete.

8.1 Inclusion Criteria

Consecutive patients suspected of having MS were recruited irrespective of their presenting symptoms and were referred for the routine standard of care investigations including VEP and PERG. Where optic neuritis was the presenting symptom, testing was not performed in the acute stage of the disease due to reduced visual acuity. Patients were asked to bring their current spectacles where appropriate, and testing was performed with optimum correction, i.e., with or without glasses.

Recruiting from the MS team had the advantage of previous triage by a specialist in the field, thus removing unsuitable referrals from general practice and non-specialists.

No participant had been commenced on disease modifying treatments at time of testing.

Healthy volunteers were recruited through internal advertising, with similar age and sex distribution.

Clinical outcomes were obtained from the patient records and in consultation with the specialist team if there was any diagnostic doubt. This meant that outcomes were recorded at approximately 18 months after data collection started, which was approximately 6 months after the last patient was recruited. Recruitment was interrupted and data collection ceased on several occasions during the COVID-19 pandemic.

8.2 Exclusion Criteria

Exclusion criteria included a history of diabetes with peripheral neuropathy or retinopathy, ocular disease or injury, neurological disease, or pathology that may affect the visual system e.g., cranial nerve palsy affecting vision.

High refractive errors, in particular, high myopia >6 diopters, was regarded as contraindication as it can result in altered retinal structure.

These criteria were applicable to both participants and healthy volunteers.

8.3 Statistical Analysis

Sample sizes were determined by a priori power calculation which provided the minimum numbers based on the predicted mean differences in the outcome measures.

Statistical analysis compared structural and functional variables between the participants and healthy volunteers. This was followed by comparison between participants with prolonged VEP measures and those with normal VEP measures and healthy volunteers.

Right and left eyes were subject to the same separate analysis for each healthy volunteer and participant. This was due to the reported high incidence of subclinical optic nerve involvement and the risk of missing or wasting data if a single eye was analysed. There is acknowledgement that most statistical tests assume independence of measurements and do not account for the high correlation between measurements from eyes of the same subject, which can introduce bias. For this reason, measurements from each eye were not combined to increase the number of measurements.

Each participant's medical record was checked for duration of disease and if there had been any prior presentations suggestive of MS. The nature of MS often means that, in hindsight, symptoms may have started some time before presentation and were dismissed due to resolution and a return to normal function.

8.4 Blinding

Each participant was given a unique study number, only known to the principal investigator. Testing was performed at the time of the patient's diagnostic work-up, and neither the clinical scientist nor the optometrist was aware of the final diagnosis at the time of testing.

8.5 Data Management

The OCT machine and the electrophysiology equipment are located in the Royal Eye Infirmary and neurophysiology departments with University Hospitals Plymouth NHS Trust, respectively. Both machines are password protected and stored in locked rooms. Data is acquired to the local data base then transferred to dedicated Trust servers that are backed up overnight. Access to review the data is granted by named individuals and subject to local governance guidelines and requires review software (University Hospitals Plymouth NHS Trust, 2022a).

8.6 Approvals

Ethical approval was granted by NHS HRA (South-West) committee on 10 August 2020, IRAS project ID: 271835. Manchester Metropolitan University EthOS approval was also granted, reference: 35530. The study adheres to the Declaration of Helsinki.

8.7 OCT Imaging

All patients underwent imaging with Carl Zeiss Cirrus 5000 HD-OCT, software version 11.5.2.54532, firmware 1.100.0.11.

Technical specifications include 27–68 k A-scans per second, A scan 2.0 mm (in tissue), 1024, superluminescent diode (SLD) 840 nm, axial resolution: 5μm (in tissue), transverse resolution 15μm (in tissue) (Zeiss, 2015). The equipment is maintained and serviced by a Zeiss representative annually.

Automated eye tracking was used to reduce movement artefacts and increase image quality. Measures of macula, retinal ganglion cell layer and the RNFL thickness were made with attention to fixation and signal quality. If necessary, scans were repeated to ensure adequate signal to noise ratio using the equipment's quality indictor. All scans were recorded with a quality score of greater than 6/10.

Normative data acquired by the manufacturer is included in the software that is used in routine clinical practice; this was acquired in a diversified population divided into decades (Zeiss, 2015).

Three routine scan protocols were used on each eye:

1. A scan of the optic disc measuring the thickness of the RNFL and optic nerve head (ONH).
2. A macular thickness scan of the macular cube 512 x 128 inner limiting membrane (ILM) RPE horizontal scan
3. Measurement of the RGC layer which by default includes the inner plexiform layer (IPL).

8.8 Electrophysiology

Pattern reversal VEPs were recorded using a 1-degree check and field size of 24.8 degrees, in the same environment for all patients. Ambient light levels were monitored over consecutive days using a digital Lux meter (BT -881D) and found to be constant at 520 Lux (Appendix 3).

Measures of the visual system were made using Diagnosy's 'Espion' visual electrophysiology equipment with CRT and ganzfeld stimulators. Equipment was maintained and calibrated annually by a Diagnosys representative in the UK (Appendix 4). Consistent with the routine standard of care, the 'Queen's Square' montage was used that utilises a midfrontal reference electrode (Fz) and active electrodes over the midline and lateral occipital areas (Blumhardt et al., 1977). Each test was repeated to check for reproducibility with traces superimposed before measurements were made.

VEPs were performed with corrected vision where appropriate to enable adequate fixation as per recommended guidelines (Odom et al., 2016).

Mydriasis was administered by the PI for ERG testing in line with routine standards of care for ERG testing.

Participation was monitored during flash ERGs with the equipment's built-in camera and with a webcam during PERG and VEP testing. Attention to the task and cooperation was encouraged throughout by the PI as per normal testing procedures (University Hospitals Plymouth NHS Trust, 2022b).

8.9 Data Security

Data was stored on a designated server accessible only with organisational permissions. All clinical records are kept in accordance with the NHS records management code of practice (2021) and local policy (University Hospitals Plymouth NHS Trust, 2022a).

8.10 Data and Statistical Methods

Data was analysed using SPSS version 27; figures of analysis were created in Microsoft Excel version 16.66.1.

Continuous variables were tested for normality using the Shapiro-Wilkes test and described using the mean and median along with the standard deviation (SD) and inter-quartile ranges (IQR), respectively. Comparison of the groups was made using the appropriate analyses depending on the distribution of the data, e.g., Student's t-test or Mann Whitney.

Results are graphically represented with boxplots that include outlying values in order to identify possible errors and interesting cases. In this version of SPSS, boxplots are used to represent the interquartile range. Mild outliers are those data points that are 1.5 times below the first quartile or above the third quartile and are represented with a circle in in this software. No extreme outliers were identified in the study.

Where continuous data is subdivided into more than two groups, the Kruskal Wallis test is used for comparison.

Categorical variables are illustrated as percentages and frequencies. The Pearson Chi squared test is used to compare the number of males and females between the two groups. Bar charts are used to represent this data.

For intra-subject comparisons, the paired t-test was used for normally distributed variables, and the related samples Wilcoxon signed-rank test for non-normally distributed variables.

Logistic regression was used to further investigate the association between those variables that are significantly different between the groups.

ROC curves were plotted to assess the sensitivity and specificity of any variables that are found to be different between the groups.

For all statistical tests, a p value of below 0.05 was considered statistically significant. All tests also assume a two-sided 95% confidence limit.

Summary: Results

- Patients presented with a variety of symptoms, some of which were subsequently attributed to alternative causes.
- Subject measures of Visual Acuity (VA) were significantly different between participants and healthy volunteers.
- No significant differences were found in structural OCT measures between healthy volunteers and participants.
- Functional measures were different across several variables involving the optic nerve (VEP) and some retinal measures (ERG) between the groups.
- MS participants when divided by VEP P100 peak-time did show differences in both rod and cone mediated ERG amplitude variables.

Chapter 9. Results

A total of 36 participants were recruited from new referrals to the specialist neurology service with suspected MS. One participant was excluded from the study due to poor cooperation during the OCT recordings. Of the remaining 35 participants, 13 were male and 22 were female, reflecting the female predominance of MS (Rolak, 2002), with a median age of 42 years (range 24–65 years). An equal number of healthy volunteers were recruited locally through internal advertising and word of mouth. There were no significant differences in the number of males and females between the two groups, p = 0.61 or their mean ages, p = 0.60 (Table 5).

	Participant	***Healthy Volunteer***	***Statistical Comparison***
Sex ***Male*** ***Female***	 13 (37.1%) 22 (62.9%)	 11 (31.4%) 24 (68.6%)	X^2 0.25[a] p 0.61
Age (years) ***Mean (SD)*** ***Median (IQR)***	 42 (12.5) 42 (23)	 40.3 (14.1) 35 (28)	P 0.60[b]

Table 5 Age and Sex distribution of participants and healthy volunteers

[a] Pearson Chi Squared [b] Mann Whitney

Presenting symptoms encompassed a variety of clinical signs and were grouped by type into cognitive, visual, sensory, and motor that encompassed a variety of clinical signs. Most participants presented with sensory symptoms that included pins and needles, paraesthesia, and numbness, typically localised to one limb or the trunk. Visual symptoms were often associated with pain in the affected eye but also included double or blurred vision. Cognitive difficulties included poor concentration, dysphasia, and general fatigue, leading to difficulties in performing day to day tasks. Motor symptoms were confined to weakness, usually isolated to a hand or limb. Some participants presented with a combination of symptoms in multiple areas (Figure 32). All clinical data was collected from the specialist team's patient record system (iMed).

Retrospective review of patient records revealed a positive family history in 5 patients (first or second degree relative). Ten patients had had a prior episode that was suggestive of a possible visual or sensory disturbance but had either not sought care or had been discharged from medical care with no further follow-up.

Of the 35 participants with suspected MS, 10 were eventually given alternative diagnoses: comprising vascular disease (n=4), functional disease (n=2), migraine (n=2) and no clear diagnosis at the point of reviewing (n=2). This group were excluded from subsequent statistical analysis. The remaining 25 were diagnosed as 'definite MS' or 'probable MS' based on the McDonald diagnostic criteria (Thompson et al., 2018b) and treated as a single group for statistical purposes.

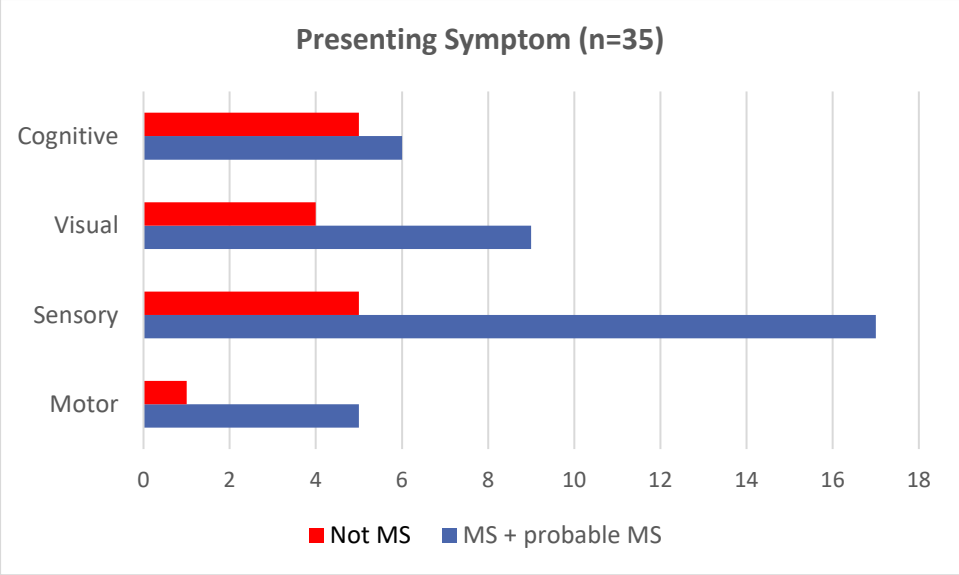

Figure 32. Participants presenting symptoms.
MS and probable MS (blue: n=25) and those who were not given an MS diagnosis (red: n=10).

MRI imaging across the whole participant group (n=35) showed abnormal findings in every subject, with some participants exhibiting multiple lesions in various locations (Figure 33). Typical findings in keeping with suspected diagnosis included white matter lesions within the spinal cord, or periventricular or juxta cortical regions of the brain. Some participants were found to have 'Dawson's fingers' phenomena, where inflammation is found along the axis of the medullary veins (as opposed to isolated lesions) that are found perpendicular to the lateral ventricles. This is considered a common but not specific finding in MS (Kaschka et al., 2014; Lv et al., 2020).

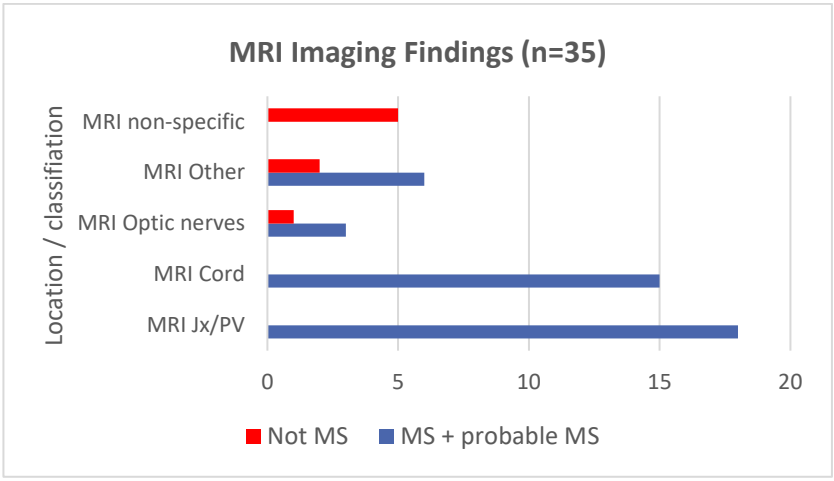

Figure 33. Location of MRI abnormalities at presentation
(Cord – spinal cord, Jx, Juxtacortical, PV periventricular)

The group of participants given alternative diagnoses had abnormal MRI findings, often described as 'non-specific' or where brain volume appeared to be reduced in the absence of typical demyelinating lesions. One participant had inflammation of the left optic nerve but was ultimately diagnosed with MOG antibody disease.

Reducing the healthy volunteer group to an equal number (n=25), did not alter the demographic data (age: mean 40.9years, SD 13.9years, median 43.0years IQR 28years).

9.1 EDSS

Patient's EDSS score was recorded at the time of diagnosis for 22 MS patients (Figure 34). There was no score recorded for 3 MS patients until they subsequently started treatment. Of the patients given an alternative diagnosis, only three had an EDSS recorded (P16: 1.5, P26: 4.5, P52: 6).

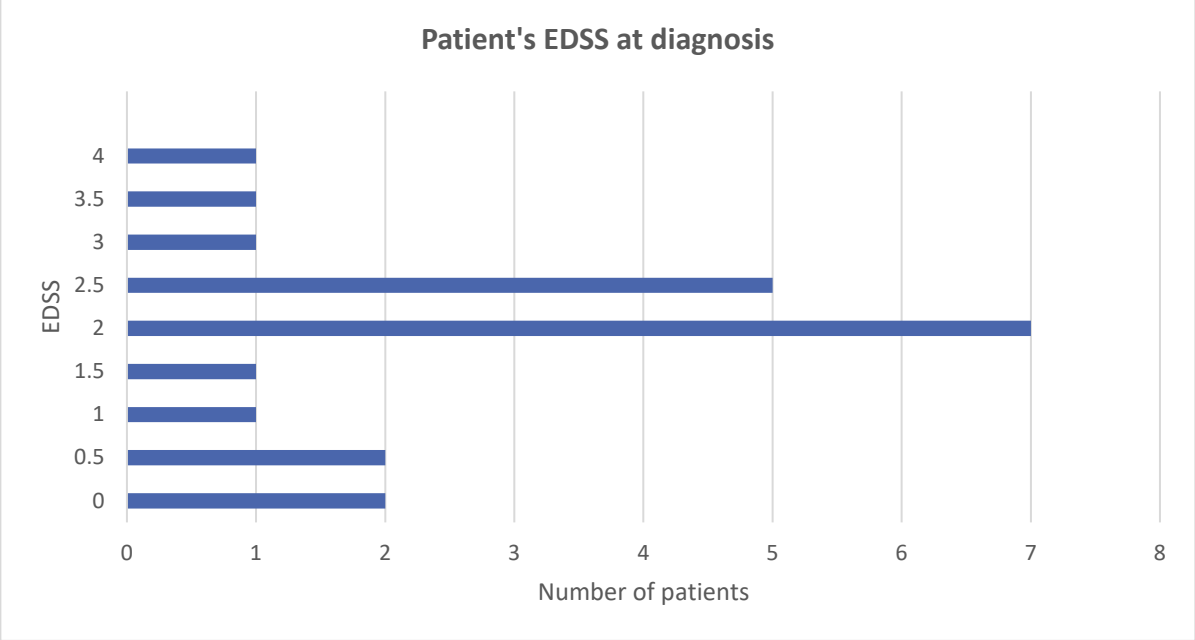

Figure 34. Extended disability score (EDSS) at time of diagnosis (4 patients had no EDSS recorded at the time of diagnosis).

A normal EDSS score of 0 indicates no loss of function (n=2). The most frequently occurring score of 2 in this sample (n=7), indicates a very small disability in one functional area. Mild disability is indicated by a score of 2.5 (n=5). Moderate disability is indicated by a score of 3 (n=1), but patients retain the ability to walk independently. A score of 4 indicates significant disability (n=1), but patients retain the ability to walk 500 m unaided (Kurtzke, 1983). There

are two patients with a score of zero and two with a score of 0.5, indicating no disability or minimal disability.

9.2 Visual Acuity (VA)

Patients were asked to bring any current prescription glasses with them prior to attending. All participants that wore glasses had had a routine sight test within 2 years of attending and wore their glasses as appropriate.

Nine of the participants with a diagnosis of MS or probable MS (10 eyes) presented with visual disturbance prior to testing, suggestive of ON (4 of the excluded participants also presented with visual disturbance). Of those included in the analysis, five presented with symptoms in their right eye, three in their left, and one presented with bilateral symptoms.

LogMAR charts were used to subjectively assess corrected VA for each eye. Mean LogMAR scores were significantly better in healthy volunteers than participants for the right eye ($p=0.007$) and the left eye ($p=0.048$) (Figure 35). Both the participant and the healthy volunteer groups included some outliers. Only one participant from the MS/probable MS group had a difference of VA >0.2 LogMAR between their 2 eyes. Two of the participants from that group also had >0.2 LogMAR difference between their two eyes but were subsequently removed from the analysis due to not having the diagnosis.

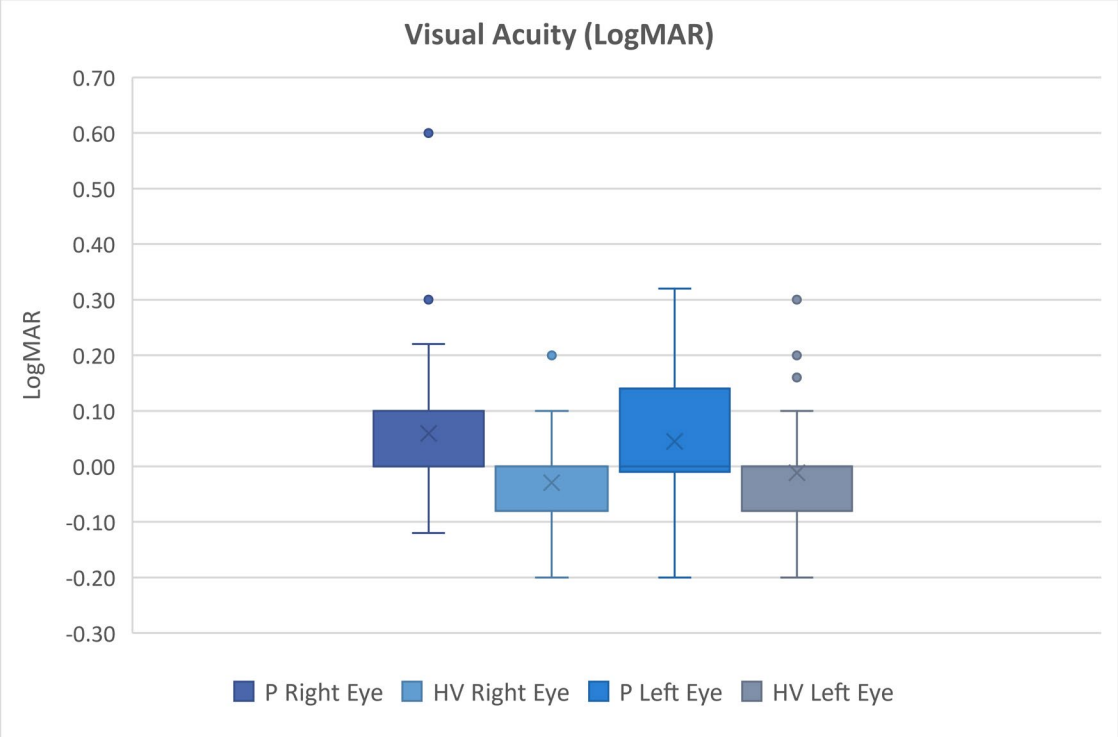

Figure 35. LogMAR visual acuity scores
P – participant (n=25), HV – healthy volunteer (n=35).

9.3 Structural Measures:

9.3.1 Macular Thickness

	Side	Participant Mean (SD)	Participant Median (IQR)	Healthy Volunteer Mean (SD)	Healthy Volunteer Median (IQR)	Test statistic	t-test p
Macular Thickness (μm)	Right	268.4 (17.0)	269.0 (22.5)	264.1 (23.1)	265.0 (31.0)	0.789	0.433
	Left	267.5 (16.7)	265.0 (25.5)	263.3 (23.5)	264.0 (35.0)	0.766	0.447
RNFL (μm)	Right	91.6 (9.1)	91.0 (10.0)	92.1 (11.5)	91.0 (15.0)	-0.200	0.842
	Left	89.6 (11.2)	89.0 (13.5)	92.2 (12.1)	91.0 (13.0)	-0.861	0.393

Table 6. Average macula and RNFL thickness
Healthy volunteers (n=35) and participants (n=25)

There were no statistically significant differences detected in macular thickness between the groups (t-test: RE p=0.433, LE p= 0.447), (Table 6). Although not significant, the standard deviation and IQR was greater in both the left and right eyes for the HV group. There were no outlying values recorded for either group (Figure 36).

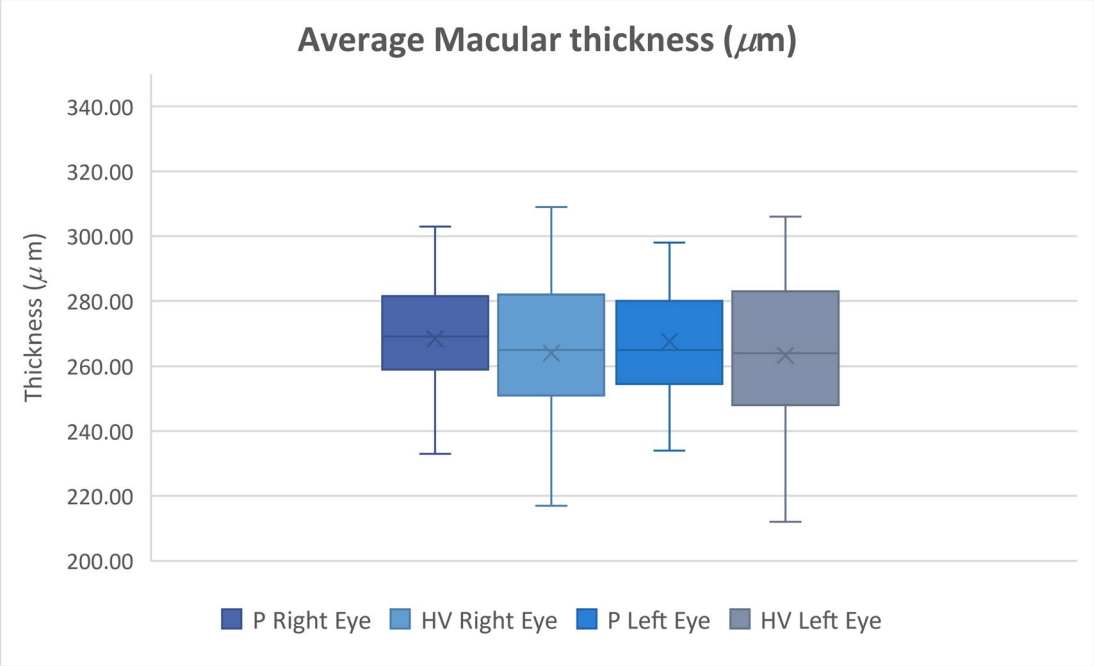

Figure 36. Average macular thickness

Healthy volunteers (n=35) and participants (n=25)

9.3.2 Retinal Nerve Fibre Layer

RNFL measures were also not considered significantly different between groups (t-test: RE p= 0.842, LE p=0.393). Outliers were all in the positive direction, indicating a greater thickness for both groups, apart from one outlier in the heathy volunteer group (HV 20:LE), (Figure 37). Similar to the macula thickness results, the range of values was mostly greater for the HV group compared to participants but did not reach statistical significance.

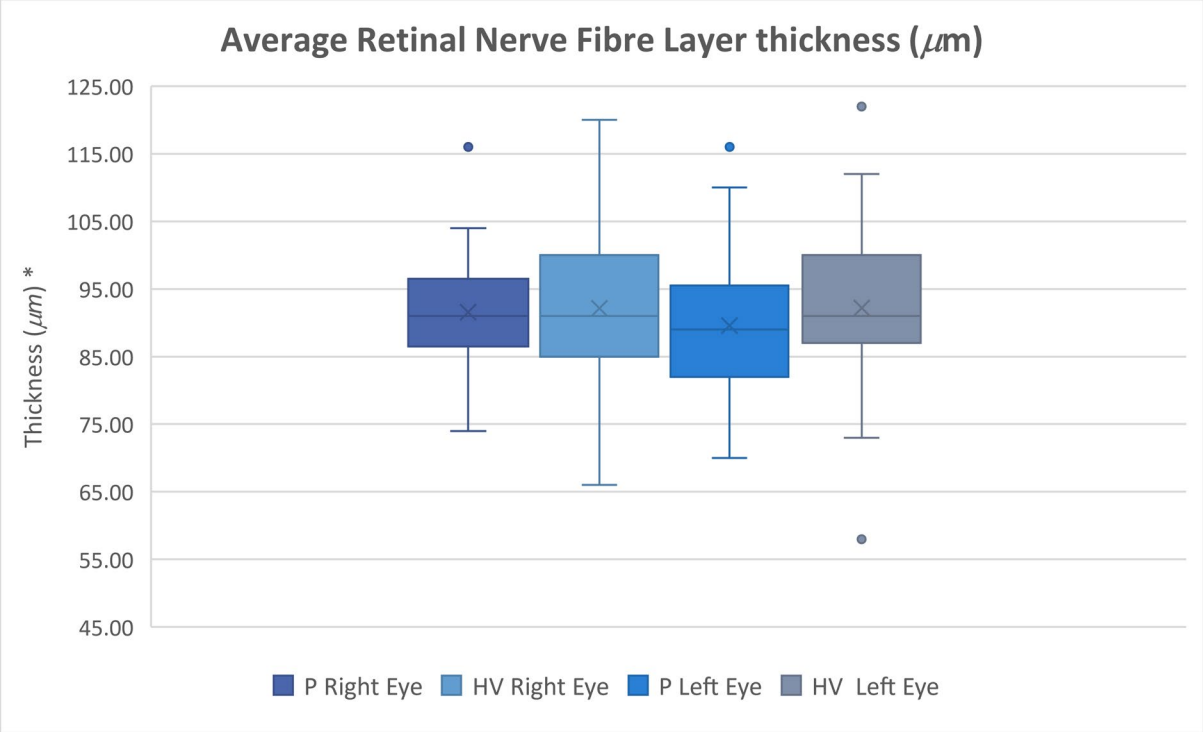

Figure 37. Average RNFL thickness.

Healthy volunteers (n=35) and participants (n=25) Y axis – scale adjusted.*

RNFL measurements were further subdivided into average thickness per quadrant (superior, inferior, temporal, and nasal) for each eye, looking for localised structural change (Figure 38). No significant difference was found between the two groups for any of the four quadrants. A clear average thickness distribution is seen for each quadrant for both groups that ranges from thickest to thinnest in the order: inferior, superior, nasal and temporal. This was consistently found for both healthy volunteers and participants. Outlying results were again mostly in a positive direction, apart from 2 values recorded from the superior quadrant in HV group (HV 20 RE and LE)

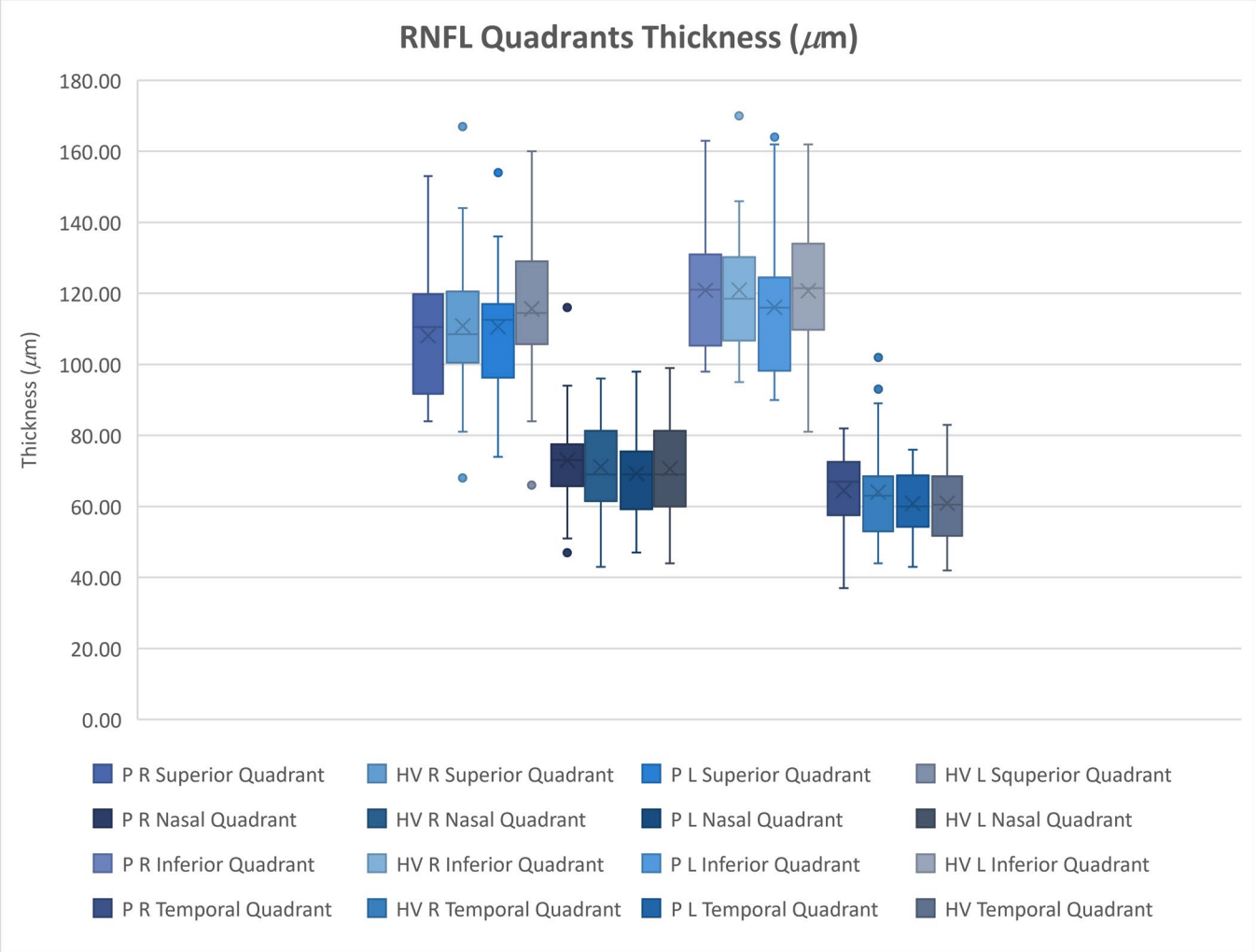

Figure 38. Average RNFL divided into quadrants.

Healthy volunteers (n=35) and participants (n=25) R- right, L- left

9.3.3 Retinal Ganglion Cell Layer

	Side	*Participant Mean (SD)*	*Healthy Volunteer Mean (SD)*	*Participant Median (IQR)*	*Healthy Volunteer Median (IQR)*	*Test Statistic*	*t-test p*
RGC (+IPL) Layer (μm)	Right	79.5 (7.6)	82.2 (6.2)	79.0 (8.5)	82.0 (6.2)	-1.539	0.129
	Left	80.2 (7.8)	81.1 (6.1)	80.0 (8.5)	82.0 (9.0)	-0.627	0.533

Table 7. Average GCL thickness

Healthy volunteers (n=35) and participants (n=25)

There were no significant differences in the mean thickness of the retinal ganglion cell layer between the groups (Table 7). The test protocol records a combined thickness of the RGC and inner plexiform layers (IPL).

9.4 Electrophysiological Measures

9.4.1 Visual Evoked Potential (VEP)

	Side	*Participant Mean (SD)*	*Healthy Volunteer Mean (SD)*	*Participant Median (IQR)*	*Healthy Volunteer Median (IQR)*	*Test Statistic*	*p*
VEP Peak-time (ms)	Right	114.4 (15.8)	103.5 (4.7)	108 (20)	104 (7.0)	257	**0.007**[a]
	Left	109.5 (12.3)	104.5 (5.4)	107.0 (12.7)	104.0 (7.0)	350	0.191[a]
VEP Amplitude (μV)	Right	11.2 (4.9)	12.5 (5.2)	10.9 (14.4)	11.1 (7.1)	-1.032	0.306[b]
	Left	10.4 (5.1)	12.4 (4.2)	8.6 (8.9)	11.5 (6.2)	-1.701	0.094[b]

Table 8. VEP peak-time and amplitude measures

Comparison of peak-time and amplitudes of the VEP P100 of both eyes were made for the both groups (Table 8); a significant delay in peak-time was recorded for the participant group, right eye P100 peak-time (t-test: p=0.007). No other VEP measurements were found to be significantly different between the two groups. The range of values for the participant group was greater for both eyes compared to the HV group.

There were two peak-time outlying measures recorded from two different participants (P53 and P41) for the right and left eyes, respectively (Figure 39.). Interestingly, P53 did not present with any visual symptoms and yet recorded the longest VEP P100 peak-time of the group. P41 presented with symptoms in both eyes. Although not significantly different, the left eye

median VEP peak-time from the participant group was longer than the same left measurement in the HV group.

Local departmental normative data suggests a cut-off value of 113ms, which is applied in clinical practice for the upper limit of the P100 peak-time, which was based on previously gathered data in normal volunteers (University Hospitals Plymouth NHS Trust, 2022b). A total of 12 participants recorded a P100 peak-time greater than 113ms, of which 6 were accompanied by visual symptoms while 6 participants were not (P13, P25, P27, P31, P33, P53). Some participants had bilateral VEP P100 delays (P13, P25, P27, P32, P41).

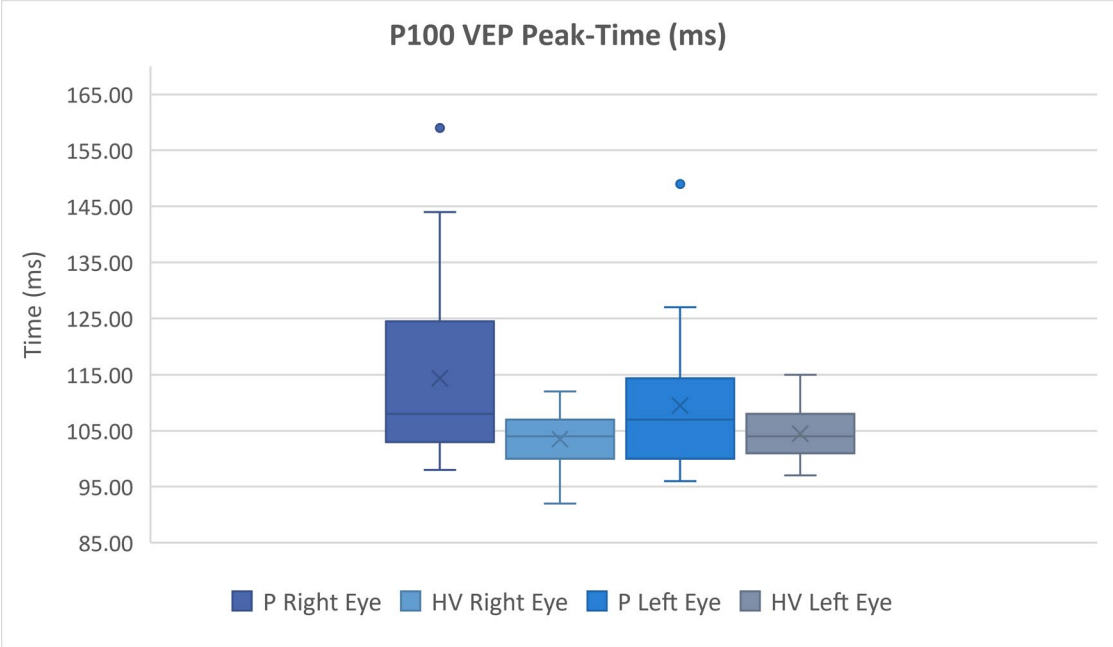

Figure 39. VEP P100 Peak-times for the right and left eye for P – participant (n=25), HV – healthy volunteer (n=35)

The P100 amplitude measured from N75 to P100 was not significantly different between the two groups (t-test: RE p= 0.306, LE p = 0.094) (Figure 40).

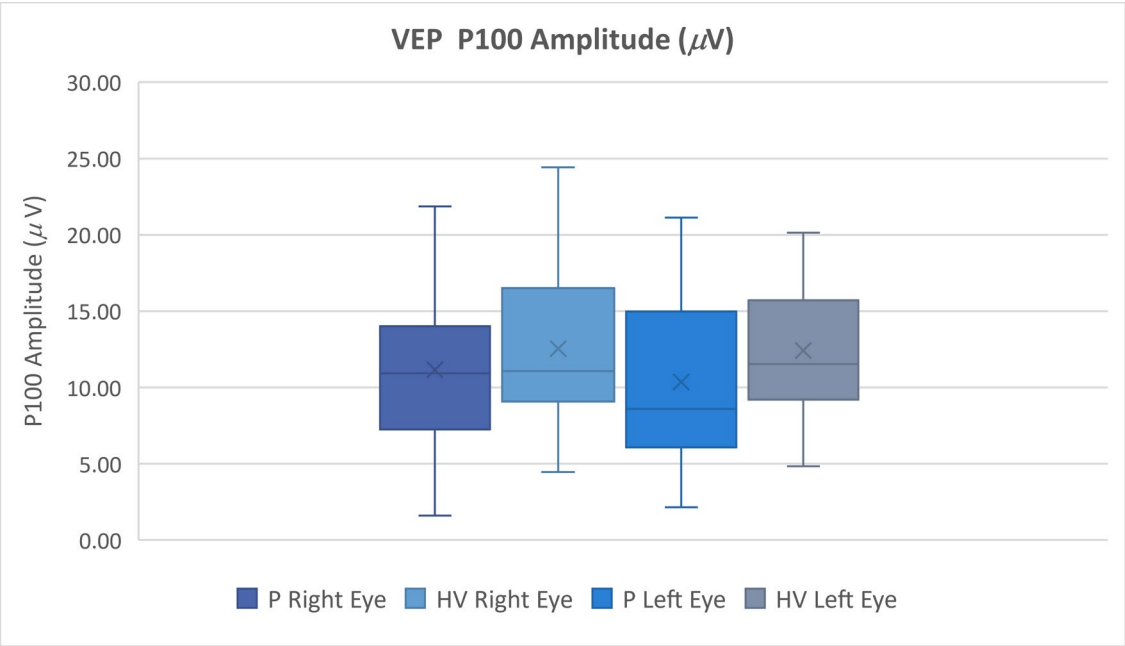

Figure 40. VEP P100 Amplitudes

Healthy volunteers (n=35) and participants (n=25) R- right, L- left

Figure 41 (overleaf) shows representative VEP traces from two trials (red and green) from a healthy volunteer (HV43-RE), demonstrating normal peak-times, amplitudes, and distribution across the hemispheres. An additional channel (left occipital referred to right occipital, i.e., O1-O2) is used in clinical practice to help identify polarity changes and asymmetries when looking for post chiasmal and hemispheric changes. For comparison, the VEP from P25-RE (two trials is shown below, demonstrating a VEP P100 peak-time delay indicated by the arrow with the P100 peak-time shifted to the left (Figure 42). The amplitude is unaffected, which is characteristic for demyelination.

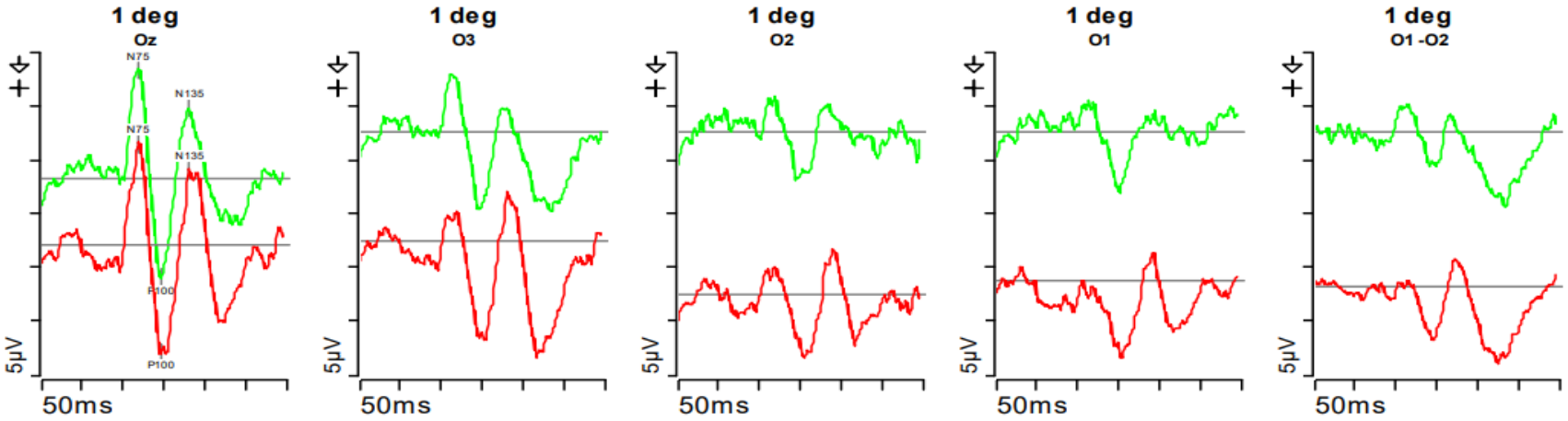

Figure 41. Normal VEP traces

(HV42-RE) demonstrating normal peak-time, amplitude and distribution (positivity is downwards)

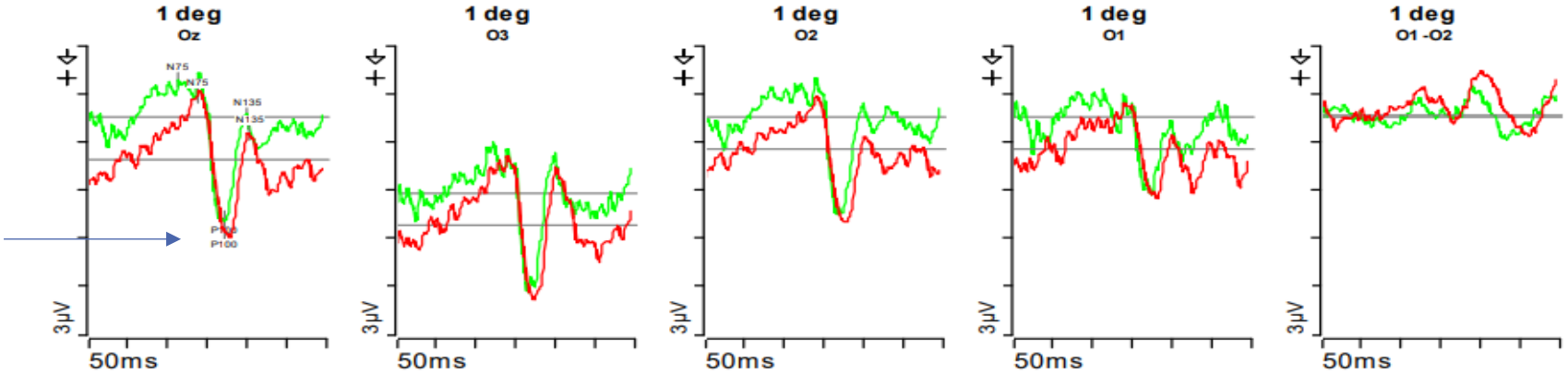

Figure 42. Abnormal VEP

(P25-RE), VEP P100 shows a peak-time delay shown with blue arrow and the P100 shifted to the left (positivity is downwards)

9.4.2 Pattern Electroretinogram (PERG)

Macular function was assessed for each eye with PERG testing (Figure 43). P50 and N95 absolute values along with P50:N95 ratios were compared for each eye (Figure 44). No significant differences were found for any of the measures between the groups (Table 9). There was one negative outlying value in the HV group (HV64-RE) with a ratio of 0.82. On further inspection of the raw traces, there were some artefacts present that distorted the measurement. This measurement was subsequently removed from the analysis. Additionally, the P50:N95 ratio is regarded as outlying in one HV (HV20-LE); however, the ratio is greater than 1:1.1 reported in the literature (Holder, 1997), so this was retained in the analysis. The remaining outlying values were in a positive direction, indicating a ratio of greater than 1:1.1 and would not be considered abnormal (P33-RE and HV40-RE).

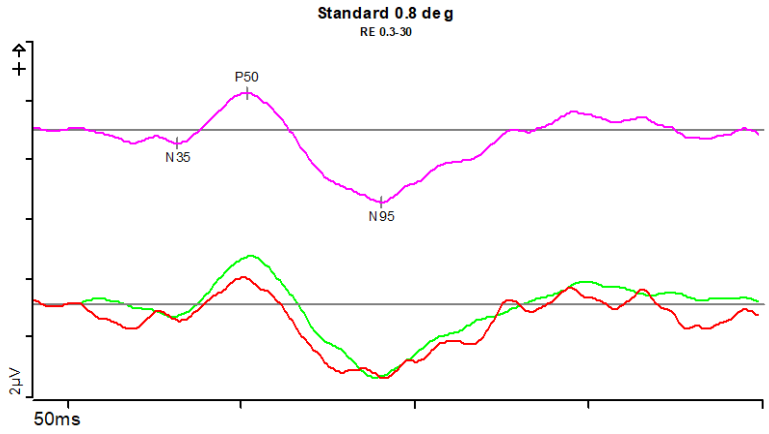

Figure 43. Normal PERG recording.

Upper trace is a grand average of the two single replications below (P53-RE)

Measure	*Side*	*Participant Mean (SD)*	*Participant Median (IQR)*	*Healthy Volunteer Mean (SD)*	*Healthy Volunteer (IQR)*	*Test Statistic*	*p*
P50 Amplitude (μV)	R	2.59 (1.24)	2.38 (1.17)	2.99 (1.35)	2.68 (1.44)	542.0	0.117 [a]
	L	2.61 (1.43)	2.18 (1.38)	3.08 (1.53)	2.86 (1.91)	539.0	0.126 [a]
N95 Amplitude (μV)	R	5.12 (2.34)	4.42 (2.37)	5.38 (2.15)	5.26 (2.54)	487.0	0.453 [a]
	L	4.71 (2.50)	4.10 (2.88)	5.35 (2.20)	5.05 (2.42)	537.0	0.136 [a]
P50 Peak-time (ms)	R	51.92 (2.66)	52.00 (3.75)	52.14 (3.06)	52.50 (4.50)	-0.290	0.773 [b]
	L	51.96 (3.62)	51.50 (4.25)	51.12 (3.20)	52.00 (4.00)	-0.174	0.860 [b]
N95 Peak-time (ms)	R	93.10 (6.31)	92.50 (8.25)	92.33 (6.47)	91.00 (8.00)	385.0	0.435 [a]
	L	93.04 (6.00)	94.00 (10.75)	92.80 (6.75)	92.50 (4.50)	379.0	0.380 [a]
P50:N95 Ratio	R	2.00 (0.31)	1.92 (0.39)	1.92 (0.53)	1.86 (0.43)	351.00	0.195 [a]
	L	1.83 (0.28)	1.82 (0.44)	1.82 (0.35)	1.89 (0.33)	473.50	0.589 [a]

Table 9. PERG Peak-time and amplitude measures

([a] Mann-Whitney, [b] t-test,)

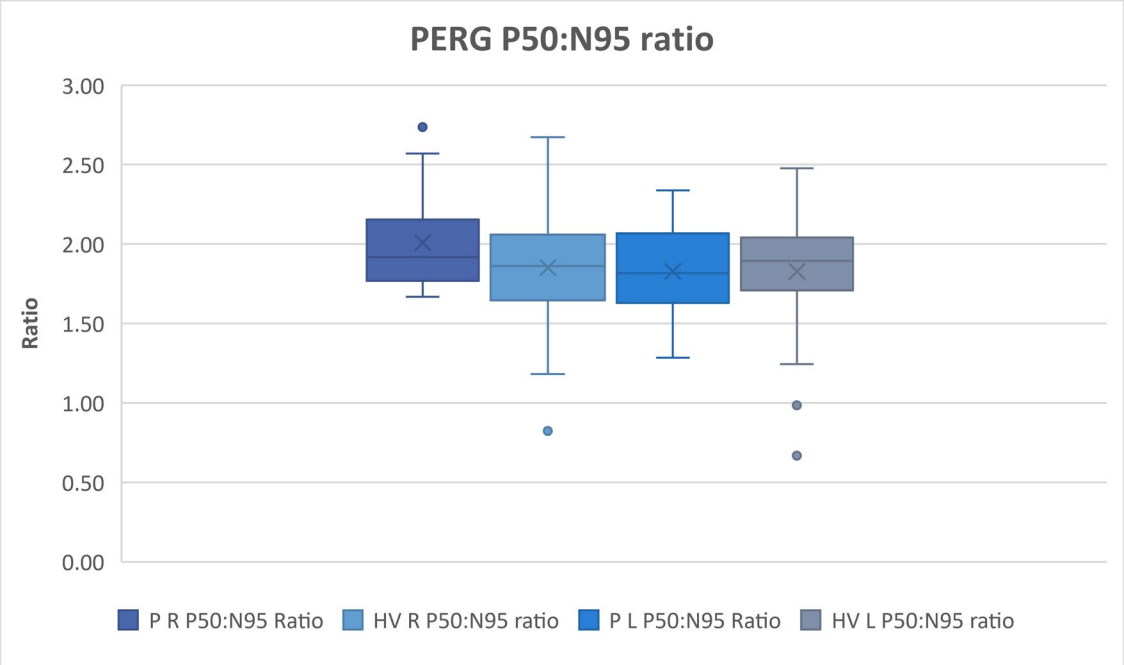

Figure 44.PERG P50:N95 ratios
Participants – P (n=25) and healthy volunteers-HV (n=35)

9.4.3 Flash Electroretinograms (ERGs)

The majority of the full field flash ERGs did not show any differences between the groups under DA or LA conditions using ISCEV standard flash intensities (McCulloch et al., 2015; Robson et al., 2022). Normal responses are illustrated in (Figure 46) from HV43-RE.

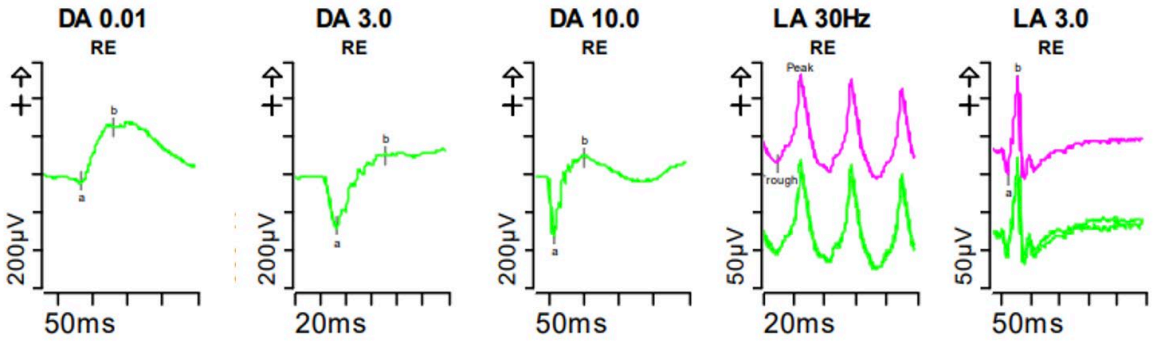

Figure 45. Normal flash ERGs.

(HV43-RE) in dark adapted conditions (DA) and light adapted conditions (LA)

The high intensity dark adapted flash, DA10.0, was the only stimulus condition where a difference was recorded between the groups, with the DA 10.0 a-wave amplitude on the right (p=0.021) (Figure 46), and the DA 10 b-wave peak-time (p=0.019) (Figure 47), on the left being statistically different between the two groups.

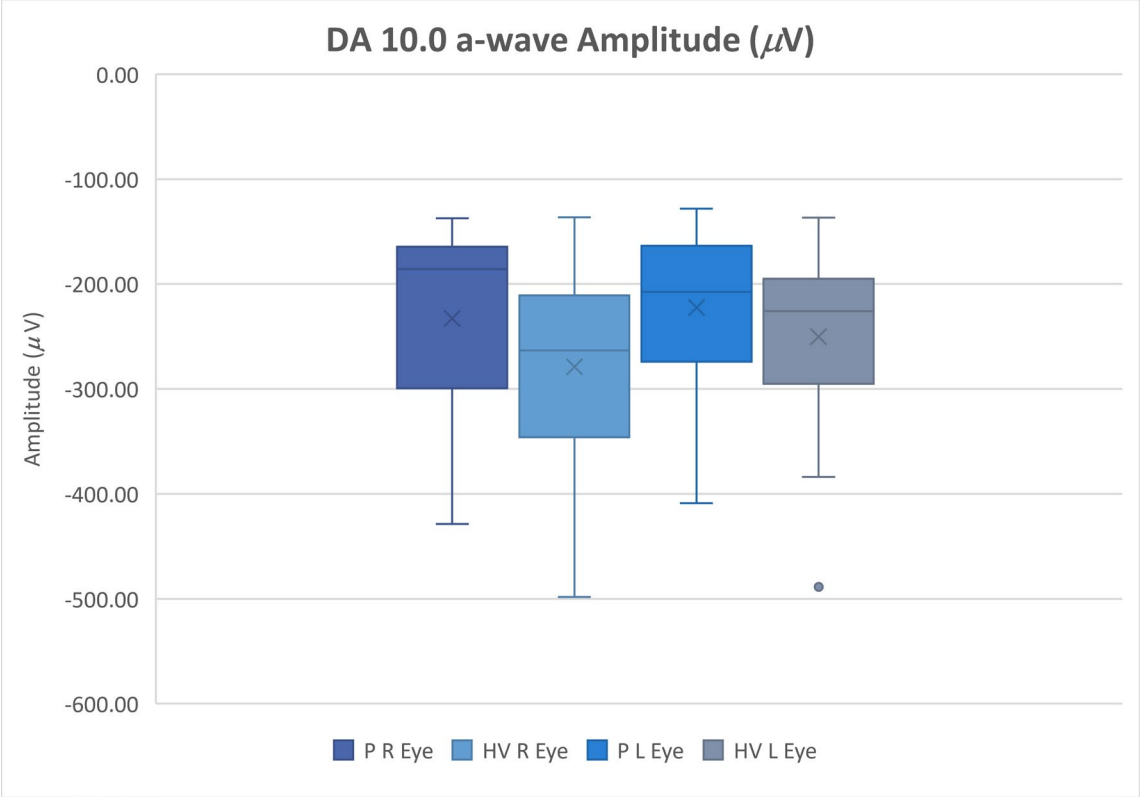

Figure 46. DA 10 a-wave amplitudes

Participants – P (n=25) and healthy volunteers-HV (n=35)

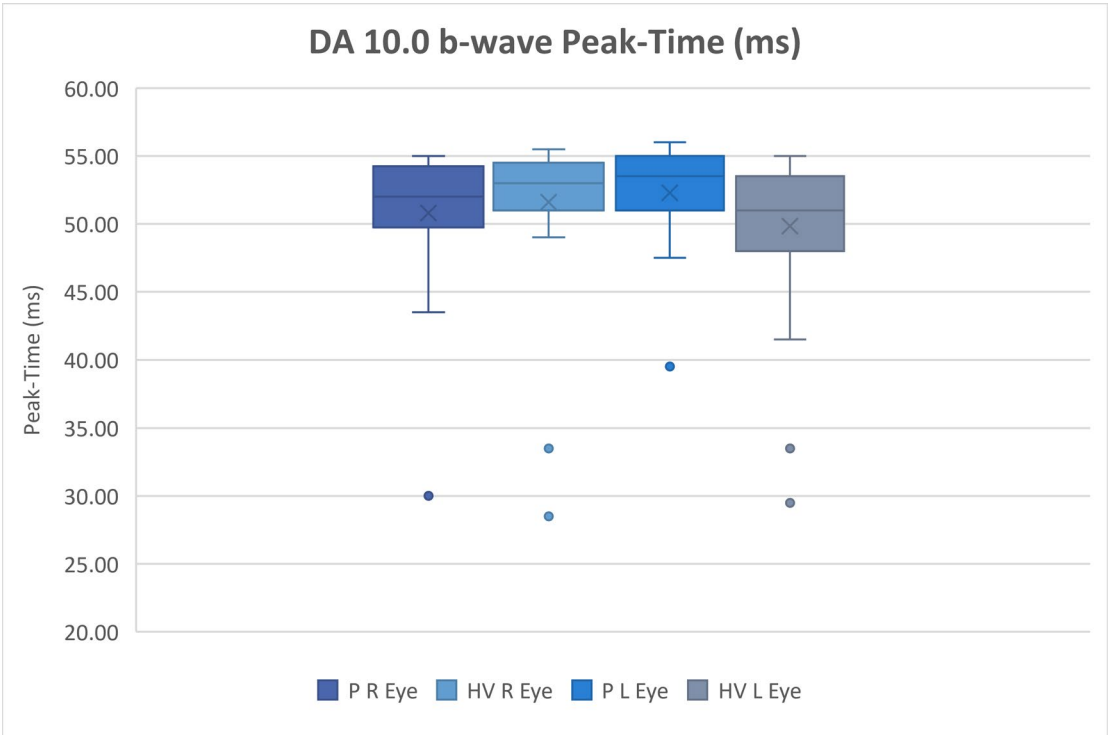

Figure 47. DA 10.0 b-wave peak times.

Participants – P (n=25) and healthy volunteers-HV (n=35). (The y-axis has been adjusted)

Differences between the right and left eyes within participants were also checked statistically for all variables. No variable was significantly different when using the appropriate test (paired t-test or the related-samples Wilcoxon signed ranked test).

In summary, when comparing all the variables between the two groups, RE-VA (p=0.007) and RE-VEP P100 peak-time (p=0.007) were found to be the most significantly different measures, followed by the LE-DA 10.0 b-wave peak-time (p=0.019), RE-DA 10.0 a-wave amplitude (p=0.021) and LE-VA (0.48). These variables were used to inform the subsequent analysis.

9.5 Further Statistical Analysis

When comparing the groups, the right VA, left VA, right VEP P100 peak-time, right DA 10.0 a-wave amplitude and left DA 10.0 b-wave peak-time variables were significantly different between the healthy volunteer and participant groups.

Logistic regression analysis was used to look at the effect each of these variables had on the outcome. The VA and VEP peak-time from the right side were the only two variables that had a significant effect (p= <0.05) (Table 10). The odds ratio illustrates that as the RE-VEP P100 peak-time increases by 1, the odds of being in the participant group increases by approximately 0.11 (1–0.89), or 11%.

Test Variable	*Side*	*p*	*Odds Ratio*
VEP P100 Peak-time	R	0.014	0.887
VA	R	0.038	0.000154

Table 10. Logistic regression results.

These values (along with the other significantly different variables) were plotted as a receiver operating characteristic (ROC) to show their individual sensitivity and specificity (Figure 49) along with the area under the curve (AUC) (Table 11). The VA and VEP P100 peak-time from the right eye have the greatest AUC, as expected.

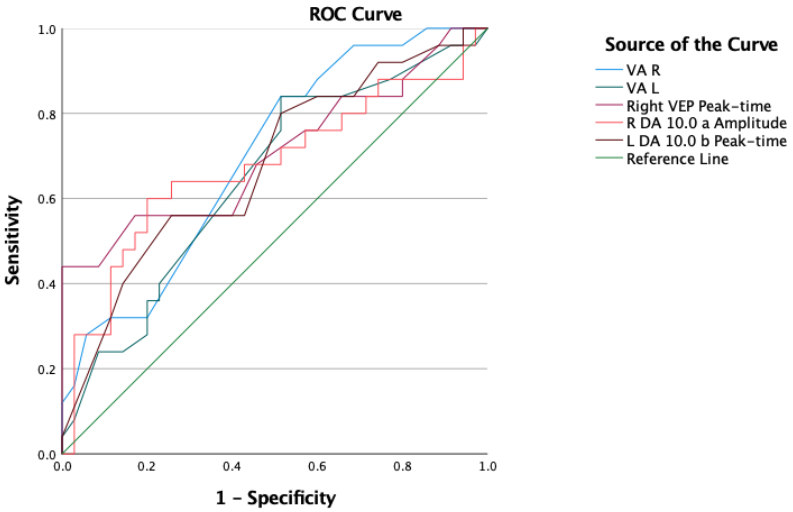

Figure 48. ROC curve.

Test variable	***Side***	***Area under the curve (AUC)***
VEP P100 peak-time	Right	0.706
VA	Right	0.697
DA 10-b wave peak-time	Left	0.678
VA	Left	0.648
DA 10.0-a wave amplitude	Right	0.607

Table 11. Area under the curve

The participant group was subdivided based on the local clinical parameter of P100 peak-time upper limit of normal being <113ms, regardless of clinical signs. This divided the participant group from 25 into a group of 12 with a VEP peak-time P100 delay (>113ms), and a group of 13 with a normal VEP peak-time (<113ms), which were compared to each other and the healthy volunteer group (Table 12). All recorded variables were analysed across the three groups. The rationale was to see if there were any diffuse inflammatory changes in the retina of either group at this early stage that might be identifiable relative to the optic nerve dysfunction. Any differences might provide evidence of a potential way of identifying those at risk of retinal damage that may serve as a biomarker. The Kruskal Wallis test was used to look for differences between the three groups and found 9 variables including the previously highlighted VA (right and left) that were significantly different between the

participant >113ms group and HV. Five of these variables were also significantly different between the participant <113ms and >113ms groups (Table 13).

	Side	*>113ms Mean (SD)*	*>113ms Median (IQR))*	*<113ms Mean (SD)*	*<113ms Median (IQR)*	*HV Mean (SD)*	*HV Median (IQR)*
VA (LogMAR)	Right	0.118 (0.19)	0.040 (0.22)	0.05 (0.54)	0.00 (0.00)	-.0.03 (0.09)	0.00 (0.08)
VA (LogMAR)	Left	0.09 (0.13)	0.03 (0.20)	0.00 (0.11)	0.00 (0.13)	-0.01 (0.11)	0.00 (0.13)
DA 0.01 b-wave amplitude (μV)	Left	193.0 (53.3)	182.7 (78.9)	303.7 (101.6)	322.7 (161.1)	265.7 (109.7)	227.7 (143.6)
DA 3.0 a-wave amplitude (μV)	Left	-154.1 (41.7)	-147.8 (51.0)	-231.2 (72.1)	-207.7 (119.3)	-211.2 (75.2)	-199.2 (105.9)
DA 3.0 b-wave amplitude (μV)	Left	256.3 (68.2)	250.7 (103.4)	400.3 (165.7)	389.2 (264.1)	353.0 (124.1)	320.7 (148.2)
DA 10.0 a-wave amplitude (μV)	Right	-216.4 (86.0)	-178.3 (115.4)	-248.5 (97.5)	-203.1 (148.7)	-279.0 (84.5)	-263.4 (135.0)
DA 10.0 a-wave amplitude (μV)	Left	-177.7 (42.7)	-168.2 (85.0)	-263.9 (83.7)	-259.1 (133.6)	-250.2 (85.3)	-225.9 (100.0)
LA 3.0 a-wave amplitude (μV)	Left	-23.6 (7.2)	-23.5 (10.0)	-31.7 (15.1)	-29.5 (23.1)	-32.8 (12.3)	-30.4 (14.7)
LA 3.0 b-wave amplitude (μV)	Left	103.7 (27.2)	97.1 (33.9)	151.1 (63.6)	153.8 (102.1)	133.8 (41.6)	127.3 (62.0)

Table 12. Significantly different variables when participants were grouped by VEP peak-time.

		Kruskal Wallis p value			
Measure	***Side***	***All Categories p***	***HV-<113ms p***	***HV->113ms p***	***<113ms->113ms p***
VA	R	**0.013**	0.154	**0.004**	0.216
VA	L	**0.042**	0.529	**0.010**	0.103
DA 0.01 b-wave amplitude	L	**0.012**	0.236	**0.021**	**0.004**
DA 3.0 a -wave amplitude	L	**0.014**	0.341	**0.016**	**0.005**
DA 3.0 b-wave amplitude	L	**0.013**	0.485	**0.010**	**0.006**
DA 10.0 a-wave amplitude	R	**0.036**	0.241	**0.012**	0.249
DA 10.0 a-wave amplitude	L	**0.007**	0.580	**0.004**	**0.004**
LA 3.0 a-wave amplitude	L	**0.049**	0.559	**0.014**	0.115
LA 3.0 b-wave amplitude	L	**0.043**	0.549	**0.028**	**0.020**

Table 13. Kruskal Wallis results comparing groups according to VEP peak-time.

(P >113ms, n=12, P <113ms, n=13, HV =35)

Additionally, a number of ERG amplitude variables that were largely confined to the left eye (despite the majority of participants presenting with visual symptoms and VEP P100 delays in their right eye). No peak-time ERG, PERG or structural measures were significantly different between the 3 groups.

For all significant measures, the participant group with a VEP P100 peak-time >113ms median values were seen to be reduced and the range of values was often narrower, compared to the other two groups. This is illustrated by the left eye DA0.01 b-wave amplitude (Figure 50).

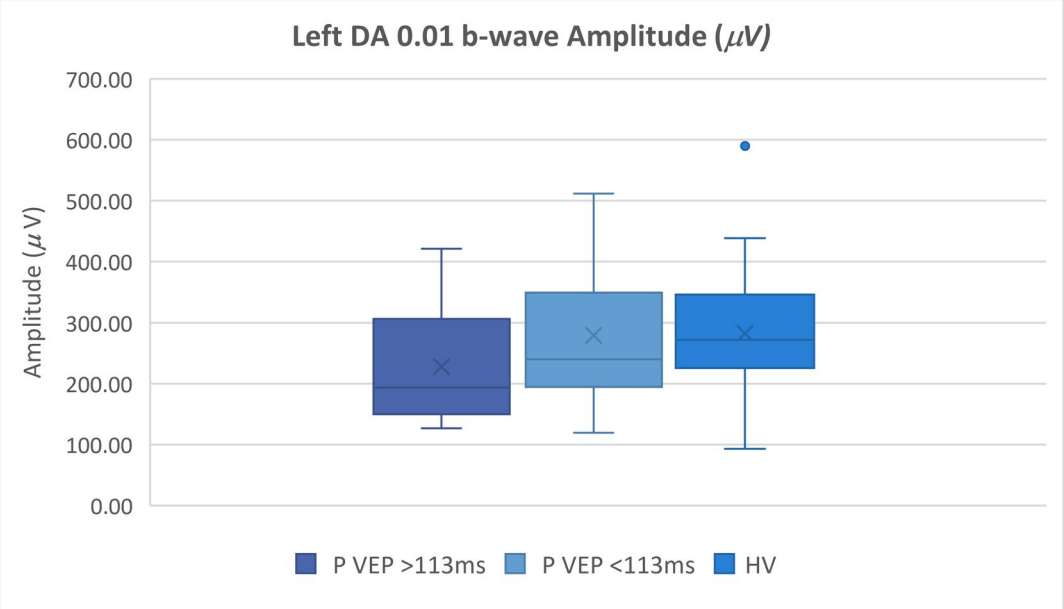

Figure 49. LE DA 0.01 b-wave amplitude.
(P >113ms, n=12, P <113ms, n=13, HV =35)

The group with a peak-time of <113ms was seen to have a median value that was often intermediate to the two other groups, the distribution of results tended to be similar to the HV group (Figure 51). Some of the variables were found to be significantly different to the >113ms group.

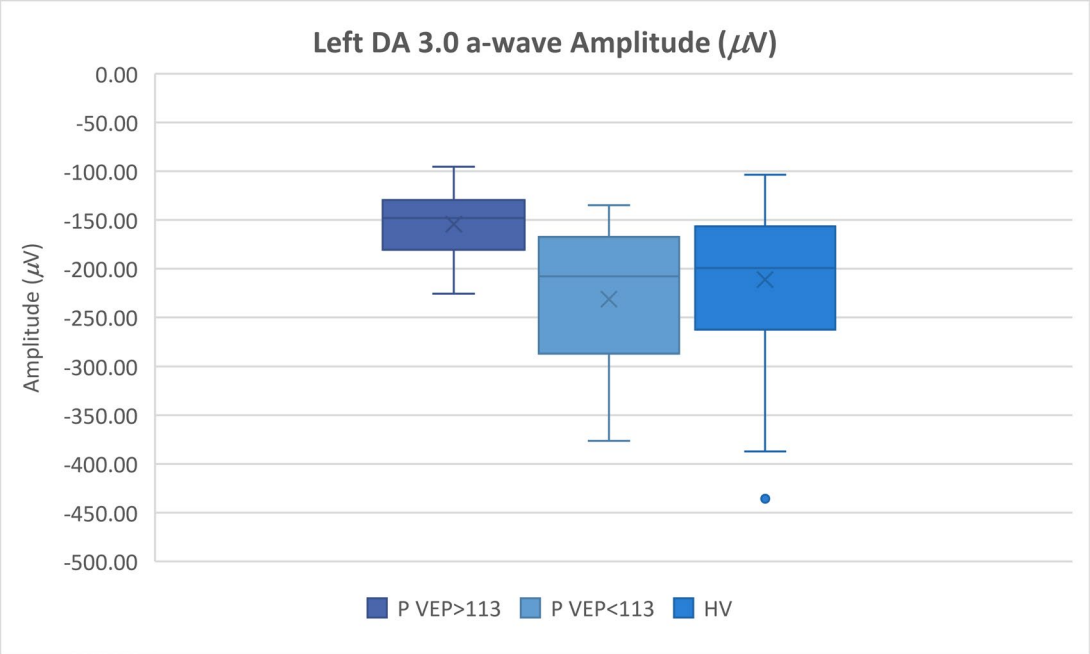

Figure 50. LE DA 3.0 a-wave amplitude.

(P >113ms, n=12, P <113ms, n=13, HV =35)

No differences were found between the HV group and the <113ms group (Table 13).

There were several measures where the median value was greater in the <113ms group than the HV and >113ms groups, for example, the left DA 3.0 b-wave amplitude (Figure 52), left DA10.0 a-wave amplitude (Figure 53) and left DA 10.0 b wave amplitude (Figure 54).

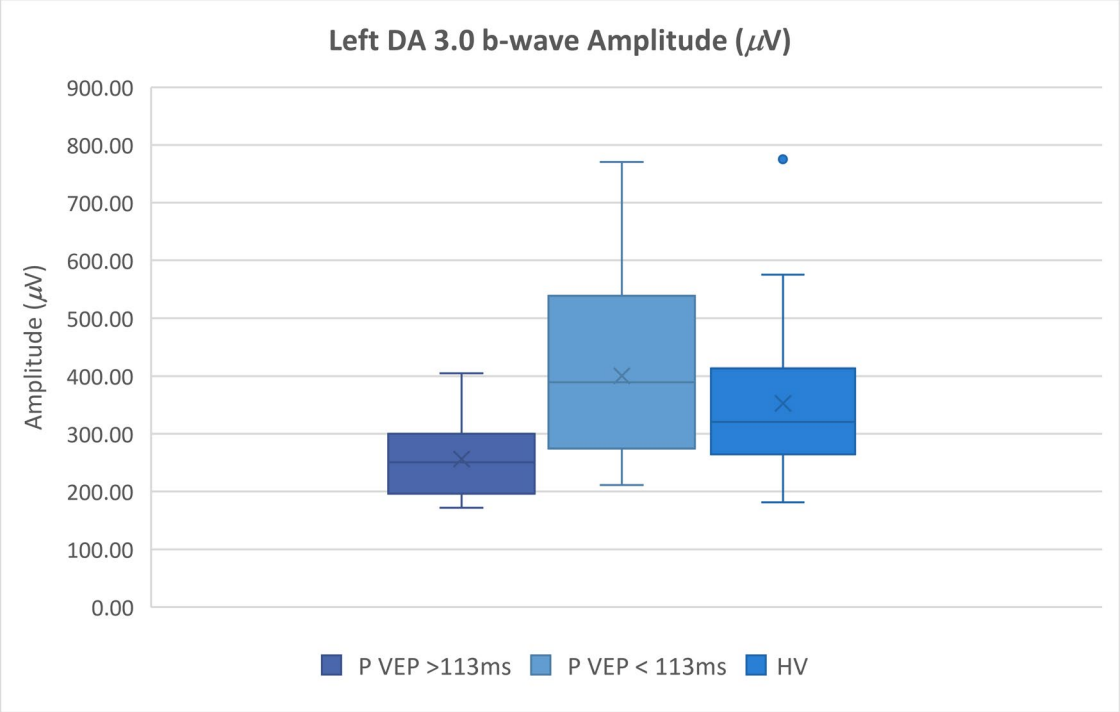

Figure 51. LE DA 3.0 b-wave amplitude.

(P >113ms, n=12), P <113ms, n=13, HV =35)

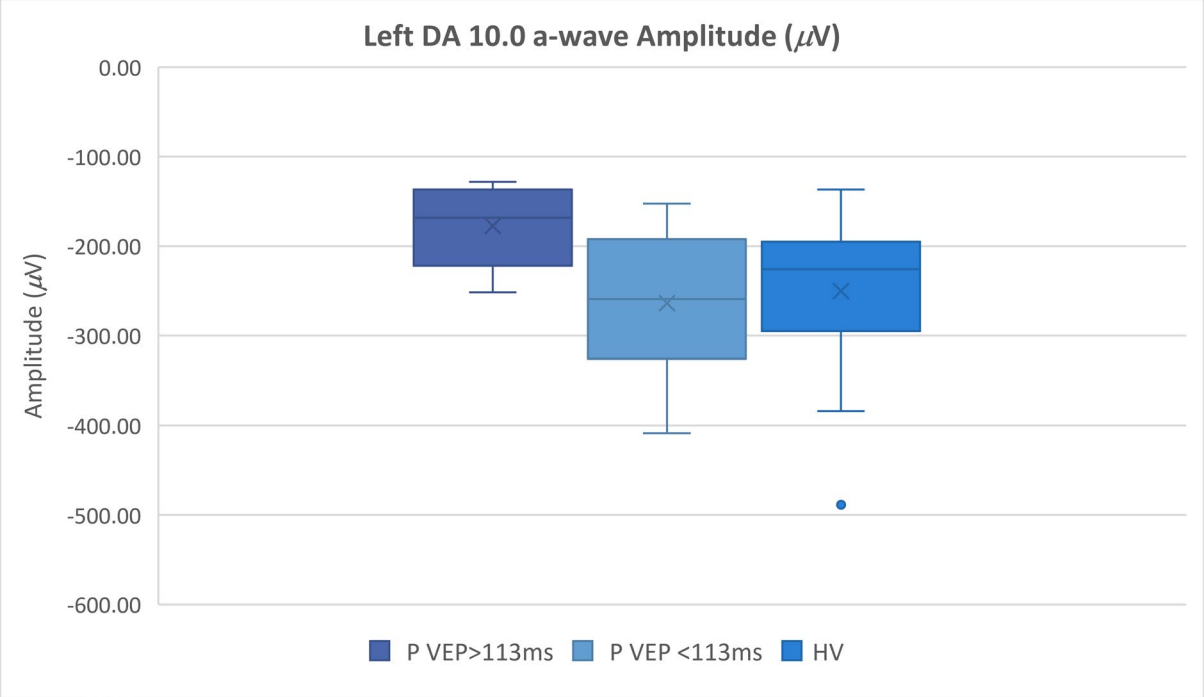

Figure 52. LE DA 10.0 a-wave amplitude.

(P >113ms, n=12, P <113ms, n=13, HV =35)

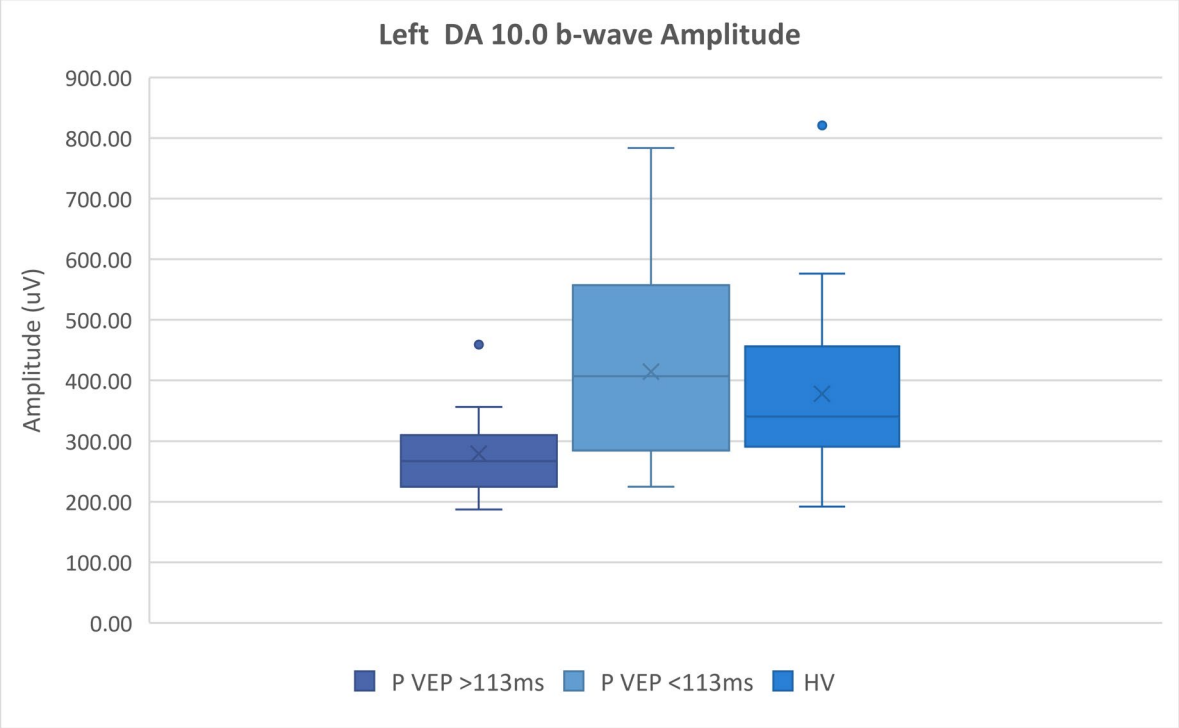

Figure 53. LE DA 3.0 b-wave amplitude.

(P >113ms, n=12, P <113ms, n=13, HV =35)

ROC curves were plotted for those variables that were significantly different for the group with a VEP P100 peak-time >113ms (variables that were found to not have any discriminatory value were not plotted) (Figure 55). The AUC was also calculated for the significant variables (Table 14).

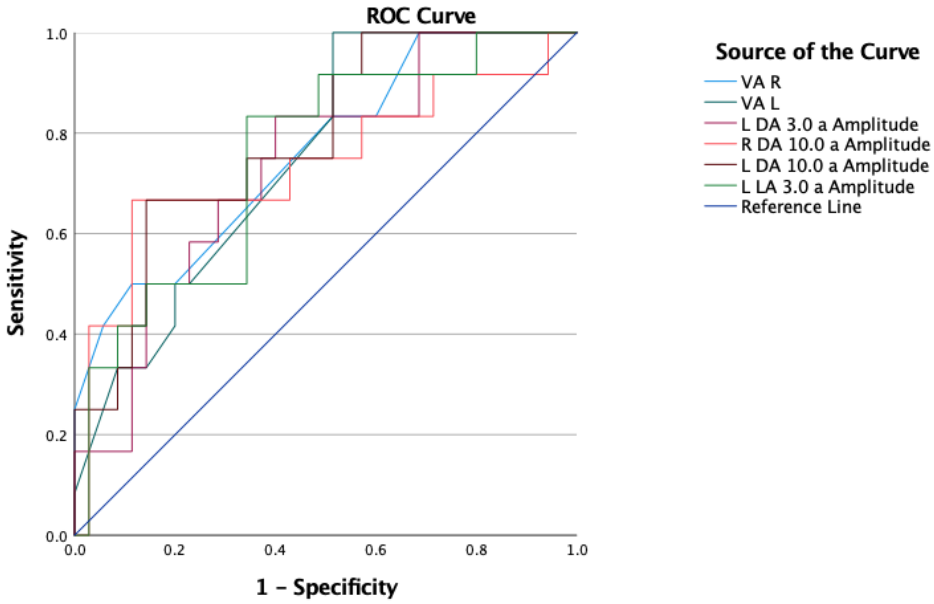

Figure 54. ROC curve (VEP >113ms).

Test variable	*Side*	*Area under the curve (AUC)*
DA 10.0 a -wave amplitude	Left	0.786
VA	Right	0.762
LA 3.0 a-wave amplitude	Left	0.750
VA	Left	0.749
DA 10 a-wave amplitude	Right	0.738
DA 3.0 a-wave amplitude	Left	0.736

Table 14. Area under the curve values (VEP >113ms).

A similar process was followed for the <113ms participant group with all variables showing less discriminatory power compared to the >113ms group (Figure 56 and table 15).

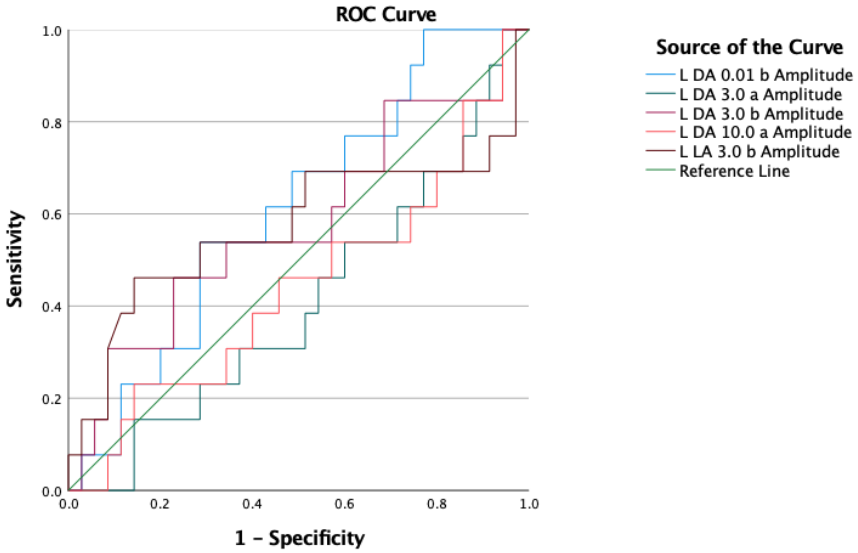

Figure 55. ROC curve (VEP <113ms, n=13).

Test variable	*Side*	*Area under the curve (AUC)*
DA 0.01 b-wave amplitude	Left	0.611
DA 3.0 b-wave amplitude	left	0.578
LA 3.0 b-wave amplitude	Left	0.573
DA 10.0 a-wave amplitude	Left	0.442
DA 3.0 a-wave amplitude	left	0.409

Table 15. Area under the curve values (VEP <113ms, n=13).

Lastly, the effect of varying the VEP P100 peak-time was investigated to see if this increased the discriminatory power of the variables when adjusted by 1ms increments in both directions from the 113ms used in clinical practice. This inversely altered the number of participants in the group, with participants reducing as P100 peak-time increased (Table 16).

	VEP P100 Peak-latency (ms)								
	>116	***>115***	***>114***	***>113***	***>112***	***>111***	***>110***	***>109***	***>108***
n	9	9	11	12	13	13	13	13	14

Table 16. Frequency of patients with variable VEP P100 peak-time.

The effect of this change was investigated using the AUC for each of the variables that were originally found to be different between the groups (Table 17).

		AUC ***VEP P100 Latency (ms)***								
Variable	***Side***	***>116***	***>115***	***>114***	***>113****	***>112***	***>111***	***>110***	***>109***	***>108***
VA	R	0.729	0.729	0.702	0.734	0.759	0.759	0.759	0.759	0.750
VA	L	0.664	0.664	0.700	0.729	0.755	0.755	0.755	0.755	0.746
DA 0.01 b-Amplitude	L	0.333	0.333	0.265	0.245	0.283	0.283	0.283	0.283	0.315
DA 3.0 a-Amplitude	L	0.662	0.662	0.735	0.759	0.715	0.715	0.715	0.715	0.682
DA 3.0 b-Amplitude	L	0.325	0.325	0.243	0.231	0.273	0.273	0.273	0.273	0.301
DA 10.0 a-Amplitude	R	0.651	0.651	0.701	0.715	0.732	0.732	0.732	0.732	0.700
DA 10.0 a-Amplitude	L	0.695	0.695	0.761	0.793	0.746	0.746	0.746	0.746	0.710
LA 3.0 a-Amplitude	L	0.612	0.612	0.696	0.724	0.674	0.674	0.674	0.674	0.640
LA 3.0 b-Amplitude	L	0.336	0.336	0.271	0.241	0.322	0.322	0.322	0.322	0.370

Table 17. AUC for varying VEP P100 peak-time cut-off values.

(<113ms is used in local clinical practice as the upper limit of normal latency)*

Summary of chapter 9

- Logistic regression found that the only variables that were found to be different when considering the participant group as a whole were the VA and VEP from the right eye.
- Dividing the participants based on VEP P100 peak-time did highlight differences in various ERG measures as well as VA.
- The differences in ERG measures were confined to amplitude and not peak-times.
- Varying the upper limit of normal for the P100 peak-time for each variable did not suggest a better VEP P100 peak-time cut-off as measured with AUC.

Summary discussion

- The study is in keeping with the literature with regards involvement of the optic nerve and P100 peak-time in MS.
- Differences in ERG variables were found that appear to be related to optic nerve dysfunction (clinical or subclinical).
- The study does not support OCT as a biomarker early on in the disease.

Chapter 10. Discussion and Conclusion

Multiple sclerosis is a common neurological condition that may provide a diagnostic dilemma for clinicians, causing anxiety for patients and delaying treatment. Despite international efforts, misdiagnosis is common (Thompson et al., 2018b). The impact of the disease is significant and unpredictable, and much is still unknown about the triggers and pathogenesis. The temporal relationship between structure and function is poorly understood, but it is suggested that electrophysiological disturbance in function occurs before, or in the absence of, structural changes (Hanson et al., 2018). This study aimed to further understand the disease process with regards to the visual pathway, so that it may direct further study in the search for a reliable biomarker.

The limited studies to date have produced conflicting evidence as to the retinal changes measured with electrophysiology observed in MS. Electrophysiological measures are uniquely placed to record neuronal function in a quantifiable way that no other paraclinical measure is able, which may indicate disease activity at the disease onset, or in those who are treatment naïve. It was based on the clinical observation that the visual pathway is frequently affected both at the start and during the course of the disease and may be clinically 'silent'. By combining ERG and OCT structural measures with the routine standard of care tests, it was hoped that this would add new insights as to the nature and characteristics of the disease that may further implicate the visual pathway.

Neurophysiological measures have reliably been used as a biomarker for MS in the past with the VEP (Halliday et al., 1973), which was largely superseded when MRI became widely available. However, there has been some renewed interest more recently. Different authors

have taken different approaches, for example, some have used VEPs in combination with other modalities of EP as a paradigm to correlate with disability over time (Pelayo et al., 2010; Canham et al., 2015). The goal of finding a reliable method of monitoring response to treatment has been a consistent feature of the literature with some promise (Hardmeiser et al., 2017), with VEPs being correlated with brain volume and lesion load (Covey et al., 2022). There have been a small number of studies examining the function of the retina with various ERG findings (Sriram et al., 2014; Hanson et al., 2018; You et al., 2018b; Hanson et al., 2021).

10.1 Key Findings

This research confirms the reports in the literature that the optic nerves are commonly affected in MS, that is, irrespective of symptoms. When considering the participant group as a whole (n=25), the findings showed that 48% of patients had evidence of optic nerve dysfunction with VEP P100 peak-time delays, involving 17 eyes (68%). This corresponds very well with reports that state the optic nerve is commonly affected in MS, which may be subclinical. This was useful in categorising the participants with regards to objective evidence of demyelination that was independent of subjective measures such as VA. In this study, VA from the right eye was found to be a significantly different between healthy volunteers and participants, which can be explained by the number of participants that presented with symptoms on that side. This also confirms that even after clinical recovery as measured with VA (apart from the two outlying VA measures for the right eye, P4 and P41), conduction delays may persist.

10.1.1 Group Analysis

When comparing the whole MS cohort (n=25), with healthy volunteers (n=35), two ERG parameters; RE DA 10.0 a-wave and LE DA 10.0 b-wave, were significantly different between the groups, however, this did not remain significant with further analysis (logistic regression). All of the other light and dark-adapted a-wave peak-time and amplitude values did not significantly differ when compared to healthy volunteers, suggesting both rod and cone photoreceptor function is comparable between the two groups. Equally, the b-wave responses generated by the inner retinal layers under each stimulus condition were also

comparable. Collectively, these findings suggest no significant differences in either phototransduction or the inner retinal function from both eyes between MS patients and heathy volunteers.

Feinsod et al. (1971); Feinsod et al. (1973), contradict these findings reporting increases in ERG amplitudes, which has not been reported in any other studies since. In contrast, Hamurcu and colleagues (2017), found a mixture of decreased amplitudes and delayed peak-times that were confined to the cone mediated responses that partially support these research findings.

Despite the VEP P100 delays, there was no evidence of axonal damage in the electrophysiology, with the VEP P100 amplitudes not being significantly different between the groups when considered as a whole (n=25), or when divided. This validates the normal PERG N95 and P50:N95 ratio findings, measures that are generally accepted to be generated by the RGCs. It therefore suggests that, at this stage in the disease, there is no retrograde degeneration of the optic nerve affecting the RGC, making the observed retinal changes independent of optic nerve damage. This is further supported by the normal OCT imaging findings. This would suggest that retrograde degeneration may be independent of the length of VEP P100 delay, as this varied greatly between individuals in the MS group, and the one participant ultimately diagnosed with MOG disease. Or alternatively, the temporal effects of the disease are not present in this cohort, but further investigation with subgroup analysis would be needed to confirm this.

The study did not find any evidence of structural abnormalities in any of the parameters measured with OCT, which may be due to patients being newly diagnosed and inflammation not being sufficient to produce measurable changes. Therefore, despite the claims in the literature that OCT is a suitable biomarker for MS, this study suggests that it is unsuitable at disease onset but may make it more suited to disease monitoring. Alternatively, the disparity between structure and function may be due to OCT being confined to the central 30 degrees of retina, whereas ERG is a measure of diffuse retinal function. Should pathology selectively affect the peripheral retina, this may not be detected by OCT.

10.1.2 Subgroup Analysis

When the participants were divided based on evidence of optic nerve dysfunction suggestive of demyelination (VEP P100 delays), as applied in clinical practice, a number of ERG variables were significantly different between the groups. These were a mixture of ERG amplitude measures generated in both the inner and outer retinal (both dark and light adapted). The limited literature to date has shown variable ERG findings in MS, with increased peak-times and both amplitude increases and reduction. The current study is discordant with the study by Hanson and colleagues (2018; 2021), who found ERG peak-time delays (DA 3.0 a-wave, LA 3.0 a-wave and LA 3.0 b-wave), at baseline, that remained largely stable over a 3-year period. They did not observe any amplitude changes at any stage which may be expected in this established cohort, although it is not clear if DMTs may have slowed or prevented the disease progression. There are some notable differences in their study design, including the patient cohort, who were recruited from an existing MS study, and it is not clear if they were receiving DMTs. They also used a novel a-wave to b-wave ratio measurement method (in addition to absolute values), which may have not highlighted abnormalities when both a and b waves were reduced. They confirm the reports of others that the changes are independent of previous ON (clinically determined), which is similar to others (You et al., 2018a), although they admit to a reduction in participants over the course of the studies.

The MS subgroup analysis suggests that there is a difference in outer and inner retinal function as measured with a and b wave ERG amplitude variables that is present in both participant groups, that is not entirely related to optic nerve dysfunction, as measured with VEP P100 delays. Although it was most prominent in the MS group with a VEP P100 delay, with 9 variables being significantly different, compared to 5 (of the same 9) in the group without a VEP P100 delay. This may represent an opportunity for identifying patients who are at greater risk of developing MS without a VEP delay.

It is interesting that all the significantly different ERG variables are amplitude measures (a and b waves), and no peak-time measures were found to be different. This suggests a retinal process in MS that affects both the photoreceptors (rods and cones) and inner retina (predominantly the bipolar cells) that contribute to the a and b waves, respectively. This is

with the caveat that there is a loss of statistical power with the participant group being split almost in half. A larger study would be required to confirm this finding.

10.2 Study Design

The study was deliberately designed to ensure capture of appropriate referrals to the neurophysiology service by only taking referrals from the specialist neurology team and not general practice or other medical specialities. In keeping with local best practice, all referrals of suspected MS should be referred to the specialist team for review. The study was prospective, with participants being recruited at the same time as their routine standard of care investigations were requested in order to maximise participation. Patient outcomes were not known until after investigations were complete and patients had been seen for medical follow-up.

Technical confounds were limited as much as possible for the electrophysiology by using the same equipment operated by the same suitably qualified person throughout the study. The ISCEV guidelines were adhered to for performing the electrophysiology, and room luminance was additionally monitored to ensure constant testing conditions (Appendix 2). Equipment was maintained and calibrated as suggested by the manufacturer (Appendix 3).

OCT scans were performed on the same piece of equipment by trained staff within the ophthalmology department. The majority of scans were performed by the same person (lead optometrist), but there were occasions when this was not possible which may have introduced some variation. Equipment was maintained as per the routine service contract provided by the manufacturer for the NHS Trust.

All individuals entering the test were fully informed both in writing and verbally prior to attending and gave their consent. Participation and cooperation with the individual tests were encouraged throughout. In one case, participation was not adequate and not all studies could be performed; the studies that were performed were not included in the analysis. In the case of participants presenting with ON, adequate time was allowed for the acute phase to resolve, enabling good fixation (12 weeks). No participant had commenced DMT or received oral

steroids within 6 weeks of testing. One participant consented to being filmed talking about his patient experience for the lay person presentation that formed part of the earlier academic assessment of C1:6ACP80424.

10.3 Strengths and Limitations

10.3.1 Strengths

This is the first study to characterise visual function in patients that are newly diagnosed with MS. Disease onset had been defined in this study as presentation to a specialist service however, current evolving research points to a possible, often prolonged prodromal phase making it difficult to define. The effects of advanced disease and recurrent relapse are there for minimised in this study. The effects of DMTs are also removed from this study which is also unique when reviewing the literature.

The visual system was characterised by both quantitative and subject methods of assessment in addition to clinical examination. This ensured that in participants with clinically silent ON cases were included in the analysis which not been considered in some previous studies.

The study design could have introduced some selection bias as referrals were coming from a single source, however, to prevent this, only new referrals were accepted and not repeat investigations or referrals with a pre-existing diagnosis, for instance, when participants had moved into the area. Although this may be considered a limitation, it prevented inappropriate referrals from non-specialists and general practice. To further mitigate this, there were two specialist MS specialist clinicians from the team that were making referrals for neurophysiological testing. Ultimate diagnosis of MS was based in each case on the outcome of multidisciplinary findings where specialist neurologists, neuroradiologists, nurses and physiologists contributed. The prospective nature of the study meant that the outcome was not known until after all investigations had been performed.

The ISCEV guidelines were adhered to for neurophysiological testing which standardised practice. Mydriasis was used for ERGs to ensure maximal stimulation of the retinas and testing conditions were standardised to minimise variation.

OCT measures were performed by senior staff from the ophthalmology department as per standard clinical protocols with all scans being above the recommended quality scores. If fixation was poor or scans did not meet the quality criteria they were repeated.

10.3.2 Limitations

Technical limitations may have affected measurements. The OCT machine is capable of 5mm resolution, and the electrophysiology equipment has a temporal resolution of 5ms and amplitude resolution of 1mV. This is considerably better than some of the earlier studies in the literature but may not detect abnormalities below these limits.

All measurements were made firstly with automated analysis but checked by the recording optometrist or principal investigator and adjusted as necessary. This was not verified by a second person due to the limitations of the study.

Measurement of the VEP P100 peak-time was a key aspect of the analysis. This failed to capture one participant who had an interocular peak-time difference >9.5ms with absolute latencies below 113ms. They were symptomatic in the eye with the longer peak-time, suggesting ON, which is not reflected when the group is broken down according to VEP P100 peak-time.

Consecutive participants were invited to participate; however, the effect of the COVID-19 pandemic did on occasions deter participants from adding extra time to their appointments, or additional visits, so some patients declined to be a part of the study for this reason. In keeping with the prior patient interviews, several patients felt they did not want to participate for social or personal reasons. Their wishes were respected, and this did not affect the routine standard of care that they received.

The cohort wholly consisted of white individuals the ethnicity of which was unknown, but reflective of the local patient population, the findings therefore may not transfer to other populations.

10.4 Study Summary

To summarise, the study recruited 35 participants and 35 healthy volunteers. Of the 35 participants, 10 were subsequently removed from some of the analysis due to not being diagnosed with MS after examination and investigation. Seven patients presented with visual symptoms consistent with ON. Additionally, five participants had P100 VEP peak-time delays without clinical accompaniment.

When considering the participant group as a whole (n=25), visual acuity and the VEP P100 peak-time from the right were the only significantly different variables in the logistic regression that could predict the odds of being in the participant group. This is perhaps related to the observation that more patients presented with visual symptoms on that side. The other electrophysiological measures: the cortically driven VEP P100 from the left, the macula driven PERGs from both eyes, and the ERG diffuse retinal responses did not show any differences between the groups. The results did not show any statistical differences in any of the OCT structural measures between the groups. The VA measure from the left eye was not statistically different between groups.

When dividing the participant group (n=25) into those with and without a VEP P100 peak-time delay indicating electrophysiological evidence of optic nerve dysfunction (clinical or subclinical), as defined in clinical practice, a number of ERG variables were significantly different when comparing the two participant groups and HVs. These were confined to amplitude measures of some of the dark and light adapted responses from a selection of a and b wave measures, mainly of the left eye. The reduction in amplitude could suggest cell loss or atrophy in both the outer and inner retina reflected, with both the a and b waves (respectively) being affected.

The finding that these variables were more commonly seen in the left eye than the right is unexpected given that more participants presented with visual symptoms on the right, which is of uncertain significance. Further work would be needed to explore the possibility of an inflammatory retinopathy in this cohort that may represent a new phenotype.

Of the excluded participants, two had prolonged VEP P100 peak-time latencies >113ms. One (P 19) was subsequently diagnosed with MOG disease and recorded bilateral P100 delays (RE-115ms LE-158ms). This patient had been symptomatic in her left eye prior to the time of testing and reported that this was much improved but was not quite back to normal (VA RE-0.0, LE-0.54). These findings would therefore be in keeping with the diagnosis, given that MOG disease is also a demyelinating disease. It also illustrates that VEPs, and VA are non-specific in nature. The other participant (P 26) was diagnosed with myelopathy and recorded P100 peak-times of 115ms from both eyes (VA RE- 0.12, LE-0.0). The reason for this delay is uncertain. The remainder of the excluded group recorded normal VAs and VEP P100 peak-times from both eyes.

The effect of shifting the VEP P100 peak-time cut-off from 113ms (in both directions) did not suggest an alternative peak-time would increase the AUC measures significantly.

10.5 Contribution to Knowledge and Future Work

This is the first study to examine diffuse retinal function in newly diagnosed MS patients with and without previous optic neuritis that is quantified with electrophysiology. It supports the findings in the literature that the optic nerve is frequently involved in this cohort of adults recruited from the south-west of England with new disease. VEP peak-time and VA were the only significant variables in the logistic regression when comparing the two groups (p 91). This supports the debate that future clinical criteria should include functional measures of the visual pathway irrespective of symptoms.

The subgroup analysis provides some support of a primary retinopathy in MS patients with both with confirmed ON and those without, as defined by VEP peak-time delays in the form of reduced ERGs (p 94). This was more apparent in those with confirmed delays. The consistent reduction in ERG amplitudes is a novel finding that needs further exploration, with a larger cohort in order to confirm these changes. This is interesting as it is consistent findings across a number of ERG variables and persists despite the reduced group size. Although not significant in the logistic regression, the DA 10 a-wave measure was found to be different when considering the group as a whole (n=25). The presence of these 'clinically silent'

abnormalities that were not found in the HV group also provides electrophysiological evidence of dysfunction that might be utilised as a biomarker at least in a subset of patients.

Further work may include additional neurophysiological measures such as focal ERG or extended ISCEV protocols in order to further localise dysfunction. Additionally, new technologies such as the hand-held 'Reteval' device may provide opportunity for point-of-care screening if ERG reductions could be confirmed.

The cause of retinal dysfunction is beyond the scope of this study but may direct future work to explore autoimmune or neurotransmitter systems as possible causes that may represent an additional phenotype in MS.

It did not support the widely reported theory that OCT structural measures are helpful as a biomarker in MS, at disease onset, with no structural measures being different to healthy volunteers. This may be due to the short duration of the disease and lack of inflammatory episodes. It does not exclude it being useful as a monitoring tool.

Longitudinal studies would be helpful in assessing how the disease affects the visual system over time given the predilection for visual involvement. The characteristic fluctuating time course followed by progression and eventual 'burn-out' (p.29), could suggest an equally fluctuating effect on vision that may be a primary retinal process or secondary to optic nerve pathology. The subgroup analysis suggests alterations at retinal level however, further studies would be needed to explore this.

The current diagnostic criteria for MS (Thompson et al., 2018) does not include evidence of optic nerve dysfunction with clinical or qualitative measures, which has been a topic of discussion over the years. The expert panel have considered adding the optic nerve as an additional region to demonstrate DIS but decided against it due to lack of evidence. However, the studies they considered did not combine measures, with some only considering clinical assessment, MRI or VEP (Vidal-Jordana et al., 2021). The current study would suggest that in addition to VEPs, ERGs might provide an opportunity to identify an early biomarker in at least a subgroup of MS patients, following further research. All participants in the study had abnormal MRI findings, some of which were non-specific. Of those excluded, it may be possible that some go on to have further episodes suggestive of inflammation in the future,

therefore fulfilling the dissemination in time and space diagnostic criteria. Two participants were not given a diagnosis at the time of review. Although diagnostic criteria have been updated, MRI specificity has been shown to be variable which is consistent with this study, with 10 participants without MS having abnormal imaging (Hemond and Bakshi, 2018; Filippi et al., 2019).

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Appendices

Appendix 1a. Literature Search 2019

#	Database	Search term	Results
1	EMBASE	(multiple sclerosis OR MS OR "clinically isolated syndrome").ti,ab,if	494188
2	EMBASE	"DEMYELINATING DISEASE"/	13844
3	EMBASE	exp "DEMYELINATING DISEASE"/	163834
4	EMBASE	*"DEMYELINATING DISEASE"/	4483
5	EMBASE	"MULTIPLE SCLEROSIS, CHRONIC PROGRESSIVE"/	62721
6	EMBASE	exp "MULTIPLE SCLEROSIS, CHRONIC PROGRESSIVE"/	122850
7	EMBASE	*"MULTIPLE SCLEROSIS, CHRONIC PROGRESSIVE"/	43867
8	EMBASE	"MULTIPLE SCLEROSIS, RELAPSING-REMITTING"/	71234
9	EMBASE	exp "MULTIPLE SCLEROSIS, RELAPSING-REMITTING"/	122850
10	EMBASE	*"MULTIPLE SCLEROSIS, RELAPSING-REMITTING"/	49134
11	EMBASE	(1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10)	552204
12	EMBASE	(electroretinogra* OR FERG).ti,ab,if	15230
13	EMBASE	exp ELECTRORETINOGRAM/	9212
14	EMBASE	(12 OR 13)	18718
15	EMBASE	(11 AND 14)	884

16	*EMBASE*	*15 [English language] [Humans]*	*537*
17	*Medline*	*(multiple sclerosis OR MS OR "clinically isolated syndrome").ti,ab,if*	*356329*
18	*Medline*	*exp "MULTIPLE SCLEROSIS"/ OR exp "DEMYELINATING AUTOIMMUNE DISEASES, CNS"/*	*71737*
19	*Medline*	*(17 OR 18)*	*374065*
20	*Medline*	*(electroretinogra* OR FERG).ti,ab,if*	*12855*
21	*Medline*	*ELECTRORETINOGRAPHY/*	*15907*
22	*Medline*	*(20 OR 21)*	*18797*
23	*Medline*	*(19 AND 22)*	*539*
24	*Medline*	*23 [Languages English] [Humans]*	*308*
25	*CINAHL*	*(multiple sclerosis OR MS OR "clinically isolated syndrome").ti,ab,su*	*39738*
26	*CINAHL*	*exp "MULTIPLE SCLEROSIS"/ OR exp "DEMYELINATING AUTOIMMUNE DISEASES, CNS"/*	*17554*
27	*CINAHL*	*(25 OR 26)*	*40302*
28	*CINAHL*	*(electroretinogra* OR FERG).ti,ab,su*	*799*
29	*CINAHL*	*ELECTRORETINOGRAPHY/*	*568*
30	*CINAHL*	*(28 OR 29)*	*799*
31	*CINAHL*	*(27 AND 30)*	*17*

Search via NHS Healthcare Databases Advanced Search interface (https://hdas.nice.org.uk/) on 29/10/19:

CINAHL *1981 to present dataset (native interface – Ebsco)*
EMBASE *1974 to present dataset (native interface – Ovid)*
Medline *1946 to present dataset (native interface – Proquest)*

Appendix 1b. Literature Search Update 2022

Search via NHS Healthcare Databases Advanced Search interface (https://hdas.nice.org.uk/) on 01/02/2022:

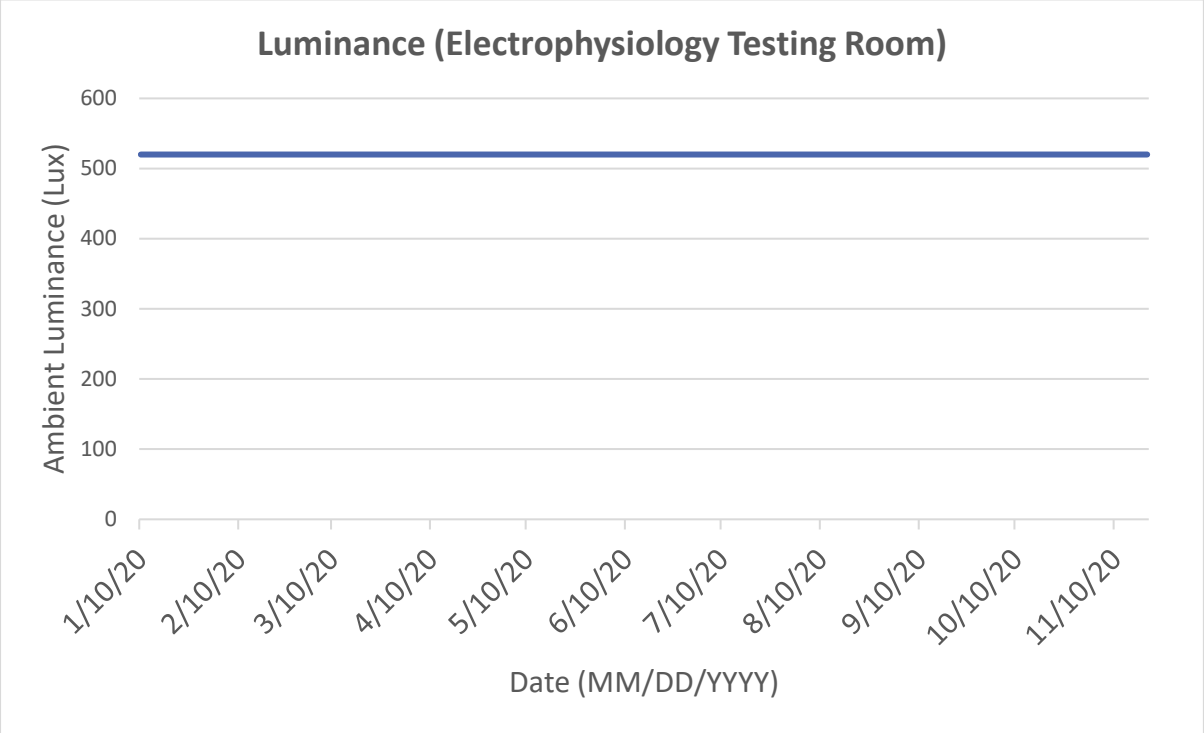
CINAHL *1981 to present dataset (native interface – Ebsco)*
EMBASE *1974 to present dataset (native interface – Ovid)*
Medline *1946 to present dataset (native interface – Proquest)*

#	Database	Search term	Results
1	EMBASE	(multiple sclerosis OR MS OR "clinically isolated syndrome" OR "neuromyelitis optics").ti,ab,if	541439
2	EMBASE	"DEMYELINATING DISEASE"/	15572
3	EMBASE	exp "DEMYELINATING DISEASE"/	176363
4	EMBASE	*"DEMYELINATING DISEASE"/	4973
5	EMBASE	"MULTIPLE SCLEROSIS, CHRONIC PROGRESSIVE"/	88431
6	EMBASE	exp "MULTIPLE SCLEROSIS, CHRONIC PROGRESSIVE"/	142784
7	EMBASE	*"MULTIPLE SCLEROSIS, CHRONIC PROGRESSIVE"/	59543
8	EMBASE	"MULTIPLE SCLEROSIS, RELAPSING-REMITTING"/	96411
9	EMBASE	exp "MULTIPLE SCLEROSIS, RELAPSING-REMITTING"/	142784
10	EMBASE	*"MULTIPLE SCLEROSIS, RELAPSING-REMITTING"/	64698
11	EMBASE	(1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10)	631564

12	*EMBASE*	*(electroretinogra* OR FERG).ti,ab,if*	*16456*
13	*EMBASE*	*exp ELECTRORETINOGRAM/*	*10314*
14	*EMBASE*	*(12 OR 13)*	*20924*
15	*EMBASE*	*(11 AND 14)*	*1005*
16	*EMBASE*	*15 [English language] [Humans]*	*617*
17	*Medline*	*(multiple sclerosis OR MS OR "clinically isolated syndrome" OR "neuromyelitis optica").ti,ab,if*	*424188*
18	*Medline*	*exp "MULTIPLE SCLEROSIS"/ OR exp "DEMYELINATING AUTOIMMUNE DISEASES, CNS"/*	*82059*
19	*Medline*	*(17 OR 18)*	*441464*
20	*Medline*	*(electroretinogra* OR FERG).ti,ab,if*	*13616*
21	*Medline*	*ELECTRORETINOGRAPHY/*	*17081*
22	*Medline*	*(20 OR 21)*	*20521*
23	*Medline*	*(19 AND 22)*	*611*
24	*Medline*	*23 [Languages English] [Humans]*	*351*
25	*CINAHL*	*(multiple sclerosis OR MS OR "clinically isolated syndrome" OR "neuromyelitis optica").ti,ab,su*	*52186*
26	*CINAHL*	*exp "MULTIPLE SCLEROSIS"/ OR exp "DEMYELINATING AUTOIMMUNE DISEASES, CNS"/*	*20966*

27	*CINAHL*	*(25 OR 26)*	*52659*
28	*CINAHL*	*(electroretinogra* OR FERG).ti,ab,su*	*1067*
29	*CINAHL*	*ELECTRORETINOGRAPHY/*	*743*
30	*CINAHL*	*(28 OR 29)*	*1067*
31	*CINAHL*	*(27 AND 30)*	*26*

Appendix 2. Room Luminance Measures



Appendix 3. Diagnosys Calibration Certificates 2019–22

Diagnosys UK Ltd
5 Trust Court
Histon
CB24 9PW
UK

ColorDome Calibration Certificate

Cal file name:	COLD831.CAL	**ILT1700:** 1
Serial number:	831	Customer Details:
Model number:	201	University Hospitals Plymouth
Firmware:	v1.90	Plymouth
Calibration date:	04/01/2019	
Signature:	LM	

ColorDome Calibration (Photopic White-6500K)			
LED Flash (cd.s/m2)	***Original***	***Calibrated***	***Pass***
0.01	0.11		✔
3	3.15		✔
10	10.53		✔
30	31.7		✔
Max Original: 102.3 New:	107.1		✔
LED Background (cd/m2)	***Original***	***Calibrated***	***Pass***
30	32.3		✔
100	106.2		✔
500	525		✔
Xenon Flash (cd.s/m2)	***Original***	***Calibrated***	***Pass***
3			✔
10			✔
30			✔
500			✔
1000			✔
Max Original: New:			✔

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UK

ColorDome Calibration Certificate

Description	*Pass*
Fixation LEDs (9 LEDS)	✔
Camera Image	✔
Camera IR LEDs	✔
Buzzer	✔
Adaptation Leds	✔

LED system check	*Bright*	*Dim*	*Low*	*Pass*
Red	✔	✔	✔	✔
Blue	✔	✔	✔	✔
Green	✔	✔	✔	✔
Amber	✔	✔	✔	✔

General comments (e.g. conditions of cables....)

N/M=Not measured, N/A=Not applicable or measurable

DOC # 13528 REV B Date 23 Mar 2012

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UK

Pattern Calibration Certificate

Cal file name: PSG206G.CAL	ILT1700: 1
Pattern (PSG) Serial number: interna;	University Hopsital of Plymouth
Model number:	Plymouth
Firmware:	
Calibration date: 04/01/2019	
Signature: LM	

Lum	White		
Cd/m2	Original	Calibrated	Pass
10	10.13		✔
20	20.2		✔
50	50.8		✔
100	102.5		✔
Max	120/121.9		✔

Lum	Red		
Cd/m2	Original	Calibrated	Pass
5	5.1		✔
10	10.27		✔
15	15.33		✔
20	20.8		✔
Max	23/24.1		✔

Lum	Green		
Cd/m2	Original	Calibrated	Pass
5	5.34		✔
10	10.58		✔
20	21.1		✔
50	52.0		✔
Max	86/89.5		✔

Lum	Blue		
Cd/m2	Original	Calibrated	Pass
1	1.09		✔
2	2.1		✔
5	5.17		✔
10	10.44		✔
Max	13/13.36		✔

General comments (e.g. conditions of cables....)

Brightness: 70%

Contrast: 100%

Color Temp: USER

Interline Width: 410

N/M = Not Measured, N/A=Not applicable or measurable

DOC # 13531 REV B Date 23 Mar 2012

Diagnosys UK Ltd
5 Trust Court
Histon
CB24 9PW
UK

ColorDome Calibration Certificate

Cal file name:	COLD831.cal	**ILT1700:** 1
Serial number:	831	Customer Details:
Model number:	201	Plymouth
Firmware:	1.9	Nicola Broomfield
Calibration date:	14-Apr-2021	
Signature:	LM	

ColorDome Calibration (Photopic White-6500K)			
LED Flash (cd.s/m2)	***Original***	***Calibrated***	***Pass***
0.01	0.01		✔
3	3.04		✔
10	10.13		✔
30	30.5		✔
Max	102 / 103	/	✔
LED Background (cd/m2)	***Original***	***Calibrated***	***Pass***
30	31.6		✔
100	102.2		✔
500	506		✔
Xenon Flash (cd.s/m2)	***Original***	***Calibrated***	***Pass***
3			✔
10			✔
30			✔
500			✔
1000			✔
Max =			✔

DOC # 13528 REV B Date 23 Mar 2012

Diagnosys UK Ltd
5 Trust Court
Histon
CB24 9PW
UK

ColorDome Calibration Certificate

Description	*Pass*
Fixation LEDs (9 LEDS)	✔
Camera Image	✔
Camera IR LEDs	✔
Buzzer	✔
Adaptation Leds	✔

LED system check	*Bright*	*Dim*	*Low*	*Pass*
Red	✔	✔	✔	✔
Blue	✔	✔	✔	✔
Green	✔	✔	✔	✔
Amber	✔	✔	✔	✔

Xenon Tube	*Value*	*Pass*
Low Tube - Low Cap		
Low Tube - High Cap		
Low Tube - Time		
Mid Tube - Low Cap		
Mid Tube - High Cap		
Mid Tube - Time		
High Tube - Low Cap		
High Tube - High Cap		
High Tube - Time		

General comments (e.g. conditions of cables....)

N/M=Not measured, N/A=Not applicable or measurable

DOC # 13528 REV B Date 23 Mar 2012

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Pattern Calibration Certificate

Cal file name: PSG166.cal	Customer Details:
Pattern (PSG) Serial number: 166	Plymouth
Model number: GW MP16B 350002420	Nicola Broomfield
Firmware:	
Calibration date: 14-Apr-21	
Signature: LM	

Lum	White		
Cd/m2	Original	Calibrated	Pass
10	10.0		✔
20	19.8		✔
50	47.8		✔
100	96.4		✔
Max	120/112		✔

Lum	Red		
Cd/m2	Original	Calibrated	Pass
5	5.37		✔
10	10.4		✔
15	15.2		✔
20	20.8		✔
Max	24/23.5		✔

Lum	Green		
Cd/m2	Original	Calibrated	Pass
5	5.47		✔
10	10.9		✔
20	20.9		✔
50	49.5		✔
Max	86/83.9		✔

Lum	Blue		
Cd/m2	Original	Calibrated	Pass
1	1.1		✔
2	2.2		✔
5	5.3		✔
10	10.49		✔
Max	13/12.9		✔

General comments (e.g. conditions of cables....)

Brightness: 65%

Contrast: 100%

Color Temp: USER

Interline Width:

N/M = Not Measured, N/A=Not applicable or measurable

DOC # 13531 REV B Date 23 Mar 2012

Diagnosys UK Ltd
5 Trust Court
Histon
CB24 9PW
UK

ColorDome Calibration Certificate

Cal file name:	COLD831.cal	**ILT1700:**1
Serial number:	831	Customer Details:
Model number:	201	Plymouth University Hospital
Firmware:	1.9	Nicola Broomfield
Calibration date:	31-May-2022	
Signature:	LM	

ColorDome Calibration (Photopic White-6500K)			
LED Flash (cd.s/m2)	***Original***	***Calibrated***	***Pass***
0.01	0.01		✔
3	3.0		✔
10	10.0		✔
30	30.1		✔
Max	102 / 101.9	/	✔
LED Background (cd/m2)	***Original***	***Calibrated***	***Pass***
30	31.1		✔
100	100.8		✔
500	499		✔
Xenon Flash (cd.s/m2)	***Original***	***Calibrated***	***Pass***
3			✔
10			✔
30			✔
500			✔
1000			✔
Max =			✔

Diagnosys UK Ltd
5 Trust Court
Histon
CB24 9PW
UK

ColorDome Calibration Certificate

Description	*Pass*
Fixation LEDs (9 LEDS)	✔
Camera Image	✔
Camera IR LEDs	✔
Buzzer	✔
Adaptation Leds	✔

LED system check	*Bright*	*Dim*	*Low*	*Pass*
Red	✔	✔	✔	✔
Blue	✔	✔	✔	✔
Green	✔	✔	✔	✔
Amber	✔	✔	✔	✔

Xenon Tube	*Value*	*Pass*
Low Tube - Low Cap		
Low Tube - High Cap		
Low Tube - Time		
Mid Tube - Low Cap		
Mid Tube - High Cap		
Mid Tube - Time		
High Tube - Low Cap		
High Tube - High Cap		
High Tube - Time		

General comments (e.g. conditions of cables....)

N/M=Not measured, N/A=Not applicable or measurable

DOC # 13528 REV B Date 23 Mar 2012

diagnosys
leading the wave

Pattern Calibration Certificate

Cal file name: PSG166.cal	Customer Details:
Pattern (PSG) Serial number: 166	Plymouth University Hospital
Model number: GW MP16B 350002420	Nicola Broomfield
Firmware:	
Calibration date: 31-May-22	
Signature: LM	

Lum	White		
Cd/m2	Original	Calibrated	Pass
10	11.2	10.0	✔
20	21.8	19.7	✔
50	52.4	48.5	✔
100	105.9	99.6	✔
Max	120/119	123/120	✔

Lum	Red		
Cd/m2	Original	Calibrated	Pass
5	4.17	4.9	✔
10	9.4	9.86	✔
15	14.4	14.6	✔
20	20.3	19.8	✔
Max	23/23.2	24/24.3	✔

Lum	Green		
Cd/m2	Original	Calibrated	Pass
5	5.8	4.88	✔
10	11.18	9.77	✔
20	21.6	19.8	✔
50	51.3	49.2	✔
Max	86/86.4	89/88.2	✔

Lum	Blue		
Cd/m2	Original	Calibrated	Pass
1	0.8	0.99	✔
2	1.7	1.98	✔
5	3.6	4.93	✔
10	9.0	10.28	✔
Max	13/11.2	13/13.2	✔

General comments (e.g. conditions of cables....)

Brightness: 65%

Contrast: 100%

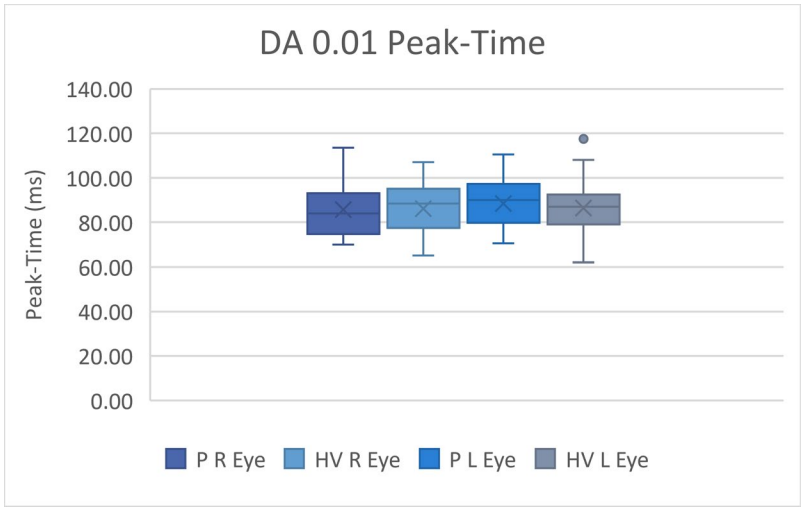
Color Temp: USER

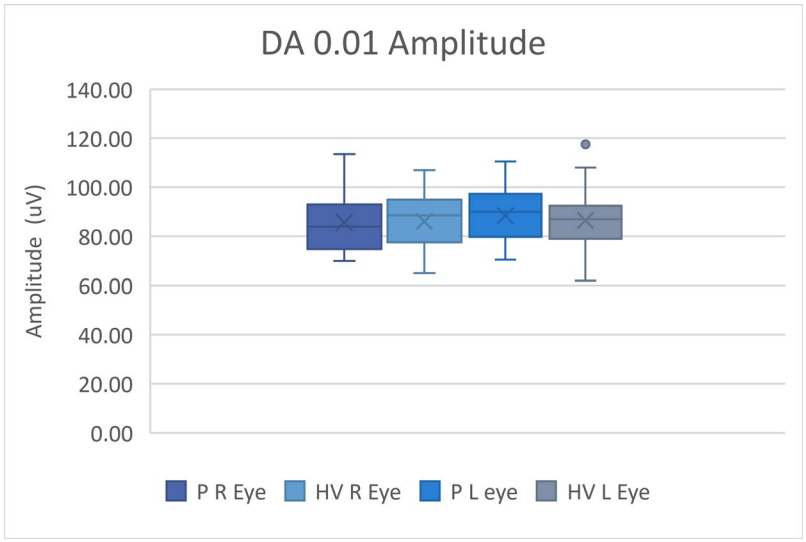
Interline Width:

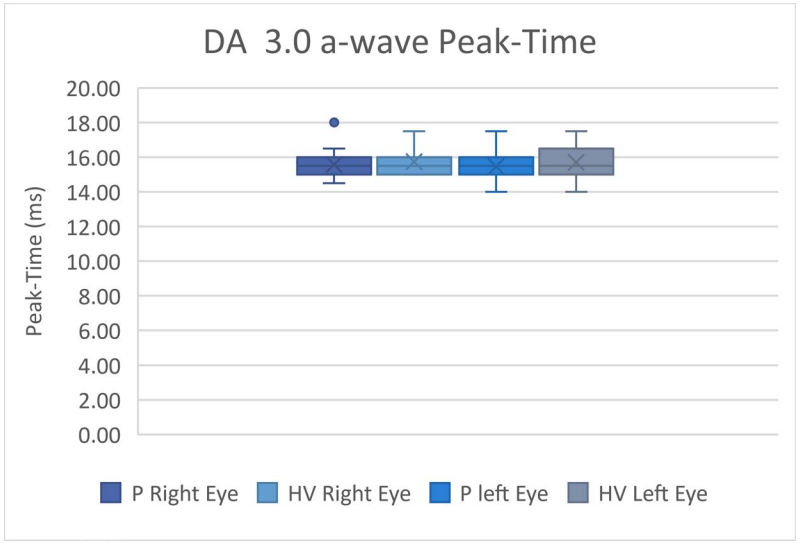
N/M = Not Measured, N/A=Not applicable or measurable

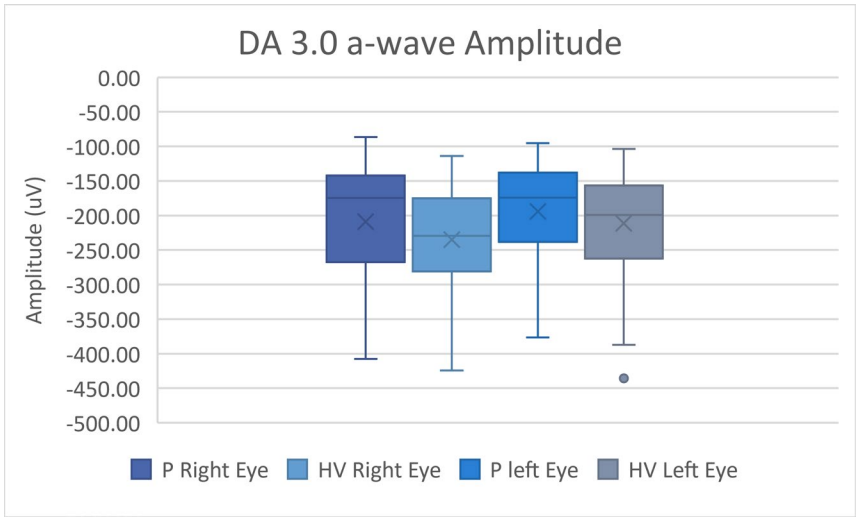
DOC # 13531 REV B Date 23 Mar 2012

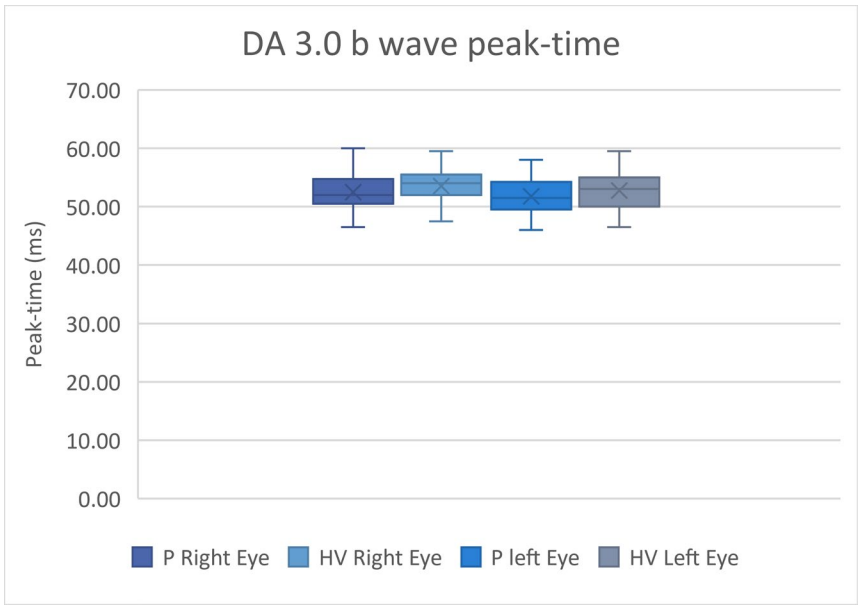
Appendix 4 Additional Graphs (not included in the main body)

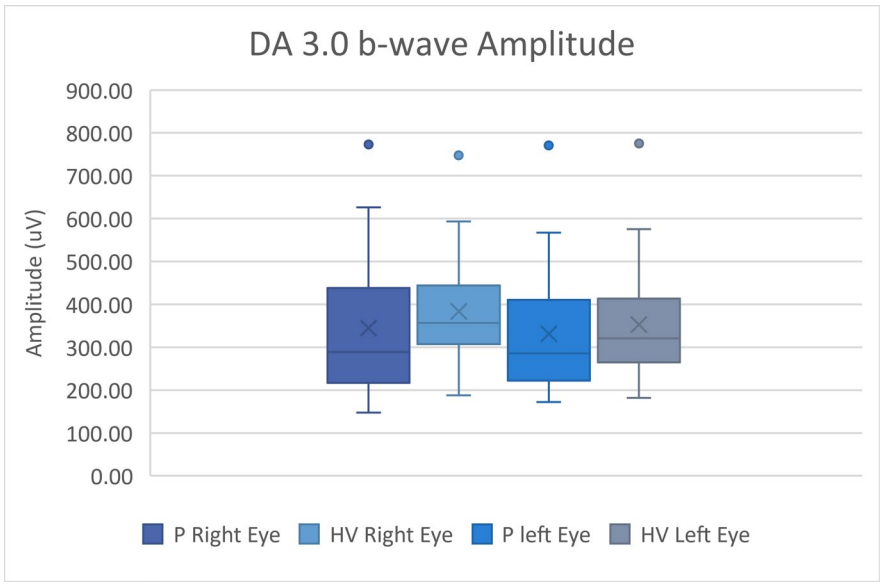


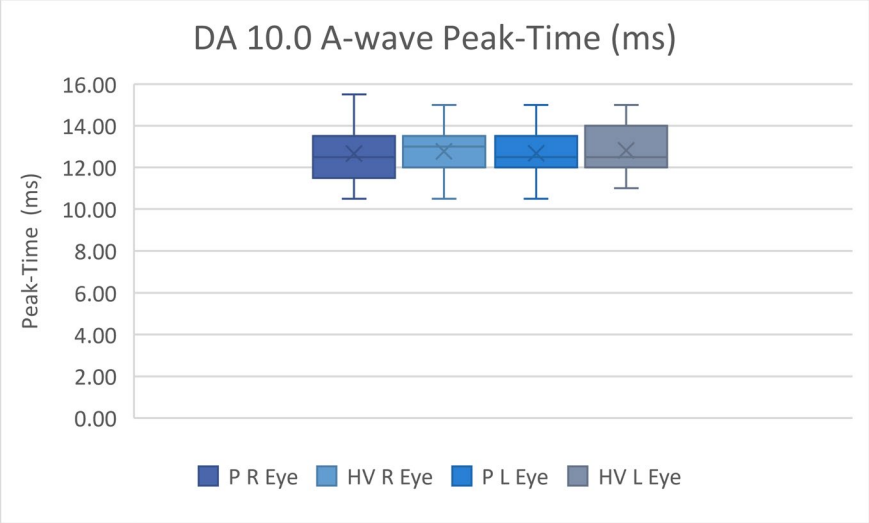


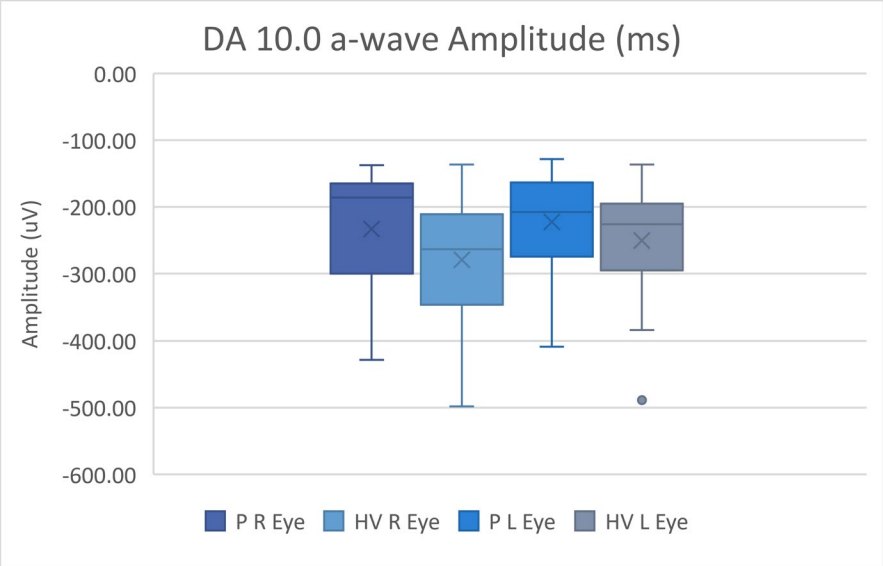


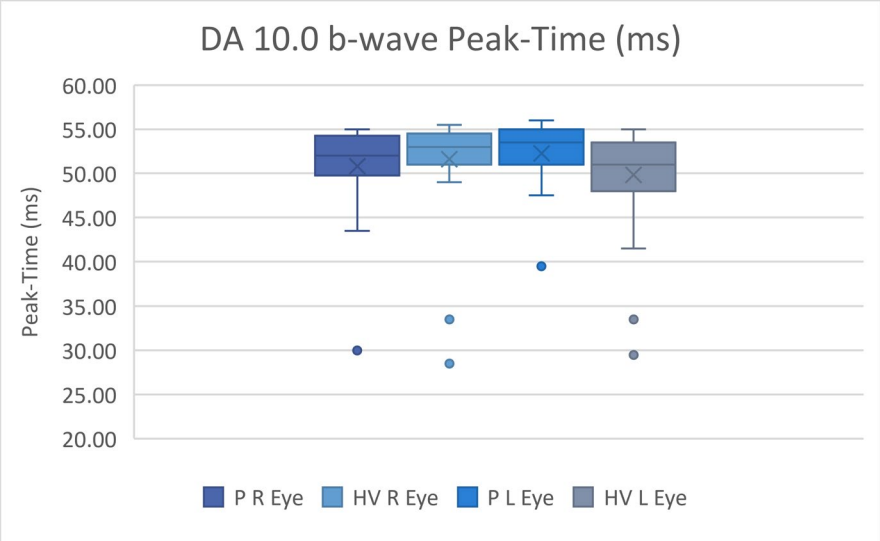

DA 3.0 a-wave Amplitude
0.00
-50.00
-100.00
-150.00
-200.00
-250.00
-300.00
-350.00
-400.00
-450.00
-500.00
Amplitude (uV)
P Right Eye
HV Right Eye
P left Eye
HV Left Eye

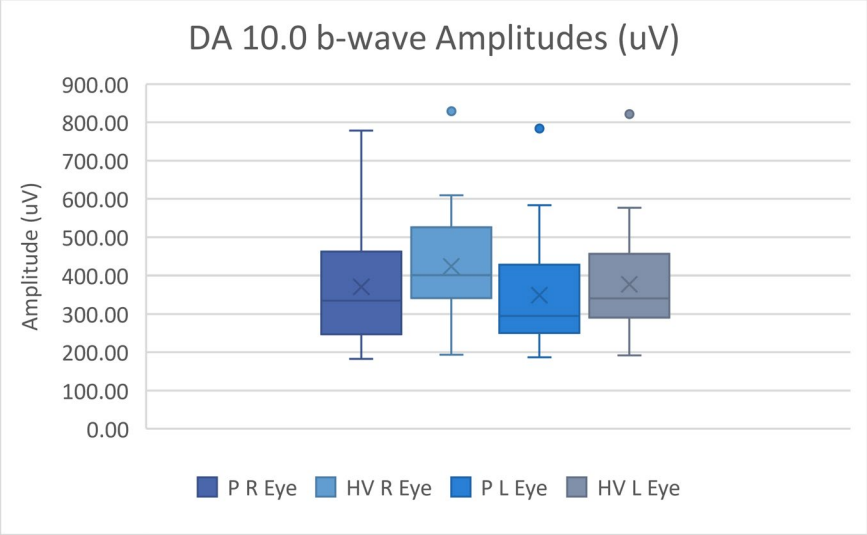

DA 3.0 b wave peak-time
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Peak-time (ms)
P Right Eye
HV Right Eye
P left Eye
HV Left Eye

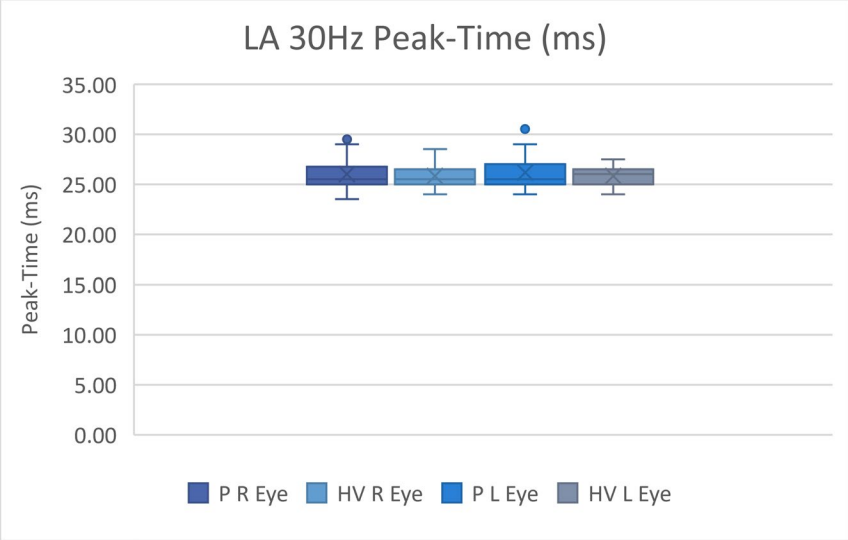

DA 3.0 b-wave Amplitude
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
Amplitude (uV)
P Right Eye
HV Right Eye
P left Eye
HV Left Eye

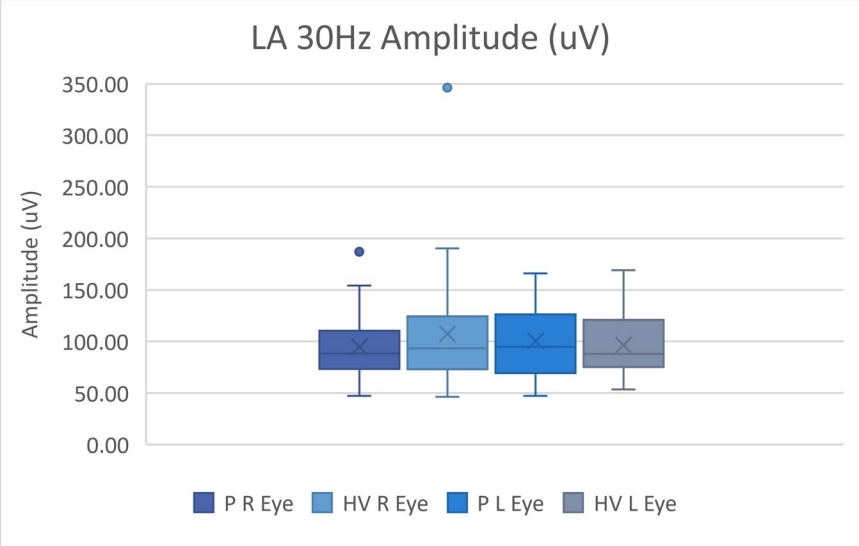

DA 10.0 A-wave Peak-Time (ms)
Peak-Time (ms)
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
P R Eye
HV R Eye
P L Eye
HV L Eye

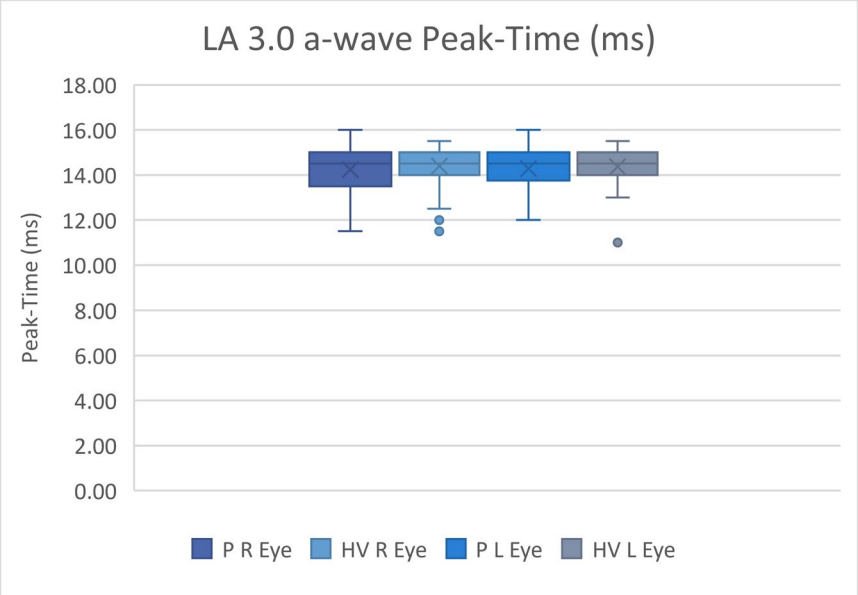

DA 10.0 a-wave Amplitude (ms)
Amplitude (uV)
0.00
-100.00
-200.00
-300.00
-400.00
-500.00
-600.00
P R Eye
HV R Eye
P L Eye
HV L Eye

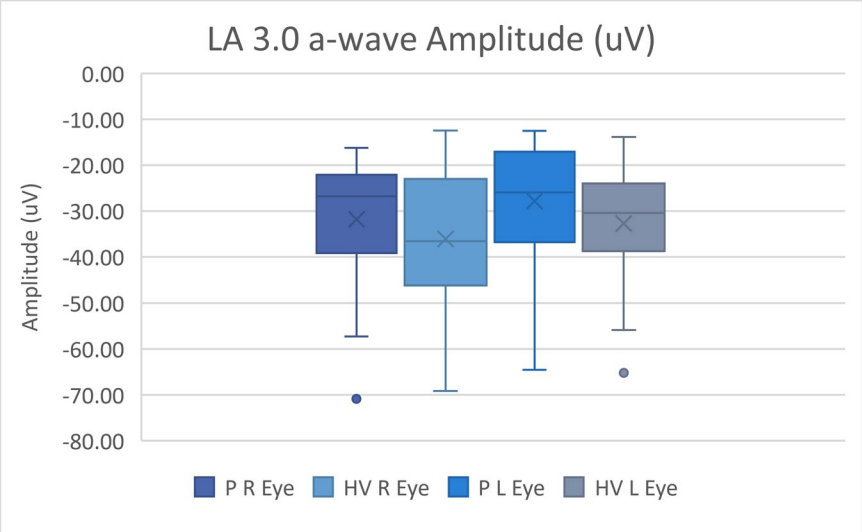

DA 10.0 b-wave Peak-Time (ms)
Peak-Time (ms)
60.00
55.00
50.00
45.00
40.00
35.00
30.00
25.00
20.00
P R Eye
HV R Eye
P L Eye
HV L Eye

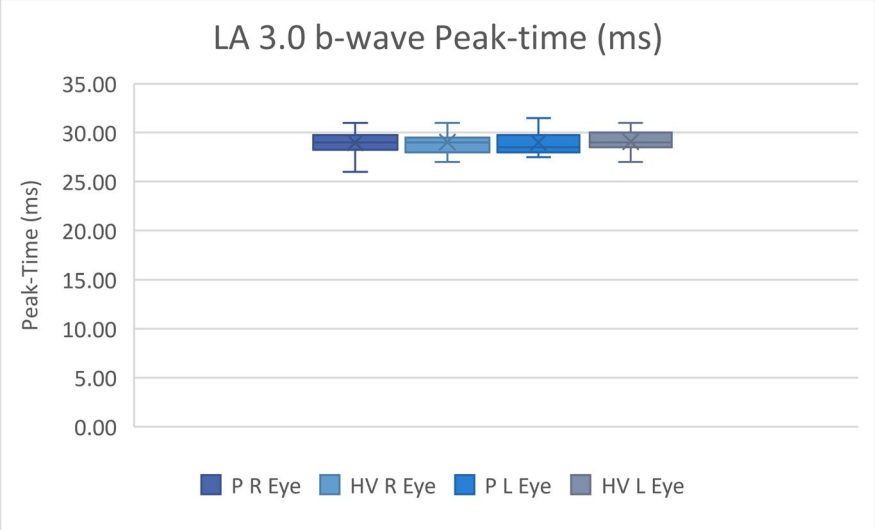

DA 10.0 b-wave Amplitudes (uV)
Amplitude (uV)
900.00
800.00
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
P R Eye
HV R Eye
P L Eye
HV L Eye

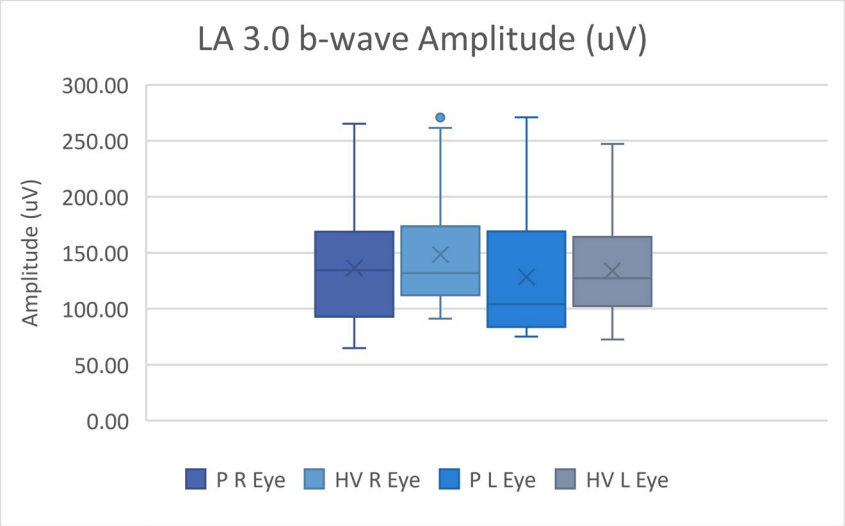

LA 30Hz Peak-Time (ms)
Peak-Time (ms)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
P R Eye
HV R Eye
P L Eye
HV L Eye


LA 30Hz Amplitude (uV)
Amplitude (uV)
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
P R Eye
HV R Eye
P L Eye
HV L Eye


LA 3.0 a-wave Peak-Time (ms)
Peak-Time (ms)
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
P R Eye
HV R Eye
P L Eye
HV L Eye


LA 3.0 a-wave Amplitude (uV)
Amplitude (uV)
0.00
-10.00
-20.00
-30.00
-40.00
-50.00
-60.00
-70.00
-80.00
P R Eye
HV R Eye
P L Eye
HV L Eye


LA 3.0 b-wave Peak-time (ms)
Peak-Time (ms)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
P R Eye
HV R Eye
P L Eye
HV L Eye


LA 3.0 b-wave Amplitude (uV)
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Amplitude (uV)
P R Eye
HV R Eye
P L Eye
HV L Eye

Appendix 5: Recruitment documents

University Hospitals Plymouth NHS Trust

Healthy Volunteer Consent Form
Structure and function of the retina in Multiple Sclerosis

Principal Investigator: Nicola Broomfield

Participant Identification Number for study

Please initial each statement as appropriate	
1. I confirm that I have read the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	Please Initial
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected	Please Initial
3. I understand that I am not consenting for the research team to access my medical records.	Please Initial
4. I agree to take part in the above study	Please Initial

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person receiving Consent	Date	Signature
Time of Consent (24hr clock)	_____ : _____	

1 copy for participant and original copy for researcher site file.

Healthy Volunteer Information Sheet

Structure and function of the retina in Multiple Sclerosis

You are being invited to take part in a research study as part of an educational doctorate programme (DClinSci). Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully, and discuss it with others if you wish.

Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?

This study aims to investigate how the eyes of patients suspected of having multiple sclerosis work in both light and dark conditions and compare them to healthy volunteers. Multiple sclerosis is a condition that is known to affect the optic nerves that take information between the eye and the brain. What is not known, is the possible effects on the retinas at the back of the eye.

2. Why have I been invited?

You have been invited to participate in this study because you have no known conditions that would affect the study, these are; significant shortsightedness (myopia) that requires a high prescription, co-existing neurological disease, diabetes, history of glaucoma, untreated cataract or symptoms of optic neuritis within the last 12 weeks.

We plan to include 35 participants with suspected multiple sclerosis from University Hospitals Plymouth NHS Trust and 35 healthy volunteers. We will compare the responses of the eye to flashes of light and images of the retinas of the eyes to look for differences that may be associated with the disease that are not present in healthy volunteers.

Should there be any abnormal findings that require clinical input you will be referred back to your GP.

3. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form to confirm that you understand what is involved when taking part in this study. If you decide to take part you are free to leave the study at any time and without giving a reason. If you withdraw, unless you object, we will still keep records relating to the treatment given to you, as this is valuable to the study. A decision to withdraw at any time, or a decision not to take part, will not affect the quality of care you receive.

4. What will happen to me if I take part?

If you agree to participate in the study, you will be asked to sign the Informed Consent Form and be given a copy of this information sheet to take away and refer to later.

The tests use flashes of light to record the function of your eyes in both light and dark conditions. These are tests that we perform routinely, but normally in different circumstances. Much of the time required for extra tests is spent waiting for your eyes to adjust to the darkness (20 minutes), the actual tests are very quick to perform. In total these tests will take 45 minutes. In addition, you will also have some pictures taken of the back of the eye which will take approximately 15 minutes. You will not be required to come back again once all the tests are done.

5. What do I have to do?

In order to perform the tests, it will be necessary to dilate your pupils with eye drops called Tropicamide. This will mean that you cannot drive home after the tests and your vision will be blurred for up to several hours after the tests.

6. What is the procedure that is being performed?

The additional tests are called 'flash electroretinograms' which are the eyes response to flashes of light and need to be performed in light and dark conditions. The imaging is called 'optical coherence tomography' which is a picture of the retina at the back of the eye.

7. What are the side effects of any treatment received when taking part?

Closed angle glaucoma is an uncommon condition that affects less than 1% of the population. It is most common in adults over the age of 60 years and you may have it without knowing. This condition can cause the following symptoms when pupils are dilated:

- *severe eye and head pain*
- *nausea or vomiting*

This can be serious and lead to long term loss of vision if not treated immediately. For this reason, a trained ophthalmologist will inspect your eye when the images are taken to make sure that you don't have the condition before the dilating drops are given. This is a precaution to ensure your safety.

If you have a family history of closed angle glaucoma, you will not be able to participate.

There are no risks or side effects associated with the imaging tests.

If you do decide to take part in the study, you must report any problems you have to the healthcare scientist. Any adverse reaction is immediate and you will be taken to the eye infirmary for treatment. There is also a contact number given at the end of this information sheet for you to phone if you become worried at any time. In the unlikely event of an emergency occurring during the conduct of the study, we may contact your nominated next of kin.

8. What are the possible disadvantages and risks of taking part?

Participation in the study will require your time in order to undertake the additional tests (approximately 45 to 60 minutes). We will also have to dilate your pupils which can be uncomfortable when you go outside, especially on bright and sunny days (sunglasses may help). This may last for several hours after testing, during which, you cannot drive or undertake tasks that require normal vision.

Unfortunately, we cannot pay you for agreeing to take part.

9. *What are the possible benefits of taking part?*

As a normal healthy volunteer, this study will not directly help you. However, information collected as part of your participation in this study may benefit patients with multiple sclerosis, in the future.

10. *What if there is a problem?*

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you have any concerns about any aspect of this study you should speak to your study doctor who will do their best to answer your questions.

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during this study, you can do this through the NHS complaints procedure. In the first instance it may be helpful to contact the Patient Advice and Liaison Service (PALS) at your hospital, contact details can be found at the end of this information sheet.

11. *Will my taking part in this study be kept confidential?*

Yes. All the information about your participation in this study will be kept confidential. If you consent to take part, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital, University Hospitals Plymouth

NHS Trust, under the provisions of the 2018 Data Protection Act. Your name will not be passed to anyone else outside the research team or the sponsor, who is not involved in the study. You will be allocated a study number, which will be used as a code to identify you on all study forms and your name will only feature on the Informed Consent Form.

Your records will be available to people authorised to work on the study but may also need to be made available to people authorised by the Research Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. A copy of your consent form may be sent to the Research Sponsor during the course of the study. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

In line with Trust policy, at the end of the study, your data will be securely archived for a minimum of 5 years. Arrangements for confidential destruction will then be made. 12.

How will we use information about you?

University Hospitals Plymouth NHS Trust is the sponsor for this study and is based in the United Kingdom. We will need to use information from you and from your medical records for this research project.

This information will include your initials, NHS number, name, contact details and study number. People will use this information to do the research or to check your records to make sure that the research is being done properly.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

13. *What are your choices about how your information is used?*

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

If you choose to stop taking part in the study, we would like to continue collecting information about your health from your hospital. If you do not want this to happen, tell us and we will stop.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

14. *Where can you find out more about how your information is used?*

You can find out more about how we use your information

- *at https://www.hra.nhs.uk/information-about-patients/*
- *by asking one of the research team, or*
- *by visiting https://www.plymouthhospitals.nhs.uk/privacy-notice-for-patients-*

15. *What if new information becomes available?*

Sometimes during the course of a clinical trial, new information becomes available on the drugs that are being studied. If this happens, we will tell you about it and discuss with you whether you want to or should continue in the study. If you decide to withdraw, we will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

On receiving new information, we might consider it to be in your best interests to withdraw you from the study. If so, we will explain the reasons and arrange for your care to continue.

If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

16. *What will happen if I don't want to carry on with the study?*

If you decide you do not want to carry on with the study you may withdraw at any time and without giving a reason (although we may ask you for a reason, to help us design better studies for the future, it is up to you whether you are happy to supply a reason or not). If you withdraw, we will still keep records relating to the treatment given to you, as this is valuable to the study and your safety. A decision to withdraw at any time, or a decision not to take part, will not affect the quality of care you receive.

17. *Will the study information help with other research projects?*

It is important that good quality research data can be shared with others in order to advance clinical research and to benefit patients in the future. After the end of the study, de-identified information collected during the study may be made available to other researchers under an appropriate data sharing agreement, but it will not be possible to identify you or your family personally from any information shared.

18. *What will happen to the results of this study?*

The results of the study will be available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the patients or volunteers involved in the study will be identified in any report or publication.

Should you wish to see the results, or the publication, please ask your study scientist.

19. *Who is organising and funding this study?*

The study is being funded by Health Education England as part of a doctorate research project.

20. *Who has reviewed the study?*

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Health Research Authority, the Research Ethics Committee and the Research Development and Innovation team at Derriford Hospital.

21. *Further information and contact details*

You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your healthcare scientist or study doctor, who will be able to provide you with up to date information about the procedures involved. If you wish to read the research on which this study is based, please ask your healthcare scientist or doctor.

Doctor: *Professor Hobart* *Healthcare Scientist: Nicola Broomfield*

Tel. Number: *01752 437612* *Tel. Number: 01752 437986*

If you have concerns while on the study

Whilst it is something we hope will not happen, if you have concerns about any aspect of research please speak to the researchers using the contact details above. Alternatively, you may wish to contact the hospital's Patient Advice and Liaison Service (PALS) who offers support, information and assistance to patients, relatives and visitors.

PALS can be contacted at:

Patient Advice & Liaison Service

Level 7

Derriford Hospital

Plymouth

PL6 8DH

Tel: 01752 439884

Email: plh-tr.PALS@nhs.net

If you decide you would like to take part then please read and sign and date the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this information sheet and to consider this study.

Participant Consent Form
Structure and function of the retina in Multiple Sclerosis

Principal Investigator: Nicola Broomfield

Participant Identification Number for study

Please initial each statement as appropriate	
1. I confirm that I have read the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	Please Initial
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected	Please Initial
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from University Hospitals Plymouth NHS Trust, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	Please Initial
4. I agree to take part in the above study	Please Initial

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person receiving Consent	Date	Signature
Time of Consent (24hr clock)	_____ : _____	

1 copy for participant; 1 copy in medical notes and original copy for researcher site file.

Participant Information Sheet

Structure and function of the retina in Multiple Sclerosis

You are being invited to take part in a research study as part of an educational doctorate programme (DClinSci). Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully, and discuss it with others if you wish.

Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?

This study aims to investigate how the eyes of patients suspected of having multiple sclerosis work in both light and dark conditions. These tests will be in addition to the evoked potential tests that you will have as part of your normal care, to look in greater detail at the function to your eyes.

2. Why have I been invited?

You have been invited to participate in this study because you are being investigated for a condition that is known to affect the optic nerves that take information between the eye and the brain. What is not known, is the possible effects on the retinas at the back of the eye. This is what will be investigated.

We plan to include 35 participants with suspected multiple sclerosis from University Hospitals Plymouth NHS Trust and 35 healthy volunteers. We will compare the responses of

the eye to flashes of light and images of the retinas of the eyes to look for differences that may be associated with the disease that are not present in healthy volunteers.

Should there be any abnormal findings that require clinical input you will be referred back to your GP.

3. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form to confirm that you understand what is involved when taking part in this study. If you decide to take part you are free to leave the study at any time and without giving a reason. If you withdraw, unless you object, we will still keep records relating to the treatment given to you, as this is valuable to the study. A decision to withdraw at any time, or a decision not to take part, will not affect the quality of care you receive

4. What will happen to me if I take part?

If you agree to participate in the study, you will be asked to sign the Informed Consent Form and be given a copy of this information sheet to take away and refer to later.

The tests use flashes of light to record the function of your eyes in both light and dark conditions. These are tests that we perform routinely, but normally in different circumstances. Much of the time required for extra tests is spent waiting for your eyes to adjust to the darkness (20 minutes); the actual tests are very quick to perform. In total these tests will take 45 minutes. In addition, you will also have some pictures taken of the back of the eye which will take approximately 15 minutes. You will not be required to come back again once all the tests are done. Your medical records will be viewed at a later date, by a member of the research team to check your diagnosis.

5. What do I have to do?

You will have the choice of having the additional tests performed at the time of attending for the routine evoked potential tests, which are detailed on your appointment letter. However, if this is not convenient then an alternative time will be arranged. If you would rather come back at an alternative time, we will reimburse you for any travel costs incurred (you will need

to provide receipts). The additional tests will take approximately 45 - 60 minutes in total. In order to perform the additional tests, it will be necessary to dilate your pupils with eye drops called Tropicamide. This will mean that you cannot drive home after the tests and your vision will be blurred for up to several hours after the tests.

6. What is the procedure that is being performed?

The additional tests are called 'flash electroretinograms' which are the eyes response to flashes of light and need to be performed in light and dark conditions. The imaging is called 'optical coherence tomography' which is a picture of the retina at the back of the eye.

7. What are the side effects of any treatment received when taking part?

Closed angle glaucoma is an uncommon condition that affects less than 1% of the population. It is most common in adults over the age of 60 years and you may have it without knowing. This condition can cause the following symptoms when pupils are dilated:

- *severe eye and head pain*
- *nausea or vomiting*

This can be serious and lead to long term loss of vision if not treated immediately. For this reason, a trained ophthalmologist will inspect your eye when the images are taken to make sure that you don't have the condition before the dilating drops are given. This is a precaution to ensure your safety.

If you have a family history of closed angle glaucoma, you will not be able to participate.

There are no risks or side effects associated with the imaging tests.

If you do decide to take part in the study, you must report any problems you have to the healthcare scientist. Any adverse reaction is immediate and you will be taken to the eye infirmary for treatment. There is also a contact number given at the end of this information sheet for you to phone if you become worried at any time. In the unlikely event of an emergency occurring during the conduct of the study, we may contact your nominated next of kin.

8. *What are the possible disadvantage\s and risks of taking part?*

Participation in the study will require your time, in order to undertake the additional tests (approximately 45 to 60 minutes). We will also have to dilate your pupils which can be uncomfortable when you go outside, especially on bright and sunny days (sunglasses may help). This may last for several hours after testing, during which, you cannot drive or undertake tasks that require normal vision.

Unfortunately, we cannot pay you for agreeing to take part.

9. *What are the possible benefits of taking part?*

We cannot promise the study will help you but the information we get might help improve the treatment of people with multiple sclerosis.

There is no guarantee that you will benefit from taking part in this study. However, information collected as part of your participation in this study may benefit patients with multiple sclerosis, in the future.

10. *What if there is a problem?*

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. If you have any concerns about any aspect of this study you should speak to your study doctor who will do their best to answer your questions.

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during this study, you can do this through the NHS complaints procedure. In the first instance it may be helpful to contact the Patient Advice and Liaison Service (PALS) at your hospital, contact details can be found at the end of this information sheet.

11. Will my taking part in this study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. If you consent to take part, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital, University Hospitals Plymouth NHS Trust, under the provisions of the 2018 Data Protection Act. Your name will not be passed to anyone else outside the research team or the sponsor, who is not involved in the study. You will be allocated a study number, which will be used as a code to identify you on all study forms and your name will only feature on the Informed Consent Form.

Your records will be available to people authorised to work on the study but may also need to be made available to people authorised by the Research Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. A copy of your consent form may be sent to the Research Sponsor during the course of the study. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

In line with Trust policy, at the end of the study, your data will be securely archived for a minimum of 5 years. Arrangements for confidential destruction will then be made.

With your permission, your neurologist who will be treating you will be notified that you are taking part in this study.

12. How will we use information about you?

University Hospitals Plymouth NHS Trust is the sponsor for this study and is based in the United Kingdom. We will need to use information from you and from your medical records for this research project.

This information will include your initials, NHS number, name, contact details and study number. People will use this information to do the research or to check your records to make sure that the research is being done properly.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

13. *What are your choices about how your information is used?*

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

If you choose to stop taking part in the study, we would like to continue collecting information about your health from your hospital. If you do not want this to happen, tell us and we will stop.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

14. *Where can you find out more about how your information is used?*

You can find out more about how we use your information

- *at https://www.hra.nhs.uk/information-about-patients/*
- *by asking one of the research team, or*
- *by visiting https://www.plymouthhospitals.nhs.uk/privacy-notice-for-patients-*

15. *What if new information becomes available?*

Sometimes during the course of a clinical trial, new information becomes available on the drugs that are being studied. If this happens, we will tell you about it and discuss with you whether you want to or should continue in the study. If you decide to withdraw, we will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

On receiving new information, we might consider it to be in your best interests to withdraw you from the study. If so, we will explain the reasons and arrange for your care to continue.

If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

16. *What will happen if I don't want to carry on with the study?*

If you decide you do not want to carry on with the study you may withdraw at any time and without giving a reason (although we may ask you for a reason, to help us design better studies for the future, it is up to you whether you are happy to supply a reason or not). If you withdraw, we will still keep records relating to the treatment given to you, as this is valuable to the study and your safety. A decision to withdraw at any time, or a decision not to take part, will not affect the quality of care you receive

17. *Will the study information help with other research projects?*

It is important that good quality research data can be shared with others in order to advance clinical research and to benefit patients in the future. After the end of the study, de-identified information collected during the study may be made available to other researchers under an appropriate data sharing agreement, but it will not be possible to identify you or your family personally from any information shared.

18. *What will happen to the results of this study?*

The results of the study will be available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the patients or volunteers involved in the study will be identified in any report or publication.

Should you wish to see the results, or the publication, please ask your study scientist.

19. *Who is organising and funding this study?*

The study is being funded by Health Education England as part of a doctorate research project.

20. *Who has reviewed the study?*

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Health Research Authority, the Research Ethics Committee and the Research Development and Innovation team at Derriford Hospital.

21. *Further information and contact details*

You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your healthcare scientist or study doctor, who will be able to provide you with up to date information about the procedures involved. If you wish to read the research on which this study is based, please ask your healthcare scientist or doctor.

Doctor: *Professor Hobart*	*Healthcare Scientist: Nicola Broomfield*
Tel. Number: *01752 437612*	*Tel. Number: 01752 437986*

If you have concerns while on the study

Whilst it is something we hope will not happen, if you have concerns about any aspect of research please speak to the researchers using the contact details above. Alternatively, you may wish to contact the hospital's Patient Advice and Liaison Service (PALS) who offers support, information and assistance to patients, relatives and visitors.

PALS can be contacted at:

Patient Advice & Liaison Service

Level 7

Derriford Hospital

Plymouth

PL6 8DH

Tel: 01752 439884

Email: plh-tr.PALS@nhs.net

If you decide you would like to take part then please read and sign and date the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the

consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this information sheet and to consider this study.